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Fifth National Symposium on Stand Establishment

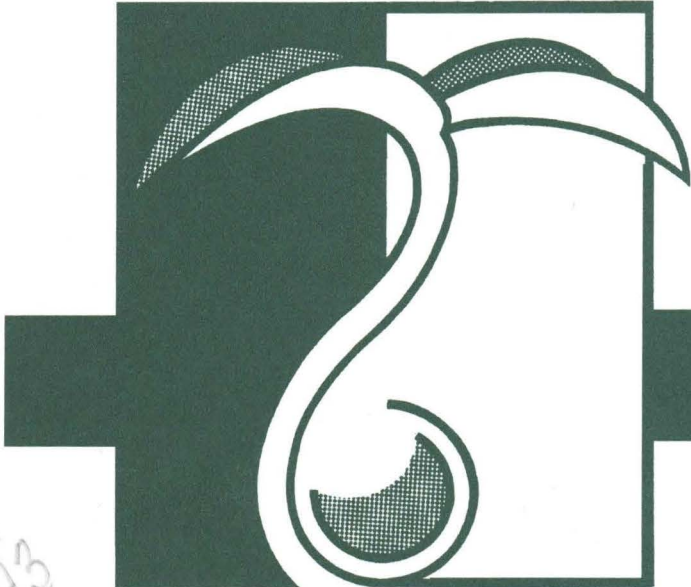


June 1 - 4, 1997
Holiday Inn on the Lane
Columbus, Ohio

Hosted by
Department of Horticulture and Crop Science
The Ohio State University, Columbus

Includes manuscripts from Transplant
Workshop and 1997 Symposium

Mark A. Bennett and James D. Metzger, editors
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SYMPOSIUM PROGRAM ORDER

FIFTH NATIONAL SYMPOSIUM ON STAND ESTABLISHMENT

**Holiday Inn on the Lane
June 1-4, 1997 - Columbus, Ohio**

All Sessions to be Held in Salon D

SUNDAY, JUNE 1, 1997: 2:00 - 5:00 PM

Welcome and Plenary Session:

Moderators: Miller McDonald and Mark Bennett

- 2:00 - 2:10** **Ohio Agriculture**
Bob Moser, Vice President and Dean of the College of Food, Agricultural and Environmental Sciences
- 2:10 - 2:20** **The Ohio Agricultural Research and Development Center**
Tom Payne, Director of OARDC
- 2:20 - 2:30** **Stand Establishment Research at OSU**
Luke Waters, Chair, Department of Horticulture and Crop Science
- 2:30 - 3:00** **Relationship Between Seed Companies and Public Universities in the Conduct of Research**
Bob Wych, Pioneer Hi-Bred, Intl. Inc.
- 3:00 - 3:30** **The Role of the National Plant Germplasm System in Stand Establishment**
Eric Roos, National Seed Storage Laboratory
- 3:30 - 4:00** **Stand Establishment Concerns of Agronomic and Vegetable Crops Commonly Encountered in Europe**
Stan Matthews, University of Aberdeen
- 4:00 - 4:30** **Stand Establishment Concerns of Herbaceous Perennial Crops**
Art Cameron, Michigan State University
- 4:30 - 5:00** **Priming and Synthetic Seed Applications to Stand Establishment Problems**
Dan Cantliffe, University of Florida
- 5:00** Dinner/Break (on your own)
- 7:00** Reception - Chadwick Arboretum (Ag Administration Auditorium if inclement weather)

MONDAY, JUNE 2, 1997

7:30 am Depart for tours at Ohio Seed Improvement Association, Dublin and
Scotts Seed and Fertilizer Co., Marysville

12 Noon Lunch sponsored by Scotts

1:30 pm Return to Holiday Inn

Oral Presentations: Seed Treatments/Physiology

Moderator: David James, Ohio State University

2:00 - 2:15 **Dividend AP: a New Cereal Seed Treatment**
Larry Zang, Ciba-Geigy Corp.

2:15 - 2:30 **Maxim: a New Potato Seed Piece Treatment**
Larry Zang, Ciba-Geigy Corp.

2:30 - 2:45 **Control of Soybean Seed and Soil-borne Diseases**
Vince Morton, Ciba-Geigy Corp.

2:45 - 3:00 **Effect of Crop Additive on Hard Red Spring Wheat Seed
Performance**
LeRoy A. Spilde, North Dakota State University

3:00 - 3:15 **Effect of Greenhouse-applied PGRs on Landscape Performance of
Perennial Bedding Plants**
Joyce G. Latimer, The University of Georgia

3:15 - 3:30 **Embryo Development and Germination Behavior of *su* and *sh₂* Sweet
Corn Seed and New Approaches for Improving Seedling Vigor**
Xianming Duan and J.S. Burris, Iowa State University

3:30 - 3:45 **Break**

3:45 - 4:00 **The Influence of an Invigoration Treatment Involving Hydration on
Seed Storage Potential**
Alison A. Powell and Louise J. Yule, University of Aberdeen, Scotland

4:00 - 4:15 **Quality Standards for Seed Performance: The Use of Nuclear DNA
Amounts to Predict Storage Behavior and Germination Rate**
Raoul Bino, S.P.C. Groot, R.D. de Castro and J.G. van Pijlen,
CPRO-DLO, The Netherlands

4:15 - 4:30 **Hydrothermal Time and Cell Cycle Activity During Priming of
Tomato Seeds**
Zhiyuan Cheng, Sunitha Gurushighe, and Kent J. Bradford,
University of California - Davis

- 4:30 - 4:45 **Dehydration Rate of Priming Seeds Can Alter Rate of Subsequent Radicle Emergence**
Phil S. Allen, Brigham Young University
- 4:45 - 5:00 **Effectiveness of *Pseudomonas aureofaciens* AB254 in Controlling *Pythium ultimum* on Tomato Seed**
Jabe Warren and Mark Bennett, Ohio State University
- 5:00 - 5:15 **Hydrotime Analysis as a Vigor Test of Primed and Pelleted Lettuce Seed**
David Still, University of Arizona
- 5:15 Dinner (on your own)

TUESDAY, JUNE 3, 1997

Scarlet and Grey Tour

-OR-

Buckeye Tour

- 7:30 am Depart Holiday Inn
8-9:30 John Deere Equipment, Dublin
10:30-12 IMC Agribusiness Seed Division, Midway
12-1 pm Lunch, IMC Agribusiness Seed Division
1:30 - 4 Farm Science Review, London

4-5:30 Return to Holiday Inn

- 7:30 am Depart Holiday Inn
9-11 am Buurma Farms, Willard
11:15 -12:30 Lunch (on coach) and travel to Toledo
12:30-2 pm SchmidtBrothers Bedding plants
2:30-3:30 Rupp Seeds, Inc., Wauseon
3:30-5:45 Return to Holiday Inn

6:30 pm Social - Holiday Inn

8:00 pm Dinner (on your own)

WEDNESDAY, JUNE 4, 1997

Oral Presentations: Equipment/Flooding/Crop Production

Moderator : Andy Evans, Ohio State University

- 9:00 - 9:15 **Evaluation of a Bulk Planting System for Low-cost Seeding of Cabbage**
Wayne C. Porter and Richard L. Parish, Louisiana State University

9:15 - 9:30 **Evaluation of Covering Devices and Presswheels for Direct Seeding of Mustard and Cabbage**
Richard L. Parish and Regina P. Bracy, Louisiana State University

9:30 - 9:45 **Using Blends in High Oil Corn Production**
P.R. Thomison, A. Geyer, T. Krill, S. Lichtensteiger, L. Lotz and H. Siegreist, Ohio State University

9:45 - 10:00 **Banded Phosphorus Effects on Alfalfa Seedling Growth and Subsequent Productivity after Temporary Waterlogging**
C.D. Teutsch, R.M. Sulc, and A.L. Barta, Ohio State University

10:00 - 10:30 **Break /View Posters**

10:30 - 10:45 **Identification of Quantitative Trait Loci for Tolerance to Submergence in Soybean**
Tara T. VanToai and Getachew Boru, USDA-ARS and Ohio State University

10:45 - 11:00 **Planting Date, Fungicide, and Cultivar Effects on Sclerotinia crown and Stem Rot Severity in Alfalfa.**
R.M. Sulc and L.H. Rhodes, Ohio State University

11:00 - 11:15 **Emergence of *Zea mays* L. on Two Soil Types at Varying Planting Depths**
Amy Barr and Mark Bennett, Ohio State University

11:15 - NOON **Visit Posters**

Noon Lunch (on your own)

Oral Presentations: Transplant Production

Moderator: Peg McMahon, Ohio State University

1:30 - 1:45 **Vegetable Transplant Plug Mix Nutrient Additions: Are They Really Necessary?**
C.S. Vavrina, University of Florida

1:45 - 2:00 **Development of a Strawberry Plug Transplant System**
Eric B. Bish, Daniel J. Cantliffe, and Craig K. Chandler, University of Florida

2:00 - 2:15 **Integrated Transplant Production Systems**
Subhas C. Mohapatra, North Carolina State University

- 2:15 - 2:30** **Effect of Herbicides on Pepper Transplants Produced Using Various Irrigation Systems**
Bethany A. Galloway, David W. Monks and Jonathan R. Schultheis, North Carolina State University
- 2:30 - 2:45** **Factors Affecting Stand Establishment of Containerized Vegetable Transplants in the Field**
Amon Koren, Israel
- 2:45 - 3:15** **Break/View Posters**
- 3:15 - 3:30** **Plant Growth Regulators, Dates and Numbers of Treatments in Tomato Transplant Production**
Giovanni Damato, Luigi Trotta and Salvatore Biancofiore, Universita di Bari, Italy
- 3:30 - 3:45** **Growth and Yield of Containerized Onion Transplants Affected by Cell Size, Age and Clipping**
D.I. Leskovar and A.K. Boales, Texas A&M University
- 3:45 - 4:00** **Comparison of Supersweet Corn Establishment by Direct-seeding and Using Ebb-and-flood, Float Bed, and T-rail Transplants**
Malkanthe Gunatilaka, Jonathan Frantz and Gregory E. Welbaum*, Virginia Tech
- 4:00 - 4:15** **Managing Light to Control Vegetable Transplant Growth in a Greenhouse**
Dr. Peg McMahon, Ohio State University

POSTER PRESENTATIONS

Fifth National Symposium on Stand Establishment
Sunday, June 1 - Wednesday, June 4, 1997
Salon C

- 1) **Early Season Sweet Corn Emergence Using Open Furrows, Black Plastic Mulch, and Solid Matrix Priming**
Vince A. Fritz, University of Minnesota
- 2) **Chemical Inhibition of Cool-Season Turfgrass Germination**
G.E. Bell, T.K. Danneberger, and M.B. McDonald, Ohio State University
- 3) **Microorganism Growth During Seed Priming**
Warley M. Nascimento and S.H. West, University of Florida
- 4) **Influence of Growth Stage on Flooding Injury in Alfalfa**
C.D. Teutsch and R.M. Sulc, Ohio State University
- 5) **Stand Establishment Problems in Soybeans: Invertebrate Pest Problems**
Ronald B. Hammond, Ohio State University
- 6) **Marigold Seedling Development Following Transplanting from Plugs Various Times after Seeding**
Robert L. Geneve, Kay Oakley, Myra Stafford and Sharon Kester, University of Kentucky
- 7) **The Effects of Soil Pathogens and Seed Quality on Soybean Seedling Emergence**
B. Hamman, G. Koning, D.M. TeKrony and D.B. Egli, University of Kentucky
- 8) **Developmental Changes Associated with Acquisition of Desiccation Tolerance in Maize**
J.M. Peterson and J.S. Burris, Iowa State University
- 9) **Morphological and Physiological Changes Associated with Desiccation in Maize Embryos**
J.S. Burris, J.M. Peterson*, A.J. Perdomo and D.S. Feng, Iowa State University

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Charles S. Vavrina, University of Florida 312 - 321

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TRANSPLANT PRODUCTION AND PERFORMANCE: MEDIA PHYSICAL PROPERTIES

Bill Argo
Blackmore Company
Belleville, MI 48111

INTRODUCTION

One of the most important aspects of plug production is media quality (Bierbaum, 1992; Styer and Koranski, 1997). For proper shoot and root growth, a root media must serve four functions: 1) to provide water, 2) to supply nutrients, 3) to permit gas exchange to and from the roots, and 4) provide support for the plants (Nelson, 1991). Thus, acceptable physical properties are an integral part of media quality. However, there is no one growing medium that works best in all situations because many of the key aspects of a medium's physical property are not constant, but rather can be affected by the grower. The objective of this review is to consider key aspects of medium physical properties which include air : water : solid space ratios, water absorption and rewettability, moisture release characteristics, and water loss due to evaporation from the root-medium surface.

Air : Water : solid Ratios

The distribution of air, water, and solid in a container root media is dependent on several factors including pore space, particle size distribution, container height, and media settling.

Pore Space. The amount of total pore space (TPS) in a root media is inversely proportional to the bulk density (BD) (Beardsell et al., 1979a; Bunt, 1983; Hanan et al., 1981). As the BD decreases, TPS increases linearly. For example, Bunt (1983) tested 32 combinations of peat and either vermiculite, calcined clay or sand with BD's ranging from 90 to 1500 kg m⁻³. Bunt obtained the following relationship between the BD of the root media combination and the TPS:

$$\text{Total Pore Space} = 98.39 (\pm 0.26) - 0.03655 (\pm 0.00036) * \text{Bulk Density}$$

Sphagnum peat and vermiculite, components of the Cornell Peat-lite A mix, would have a BD of approximately 125 kg m⁻³ or less. Using the above equation, the calculated TPS of the Peat-lite A mix would be approximately 93%. In comparison, a loam based soil can have a BD of 1400 kg m⁻³ and a calculated pore space of 47%. It is commonly reported that mineral soils contain about 50% solid and 50% pore space. In contrast, in a soilless peat-based root media, only 7%-15% of the volume may be solid with the remaining 85%-93% being occupied by pore space (Blom, 1983; DeBoodt and Verdonck, 1971; Fonteno, 1988).

Pore space is occupied by either air or water. For field soil with a column height of over 1 meter, pore space (50% of the total volume) after drainage is typically reported to be 50% air (25% of the total volume) and 50% water (25% of the total volume). For a peat based container root media in a 15-cm-tall pot at container capacity, the reported ideal pore space (85% of the total volume) is 30% air (25% of the total volume) and 70% water (60% of the total volume) (Deboodt and Verdonck, 1972). Fonteno (1988) found that the average air space in five commercially available root media was 21% and the average water space was 65% in 15-cm-tall pots.

Particle size and pore space distribution. Particle size and pore space distribution influence the ratio of water to air held in the root media. Two types of pores exist within a root media, capillary and non-capillary pores. Capillary pores are smaller (< 0.3 mm) and retain much of the water after an irrigation. Non-capillary pores are larger (> 0.3 mm) and provide the aeration for the roots. It is normally reported that the water held in a root media that is available to the plant is held at a tension between 1 and 10 kPa (DeBoodt and Verdonck, 1971) (see moisture release discussion). This range of moisture tension corresponds to pore space diameters of between 0.3 and 0.03 mm (Bunt, 1988). Thus, the smaller the particle size, the greater percentage of smaller pore spaces and the greater amount of water held in the root media after an irrigation.

Puustjarvi and Robertson (1975) reported on the relationship of particle size and water-holding capacity of peat. If the particle size is less than 0.01 mm, the pore space diameter is so narrow that the water is held at tensions that make the water unavailable to the plant. Particle size between 0.01 mm and 0.8 mm retain most of the water applied and so most of the pore space within these particles would be termed capillary pores. Non-capillary pores may still contain water, but the water is held as a film along the sides of the pore space. Both water and air can exist in non-capillary pores at the same time. As particle size increases from 0.8 mm to 6.0 mm, the proportion of large non-capillary pores

increases thus increasing the amount of space occupied by air after an irrigation. Above 6.0 mm, large non-capillary pores predominate (Puustjarvi and Robertson, 1975)

The type of peat used in a root media will greatly effect the physical properties. In general, the more degraded the peat, the greater the BD, which in turn reduces pore space (Puustjarvi and Robertson, 1975). More degraded types of peat also contain a greater percentage of fine particles which reduces the amount of large pore spaces (non-capillary pores) in the root media. More degraded types of peats also maintain a lower percentage of air space in a root media due to the lower percentage of non-capillary pores.

The handling and preparation of peat based root media can have a great effect on the distribution between capillary and non-capillary pores (Milks et al., 1989). Excess shredding or mixing can break down the structure of peat by reducing particle size. In greenhouse operations where root media is prepared on site in batch mixers, it is not uncommon for the first bale of peat to be in the mixer for 20 to 30 minutes before the root media is ready for the pot filling machine. By reducing the particle size of the peat, the distribution between capillary pores and non-capillary pores is changed. Increasing the percentage of small pores will increase the water holding capacity but will decrease the air space (Bunt, 1988; Fonteno, 1988).

Container height. Container height also affects the ratio between air and water in a given root media. The greater the container height, the less water that will be held in the root medium. After saturation and drainage, a perched water table exists at the bottom of the pot (Spomer, 1975). For every 1 cm increase in height above the bottom of the pot, there is a 0.1 kPa increase in moisture tension and less water held. Milks et al. (1989) showed that the percent moisture held in a 17 cm tall pot decreased from 69% at the bottom of the pot to 32% at the top of the pot. The overall container capacity of the root media within the pot was the average water held by the root media throughout the column.

An illustration of how container height affects the water content of a root media is presented by Fonteno (1988). At container capacity, the average water content of 5 different commercially available root media in a 15 cm pot was 64%, in a 10 cm pot was 70%, a 48 cell bedding flat was 76%, and a 273 plug tray (5 cm tall) was 82% water by volume. The percentage of solid material in the root media remained relatively constant in the different container sizes. It was the ratio of air space to water space that changed with the different container heights.

Settling. Root-medium settling affects the physical properties of a root media by decreasing column height and changing the distribution between capillary and non-capillary pores (Nash and Pokorny, 1990). Settling occurs when the small particles settle into the large non-capillary pores located between the larger particles (Spomer, 1974). Nash and Pokorny (1990) found that excess settling occurred in a two component root media when there was a large difference in the particle size of the two components. The greatest amount of settling occurred when the components were mixed in equal volumes (50% each by volume). Settling could be reduced or eliminated by using similar size components in the root media (Nash and Pokorny, 1990). Argo and Biernbaum (1993) and Blom and Piott (1992) found that most of the settling occurs with the first irrigation.

Water absorption and rewettability

The currently used method of determining root media air and water space at container capacity (Fonteno, 1988; Milks et al., 1989; White and Mastalerz, 1966) has little relationship with a normal irrigation under commercial conditions. With current methods, the root media remain submerged in water for 24 hours. Following drainage, a perched water table is present at the bottom of the pot. Under production conditions, the root media is typically dry at the start of an irrigation and may be irrigated for a period of one to five minutes. Lateral distribution of the water is slow and saturation often does not occur (Argo and Biernbaum, 1993; 1994b).

Organic materials such as peat tend to be hydrophobic and may be difficult to rewet if allowed to become too dry. Airhart et al. (1978) and Beardsell and Nichols (1982) found that when the water content of pine bark was allowed to decrease below 35%, little of the water applied was retained. As moisture levels increased to 50%, the bark became progressively easier to rewet. Argo and Biernbaum (1994b) found that peat-based media became more efficient at absorbing applied water as the moisture content of the medium prior to the irrigation increased.

The state of decomposition of the peat may also affect the ability to rewet after drying. Peats in a greater state of degradation also have a greater amount of humic acid. Humic acid plays an important role in cation exchange capacity of peat based root media. However, if peat is allowed to dry, the humic acid may form hard granules that have lost their initial capacity to absorb water and nutrients and may ultimately have an adverse affect on the structure of the peat (Puustjarvi and Robertson, 1975).

Other components can be added to a root media to increase water absorption. Beardsell and Nichols (1982) found that water absorption by coarse sand was not dependent on the moisture content prior to water being applied. This water absorption characteristic could be transferred to a root media in proportion to the amount of coarse sand used. Beardsell and Nichols concluded that a minimum of 30% of the volume of the root media be made up of coarse sand to

achieve acceptable levels of rewettability (> 80% of initial container capacity). However, the large percentage of sand reduced the water holding capacity of the root media and, therefore, was less effective than preventing the root media from drying out (Beardsell and Nichols, 1982). Vermiculite and perlite may also improve the rewettability of root media (Bunt, 1988).

Irrigation method also can affect water absorption. Argo and Biernbaum (1994b) found that with the same five media, an average of 0.5 L was absorbed with top watering, 0.38 L was absorbed with drip irrigation, and 0.19 L was absorbed flood subirrigation. Under the conditions of the experiment, a maximum of 0.60 L needed to be absorbed by the medium to reach the air : water ratio measured in the laboratory with standard physical property testing methods.

The volume of water applied to the medium also influences water absorption. Many growers only apply small quantities of water to the medium at any one time in order to control shoot growth. Under these conditions, the air : water ratio never gets close to that of the minimum value measured in the laboratory.

Much of the research on the rewettability of peat has dealt with the effect of wetting agents or surfactants. Many surfactants exist but relatively few are not phytotoxic to plants (Sheldrake and Matkin, 1969). Wetting agents are nonionic materials that bind to the surface of the root media particle and decrease the surface tension of the water, thus increasing the penetration of water into the root media which increases rewetting (Valoras et al., 1976; Templeton, 1987). Wetting agents are commonly added to commercial peat based root media to aid in rewetting (Templeton, 1987). One application of AquaGro L ($1500 \text{ mg} \times \text{L}^{-1}$) increased the amount of water absorbed by air dried peat (17% moisture content) by 90% at one irrigation (Aquatrols Corporation, 1992).

The effect of a wetting agent can be relatively long lasting. Valoras et al. (1976) found that a nonionic surfactant did not degrade quickly in sphagnum peat. After 270 days, only 30% of the surfactant had decomposed in the peat compared to 70% degradation in a water repellent sandy loam soil. Argo and Biernbaum (1993) found no increase in water absorption by reapplying a wetting agent to six month old impatiens hanging baskets grown using long-fibered peat-based media compared to that of the same media not given the wetting agent. In all cases, a wetting agent was added to the medium at mixing (six months prior). However, in media containing more degraded peats, the reapplication of a wetting agent was necessary to increase the rewetting of the medium six months after planting.

Moisture Release Characteristics

The water held in the root media after an irrigation can be divided into water available to the plant (available water) and water that remains in the root media even when the plant is wilted (unavailable water). The available water is reportedly held at moisture tensions of between 1 and 1467 kPa, 1 kPa would be equivalent to a root media at container capacity and 1467 kPa would be the same root media at permanent wilt (Bunt, 1988; Milks et al., 1989) (1 kPa = 10 cm water = 10 mbars).

A reduction in plant growth is observed long before the moisture tension reaches 1467 kPa (Bunt, 1988). For example, Spomer and Langhans (1975) measured an increase in the growth of bench chrysanthemums as the water content of the root media was increased to approximately 90% of pore saturation. When Kiehl et al. (1992) grew chrysanthemums at different moisture tension levels, there was a decrease in fresh and dry weight as the constant moisture tension the plants were grown at increased from 0.8 to only 16 kPa.

Moisture tensions for container root media that are easily available to the plant are often reported between 1 and 5 kPa and moisture tensions between 5 and 10 kPa are termed water buffering capacity (DeBoodt and Verdonck, 1972). Milks et al. (1989) termed moisture tensions levels above 30 kPa as being unavailable water. Verdonck et al. (1983) recommended that for optimal growth conditions, 30-45% of the water held in a root media after an irrigation should be easily available water. Fonteno and Nelson (1990) found that two commercial root media (Metro Mix 350 and Ballmix #2) had available water contents of approximately 35%.

Peat type and particle size also affect moisture release. As with water holding capacity, the more degraded the peat, the greater the percentage of water held at higher moisture tensions (Puustjarvi and Robertson, 1975). The higher moisture tensions are due to the greater percentage of fine particles (<0.1 mm) and capillary pores small enough to retain water even at the high moisture tensions. Thus for more degraded peats, it may be necessary to maintain moisture levels closer to saturation than for less degraded peats in order to maintain optimal growth levels, similar to the results found by Spomer and Langhans (1975) for greenhouse bench soils.

The difference between available water-holding capacity (AWHC) and water release from a root media to the plant was illustrated by Beardsell et al. (1979b). In the experiment, different organic and inorganic root media components were evaluated for both water holding capacity and days to wilt (water release). Marigold seedlings were transplanted into the different components and allowed to acclimate. The components were then saturated with water and allowed to dry until wilt was observed. Of the organic materials, peat held the greatest amount of water after an irrigation but went the shortest period of time to wilt. Pinebark held 30% less available water but went 80% longer

before wilt was observed. Transpiration rates (measured gravimetrically) for plants grown in peat were higher than for plants grown in the other materials tested. As available water became limiting in other materials, transpiration rates of the plants gradually decreased. This would indicate that for materials such as pinebark or sandy loam, there was a relatively small percentage of easily available water, but a large percentage of less available water (water buffering capacity) that could be absorbed by the plant, but not as quickly as easily available water. Peat contained a large percentage of easily available water but once used up, there was relatively little water buffering capacity and the plants wilted (Beardsell et al., 1979b)

Evaporation of water from the surface of the root media

Laurie (1950) commented on the large amount of water lost by peat due to surface evaporation. The peat fibers act as a wick, moving the internal moisture by capillarity to the surface where evaporation is most rapid. The more fibrous the peat, the greater the wicking effect and the greater amount of water lost due to surface evaporation.

In an experiment by Beardsell et al. (1979b), different materials were placed in 13-cm-tall pots and saturated with water. After draining, the pots were weighed to determine the amount of total water held in the pot. Weights were taken daily for the first 5 days and every other day for the remaining 8 days to determine the amount of water lost by evaporation from the surface of the media. Peat took seven days to lose 0.25 L or 50% the water held at container capacity by evaporation. In comparison, pine bark lost 0.10 L or 22% of the total water held at container capacity over the same time period. Thus, the high water holding capacity of peat compared that of other material used in container media is offset in part by the large amount of water lost because of evaporation from the surface (Beardsell et al., 1979b).

Various researchers have estimated the amount of water lost from the pot due to evaporation from the surface of the root media during plant production to be 25%-30% of the total amount of water used by the plant on a per day basis (Argo and Biernbaum, 1994a; Argo and Biernbaum, 1995b; Furuta et al., 1977; Van de Werken, 1989; Yelanich, 1995). Evapotranspiration can be reduced by simply placing a barrier over the surface of the root media to block evaporation. Furuta (1976) reduced evapotranspiration of Monterey pines grown in 3.8 L pots by 26% with the use of a plastic disk placed over the surface of the root media. Argo and Biernbaum (1994a) reduced evapotranspiration of easter lilies grown in 15-cm-tall (1.7 L) pots by 35% in the greenhouse and 56% in the postproduction environment by placing a saran cover on the surface of the root media. Argo and Biernbaum (1995b) reduced evapotranspiration of poinsettias grown in 15-cm-tall (1.7 L) pots by 46% by placing a polystyrene disk on the surface of the root medium.

CONCLUSION

In the laboratory, root-medium physical properties are influenced by bulk density (Bunt, 1983; Beardsell et al., 1979a; Hanan et al., 1981), particle size (Puustjarvi and Robertson, 1975) and container height (Fonteno, 1988; Milks et al., 1989). In the greenhouse, physical properties also are influenced by irrigation method, applied water volume, and media moisture content (Argo and Biernbaum, 1994b; Beardsell and Nichols, 1982; Bunt, 1988; Airhart et al., 1978). Finally, the amount of time the media is at or near container capacity may be relatively small because of the evapotranspirative demands of the plant and the root medium surface (Argo and Biernbaum, 1994a; 1995b; Furuta, 1976). Understanding the root environment under production conditions requires an understanding of the dynamic nature of air : water ratio in the medium and the limitations of static laboratory physical property measurements.

Many experiments have been conducted to determine plant responses to root media with different physical properties. Often, the different root media are watered and fertilized identically (Bilderback et al., 1982; Brown and Emimo, 1981; Fonteno and Nelson, 1990; Fonteno et al., 1981). The conclusions of these experiments may be biased because the experimental methods were optimized for a single medium or container size. In order to compare root media with different water-holding capacities or the same root media in different size containers, the total water-holding capacity and the amount of available water must be determined for each root medium individually, and irrigations must be scheduled accordingly (Argo and Biernbaum, 1994a; 1995a).

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TRANSPLANT PRODUCTION AND PERFORMANCE: ROOT MEDIUM CHEMICAL PROPERTY

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INTRODUCTION

A plug or small containerized transplant is the primary method of producing ornamental and vegetable transplants in the United States. Styer and Koranski (1997) estimated that about 25 billion ornamental and vegetable plug transplants were produced in the United States in 1996. The advantage of growing plug transplants include greater transplant uniformity, a reduction in labor when transplanting, and increased production per m³ of bench space in the greenhouse (Nelson, 1991; Styer and Koranski, 1997).

In plug production, the most common component of a plug medium is sphagnum peat (Adams, 1992; Fonteno et al., 1996; Koranski and Kessler, 1991; Styer and Koranski, 1997). In peat-based media, a number of sources interact to affect the nutrient supply in container root media throughout crop production. However, these sources may not affect the nutrient supply simultaneously or with equal intensity. For example, with Ca nutrition, the sources can include cation exchange capacity (CEC), lime, preplant nutrient charge (PNC) fertilizers, irrigation-water source (IWS), and water-soluble fertilizer (WSF) (Argo, 1996). Any discussion of nutritional management in container root media also must include pH management because of the direct or indirect effects that many nutrient sources have on pH and the effect that pH has on nutrient availability (Lucas and Davis, 1963; Peterson, 1981). Finally, plant growth may directly affect medium pH as well as nutrient uptake (Argo, 1996; Argo et al., 1997; Bailey et al., 1996). The objective of this review is to consider the key chemical properties that affect pH and nutrient management of plants grown in peat-based root media.

Cation exchange capacity (CEC).

It has been suggested that an adequate CEC is desired in soilless media to buffer it from sudden changes in pH and nutrient concentrations (Bierbaum, 1992; Bunt, 1988; Nelson, 1991; Styer and Koranski, 1997). The CEC of organic materials such as peat or bark often are associated with the pH and nutrient buffering capacity and are due to the pH-dependent exchange of cations with H⁺ from organic acid functional groups on the particles. For example, Helling et al. (1964) found that the CEC of a sphagnum peat increased by 140 meq·L⁻¹ as the pH increased from 3.5 to 8.0. The ratio of H⁺ to cations bound to the peat also changes with increasing pH. At a pH of 3.7, 4.5, 5.5, or 7.8, acid sphagnum peat is 100%, 50%, 30%, or 0% H⁺ saturated, respectively (Lucas et al., 1975; Puustjarvi and Robertson, 1975). Bunt (1988) reported that the CEC of peat largely indicates the potential for divalent ions adsorption (primarily Ca²⁺ and Mg²⁺), with most monovalent cations (NH₄⁺-N, K⁺, Na⁺) remaining water-soluble.

The CEC of organic materials such as peat on a weight basis is much higher than that of mineral soils. For example, Lucas (1982) reported that the CEC of a sphagnum peat was 1000 meq·kg⁻¹ while that of a loam mineral soil was 120 meq·kg⁻¹. However, because of the low bulk density of the sphagnum peat, the effective CEC measured on a volume basis was 40% less than that of the mineral soil (80 meq·L⁻¹ for the peat vs. 140 meq·L⁻¹ for the mineral soil). Puustjarvi (1982) reported a linear increase in the CEC of sphagnum peat from 45 meq·L⁻¹ to 130 meq·L⁻¹ as the degree of decomposition increased from H1 to H5 as measured with the von Post scale (Puustjarvi and Robertson, 1975). The overall increase in CEC was associated with both a higher CEC of the more degraded peat itself (H1 peat was 1000 meq·kg⁻¹, H5 peat was 1240 meq·kg⁻¹) as well as an increase in the bulk density with greater decomposition (H1 peat was 45 kg·m⁻³, H5 peat was 105 kg·m⁻³).

Other materials such as perlite, polystyrene, or rockwool (RW) have minimal CEC and are included in container media to increase aeration or water-holding capacity (Argo and Bierbaum, 1994; Nelson, 1991). Expanded vermiculite, coconut coir, and bark are added to soilless media for aeration and water-holding capacity, but each also has significant CEC (Bunt, 1988; Nelson, 1991).

Root medium aeration

Root-medium aeration is important when plants are produced in containers (Bunt, 1988; Deboodt and Verdonck, 1971; Fonteno, 1988; Milks et al., 1989). From a chemical property standpoint, O₂ partial pressure is important because it affects the redox potential of the medium, which directly affects nitrification and denitrification rates

and the solubility and availability of micronutrients (Lindsay, 1979; Marschner, 1986). Hanan (1964) measured O₂ partial pressures (PP) in cut flower beds ranging in depths from 8 to 60 cm and containing media with various percentages of leaf mold, loam, peat, perlite, sand, and silt. At the 5 to 7 cm depth within the beds, O₂ partial pressure ranged from 9.8 to 21 kPa (ambient O₂ partial pressures are about 21 kPa), with the highest O₂ partial pressure (21 kPa) measured in the beds containing a soilless root media (1 peat:1 perlite [v:v]). Paul and Lee (1976) found that the oxygen diffusion rate correlated well with the growth of chrysanthemums in 13 root media. Fifteen percent air-filled porosity at container capacity was suggested for optimum oxygen diffusion rates and plant growth in 12-cm-tall pots. Argo et al. (1996) found that the O₂ partial pressure in three soilless medium in 12-cm-tall pots with chrysanthemum was 21 kPa. Irrigating the pots using either drip irrigation or subirrigation had minimal effect on O₂ partial pressure measured at three levels in the pot.

Medium CO₂ partial pressure also is important because of its effect on solution pH and nutrient solubility. Lindsay (1979) reported that for soils at equilibrium with CaCO₃ (calcareous), the measured pH varied from 7.3 to 8.5, depending on the CO₂ partial pressure. Soil pH values of 8.5 in calcareous soils can be obtained only when the partial pressure of CO₂ in the soil is similar to that measured in the ambient atmosphere (30 to 40 Pa). Because of factors such as root respiration, microbial activity, and organic matter degradation, average CO₂ partial pressure in the soil atmosphere are commonly reported at 300 Pa, or 10 times higher than that measured in the air (Lindsay, 1979).

In container-grown plants, root respiration is considered higher than that of plants grown in mineral soils because of faster plant growth rates (Paul and Lee, 1976) and higher microbial respiration because of the high organic matter content of most soilless container media (Bunt, 1988). Argo et al. (1996) found that the CO₂ partial pressure in three soilless medium in 12-cm-tall pots with chrysanthemum was 63 Pa (ambient CO₂ partial pressure was 46 Pa). Irrigating the pots with water containing alkalinity at 320 mg CaCO₃/L caused an increase in medium CO₂ partial pressure up to 1600 Pa. The high medium CO₂ partial pressure measured after the irrigation was not persistent, and within 180 minutes, returned to levels averaging 45% higher (100 Pa) than that measured before the irrigation. In comparison, when reverse osmosis purified water (alkalinity of <20 mg CaCO₃/L) was used instead of well water, the large increase in medium CO₂ did not occur. This indicated that the alkalinity in the irrigation water was the source of the CO₂.

In general, soilless container root media maintain high air-filled porosities in pots (Deboodt and Verdonck, 1971; Fonteno, 1988; Milks et al., 1989). This high porosity after the irrigation allows for rapid CO₂ dispersion and re-establishment of O₂ partial pressures to near pre-irrigation levels. In small containers such as plugs, air-filled porosity is less than in pots (Fonteno, 1988; Milks et al., 1989). However, small containers tend to dry out quickly, which would also lead to a high air-filled porosity and O₂ and CO₂ partial pressures similar to that of ambient levels.

Liming materials.

Liming materials (CaCO₃, CaCO₃ and MgCO₃, Ca(OH)₂, Ca(OH)₂ and Mg(OH)₂) are added to a soilless root medium to neutralize acidity, increase pH to a level acceptable for plant growth, and provide a source of Ca²⁺ (and Mg²⁺ if dolomitic lime). Incorporating sufficient lime into a soilless root medium to obtain an initial pH range of 5.5 to 6.4 is recommended (Nelson, 1991; Peterson, 1981; Warncke and Krauskopf, 1983). The amount of liming material (incorporation rate) required to obtain an equilibrium pH of about 6 in the root medium depends not only on the components used to produce the medium, but also on the liming material's reactivity and particle size (Argo and Biernbaum, 1996b; Chapin, 1980; Gibaly and Axley, 1955; Schollenberger and Salter, 1943; Sheldrake, 1980; Williams et al., 1988) as well as the surface area of the liming material (Parfitt and Ellis, 1966).

Argo and Biernbaum (1996b) found that the lime that reacted initially to increase the medium's pH had minimal effect on root-medium Ca²⁺ or Mg²⁺ (if dolomitic lime) concentrations measured with the saturated media extract (Warncke, 1986). Water-soluble Ca²⁺ and Mg²⁺ concentrations remained below levels considered acceptable for plant growth (Warncke and Krauskopf, 1983) even though the pH of the peat increased, indicating that the lime was still reacting. Argo and Biernbaum (1996a) proposed that not all the liming material incorporated into a soilless root medium may have reacted once an equilibrium pH is reached. Instead, it was found that a portion of the liming material remained unreacted in the medium after the equilibrium pH was reached. The residual lime fraction was found to have an important role in pH, Ca²⁺, and Mg²⁺ buffering under acidic conditions (Argo and Biernbaum, 1996a, 1997b). It can be speculated that the ratio of reacted : residual contained in the medium probably depends on the reactivity of the liming material.

Preplant nutrient charge (PNC) fertilizers.

In general, unamended acidic peat-based root media do not contain sufficient nutrients for plant growth (Bunt, 1988; Nelson, 1991). Current recommendations for the incorporation of fertilizer materials other than liming materials

into a soilless root media before planting include sources of N, PO₄-P, K, Ca, Mg, SO₄-S, and trace elements (Table 1). These guidelines come from the early soilless container media recommendations, including the Cornell Peat-lite media (Boodley and Sheldrake, 1972), the Pennsylvania State University media (White, 1974), Glasshouse Crops Research Institute media (Bunt, 1988), and floriculture textbooks (Nelson, 1991). The most commonly recommended macronutrient fertilizers include Ca(NO₃)₂, KNO₃, superphosphate or triple superphosphate, and gypsum (Bunt, 1988; Nelson, 1991; Warncke and Krauskopf, 1983).

The N and K content of the lime and PNC fertilizers is small compared to the total amount applied to a crop. For example, Yelanich (1991) found that a minimum of 1.0 to 1.5 g mineral N/pot was required to produce a poinsettia in a 15-cm-wide by 12-cm-wide (1.3-L) pot in 16 weeks. An initial incorporation of 0.17 kg mineral N/m³ would supply .022 g mineral N to the 1.3-L pot, or 15% to 22% of the total N requirement. In comparison, Ca, Mg, PO₄-P, and SO₄-S content of the lime and PNC fertilizers may represent a large percentage, in some cases up to 100%, of the total amount applied to the crop.

A number of studies have tested the persistence of PNC fertilizer in peat-based root media. Yeager and Barrett (1985) found that soilless media have a limited ability to retain PO₄-P against leaching. Biernbaum et al. (1995) demonstrated that all macronutrients (N, P, K, Ca, Mg, S) supplied from one blended PNC fertilizer leached very quickly from peat-based medium when placed in pots under mist irrigation. The rate of nutrient loss could be predicted by quantifying the volume of water leached from the pot. While there were minor differences in rate of loss between the individual nutrients, the concentration of all nutrients were below acceptable levels for plant growth by the time two container capacities were leached from the pot. Argo and Biernbaum (1996a, 1996b) concluded that the nutrients from PNC fertilizer (such as gypsum and 0-46-0) were soluble and easily leachable.

Fertilizer salt stratification within the pot also affects the availability of nutrients from PNC fertilizers. Fertilizer salt stratification within the pot is thought to be caused by evaporation from the root-medium surface (Argo and Biernbaum, 1994, 1995) or a water front moving into the root medium with each irrigation (Yelanich, 1995) and occurs with all methods of irrigation. Argo and Biernbaum (1994, 1995, 1996a, 1996b) found that PNC fertilizer moved rapidly from the root zone (lower 2/3 of media in the pot) and into the top layer (top 2-cm of media in the pot) within a few days after planting. With flood subirrigation, the nutrients in the top layer were unavailable to the plant, and the salt concentration in the top layer continued to increase even when the nutrient levels in the root zone were below levels considered acceptable for growth (Argo and Biernbaum, 1994, 1995, 1996a, 1996b). With top watering, the fertilizer salts contained in the top layer were found to gradually moved down into the root zone, buffering the medium from sudden changes in nutrient concentrations when application of WSF were stopped (Argo and Biernbaum, 1995). Fertilizer salt stratification also has been demonstrated in 406 plug trays (Argo and Biernbaum, unpublished data).

Irrigation-water sources (IWS).

The IWS is often considered one of the most important factors in container plant production (Bailey, 1997; Biernbaum, 1994; Bunt, 1988; Lang, 1996; Nelson, 1991; Reed, 1997; Styer, 1996; Styer and Koranski, 1997). Recommendations for acceptable levels of pH, alkalinity, electrical conductivity (EC), and nutrients concentrations exist (Table 2). In general, the most important nutrients for characterizing IWS are alkalinity, Ca²⁺, Mg²⁺, SO₄-S, Na⁺, Cl⁻, B, and F concentrations and sodium adsorption ratio (SAR) (Argo et al., 1997a).

Several studies have been conducted to quantify the nutrient content of different sources of irrigation water in the United States. Based on 4300 samples, Argo et al. (1997) found that the overall median water source in the United States had a pH of 7.1; an electrical conductivity (EC) at 0.4 dS·m⁻¹; an alkalinity of 130 mg CaCO₃/L; (in mg·L⁻¹) 40 Ca, 11 Mg, 8 SO₄-S, 13 Na, 14 Cl, 0.02 B, and <0.01 F; a Ca : Mg ratio of 3.2 and a sodium adsorption ratio (SAR) of 0.7. These values were also quantified for the ten leading states in floriculture production. More limited studies that quantified IWS were conducted by Ludwig and Peterson (1984) (all nutrients) and Reddy et al. (1994) (only SO₄-S).

Different IWS require different types of management in order to maintain an acceptable pH in the root medium (Bunt, 1988; Nelson, 1991; Styer and Koranski, 1997; Vetanovetz and Hulme, 1991). The suggest range for IWS pH and alkalinity is 5 to 7 and 40 to 100 mg CaCO₃/L, respectively. Argo et al. (1997) suggest that IWS outside these ranges are not detrimental to plant growth as long as the pH of the medium is maintained within an acceptable range. Argo and Biernbaum (1996) demonstrated that IWS alkalinity, not pH, is the primary factor influencing medium pH management. Irrigation water containing large amounts of alkalinity (>250 mg CaCO₃/liter) commonly are treated by adding strong mineral acid (HNO₃, H₂SO₄, or H₃PO₄). Researchers recommend adding sufficient acid to reduce the alkalinity to 40 to 120 mg CaCO₃/liter (depending on the crop) or reduce the solution pH to 6.0 to 6.5 (Bunt, 1988; Nelson, 1991; Spurway and Wildon, 1938). Alternative sources such as rainwater or reverse osmosis (RO) purified water are gaining popularity because of their low alkalinity (Biernbaum, 1992). However, rainwater and RO water contain minimal nutrients.

Water-soluble fertilizers (WSF).

The type of WSF applied to a root medium affects pH and nutrient concentrations two ways: directly, by nutrients applied to the root medium, and indirectly, by acidification of the rhizosphere pH. Fertilization with NH_4^+ -N causes the medium pH to decrease because of H^+ secretion during root uptake and nitrification of the NH_4^+ -N to the NO_3^- -N form, which also releases H^+ . In comparison, fertilization with NO_3^- -N causes the medium pH to increase because of OH^- or HCO_3^- secretion associated with balancing ion uptake (Barker and Mills, 1980; Bunt, 1988; Hawkes et al., 1985; Marschner, 1986; Nelson, 1991; Vetanovetz and Hulme, 1991).

The pH of soilless media affects nitrification rates. Niemiera and Wright (1986) found that the application of a dolomitic carbonate lime at 3 or 6 $\text{kg}\times\text{m}^{-3}$ before planting resulted in a $\text{pH} > 5.5$ and an increase in NO_3^- -N concentrations in the medium of plants periodically fertilized with NH_4^+ -N at 100 $\text{mg}\times\text{L}^{-1}$ from $(\text{NH}_4)_2\text{SO}_4$. In comparison, the medium pH of the 0 $\text{kg}\times\text{m}^{-3}$ lime treatments was < 5.5 and there were no measurable levels of NO_3^- -N. Lang and Elliott (1991) reported that ammonium oxidation rate in a peat-based medium was insignificant at a pH of < 5.6 . However, tissue N was not reported in either of these two studies. Argo and Biernbaum (1997a) found that the critical root-medium pH for NH_4^+ -N accumulation in the medium was between 5.4 and 5.7, and for NO_3^- -N, accumulation was between 5.3 to 5.9. Above this pH, minimal NH_4^+ -N concentrations were measured in the medium, even with 50% or 25% NH_4^+ -N WSF, while below this pH, NH_4^+ -N began to accumulate in the medium with a corresponding decrease in the NO_3^- -N concentration. Argo and Biernbaum (1997a) concluded that the ratio of NH_4^+ -N : NO_3^- -N in the WSF applied to the medium and that taken up by the plant may be different, depending upon medium pH.

Table 3 contains the analysis from several commercially available WSF. Many commercially available WSF contain a high percentage of NH_4^+ -N and PO_4 -P but little Mg^{2+} and no Ca^{2+} [examples: 21-7-7 Acid Special, 100% NH_4^+ -N, 0.05% Mg^{2+} , 0% Ca^{2+} ; 20-20-20 General Purpose, 72% NH_4^+ -N, 0.05% Mg^{2+} , 0% Ca^{2+} ; 20-10-20 Peatlite Special, 40% NH_4^+ -N, 0.05% Mg^{2+} , 0% Ca^{2+}]. Because of the high NH_4^+ -N content, the reaction produced by these WSF are acidic [21-7-7 = 0.78 kg acidity/kg, 20-20-20 = 0.30 kg acidity/kg, 20-10-20 = 0.21 kg acidity/kg]. In comparison, WSF that produce neutral or basic reactions in the root medium are typically low in NH_4^+ -N and PO_4 -P but high in Ca^{2+} and NO_3^- -N (examples: 17-5-17, 20% NH_4^+ -N, 3% Ca, 0 kg acidity/kg; 15-5-15, 28% NH_4^+ -N, 6% Ca^{2+} , 0.07 kg basicity/kg; 13-2-13 Plugcare^{Plus}, 5% NH_4^+ -N, 6% Ca, 0.19 kg basicity/kg; 15-0-15 Dark Weather Special, 13% NH_4^+ -N, 11% Ca^{2+} , 0.21 kg basicity/kg).

Nutrient Solution (NS)

The NS is the combination of the IWS and WSF. The term NS should be used whenever discussing fertilization of any crop because whenever WSF is applied, it is in conjunction with an IWS, that also affects the pH and nutrient concentrations in the medium. For example, Argo and Biernbaum (1996a) found that the an acceptable medium pH of about 6.0 could be maintained in the medium with a 50% NH_4 -N WSF and a IWS alkalinity of 320 $\text{mg CaCO}_3/\text{L}$, a 25% NH_4 -N WSF and a IWS alkalinity of 120 $\text{mg CaCO}_3/\text{L}$, or a 3% NH_4 -N WSF and a IWS alkalinity of < 20 $\text{mg CaCO}_3/\text{L}$. Thus the term acidic, neutral, or basic does not apply to the WSF because in each case, the overall reaction produced by the NS was neutral. Low levels of nutrient in the IWS (particularly Ca^{2+} , Mg^{2+} and SO_4 -S) are often supplemented with WSF containing those nutrients. Argo and Biernbaum (1996a, 1997a) found that the Ca^{2+} , Mg^{2+} , and SO_4 -S concentration measured in the root medium and shoot-tissue were better quantified by using the total concentration measured in the NS rather than discussing the concentration of those ions in the IWS or WSF separately.

Species effects

The plant may also affect pH management. With agronomic crops, some species are less susceptible to lime-induced iron chlorosis because of the plants ability to lower the rhizosphere pH through root exodation of H^+ and organic acid (citrate, malate) when grown in calcareous soils ($\text{pH} > 7.8$). In comparison, species that do not lower the rhizosphere pH are much more susceptible to lime-induced iron chlorosis (Marschner, 1986). Among cultivars of the same species, there may be considerable differences in the susceptibility of lime-induced iron chlorosis because of differences in the cultivars ability to lower the rhizosphere pH (Froehlich and Fehr, 1981; Saxena and Sheldrake, 1980). These cultivars differences have not been quantified.

In vegetable and ornamental plug production, much less is known of species or cultivar effects on medium pH and the resulting differences in nutrient uptake. In laboratory experiments on germinating seedlings, Bailey et al. (1996) found that substrate pH varied from 4.5 with tomatoes to 7.5 with zinnia under the same conditions. In greenhouse experiments, Argo et al. (1997) found that the average root-medium pH of ten potted plant species given the same WSF (20N-4.3P-16.6K Peatlite Special [Scotts, Marysville, Ohio]) ranged from 5.1 with African violets to 6.5 with gerbera. Argo (1996) found up to a 1.7 pH unit difference in the media of seven bedding plant species given the same WSF. In

general, geraniums had the lowest medium pH while pansies and petunias had the highest medium pH.

Interactive effects of multiple nutrient sources.

The factors discussed in this review (CEC, liming materials, PNC fertilizer, NS, and plant species) interact to affect the nutrient supply initially and over time. However, these factors do not affect the nutrient supply simultaneously or with equal intensity. Argo (1996) proposed that the relative importance of the nutrient sources for pH buffering and calcium and magnesium nutrition in peat based media were: nutrient solution (IWS and WSF) > plant species > residual lime > PNC fertilizers > root media. This conclusion is based on a number of experiments testing the interactive effects of nutrient sources on pH and nutrient management in container grown crops.

With Ca nutrition as an example, Argo and Biernbaum (1996a, 1997a) demonstrated that there was a linear increase in the shoot-tissue Ca concentrations as the concentration of Ca in the nutrient solution (NS) (composed of both the IWS and WSF) increased from 20 to 210 mg·L⁻¹ with hybrid impatiens. Other ions contained in the NS (NH₄, NO₃, K, SO₄) did not appear to affect Ca. Argo (1996) found a linear increase in shoot-tissue Ca in eight other bedding plant species in addition to impatiens. However, there were differences in the shoot-tissue Ca concentrations of the nine species. Given the same NS, impatiens were found to contain the highest shoot-tissue Ca, while Nonstop begonia, pansies, vinca, and wax begonias contained the lowest shoot-tissue Ca.

The lime that reacted initially to increase the medium's pH was found to have a minimal effect on root-medium Ca concentrations (Argo and Biernbaum, 1996b). However, the residual lime did influence long-term Ca management. Both root-medium and shoot tissue Ca concentrations were increased when given an acidic NS containing low Ca and Mg. Reducing the acidity of the NS by reducing the NH₄-N content and increasing the alkalinity concentration in the IWS negated the residual lime as a Ca source (Argo and Biernbaum, 1996a; 1997a).

Preplant nutrient charge fertilizer other than lime (gypsum, triple superphosphate, Ca(NO₃)₂) did increase the initial Ca concentration in the medium. However, the Ca supplied with the PNC fertilizers was found to be very soluble and easily removed from the root zone because of leaching or salt stratification within the pot (Argo and Biernbaum, 1996b; Biernbaum et al., 1995). With subirrigation, the PNC fertilizers had no effect on root-zone nutrient concentrations for longer than one week (Argo and Biernbaum, 1996a; 1996b; 1997a). With top watering, nutrients contained in the top layer would probably buffer root-zone nutrient concentrations (Argo and Biernbaum, 1995). The duration of the buffering would depend on the amount of water leached from the pot.

Historically, root media has been the primary focus of nutrient management and buffering in container grown crops (Biernbaum, 1992; Bunt, 1988; Nelson, 1991; Styer and Koranski, 1997). Argo and Biernbaum (1997a) found that root media CEC had minimal influence on both short term and long term Ca management. The Ca concentrations in the root medium and shoot tissue of plants grown in a 70% rockwool/30% perlite medium were similar to those of plants grown in a 70% highly degraded peat/30% perlite medium. However, root medium did influence lime incorporation rate which may affect the amount of residual lime remaining in a medium once the equilibrium pH was reached.

CONCLUSION

One key to successful plug transplant growing is pH and nutritional management (Styer and Koranski, 1997). Optimizing the pH and nutritional management of container grown crops such as plugs requires an understanding of how a variety of factors interact to affect nutrient supply and uptake initially and over time. All aspects of nutritional management including media, lime, IWS, WSF, and plant species should be quantified to allow for consistent and reproducible result.

Future Research Priorities.

- 1) Quantification of the effects that other components, such as vermiculite or bark, have on the pH and nutrient buffering capacity of a soilless root medium.
- 2) Quantification of the residual lime content of root medium.
- 3) Better characterization of the reactivity of liming materials.
- 4) Quantification of the acidic/basic reactions of WSF. Effect that WSF concentration has on the reaction.
- 5) Quantification of species and variety effects on pH and nutrient management.

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Table 1. Recommended types and incorporation rates for lime and preplant nutrient charge fertilizers. The nutrient content of the individual fertilizer salts was estimated from Hawkes et al. (1985) and Young and Johnson (1982).

media ^x	Cornell Peat-lite A and B ^Z	Cornell foliage media ^Z	Pennsylvania State media ^Y	Nelson potting media ^W
	GCRI-I potting media ^W	GCRI-2 potting media ^W		
All incorporation rates in kg·m ⁻³ of root media				
PNC fertilizers	0.9 KNO ₃	0.6 0-8.6-0 ^V	0.9 KNO ₃	0.6 0-8.6-0 ^V
0.6 20-8-15 ^U	0.6 KNO ₃ ^t	0.6 C ^a (NO ₃) ₂	0.3 MgSO ₄ ^t	2.7 0-8.6-0 or 1.3 0-19.8-0
NH ₄ NO ₃	1.5 0-8.6-0 ^V	0.8 KNO ₃	0.9 urea-formaldehyde	1.5 0-8.6-0 ^V
			0.6 KNO ₃	1.2 0-8.6-0 ^V
			0.8 KNO ₃	0.4
			0.9 gypsum	

Table 1. Recommended types and incorporation rates for lime and preplant nutrient charge fertilizers. The nutrient content of the individual fertilizer salts was estimated from Hawkes et al. (1985) and Young and Johnson (1982).

	Cornell Peat-lite A and B ^z	Cornell foliage media ^z	Pennsylvania State media ^y	Nelson potting media ^x	GCRI-1 potting media ^w	GCRI-2 potting media ^w	
	All incorporation rates in kg·m ⁻³ of root media						
PNC fertilizers	0.9 KNO ₃ 0.6 0-8.6-0 ^v	0.9 KNO ₃ 0.6 0-8.6-0 ^v 1.6 10-4-8 ^u	0.6 KNO ₃ 1.2 0-8.6-0 ^v 0.6 20-8-15 ^u	0.6 KNO ₃ ^t 0.6 Ca(NO ₃) ₂ 0.3 MgSO ₄ ^t 2.7 0-8.6-0 or 1.3 0-19.8-0 0.9 gypsum	0.8 KNO ₃ 0.4 NH ₄ NO ₃ 1.5 0-8.6-0 ^v	0.8 KNO ₃ 0.9 urea-formaldehyde 1.5 0-8.6-0 ^v	
	Lime rate (kg·m ⁻³)	3.0 ground limestone	4.9 dolomitic limestone	3.0 dolomitic limestone	6.0 dolomitic limestone	2.25 each ground and dolomitic limestone	2.25 each ground and dolomitic limestone
		kg nutrient per m ³ of root medium					
	Total N	0.12	0.28	0.2	0.18	0.25	0.45
PO ₄ -P	0.05	0.12	0.15	0.23	0.13	0.13	
K	0.33	0.46	0.31	0.22	0.29	0.29	
Ca	0.12	0.12	0.23	0.64	0.29	0.29	
Mg	0.00	0.00	0.00	0.03	0.00	0.00	
SO ₄ -S	0.07	0.07	0.14	0.35	0.18	0.18	

^z Boodley and Sheldrake, 1972.

^y White, 1974.

^x Nelson, 1991.

^w Glasshouse Crops Research Institute (Bunt, 1988).

^v N-P-K content of single superphosphate (3Ca(H₂PO₄)₂·H₂O + 7CaSO₄·2H₂O + 2HF).

^u N-P-K content of blended fertilizer. The exact formulation of this fertilizer is unknown and is not included in the nutrient content calculation for Ca, Mg, or SO₄-S.

^t The incorporation of these materials is optional (Nelson, 1991) but is included in the nutrient content calculations.

^s N-P-K content of triple superphosphate (10Ca(H₂PO₄)₂·H₂O + 2HF).

^f The lime recommendation is not included in the nutrient content calculation.

Table 2. Suggested minimum (Min) and maximum (Max) acceptable pH, electrical conductivity (EC), alkalinity, nutrient concentration and sodium adsorption ratio (SAR) for irrigation water used for greenhouse plant production. Units of measure are EC, $\text{dS}\cdot\text{m}^{-1}$; alkalinity, $\text{mg CaCO}_3/\text{L}$; Ca, Mg, SO_4 -S, Na, Cl, B, and F, $\text{mg}\cdot\text{L}^{-1}$.

	Biernbaum (1994) ^z		Fafard (1996) ^y		Gabriels (1978)		Nelson (1991) ^x		Rose et al. (1995)		Scotts (1996) ^w		Sungro (1996) ^v	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
pH	5.5	7.0	5.0	7.0	NA	NA	NA	NA	5.0	7.0	NA	NA	5.0	7.5
EC	0.2	0.8	0	1.0	0	0.85	0	0.75	0	1.5	0.2	1.3	0	1.0
Alkalinity	40	160 ^u	0	100	NA	200	0	40	0	100	40	150 ^t	75	150 ^s
Ca	25	75	40	120	0	120	NA	NA	40	120	25	100	40	80
Mg	10	30	6	24	0	25	NA	NA	6	24	15	50	20	40
SO_4 -S	0	40	NA	NA	NA	NA	NA	NA	0	80	NA	NA	30	60
Na	0	20	0	50	NA	NA	0	70	8	50	0	50	0	80
Cl	0	20	0	20	0	70	0	100	0	140	0	70	0	80
B	0	0.1	0	0.5 ^r	0	0.75	0	1.0 ^r	0.2	0.8	0	0.5 ^r	0	0.5
F	0	0.1	0	0.75 ^r	0	1.0	0	0.5 ^r	0	1.0	0	1.0 ^r	0	1.0
SAR	NA	NA	0	4	NA	NA	NA	NA	0	4	NA	NA	NA	NA

^{NA} Not available

^z Suggested target values from water analysis. A broader range of acceptable values was also presented.

^y Fafard Analytical Services, Athens, Ga.

^x Suggested concentrations at which no nutritional problem should occur.

^w Scotts Analytical Services, Allentown, Pa.

^v Sun Gro Analytical Services, Warwick, N.Y.

^u Average suggested alkalinity concentration. The actual acceptable suggested alkalinity concentrations are dependent on the container size. With plugs (in $\text{mg CaCO}_3/\text{L}$), 40 to 80; while with 15-cm pots, 120 to 180.

^t Average suggested alkalinity concentration. The actual acceptable suggested alkalinity concentrations are dependent on the container size. With plugs (in $\text{mg CaCO}_3/\text{L}$), 40 to 120; bedding flats, 40 to 140; 10- to 12-cm pots and large bedding flats, 40 to 160; and 15-cm pots or larger, 60 to 200.

^s If plugs are grown, alkalinity values on the lower end of the range are suggested.

^r The concentration that can cause toxicity in certain crops may be much lower.

Table 3. Macronutrient information on commercially available water-soluble fertilizers.

Formula ^z	Elemental Analysis (%)					Reaction					Macronutrient Salts
	NH ₄ -N	NO ₃ -N	Urea	P	K	Ca	Mg	Slope ^y	Type	Strength	
21-7-7	9.1	-	12.0	3.1	5.8	-	0.1	179	A	0.78	KCl, MgSO ₄ , NH ₄ H ₂ PO ₄ , (NH ₄) ₂ SO ₄ , urea
25-10-10	1.8	2.7	20.6	4.4	8.3	-	0.1	625	A	0.52	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
30-10-10	2.2	3.2	24.7	4.4	8.3	-	0.1	714	A	0.52	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
9-45-15	9.0	-	-	19.7	12.4	-	0.1	83	A	0.47	KCl, NH ₄ H ₂ PO ₄
27-15-12	3.0	3.8	20.3	6.6	9.9	-	0.1	455	A	0.47	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
20-2-20	7.1	5.9	7.1	0.9	16.5	-	1.0	161	A	0.41	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , (NH ₄) ₂ SO ₄ , urea
15-30-15	5.8	4.5	4.8	13.1	12.4	-	0.1	161	A	0.34	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
20-19-18	3.8	5.2	11.1	8.3	14.9	-	0.2	250	A	0.32	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
25-5-20	1.2	6.0	17.9	2.2	16.5	-	0.1	385	A	0.29	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
20-20-20	3.9	6.2	9.9	8.7	16.5	-	0.1	250	A	0.29	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
10-30-20	4.4	5.7	-	13.1	16.5	-	0.6	104	A	0.21	KH ₂ PO ₄ , KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄
20-10-20	8.0	12.0	-	4.4	16.5	-	0.1	152	A	0.21	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , NH ₄ NO ₃
25-0-25	-	7.3	17.8	-	20.7	-	0.1	333	A	0.20	KNO ₃ , urea
15-20-25	4.0	7.0	4.0	8.7	20.7	-	0.1	147	A	0.15	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
15-15-15	3.0	7.2	4.8	6.6	12.4	-	0.1	156	A	0.13	KNO ₃ , MgSO ₄ , NaNO ₃ , NH ₄ H ₂ PO ₄ , urea
20-15-25	2.8	7.8	9.4	6.6	20.7	-	0.2	238	A	0.12	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
15-16-17	4.5	10.5	-	7.0	14.0	-	0.1	147	A	0.08	KNO ₃ , MgSO ₄ , NaNO ₃ , NH ₄ H ₂ PO ₄ , NH ₄ NO ₃
20-5-30	1.0	8.8	10.2	2.2	24.8	-	0.1	227	A	0.08	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
15-11-29	2.2	8.6	4.3	4.8	24.0	-	0.1	147	A	0.05	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
15-5-25	4.2	10.8	-	2.2	20.7	-	1.3	114	A	0.04	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
15-10-30	2.2	9.1	3.7	4.4	24.8	-	0.1	143	A	0.04	KNO ₃ , MgSO ₄ , NH ₄ H ₂ PO ₄ , urea
20-0-20	5.0	15.0	-	-	16.5	6.0	-	147	A	0.02	Ca(NO ₃) ₂ , KNO ₃ , NH ₄ NO ₃
17-5-17	3.4	13.6	-	2.2	14.1	3.0	1.0	147	A	0.00	Ca(NO ₃) ₂ , KNO ₃ , Mg(NO ₃) ₂ , NH ₄ H ₂ PO ₄
15-5-15	1.2	11.8	2.1	2.2	12.4	5.0	2.0	128	B	0.07	Ca(NO ₃) ₂ , KNO ₃ , Mg(NO ₃) ₂ , NH ₄ H ₂ PO ₄
13-2-13	0.6	12.8	-	0.9	10.7	6.0	3.0	147	B	0.19	Ca(NO ₃) ₂ , KNO ₃ , Mg(NO ₃) ₂ , Urea Phosp.
14-0-14	1.2	12.9	-	-	10.2	5.9	2.9	116	B	0.21	Ca(NO ₃) ₂ , KNO ₃ , Mg(NO ₃) ₂
15-0-15	-	13.0	2.0	-	12.4	11.0	-	135	B	0.21	Ca(NO ₃) ₂ , KNO ₃ , urea

^z N-P₂O₅-K₂O formula

^y Units for EC slope are mg·L⁻¹ N per 1 dS·m⁻¹.

^x The potential reaction of the water-soluble fertilizer. The type of reaction is either acidic (A) or basic (B) and the strength of the reaction is given in kg of acidity or basicity per kg of fertilizer.

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TRANSPLANT PRODUCTION AND PERFORMANCE: THE EFFECT OF CONTAINER CELL SIZE

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A number of factors influence transplant production and performance. This workshop reviews much of the research in this area to date. While factors are discussed individually, it is imperative to remember that few of these factors will act singularly to influence transplant quality and performance. In fact, the transplant production process involves optimizing the many factors that govern both seedling production and post-plant performance. McKee (1981a, 1981b) provides a comprehensive review of components affecting transplant production and establishment, including effects of various cell sizes or soil volumes. This review will focus on those research efforts over the past two decades concerning the effect of container size on both transplant production and field performance. In depth discussions of other transplant topics will be minimized, as these will be discussed by others in accompanying review articles.

The issue of container cell size is extremely important to both transplant producers and consumers. A trend among many commercial transplant producers is toward more cells per tray (smaller cells), which increases production while reducing the need to develop more transplant production space (Vavrina, 1995). This trend also reduces propagation costs, since production costs are directly related to container size and type (Dufault and Waters, 1985; Marsh and Paul, 1988). While the usage of smaller cell sizes may improve the efficiency of transplant production, there is uncertainty as to how smaller root volumes will perform under post-plant field conditions. Decreasing cell size essentially increases root restricting conditions of the transplants.

There are numerous physiological and morphological changes of plants in response to rooting volume that can effect transplant quality and performance. Changes in root and shoot growth, biomass accumulation and partitioning, photosynthesis, leaf chlorophyll content, plant water relations, nutrient uptake, respiration, flowering, and yield have all been described in the literature as being affected by root restriction and varying container sizes. Plant responses to reduced soil volume have been reported for a wide range of crops with some conflicting data among them. There are differences in responses reported between species and even between cultivars within a species.

In general, as cell size increases transplant leaf area, shoot biomass and root biomass increases (Cantliffe, 1993). Growth rates of shoots and roots are interdependent (Tonutti, 1990). Roots rely upon plant aerial portions for photosynthates and various hormones; whereas, plant aerial portions rely on the roots for water, nutrients, support, and hormones. The delicate balance between roots and shoots can be upset when the root system is restricted in a small rooting volume. The resulting imbalance can have short term, as well as long term, effects on plant growth.

Optimal transplant root growth depends on favorable soil or media conditions including water, fertility, and the physical rooting environment (Leskovar et al., 1990). Transplants with large root systems generally suffer less post-plant shock and thus come into production earlier than plants with small root systems (Weston and Zandstra, 1986). Container grown plants in general have a different root morphology than field seeded crops. For example, root restricting tomatoes results in a loss of primary roots and an increase in the number of lateral roots (Peterson et al., 1991a). Also, watermelons which were transplanted were reported to have decreased taproot dominance and in some instances no taproot at all (Elmstrom, 1973). These alterations in root morphology may be more pronounced with smaller container sizes and could predispose plants to drought stress since a significant reservoir of soil water resources goes unexplored. Often, when root restricted seedlings are planted in the field they are unable to compensate for evapotranspiration even if they are well watered after transplanting (Aloni et al., 1991). Root restriction can mimic the effect of soil moisture stress even when there is sufficient soil moisture for normal plant growth (Krizek et al., 1985).

When roots are confined in a container that restrict their growth, the roots compete for essential resources. Increased root mass and decreased root space leads to competition for available oxygen (Peterson et al.,

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1991b). Container geometry and media selection also have a pronounced effect on soil moisture content and aeration. In general, as container height and width are decreased the amount of media pore space decreases, reducing both media water holding capacity and aeration (Bilderback and Fonteno, 1987). Increasing the root mass in the container further reduces the amount of pore space.

Shoot growth is greatly impacted by varying container size and root restriction. Shoot height and biomass reduction by small transplant cell sizes have been reported for tomato (Peterson et al., 1991a), marigold (Latimer, 1991), and watermelon (Hall, 1989; Liu and Latimer, 1995). Hall (1989) also noted that the rate of vine growth was greater in plants grown in larger cells than in smaller ones once transplanted to the field. Liu and Latimer (1995) found that shoot growth reductions in watermelons could occur in 4 to 5 days after seedling emergence depending on cell size. *Euonymus* grown in large containers had a higher mean relative growth rate than those grown in smaller containers (Dubik et al., 1992). Branching or lateral shoot growth of plants has been shown to decrease due to root restriction in bell pepper (NeSmith et al., 1992), salvia (van Iersel, 1997), and soybean (Krizek et al., 1985). Larger container sizes resulted in an increase in the amount of dry matter present in stems of tomato (Kemble et al., 1994) and soybean (Krizek et al., 1985) when compared to smaller containers. Marigold transplants from small cells did not grow as well as those transplants from larger cells when transplanted to an unrestricted soil volume (Latimer, 1991).

The effect of container size and root restriction on leaf growth has been documented for bell pepper (Weston, 1988; NeSmith et al., 1992), marigold (Latimer, 1991), *euonymus* (Dubik et al., 1992), soybean (Krizek et al., 1985), cabbage (Csizinszky and Schuster, 1993), tomato (Weston and Zandstra, 1986), watermelon (Liu and Latimer, 1995), salvia (van Iersel, 1997), and squash (NeSmith 1993a, 1993b). In all cases, as rooting volume decreased, less plant leaf area was produced. The reduction in leaf area was due to both smaller leaves and to fewer leaves per plant.

Reduced plant biomass under root restricting conditions could possibly be due to a lower photosynthetic rate; although, few container size or root restriction experiments have measured photosynthetic rate. Whole-plant photosynthetic rate decreased with increased root restriction in bell pepper, as did leaf photosynthetic rate, although to a lesser degree (NeSmith et al., 1992). The decline in leaf photosynthetic rate in bell pepper in response to decreased rooting volume was coupled with less leaf chlorophyll content (NeSmith et al., 1992). In contrast, no reduction in soybean photosynthetic rate was observed in response to root restriction (Krizek et al., 1985). Summer squash (NeSmith, 1993a and 1993b) and salvia (van Iersel, 1997) net assimilation rate was reduced by prolonged root restriction.

Biomass distribution has been shown to differ with container size. In root restricted *euonymus*, 46% of assimilates were partitioned into the main stem compared to 21% for the control group, with no difference in partitioning to the root system of the plants (Dubik et al., 1990). Krizek et al. (1987) found that root restricted tomato preferentially partitioned assimilates to the roots and decreased those in the leaves. Increases of top biomass for Burford holly, *euonymus*, and azalea were linearly correlated with increasing pot size as noted by Keever et al. (1985). Both root and shoot biomass of salvia increased linearly with container volume (van Iersel, 1997). Total plant biomass decreased in bell pepper and squash with increased root restriction, but there was no disproportional biomass allocation to leaves, stems, or roots (NeSmith et al., 1992; NeSmith, 1993a). Root and shoot biomass were both reduced for watermelon transplants as cell size decreased, although root-to-shoot ratio remained constant (Liu and Latimer, 1995).

Plant development can be influenced by container size and increased or prolonged root restriction. The flowering period was reduced due to increased root restriction in tomatoes (Peterson et al., 1991a). As rooting volume increased, the time from sowing to anthesis was shortened for tomato (Kemble et al., 1994, Ruff et al., 1987) and salvia (van Iersel, 1997). Also, a delay in fruit maturation was shown for root restricted tomatoes (Ruff et al., 1987). In contrast, root restriction resulting from small containers did not have an influence on duration of flowering or time to anthesis in summer squash (NeSmith, 1993a). In bell pepper increased root restriction decreased the time necessary to begin and halt flowering (NeSmith et al., 1992). Root restriction has been viewed as a possible means to accelerate flowering and harvest of cotton (Ruff et al., 1987).

Many morphological and physiological responses of plants to varying container sizes and root restricting conditions have been reported. However, of most concern to the end user of the transplant is the post-plant performance of the seedlings. Of particular concern is crop yield resulting from various container sizes. Varying container cell size has shown mixed results on harvested yield. No reduction in yield was shown for watermelon (Hall, 1989; Vavrina et al., 1993), broccoli (Dufault and Waters, 1985), and cauliflower (Dufault and Waters, 1985) with regard to container size used for transplant production. However, yields were increased in tomato (Weston and Zandstra, 1986), cauliflower (Csizinszky and Schuster, 1988), cabbage

(Marsh and Paul, 1988), watermelon (Liu and Latimer, 1995), and bell pepper (Weston, 1988) as transplant container size increased. Marigold flower cover was increased for plants transplanted from larger cell trays (Latimer, 1991). Differing observations between yields of species and cultivars in response to transplant container size have not been thoroughly explained.

Contradictory evidence and differing responses between species and cultivars in response to rooting volume need further experimentation. It is difficult to optimize transplant production with regard for both the propagator and the end user without such information. There is no doubt that reducing transplant cell size increases the probability for root restriction, but the length of time a plant remains in the container is also a major factor to be considered. Determining when root restriction occurs, along with the identification of the consequences of prolonged restriction, is important in developing improved transplant production systems. Transplant age, a topic to be discussed elsewhere, must be considered when selecting cell sizes for production units. One goal for the transplant industry could be to develop production systems that minimize the time in which plants are under root restricting conditions. Continued experimentation on the interaction of container size, transplant age, and other factors is needed. Experiments need to particularly measure crop yield, as this component will ultimately govern acceptance of transplants by consumers. Research integrating economics into transplant production and performance would be beneficial in developing optimum production systems for both the transplant producer and consumer.

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SEED GERMINATION FOR TRANSPLANTS

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Abstract: Seed germination is the most important step to insure economic success in a transplant operation. Total germination of a seed lot dictates plant sales by the producer, while uniformity of germination dictates the quality of the transplant crop. Insuring high vigor seed will help to achieve uniform as well as maximum stands in the transplant house or field. In order to maintain the highest seed quality, transplant producers should store unused seeds under recommended temperature and relative humidity for the crop species. Methods to promote uniformity and optimum stands under a wide range of conditions include the use of seed priming, film coating with fungicides, and pelleting for ease of planting.

With the advent of precision seeding and mechanical harvesting, growers and seedsmen have become aware of the results of poor seed germination and seedling growth. Optimum plant stand is needed by the grower for highest economical returns. Poor quality seed will eventually lead to sales losses by the transplant producer. Seed storage conditions can adversely affect seed longevity, germination and seedling vigor. Storage at high temperature and high relative humidity can rapidly reduce germination and seedling vigor.

Germination, in the truest sense is the resumption of active growth of the embryo, usually after a state of rest. This results eventually in the breaking of the seed coat and the emergence of a young plant. The seed physiologist measures germination after he notes a biochemical change in the seed is noted or after radical emergence. The seed technologist measures germination only after a normal plant is observed, i.e. root and shoot which are normal in appearance. On the other hand, a farmer only counts emerged seedlings as germination. This may or may not be the point where a transplant producer counts a seed as germinated. Germination of crop seeds follow in a specific sequence of events. They include: imbibition of water, enzyme activation, initiation of embryo growth, rupture of the seed coat, and emergence of the seedling. Imbibition, or the movement of water into the seed, first occurs as a physical movement through natural openings in the seed coat. This water generally moves throughout all the seed tissues. The rate of water movement and total volume is dependent on seed composition. Seeds such as soybean, which contain protein as the major storage component, will reach a larger final volume than a seed that contains a large amount of starch, such as corn. After an initial burst of water movement into the seed there is a lag phase, where respiration starts and the imbibition rate slows down. After the lag phase water movement begins again and is regulated by both the physical properties of the seed and the metabolic processes going on in the seed.

Water causes the cells in the seed to become turgid, the entire seed grows in volume, and the seed coat becomes more permeable to oxygen and carbon dioxide. As the seed swells the seed coat may rupture, facilitating water and gas movement. Generally, dry seed moisture content is from 5% to 8% on a weight basis. Total seed moisture content will rise rapidly to over 60-80%. The embryonic axis will have to attain a moisture content in excess of 90% in order for the radicle to develop. Other portions of the seed may still be below 50% moisture after 12 hours of imbibition. This is especially true in starchy seeds.

As previously mentioned, there are three stages or phases of water uptake. Phase I may be the most rapid, usually lasting from 1 to 8 hours. Lettuce seeds will generally reach Phase I imbibition in 1 to 2 hours. So long as the seed coat does not interfere with water uptake, imbibition in Phase I is similar in both dead and living seeds as well as dormant and nondormant seeds. Phase II or the lag phase can last for several hours to several days and concludes generally when the radicle protrudes through the seed coat. It is during Phase II that the major metabolic events take place which lead to complete germination. Dormant seeds do not come out of Phase II until dormancy is released. Phase II metabolic events include membrane reorganization, enzyme activation, protein synthesis, storage material breakdown, RNA synthesis, and sugar metabolism for energy derivation. Many times dormant seeds will have elevated levels of respiratory activity during Phase II, as well as certain types of synthetic processes taking place. However, depending

on the type of dormancy, these seeds generally never begin cell division.

The final phase, Phase III, is also a period of rapid water uptake. This is generally related to cell division and cell expansion, radicle protrusion, and eventually hypocotyl elongation and protrusion from the seed coat. This marks the end of germination.

The length of time for germination and its various phases are dependent on many factors. In the plant house, it would be dependent upon moisture availability, media composition, oxygen and CO₂ exchange, and especially temperature. Under conditions of low temperature, these processes, especially during Phase II, would be much reduced. Under high temperature, these processes will also be reduced during Phase II. Thus, seeds which have been planted in moist media should be maintained close to their germination temperature optimum. This will provide the most rapid and generally the most uniform emergence of seedlings in the transplant house. Transplant growers should also understand that various seeds respond differently to the above-mentioned environmental conditions, and that the seed itself will predispose the seedling to its optimal growth rate. Such things as the size of the seed, the composition of the seed, the size of the embryo, seed coat permeability (in regards to water and gas exchange) are all influenced by the rate of imbibition in Phase I and the length of time that the seed remains in Phase II. Generally, again as previously stated, Phase I is more affected by water availability and inherit seed characteristics than other outside environmental conditions.

For transplant producers, it is generally easier to maintain adequate moisture levels in the transplant tray or in the transplant field than it is to control temperature. Transplant producers are encouraged to wet their seeds immediately following planting. This offsets problems of maintaining seeds in trays or in field conditions wherein soil moisture conditions are variable, and thus, initiating germination in the seed population at different times. If seeds are not wetted immediately following planting, certain seeds in the seed population may be predisposed to moisture levels which will initiate the early stages of germination. Thus, when the entire population is then wetted, it can lead to variable stand establishment.

The second factor, temperature, is extremely hard to control, especially in field plantings. In greenhouse culture of transplants, germination temperatures may be best controlled by placing the transplant trays in a controlled room so that optimum temperatures for the species in question can be maintained. The period of time in the germination room should last no longer than to the initiation of radicle protrusion through the seed coat. One of the factors that must be considered here to ensure optimum and uniform germination of all the seeds should be to maintain not only adequate temperature and moisture in the tray, but also adequate aeration. Many times transplant producers who use germination temperature control rooms do not consider this factor when trying to establish uniform emergence. In these cases, the trays are stacked one atop the other, filled with media and moistened with water to the point that aeration can be limited, especially in the trays anywhere below the top of the stack. Sometimes these stacks will be on a palette from floor to ceiling in excess of 10 feet high. Many transplant producers have developed tray styles and stacking procedures which allow aeration at the seed level in each of the trays.

A limiting step in germination may occur any time temperatures fall below the optimum for germination of any particular seed. Temperature, especially high temperature, can lead to reduction or inhibition of germination on many seeds. This is especially true of several vegetable and ornamental flower seeds when the temperature rises above 30°C. In many of these seeds, this inhibition of germination only lasts as long as the seeds are in an excessively high temperature for germination. Thus, as temperature is reduced towards germination optimum, the seeds will generally germinate. Unfortunately, because of several factors including seed quality (vigor), moisture availability, and variability in temperature within a tray, the transplant producer may once again find that seed lots may vary greatly in their emergence capacity. In some seeds such as lettuce, geranium, and impatiens, imposing a high temperature for periods in excess of approximately 72 h may impose a secondary dormancy called thermodormancy. In many cases, geranium and impatiens are inhibited by temperatures in excess of 25°C and many if not most of the lettuces are inhibited by temperatures of 30°C or more.

In summary, the key factors to optimize the germination process are moisture, temperature, oxygen, and sometimes light. Regardless of the seed species in question, it is generally necessary to cover seeds during germination with media that will maintain moisture content in the cell or obviously in the field, but allow the maximum aeration at the seed level. Such materials as coarse vermiculite, sand, perlite, styrofoam beads or calcine clay could be used to cover the seed. Smaller seeds should not be planted and covered to the depth of large seeds. Some flower seeds need wet conditions. These seeds include impatiens, begonia,

pansy, ranunculus, and cyclamen. Most vegetable seeds germinate best at field capacity. Some seeds germinate better under dry conditions. These conditions really mean high humidity, but not excessive moisture. These seeds include seedless watermelon, aster, zinnia, verbena, and delphinium. Excessive moisture can lead to anaerobic conditions, especially when media and soil types are quite dense. In a plant house situation, this condition is further exacerbated by stacking flats in the germination room. Conditions of too much wetness can reduce germination and make the seeds more sensitive to high temperatures. Thus, moisture, temperature, and gas exchange are intimately interrelated around one another.

Many of our crop species do not require light for germination, however, in some crop species light is a requirement for germination. In other crop species, light can actually inhibit radicle extension and growth, thus inducing nonuniform seedling growth. This latter case can be exemplified by such species as vinca, cyclamen, phlox, and lettuce. In other species germination may be improved through the use of added light. Examples of these are impatiens and petunia.

A seed testing laboratory can help determine the ability of a seed to establish by reporting percentage of germination. Most seed testing laboratories will test seed for purity, moisture content, and percent germination. Unfortunately, seed laboratories cannot give an accurate idea of how seeds will perform under varying field conditions. They only report how well the seeds will germinate under ideal conditions. This is because seed testing laboratories generally use paper towel tests which provide the seed with continued optimum moisture conditions after the seeds are placed on the towel and wetted. The seeds are then placed in a germination chamber wherein the temperature is set for that particular species' optimum. Thus, the requirement for providing the transplant grower with germination percent can many times be grossly misleading if conditions are not at optimum during the germination process.

Also, seed testing labs generally do not provide tests for seed vigor. Vigor can be generally defined as the ability of a seed to germinate rapidly under a wide range of conditions and to produce a normal seedling. Seed vigor is something that cannot be seen or measured until the seed germinates. Even then, vigor measurements are hard to correlate to final yield. There is no one universal vigor test for all seeds. A vigor test can only measure one phase of early seedling growth. In actuality, the best measures that a plug planter or field producer of transplants can do is to germinate a quantity of seeds under conditions that the entire lot will be germinated under and look at both uniformity of emergence as well as total emergence under those conditions. This, in most instances, relates the sum total of the seeds' ability to germinate under those conditions as well as the potential vigor of that seed lot.

There are several tests that are used by seedsmen to quantify, or at least to attempt to quantify, vigor in the seed lot. Several examples of tests for seed vigor include looking at either cool and/or warm germination stress tests, uniformity and rate of radicle protrusion, measuring radicle growth over a certain period of time, conductivity of seed leachate, accelerated aging, various seedling growth tests such as root length and seedling height, and more recently, a technique developed by the Ball Seed Company utilizing image analysis of cotyledon expansion.

Vigor tests are many times used by a seed company to determine which are their strongest seed lots, and in some instances, how long those seed lots may be predicted to store. Vigor tests can be used by the seedsmen to determine potentially on what markets those seeds should be sold. Companies using such tests will generally direct their highest quality seeds to various markets which require and will pay for that type of seed. The grower who purchases seed from these companies generally will find that total stand counts and uniformity of stand counts are improved. Many times seed companies will charge more for their higher quality lots. If a seed company has to remove poor seed from a lot, this too will increase the price of the remaining seed.

A transplant grower essentially is selling rental space in a transplant house or field. The use of the highest quality seed (highest vigor) will insure rapid uniform and optimum stands of the crop being grown. This translates into greater profits for the transplant producer. High seed vigor can determine the rate of germination, the uniformity of germination, and rate of early seedling growth, especially as it translates to plant growth under less than optimum conditions.

Many times the seeds' viability (ability to germinate) and the seeds' vigor are directly related. Both viability and vigor decline with time. Generally, vigor begins to decline before the producer sees a decline in viability. This means that a lot which germinates uniformly at 90% may be adversely affected as conditions become more stressful. If a grower is to save this seed lot and come back several months later and plant more trays, vigor may have fallen. In this case, the seed lot might germinate well (90%) at

conditions closer to the optimum, but as stressful conditions ensue, the seed lot may fail to germinate or could become very non-uniform in its germination pattern. The process of aging occurs naturally during storage, but can be artificially accelerated by high temperature and high relative humidity during storage. Optimum seed moisture contents for many of our crop species used as transplants is generally in a range of 5-8%. If moisture content drops below the 5% level, storage life and especially seed vigor may be decreased. This is generally related to a disruption of membrane organization. This is a non-reversible disorganization of membrane organization. When seed moisture contents go above 12%, various fungi and insects can grow and reproduce in and on the seed. Also, the aging process is accelerated because some of those processes mentioned in Phase II of imbibition will start to occur as seed moisture content exceeds 12%. Thus, seed storage conditions are of prime importance to maintain seed viability and seed vigor. These processes can then relate to seed longevity. Optimum storage conditions for most seeds is between 5° and 10°C at approximately 40%-50% relative humidity.

Seed companies will store and ship many types of seeds in hermetically-sealed containers. This can be in foil packets, cans, or plastic containers. Such sealed containers will provide an excellent barrier against moisture movement in and out of seeds. Unfortunately, these storage materials do not maintain a barrier of temperature moving between the inside and outside of the container. Thus, transplant producers are cautioned in all phases of seed movement from the seedsmen to the transplant tray to try to maintain temperature conditions towards the optimum. Once these containers are opened, the moisture barriers are removed, and the transplant producer will then have to maintain adequate moisture levels in the storage area in order to maintain seed longevity over an extended period of time.

Many larger transplant producers may have temperature and humidity controlled seed rooms with bells and whistles on them, should either the temperature or humidity go off the desired range. For smaller transplant producers, the use of a frost free refrigerator will substitute for a high-tech seed room. Most important would be to maintain the seeds inside of a container which can be closed and placed inside the refrigerator. When seeds are needed for use, the container should be brought out and maintained closed in a room until the container temperature has equilibrated to room temperature. This prevents moisture condensation on the container, inside the container, and on the seeds. Once the seeds are used, the seeds that remain should again be tightly sealed and put back into the refrigerator. If the transplant producer suspects that seeds are gaining moisture through the process of removal in and out of the refrigerator, then the moisture content of the seeds should be once again brought down to the 5 or 8% level. This can be done by a low relative humidity dry back of that seed lot. Ideally, seed storage should be in waterproof and vapor proof containers such as Tupperware, a mason jar, 5-gallon or smaller plastic cans where the lid can be sealed. A layer of silica gel could be placed at the bottom of the container, and seeds should always be equilibrated to room temperature before the container is opened. If the seed is to be used over an eight-hour or longer period, it is recommended that the seed be retained in the container, and only amounts needed during the time of use should be removed from the container. In summary, it is highly recommended to equilibrate said container in an air conditioned room, and then when needed, move the seed into the transplant area.

Many times transplant producers set up a seed room as part of a controlled climate room or a walk-in cooler. Home-use dehumidifiers are not suitable for controlling moisture content in these rooms, because they will freeze up at temperatures below 17°C and they also add heat into the room. Germination should be routinely checked for seeds that are stored for periods of six months or more. The transplant operator should determine whether or not a seed lot should be retained if germination and/or the ability to germinate under stressful conditions reduces too rapidly.

Transplant producers should be continually aware of the problems related to seed borne pathogens in reducing stand and potentially spreading through the transplant production area. For this reason it is recommended that seeds be used which have been treated with various fungicides recommend for those seeds. Further, the use of film coating will ensure safe conditions for the transplant operators.

Where high-volume precision placement of the seed is required, it is recommended that the seed be pelleted. The coatings used in pelleting are generally some type of clay mixed with a binder and/or water. The plug producer should only purchase pelletized seed that has been recently coated. Seed storage conditions for pelletized seeds are the same as for regular seed. In some instances, and for some species, pelletized seeds may not last as long as conventional seeds.

Certain seed enhancement treatments can improve the rate of germination and the uniformity of

germination, especially under less than ideal conditions. Treatments for seed priming are presently being sold by many seed firms. Seed priming refers to hydrating a seed under controlled conditions, permitting the initial germination processes to begin, while preventing the radicle from emerging through the seed coat. During priming, the moisture content may increase to 40 or 50%. Generally, cell division and/or cell elongation do not take place during the priming process. Most importantly, after priming the moisture in the seeds is reduced to the initial content of between 5 and 8%. Primed seeds can then be packaged as conventional seeds. They can be shipped and planted using conventional seeding equipment. Unfortunately, many times seed storage time is reduced in primed seeds. Also, seed priming increases the rate of germination and generally the uniformity of germination under a wider range of conditions, but does not increase total germination under ideal conditions. In other words, seed priming cannot make dead seeds come alive. The variables which are controlled in priming conclude the amount and the rate of water uptake, temperature, and duration of the process. Seeds which require light to germinate should be given light during the priming process. At all times, oxygen should be made available to the seeds because the seed is a living entity and requires oxygen for respiration. In summary, understanding seed germination is extremely important to the transplant producer. Seed germination is the most important step to ensure economic returns in the transplant operation. Germination is important and dictates final stands that the transplant operator will achieve, but also the uniformity of emergence will ensure a high quality crop for the transplant producer.

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TRANSPLANT PRODUCTION AND PERFORMANCE: WATER MANAGEMENT

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ABSTRACT

Successful water management depends on what is applied, when and how much is applied, and how it is applied. Key water quality interpretation considerations for greenhouse crops and transplants are: 1) alkalinity; 2) soluble salts (as measured by electrical conductivity (EC) and sodium adsorption ratio (SAR)); and 3) elemental toxicities or deficiencies. A number of factors influence the amount of available water per individual transplant cell including cell size and geometry, medium particle size, medium moisture content at tray filling, medium moisture release characteristics and wetting agents. However, scheduling of frequency of irrigation and the volume of water applied are the primary factors determining moisture availability. Water loss prediction based on VPD can be used for automated irrigation but gravimetric (weight) methods are more practical for quantifying manual scheduling of irrigations. Crop stage of development, crop moisture requirement, the desired frequency of medium drying and the effect of volume applied on leaching also must be considered when scheduling irrigation frequency and volume applied. There are five major ways to apply water to plug seedlings: 1) hand watering; 2) stationary sprinklers, 3) traveling boom sprinklers, 4) fog, and 5) subirrigation. The water droplet size and the uniformity of the water application will affect the uniformity of the finished product.

INTRODUCTION

In recent years, the trend in ornamental and vegetable transplant production is the growing of transplants in plug cell trays. Broadcast sowing of seeds in seedling flats and then hand transplanting the seedling has been replaced by placing a single seed in a small cell of a plug flat, thereby individualizing the transplant. The root system remains intact during transplant thereby reducing shock and the establishment time. Producers have decreased the amount of labor that is needed to produce a crop and increased the amount of mechanization used. Plug production has both increased the efficiency of production and complicated the process of production at the same time. The decreased size of the root zone has required more precise water and fertilization management. Some plug cells may be as small as 2 cm³ (800 plug flat).

The objective of this review is to consider the key components of water management for transplant production. The first issue addressed is *what* type of water is applied as defined by water quality. The second issue considered is *when* and *how much* water is applied, including the available methods of irrigation scheduling. The third topic is *how* the water is applied using commercial irrigation practices. For each topic, the relevant research information and popular grower related information was reviewed. The literature for production of ornamental (flower) transplants for continued growth in greenhouses or outdoor gardens far outweighs that for vegetable transplants grown for food production in the field. Research relating water management during production to crop growth or yield performance after transplanting was not found. A sampling of food crop transplant related research was reviewed to see how each of the irrigation issues was or was not considered and reported.

Water Quality

There are several characteristics of water quality that can drastically effect the quality of transplants through changes in the nutrient status and pH of the growing media. Much of the water quality related research is traced back to publications from the USDA Salinity lab (Allison et. al, 1954). In the first handbook, four major characteristics of an irrigation water that determine quality were recognized: 1) concentration of soluble salts; 2) relative proportion of sodium to other cations; 3) concentration of boron and other toxic elements; and 4) bicarbonate concentrations as related to the concentration of calcium plus magnesium. However, Spurway (1938) presented a review of water quality issues for greenhouses much earlier. The most recent complete discussions of water quality related to greenhouse crop production have been by Biernbaum (1995) and Petersen (1996) and for plugs by Styer and Koranski (1997). Water

quality related to plant production in containers is also covered by Bailey (1997), Bunt (1988), Lang (1996), Nelson (1991), Reed (1997), Styer (1996), Styer and Koranski (1997) and Vetanovetz and Knauss (1988). A characterization of water samples from commercial greenhouses in the United States including the pH, EC and macronutrient concentrations was recently published by Argo et al. (1997). Their results were similar to those of Ludwig and Peterson (1984) as reported in Rose et al. (1995).

Biernbaum (1995) provides information on sources of irrigation water and recommendations for how to have water tested and suggests that potential sources of irrigation water be tested before greenhouse construction begins. Routine irrigation water analysis is then repeated 2-4 times a year. Key interpretation considerations for greenhouse crops and transplants are: 1) alkalinity; 2) soluble salts (as measured by electrical conductivity (EC) and sodium adsorption ratio (SAR)); and 3) elemental toxicities or deficiencies.

Alkalinity. Alkalinity is a measure of bicarbonate and carbonate concentration in the water as determined by titration with dilute acids to a pH endpoint of 4.5. The water alkalinity, and not the water pH, determines the effect on changes in the pH of the root medium. Argo and Biernbaum (1996) have shown how alkalinity in combination with fertilizer reaction influences changes in media pH for impatiens grown in pots.

A number of authors warn that alkalinity levels greater than 80 mg/liter bicarbonate will cause the pH of the media in plug cells to increase (Biernbaum, 1995; Lang, 1996; Styer, 1996; Styer and Koranski, 1997). An alkalinity concentration of between 40 and 80 mg/liter is generally recommended to maintain a stable medium pH. However, Biernbaum points out that one bicarbonate concentration may not be the best guideline for all crops. When growing plugs there is a very small amount of media to manage and the effect of water alkalinity is different than for larger pots of media. The amount of water applied may influence the effect of alkaline water. Monitoring the pH of the root medium is the best method to determine the alkalinity level that will maintain a balanced medium pH for a given system. If rising medium pH is a problem during production, either an acidifying fertilizer (high in NH_4) or the addition of acid to the irrigation water can be used. High ammonium fertilizers (>20%) are not recommended for plugs due to the increased growth that may occur. Methods of acidification are covered by Biernbaum (1995).

Soluble Salts or Electrical Conductivity. High levels of soluble salts in the irrigation water or excess application of fertilizer salts can lead to a buildup of salts in the media over time and will limit water uptake due to osmotic effects. Seed germination and transplant production requires frequent, nonsaturating applications of water with little or no leaching to maintain media moisture. Consequently, the irrigation water must be fairly pure and the grower should pay close attention to the levels of salts in the root medium. Sodium and chloride are the most common non-nutrient salts in irrigation water and when levels are excessive (>60 mg/liter) some type of water purification is recommended for plug production. Reed (1997) and Biernbaum (1995) cover water treatment options. Styer recommends that sodium absorption ratio (SAR) values should be less than 2.0 and that the concentration of sodium ions less than 40 ppm. According to Styer (1996), high SAR value will cause a media to hold more water because of the increase in solute potential negativity, which will result in lower O_2 content thereby reducing potential for root growth.

Other Elemental Toxicities or Deficiencies. There are certain elements that should be tested for, because they can be phytotoxic at very low concentrations. Boron and Fluoride are two elements found in excess in irrigation water. Alkalinity from calcium and magnesium bicarbonates and iron may create problems with plugging of spray nozzles and residue on plant foliage. Sulfur concentrations in irrigation water are less than is normally recommended for crop production and sulfur is often not added to water soluble fertilizers (Reddy et al., 1994). With very pure water, calcium and magnesium may also be limiting if not supplied on a regular basis with water soluble fertilizer.

Water Treatment. If alternative water sources are not available, water purification is often justified for high value transplants (Reed, 1997). Options include filtration, acidification, softening and reverse osmosis, ozonation, bromination or chlorination. Depending on the water source, treatment may be required for plant pathogens and algae.

Irrigation Scheduling: Amount of Available Water

Before considering methods of irrigation scheduling, it is important to determine which factors influence the amount of water that is available to the plant. The volume of the cell, the shape of the cell flat, the media particle size, the medium water release characteristics and possibly the moisture content of the media at the time of flat filling can influence the available water content of the root zone.

Container Size and Geometry. The volume of plug or transplant cells ranges from 2 cc (800 cells) to 25 cc (128 cell, deep). The volume is not only a function of the number of cells but the depth and geometry. Square cell typically have a greater volume than round cells. At saturation, the amount of available water may range from 40 to 60% of the volume or from 1 to 15 ml.

Milks et al. (1989) point out that shallowness of containers results in too much water, and therefore too little air in the

root zone. A relationship exists between the ratio of air space to available water, and the shape of the plug cell. In general, deeper cells hold more air in the germination medium than the same medium in a shallower cell. The decrease in air content of shallower cells is due to the decreased effect that gravity has on the drainage of excess water of saturation. The shorter the column of water the more water will be retained in the container, because adhesive forces are greater than those of gravity.

Media Particle Size. Air space is also directly related to pore size of the media. After a container media is irrigated to saturation, the first water to drain out the bottom of the cell is that which filled the macro-pores or air space of the media. If the pore space of the media is sufficiently small enough to attract water more strongly than gravity, a capillary fringe (a zone of saturation) can form in the bottom of larger containers (Foth, 1990). Because of the reduced height of a plug cell this capillary fringe of the root zone may include the entire cell height, and cause the entire cell to remain saturated.

Bilderback and Fonteno (1987) effectively demonstrated the interaction of container size and media particle size in the determination of air space. The percent air space increased with both larger containers and larger pore space which was a result of larger media particle size. This relationship has been mathematically modeled (Fonteno, 1989; Milks et al., 1989). In very shallow containers, medium particle size will likely have very little effect on aeration at saturation.

Media Moisture Content at Tray Filling. With respect to plug cells, it has been shown that air space in plug cells (a 288 tray) can be increased from the normal 2 to 7% when the moisture content of the mix is increased from 60 to 70% before the flat is filled (Milks et al., 1989). The advisable pre-flat-filling minimum water content is 50%. These percentages are water content on a weight basis. A consistent initial media water content will increase the uniformity of plug production over time although flat filling practices and equipment may dictate media moisture content.

Media Moisture Release Characteristics. Along with air space, the amount of water that is available to the seedling is a function of media type. Even though there may be moisture in the media, it may not be available to the plant to absorb. The forces of adhesion to the soil particles may be greater than the water potential of the seedling roots. Bilderback and Fonteno (1987) show that even though two different media contain the same percentage of water on a volume basis the moisture tension can differ based on the nature of the media.

Wetting Agents. Wetting agents or surfactants are commonly added to peat-based media to aid in rewetting. The primary effect is on the absorption of water into peat fibers and is more important for very dry peat as opposed to peat that already contains some moisture. In taller containers under saturation conditions, the effect of the wetting agent on surface tension can lead to an increase in drainage and air content. Wetting agents can degrade if media are stored for several months. While commonly used materials are not phytotoxic, excessive rates or nonhorticultural wetting agents can result in effects on germination or plant growth.

Summary. Despite the effect of growing container height and root media properties on air and moisture holding capacity, in most commercial settings air space in the root medium is maintained more by limiting the application of water at each irrigation than by container or root medium selection. While in larger containers water must be added to thoroughly moisten the entire media profile, in shallow containers a less than saturating amount of water can be added without detrimental effects to roots since the water will distribute adequately.

Irrigation Scheduling: Methods

Soil Moisture Tension. Although not commonly done, in larger pots growers can measure the soil moisture tension with a tensiometer which when connected to a climate control computer can be used determine irrigation frequency (Wilkerson and Samengo, 1992). With plug production, the soil volume is so small that the tensiometer would displace the media.

Water Loss Predictions. Water loss can be predicted using data collected by environmental control computers. Total accumulated solar radiant energy or light intensity can be summed over time and used to schedule irrigations. Solid state mist controllers based on light sensor input are commercially available (Davis Engineering, CA) Vapor pressure deficit (VPD) values can also be used to estimate the amount of water that has been used by the plant, or has evaporated and the amount that remains in the plug cell media (Barret, 1996). Vapor pressure is a measure of the partial pressure of water vapor, or the concentration of water in the air. Because growing conditions are rarely at 100% relative humidity there is a potential for more water to be dissolved into the air. The leaf on the other hand, has intercellular spaces that are composed of air that is at 100% relative humidity, or completely saturated with water vapor. This difference in water potential or concentration is the driving force of transpiration. Climate control computers with automatic watering systems monitor the factors that influence both leaf temperature (combination of ambient temperature and light levels), air temperature and relative humidity. With a VPD based watering system, the computer determines VPD every few seconds; the accumulated VPD becomes an estimate of plant water use (Barret, 1996).

Based on this VPD integral, irrigation frequency is determined by how long it takes this integral to reach a target value set by the grower. The magnitude of the target is determined by plant maturity, container size, and the crop that is being grown. In order to set the correct target value some experimentation may need to be done. Close monitoring of the system is needed when first establishing the target value. Commercially available programs have been used successfully for plug production in commercial greenhouses (Miller, 1989).

Determination by weight/ gravimetric. Determining irrigation scheduling can be done consistently and reproducibly using a scale. Weight loss between the time of a typical irrigation and the point of wilting can be used as an estimate of available water and to schedule irrigations. Argo and Biernbaum (1994, 1995a, 1995b) used scales with poinsettias and Easter lilies to make comparisons between different potting media where available water and water use were not all the same. Available water is determined as the weight of the flat, medium and plants after an irrigation minus the weight at wilting. Irrigation is usually done when 75 to 85% of the available water is lost. This method is very useful to quantify the method of irrigation or medium moisture content in research situations. With the scale method, a repeatable set of parameters that anyone can follow can easily be defined.

Other Factors Influencing Irrigation Scheduling

Effect of Crop Stage. One complication to plug water level management is the different water management strategies that are needed during different stages. Commercial transplant or plug production is typically divided into four stages. During Stage 1 of plug production (from sowing to radicle emergence) plugs need to be at or near saturation depending on the species so that the seed is in constant contact with water in order to germinate. Once the radicle starts to penetrate the media surface water levels need to be decreased to allow for proper root development. Stage 1 can be completed either in a germination chamber supplied with a fog generating system to maintain near 100% relative humidity (Miller, 1989; Stryer and Koranski, 1997) or out in the greenhouse if uniform moisture can be maintained. For germination in the greenhouse either frequent application of water, covering the seed with a thin layer of medium, perlite or vermiculite, or covering the trays with a light weight, frost protection fabric is necessary to maintain the high level of moisture required.

High moisture levels during stage 1 are not recommended for all bedding plant species. Bedding plants water requirements during stage 1 have been classified as wet, moist, or dry (Koranski et al. 1991; Kuack, 1991; Stryer and Koranski, 1997). Wet medium is saturated so the seed is surrounded with moisture. Moist medium is wet but not saturated. Dry medium has little water added before or after sowing and is kept dry until germination begins. As the transplant continues to mature, moisture levels are decreased to help "harden off" the the plant to increase tolerance to drought stress conditions, and increase the chances that it survives during shipping if needed or transplanting (Koranski, 1983). Stage 2 is defined as the period from radicle emergence to formation of the cotyledonary leaves. Stage 3 is development of the true leaves. Stage 4 is the finishing or hardening stage. In some cases there is a Stage 5 which is storage at cold temperatures prior to transplanting. Keeping the foliage dry during cold storage is critical to prevent foliar pathogens. Subirrigation is recommended during stage 5. (Need a Heins reference)

Drying of the media. Over watering of plugs is the most common problem plug growers encounter (Styer and Koranski, 1996). Commercial transplant or plug producers are reportedly concerned about having the time between irrigations or the drying time to a maximum of 24 to 48 hours. A rapid drying time reportedly allows replenishment of the air/oxygen in the root zone and more frequent application of nutrients if needed. However, growth of seedlings under prolonged high moisture conditions with infrequent irrigation verses rapid drying and frequent irrigation has not been evaluated under controlled conditions. Control of plant growth in containers can be accomplished by keeping plants dry. Unfortunately, "dry growing" has not been well defined. Allowing plants to wilt and then thoroughly saturating the root media is not as effective at controlling plant size as more frequent small volume applications that do not allow for saturation of the root zone.

Water loss is also influenced by bottom heating, frequently used to maintain root medium temperatures. Hot water is circulated through pipes under the expanded metal bench tops on in concrete floors. Without bottom heating, the root medium temperature can be significantly below air temperature due to evaporative cooling. Bottom heating also contributes significantly to the drying of the root medium (Sray, 1996).

Volume Applied/Leaching. The volume of water applied can have a large effect on fertilizer retention in the root medium (Biernbaum, 1992). A small amount of leaching can remove soluble fertilizer in the medium and alter the water soluble fertilizer requirement (Argo and Biernbaum, 1996b). The concentration of nutrients from water soluble fertilizer is very dependent on whether leaching occurs. Lower water soluble fertilizer concentrations applied with no leaching can lead to the same nutrient concentration in the root zone as with higher water soluble fertilizer concentrations applied with leaching (Yelanich and Biernbaum, 1993, 1994; Nelson et al, 1996).

Irrigation Methods

There are five major ways to apply water to plug seedlings: 1) hand watering; 2) stationary sprinklers, 3) traveling boom sprinklers, 4) fog, and 5) subirrigation. Each has its advantages and disadvantages. The water droplet size and the uniformity of the water application will affect the uniformity of the finished product. General discussion of watering methods for plugs is in Styer and Koranski (1997) and Lucas ().

Hand Watering. Hand watering is the most flexible when it comes to irrigating portions of an area whether it be a part of a flat, bench or greenhouse. However, hand watering is usually the most expensive due to the cost of labor. It is also hard to achieve the uniformity and small particle size that is needed. If plant quality is reduced, in addition to the increased labor costs associated with hand watering the operation is also losing money due to lower product quality.

Stationary Sprinklers. Stationary sprinkler systems like those used to finish bedding plants are commonly used for plug irrigation. Nozzles can be on risers coming from below the bench or on suspended water lines over the crop. Once again though, uniformity is a problem. It is hard to design a sprinkler system so that there is little overlap or complete overlap. Droplet size is usually larger than with booms or fog systems.

Traveling Booms. One of the most popular choices for plug producers today is boom irrigation. By mounting the spray nozzles on a horizontal pipe moving at a constant speed down the greenhouse only a uniform line of water is applied (Lucas, 1997). New advancements like variable speed motors, selectable spray nozzles, and bar codes that can be placed along the path of the boom to control water application and speed of movement have increased the flexibility of this system. The cost is offset in large greenhouses or systems where the boom can travel from house to house.

Fog. Very fine, uniform particles (5 micron) of water produced by fog systems can allow maintenance of high moisture levels and relative humidity with very low application rates compared to fine mist (20-60 micron) or large particles typical with hand watering (300 to 500 microns). A high quality, pure water source is required. For some flower crops, plants started with fog grew faster than other watering methods with large droplet size (Ball, 1987). Fog during the first 10 days increased the germination percentage of petunia (Koranski et al. 1991). Fog is used in chambers or in the greenhouse, although it is more practical in germination chambers. In the greenhouse, the high relative humidity from fog can lead to condensation on the glazing surface and dripping. Dripping from the glazing can rapidly destroy plants and disperse media from the small cells.

Subirrigation. Sub-irrigation is a popular choice for many of the potted crops that are produced in the greenhouse due to the uniformity and low labor costs (Biernbaum, 1993), but presents specialized problems with plug production. The shallow medium columns in plug trays rapidly become saturated when water comes in contact with the medium. Less than saturating applications of water are not possible and increased particle size of the root medium does little to reduce water uptake. Root pruning is also not generally possible with subirrigation on tables or cement floors because the bottom of the flats cannot be dried out adequately. Subirrigation has been used successfully with large cell (128s), polystyrene flats that will float on the irrigation water (Leskovar et al. 1994). In this system, the plants are suspended on wires between irrigations which allows root pruning. The time between irrigation is 2 to 3 days. Transplants grown with the flotation system were found to be acceptable as long as there was minimum hardening before planting in the field (Leskovar et al. 1994). One of the advantages of subirrigation is that the foliage remains dry during an irrigation. One of the main motivations for the transplant float system was to reduce the spread of bacterial diseases in tomato transplants that occurred with overhead watering.

SUMMARY AND CONCLUSIONS

The key to successful growing in any plug tray is water management (Koranski and Karlovich, 1989). Successful water management depends on what is applied, when and how much is applied, and how it is applied. Whether in research or production situations, aspects of water management must be quantified to allow consistency and reproducibility within a tray and over time.

The effect of water quality or application method has often not been considered in experimentation regarding vegetable transplant production. The effect of container size, container geometry, and "root restriction" have been discussed with no apparent consideration for the relationship of these variables to the amount of irrigation water applied and possible effects on medium pH, EC or nutrient content (Latimer, 1991; Leskovar et al., 1994; Leskovar and Cantliffe, 1992; Liptay and Edwards, 1994; Marsh and Paul, 1988; Maynard et al., 1996; Weston, 1988; Weston and Zandstra, 1986). For example, a comparison of the effect of transplant container volumes from 7 to 70 cc can be done by seeding all the plants on the same date and then transplanting on the same date, for example 30 days later. Not only would the plants in the 7 cc cell be ready to transplant after 15 days and therefore likely sitting stalled for 15 days, but the high volume of water necessary to keep those plants alive in the small cell for the remaining 15 days could have a dramatic effect on root medium pH and EC (nutrient content) depending on whether pure water or high alkalinity water

was used for irrigations. The water quality, irrigation method (leaching) and fertilization method could have a large effect on the outcome of this experiment. Reporting medium pH and EC at the end of the transplant production phase would be very helpful. As an alternative, the experiment could also be done with different seeding dates leading up to the same transplanting date so the maturity of the plant for a given cell size is reached at the same time and the comparison is more of cell size/transplant age as opposed to maturity (root restriction) or degree of possible pH or nutrient stress.

Uniformity and a well developed root system are essential for transplants whether planting is in the greenhouse or the field. However, it may be useful to identify if a transplant going from one container size to another in the greenhouse has the same desirable characteristics as a flower or vegetable transplant that will be shipped and then sit in a retail store or outside display area with minimal care prior to ending up in a residential garden, or a vegetable transplant that will go direct from the greenhouse to field, or a flower transplant that will be planted along a roadside. Irrigation practices can have an important effect on the survival in the container and immediately following transplanting into a stressful environment.

Future research or problem areas related to water quality and irrigation method include:

1. Uniform reporting of research methods including type of water and method of irrigation.
2. Methods of controlling or eliminating algae on the medium surface. The primary effect is on fungus gnat and shore fly development which can effect disease transfer.
3. Production of a well developed root system and a transplant that is hard enough to survive mechanical transplanting but soft enough to grow rapidly after transplanting.
4. Further identification and definition of the optimum medium moisture status in combination with fertility programs during various stages of plug production that will minimize production time while maintaining transplant survival and regrowth potential, including the relation to growth retardant chemicals.
5. Methods to reduce water availability during the early stages of production while maximizing water availability in the final stage when plants are large.
6. Maintenance of plant vigor and reduction of the use of foliar and root disease control chemicals through water management techniques.

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ROOT AND SHOOT GROWTH OF VEGETABLE CROP TRANSPLANTS: MODIFICATION BY IRRIGATION

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INTRODUCTION

Transplant root systems have important physical and physiological roles from early stages of seedling emergence through subsequent seedling growth and development. The size, distribution, or architecture of a transplant root system may exert a control over the relative size and growth rate of the developing shoot system. Irrigation methods, rates, timing, and frequency may influence the physical and chemical properties of the growing media thereby affecting root initiation, elongation, branching, development and the dry matter partitioning between roots and shoots. The importance of root morphological components of vegetable seedling root systems has been reviewed by Leskovar and Stoffella (1995). The purpose of this paper is to review the influence of irrigation systems and the interaction with other production factors on root and shoot growth and development of vegetable transplants.

IRRIGATION SYSTEMS

Overhead irrigation is the most commonly used irrigation system for containerized production of vegetable transplants. In the overhead system, flats are generally supported by a T-rail system and at each irrigation water is delivered by a sprinkler system until leaching. A sub-irrigation, or commonly referred as ebb-and-flow system, was developed by Speedling, Inc. in Florida in 1984, and in California in 1991 (Thomas, 1993). Overhead and ebb-and-flow system are also used in Japan, but the former is dominant because of its simplicity (Kozai and Ito, 1993). The ebb-and-flow system, which is a variation of the continuous floatation system originally designed to grow tobacco plants to increase field survival and reduce transplant shock, is now being used to grow a large number of commercial vegetables. This system utilizes recycled stored- or collected-water, saving water and reducing fertilizer and pesticide use as compared with overhead irrigated systems. Trays are suspended on metal wires about 0.20 m above concrete floors, and every 2-3 days the irrigation water is raised to the level of the container, maintained for 15-45 min and then decreased to its original level or returned to the main reservoir until the next irrigation. In this system, water moves by capillary action generally until cells are saturated. Previous investigations reported that floatation systems improved uniformity and quality of bell pepper (*Capsicum annuum* L.), jalapeño pepper, and tomato (*Lycopersicon esculentum* Mill.) if grown with minimal nutrient and water stress (Leskovar and Boales, 1995; Leskovar and Cantliffe, 1993; Leskovar et al., 1994). However, in Europe there is a concern that viruses including tomato spotted wilt virus, tomato mosaic virus and lettuce big vein virus are released from roots of diseased plants and spread by recirculating nutrient solution of the ebb-and-flow irrigation system with the potential for contamination of the irrigation water and infection of healthy plants (Buttner et al., 1995). Similarly, several plant pathogenic fungi such as *Pythium* spp. and *Phytophthora* spp. can cause persistent problems with recycled irrigation water (Pottorff and Panter, 1997; Sanogo and Moorman, 1993).

ROOT AND SHOOT DEVELOPMENT

Bell pepper transplants grown with overhead irrigation had more basal but less lateral roots than transplants grown with the ebb-and-flow system (Leskovar and Cantliffe, 1993). In tomato, more early basal root dry matter allocation in overhead-irrigated transplants during the first two weeks after transplanting was important for the subsequent shoot growth increase three to four weeks after transplanting (Leskovar et al., 1994). These investigations reported that tomato transplants grown with floatation had excessive root growth outside the bottom of the container, and consequently roots were severely pruned in the greenhouse for ease in transplant manipulation in production fields. However, root pruning prior to transplanting delayed re-establishment in tomato transplants (McKee, 1981). Cabbage (*Brassica oleracea* var. *capitata*) transplant production using overhead, ebb-and-flow, and float bed systems was investigated relative to survival, transplant shock and growth of cabbage (Frantz and

Welbaum, 1995a). They concluded that constant availability of water in the floating systems or short drying cycles in the ebb-and-flow systems contributed to excess root growth through the bottom of the trays producing plants with less ability to overcome transplant shock. Similar conclusions were reported on sweet corn (*sh2*, *Zea mays* var. *saccharata*) transplants in a float-bed system (Frantz and Welbaum, 1995b).

The influence of irrigation timing and irrigation systems on root elongation, root morphology, shoot growth and water status of 'TAM-Mild Jalapeño-1' pepper seedlings was reported by Leskovar and Heineman (1994). Irrigation systems used were: a) floatation or ebb-and-flow (FI), b) 28 days floatation plus 14 days overhead (FI+OI); c) alternate floatation and overhead (FI/OI), and d) overhead (OI). Transplants with FI or FI/OI maintained a uniform lateral root length increase between 20 and 41 days after seeding (DAS). Lateral root elongation in transplants that were irrigated with OI or FI+OI reached a plateau ~ 31 DAS, but the number and length of basal roots increased by 33%. Providing uniform moisture levels around the hypocotyl during the last two weeks prior to field establishment is thought to enhance basal root primordia development. OI transplants had higher shoot/root ratio (S:R= 5) and maintained a higher shoot water potential ($Y_{stem} = -0.58$ MPa) than FI transplants (S:R=3; $Y_{stem} = -0.69$ MPa), respectively. The latter had lower stomatal conductance and photosynthesis, compared with OI- and FI+OI-transplants. Leskovar and Heineman (1994) concluded that floatation irrigation may be used to reduce shoot:root ratio and promote hardiness in single stem jalapeño transplants. Conversely, Galloway et al. (1996) reported that banana 'Banana Supreme', bell 'Camelot' and jalapeño 'Mitla' pepper transplants grown on ebb-and-flow systems were taller than with overhead irrigation. In the field, spatial and temporal root growth distribution of bell pepper transplants grown with ebb-and-flow and overhead irrigation systems were similar when described between 30 and 86 DAT (Leskovar et al., 1990). Currently, we are investigating the interaction of irrigation systems, ebb-and-flow and overhead, and N levels (50, 100, and 200 mg N/liter) on root and shoot growth of five vegetable species including a jalapeño pepper hybrid 'Mitla'. Based on preliminary data (Leskovar, 1997 unpublished) we were not able to measure growth responses obtained previously in 'TAM-Mild Jalapeño-1' pepper. It appears that influence of irrigation method and the interaction with N on root and shoot development are both species and cultivar dependant.

Root development is a dynamic process responding to several stress stimuli, probably as an adaptive mechanism. Roots sensing water stress synthesize chemicals and transport a 'signal' to the shoots influencing stomatal behavior, gas exchange characteristics and ultimately growth (Davies et al., 1990). When drought stress and root pruning methods were used to harden and prevent stem elongation in fresh market tomato transplants grown with floatation irrigation, there was an increase in lateral root elongation and a decrease in shoot:root ratio, but dry matter partitioning, leaf enlargement and total plant size were severely affected (Leskovar et al., 1994). Lower root dry weight, basal root number, basal root diameter, and shoot growth were measured on pepper transplants exposed to mild drought conditions as compared with optimum-watered seedlings (Leskovar and Cantliffe, 1992). Induced drought or application of abscisic acid (ABA) to control shoot growth in bell pepper plants in the greenhouse altered dry matter partitioning and stomatal behavior (Watts et al., 1981, Berkowitz and Rabin, 1988; Leskovar and Cantliffe, 1992). This response was suggested to be mediated by endogenous changes of ABA concentration acting as a signal for control of growth processes (Davis et al., 1986). A reduction of shoot:root ratio either by shoot growth reduction or by larger root growth relative to shoot growth was correlated with ABA synthesis induced by drought (Creelman et al., 1990). When ABA at 10^{-4} M was applied to pepper seedlings three weeks after seeding, there was a transient inhibition of leaf weight increase, but root growth was unaffected (Leskovar and Cantliffe, 1992). ABA at 10^{-3} M reduced relative growth rates of eggplant (*Solanum melongena* L.) seedlings (Latimer and Mitchell, 1988). Exogenous application of ABA increased leaf resistance, leaf water potential and seedling survival in peppers (Berkowitz and Rabin, 1988).

IMPLICATIONS FOR STAND ESTABLISHMENT

The ultimate goal of the nursery is to produce a quality transplant with the ability to withstand transplanting shock. Seedling establishment in any field environment depends on adequate development of a taproot, associated laterals, and basal roots for dicot species, or adventitious roots and associated

lateral roots for most monocot species (Leskovar and Stoffella, 1995). The ability of a containerized transplant to overcome transplant shock and become established in a field environment following transplanting depends on the ability of seedlings to withstand root disturbance (McKee, 1981), the water and nutrient uptake capacity of the roots, and the capacity of the pre-existing roots to rapidly regenerate new lateral, basal, or adventitious roots are affected. Transplant shock generally occurs when transpiration exceeds water uptake, resulting in a sudden or severe plant water deficit (Nitzsche et al., 1991). Other manifestations of transplant shock includes leaf abscission, burning, and detopping. When 'TAM-Mild Jalapeño-1' pepper transplants grown with overhead irrigation in Texas and ebb-and-flow irrigation in Florida were evaluated 20 days after transplanting, transplant shock [dead, detopped (absence of shoot apex), or burned (> 1 cm long hypocotyl)] was 23 % for overhead and 13% for ebb-and-flow irrigated transplants (Leskovar and Boales, 1995). This difference was due to an increase in the number of burned seedlings associated with taller and thinner stem plants obtained from the overhead system. Prior to shipping tomato seedlings grown on ebb-and-flow, drenching roots with a humectant in water (1:14 ratio) was reported to improve water retention and uptake, increasing total yields as compared with the untreated control (Ciardi et al., 1995).

Irrigation methods and irrigation timing affect temporal and spatial nutrient concentrations, pH, and root medium electrical conductivity. Evidence for these changes have been reported in flowering plants including geraniums (*Pelargonium hortorum*), Easter lilies (*Lilium longiflorum*), and poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotzsch) (Argo and Biernbaum, 1994; Argo and Biernbaum, 1995; Morvant et al., 1997), but is lacking on vegetable species. Nutrition often has a strong influence on seedling growth and carbohydrate status, affecting post-transplant recovery to shock (Aloni et al., 1991; Dufault, 1994). For example, differential water condition at the root tip may affect root responsiveness to nutrients such as calcium (Takahashi et al., 1992). Additional research is needed on the effects of irrigation systems and the interaction with nutrition, particularly on small-volume cells (> 200 cells/tray) and for a wide array of vegetable species. Information from this research will enhance our understanding of root and shoot growth dynamics in the greenhouse, post-planting transplant shock, and shoot recovery in the field.

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Transplant Production and Performance: Effect of Transplant Nutrition

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Seedling transplants has been used for decades and the advantages for their use have been outlined previously (Dufault, 1993). The character of transplants has changed tremendously since the early 1930's, as well as the methods to produce transplants. In olden days, all sorts of large containers were used such as clay pots, peat pots, paper bands, wood veneer bands and tin cans (Ware, 1937). Field soils were used to grow transplants and in many cases, compost heaps were developed two years in advance to produce a soil that was indigenously fertile for transplant growing (Work, 1945). In many cases, the resulting transplants were very large and unwieldy. Regulation of the nutrition was not a major consideration during the production period since the field soils naturally provided nutrient release.

In sharp contrast, today's transplants resemble nothing of the olden day transplants and all the methodology for growing has changed, including nutritional practices. In the last 20 years, there has been an abundance of transplant nutrition research published on a wide array of crops. However, distillation and adaptation on a commercial basis of this information is not always easy. Examination of this literature leaves one confused about what is required to produce an acceptable transplant with high yield potential. Clear cut, straight-forward application of these guidelines is difficult because of the diversity of conditions that the research was conducted. Confounding items include differences due to:

1. crops
2. cultivars within the same crop
3. microclimatic diversity of greenhouse environments used in research
4. fertilizer sources and concentrations, i.e. nitrate, ammonium, urea, and other nutrients used such as secondary and even trace elements
5. interaction of other factors studied, for example CO₂ enrichment, nutrient ratios, application timing, container type and size, etc.
6. interaction between nutrients and growth media, affecting cation exchange capacity, pH, salinity, etc. of media
7. geographical location and microclimatic diversity of field environment transplant subsequent yield performance evaluated

Commercial production of transplants needs to be as pragmatic as possible. Complex nutritional practices are only justified if the subsequent transplant performs "better" in the field. These changes include improvements of stand establishment coupled with enhancements in one or more attributes of earliness, uniform maturity, yield quantity and quality, and postharvest holding superiority. Much of the published research describes changes induced by a certain nutritional

practices upon seedling growth only, but, unfortunately, field trials are lacking. This research has merit since transplant growers are very concerned that the salable product is visually appealing and acceptable to the commercial vegetable grower. However, it is the author's view that if long term effects of transplant nutrition are not demonstrated in some improvement of field performance, the most basic fertility plan should be chosen.

The first objective of this paper is to review the historical research on nutritional practices used to grow vegetable transplants from published refereed journals and to portray this information in table form. The reader can use this table as a resource for quick comparisons of methods used and conclusions reached. The second objective of this paper is to note those studies that indicated a direct relationship between transplant nutritional practices and field performance. Conclusions that beneficially improve yield components are denoted in the tabular column labeled "Conclusions and recommendations" with ☺; studies that did not show yield suppression or no yield effect at all are denoted by ☹.

Even after review of the plethora of available information in journals, it is not possible to summarize the one "best" way to grow any transplant simply because of the milieu of interacting and confounding factors that moderate the effect of nutritional treatments. It is, however, important to recognize that all these confounding factors must be considered when developing guidelines for producing transplants. After thorough review of this information, it is concluded that transplant nutrition generally has a long term effect on influencing yield potential. Therefore, derivation of a nutritional regime to grow transplants needs to be carefully planned. It is hoped that the information that follows can be used to help guide this process.

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Table 1. Vegetable transplant nutrition research (☺ denotes yield (yld) increases with nutrition; ☹ denotes no effect of nutrition on yld)

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
asparagus 'Green Giant Select'	Adler et al., 1984	0, 100, 200 ppm N from N-urea	P anhydride at 0, 10, 20 ppm, K from KCl at 0, 100, 200 ppm	shoot & root wt & no, ht, buds no	no field work	Recommended 100 ppm NK with 20 ppm P for quality transplant production.
asparagus 'Rutgers Beacon'	Precheur & Maynard, 1983	Ratios of NO ₃ to NH ₄ at 100, 75, 50% at 15 meq/liter each from Ca(NO ₃) ₂ , KNO ₃ , (NH ₄) ₂ SO ₄	CaCO ₃ at 1% (w:w) mixture in sand media	shoots & roots wt, fern N status	no field work	Maximum transplant growth with 75% NO ₃ -N & 25% NH ₄ -N. Lime reduced NH ₄ toxicity but also transplant growth.
broccoli 'Premium Crop'	Masson et al., 1991a	100, 200, 300, 400 ppm N from 2:1:2 ratio N-NO ₃ , N-NH ₄ , N-urea	natural light versus artificial light at 10 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$	shoot & root wt, leaf area, growth ratios	in following citation	400 ppm increased transplant shoot growth but decreased root growth; supplementary light increased shoot & root growth.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
broccoli 'Premium Crop'	Masson et al., 1991b	100, 200, 300, 400 ppm N from 2:1:2 ratio N-NO ₃ , N-NH ₄ , N-urea	natural light versus artificial light at 10 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$	see previous citation	shoot wt, head diam, marketable ylds	☺ Light effect was NS, but maximum yld & head wt with transplants grown with 400 ppm N.
broccoli 'Emperor'	Tremblay & Senecal, 1988	150, 350 ppm N from 3:2:1 ratio N-urea, N-NO ₃ , N-NH ₄	K from KOH & K ₂ SO ₄ at 50, 200, 350 ppm	shoot & root wt, leaf area, growth ratios	no field work	N at 350 ppm with 200 ppm K promoted transplant shoot growth.
cabbage 'Golden Acre' 'Early Jersey' 'Wakefield'	Babb, 1940	1030 ppm N from nitrate of soda (calculated by Dufault)	estimated super-phosphate at 4150 ppm, K from muriate of potash at 2249 ppm	no data given	early & total season heads, head length, diam & density	☺ Transplants grown with N or complete nutrient solutions ylded earlier than those unfertilized. Total yld & quality unaffected by transplant nutrition.
cantaloupe 'Magnum 45'	Dufault, 1986	10, 50, 250 ppm N from N-urea	P anhydride at 5, 25, 125 ppm, K from KCl at 10, 50, 250 ppm	shoot & root wt, leaf no., stem diam, leaf area, growth ratios	transplant shock, vining, fruiting, flowering, early, mid, late season fruit yld	☺ Transplant shock increased with transplant NPK rates, but time to vine, flower, set fruit & yld earlier increased with NPK rates. Mid-season & total ylds unaffected by NPK transplant conditioning. Recommended 250N-125P-250K ppm.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
cauliflower 'Danish Giant'	Babb, 1940	1030 ppm from nitrate of soda (calculated by Dufault)	estimated super-phosphate at 4150 ppm, K from muriate of potash at 2249 ppm	no data given	early & total ylds, head depth, diam & density	☺ Transplants grown with only K or P or complete nutrient solutions ylded earlier than those fertilized with N. N lowered total yld, but K, P & complete had no effect on total yld.
cauliflower 'White Fox' 'Snowy River'	Wurr et al., 1986	52, 104 ppm K from KNO ₃	145, 290 ppm K from KNO ₃	shoot wt & no, growth rates	earliness, uniform maturity, curd wt	☹ Transplants grown with high NP rates were larger than low NP. Transplant nutrition did not affect earliness, maturity rate, or yld.
celery 'Utah 52-70R'	Dufault, 1985	10, 50, 250 ppm N from N-urea	P anhydride at 5, 25, 125 ppm, K from KCl at 10, 50, 250 ppm	shoot & root wt, leaf no, stem diam, ht, leaf area, growth ratios	no field work	Shoot & root growth increased with N rate. Shoot growth but not root growth increased with P rate. K had no effect. Suggested 250N-125P-10K ppm for quality growth.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
celery 'Utah 52-70R'	Dufault, 1987	1.25, 2.5, 5.0, 7.5, 10.0, 20.0 g·kg ⁻¹ Osmocote media (1.5 vermiculite: 1.5 perlite:7 peat)	P at 1.25, 2.5, 5.0, 7.5, 10.0 g·kg ⁻¹ media from Osmocote	shoot & root wt, leaf no, stem diam, ht, leaf area, media nutrient content	no field work	Shoot growth increased from 1.25 to 10 g·kg ⁻¹ media, but decreased from 10 to 20g·kg ⁻¹ media. Increasing P rate from 1.25 to 10.0 g·kg ⁻¹ media only increased chlorophyll content decreased. A N rate of 1.25 & 2.5 g·kg ⁻¹ media produced quality transplants only in "cool" greenhouses.
celery 'Florida 683'	Masson et al., 1991a	100, 200, 300, 400 ppm N from 2:1:2 ratio N-NO ₃ , N-NH ₄ , N-urea	natural light versus artificial light at 10 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$	shoot & root wt, leaf area, growth ratios	see following citation	400 ppm increased shoots but decreased roots; supplementary light increased shoot & root growth
celery 'Florida 683'	Masson et al., 1991b	100, 200, 300, 400 ppm N from 2:1:2 ratio N-NO ₃ , N-NH ₄ , N-urea	natural light versus artificial light at 10 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$	see previous citation	total & marketable yld	☺ Light effect was NS, but maximum yld & head wt with transplants grown with 300 ppm N.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
celery 'Florida 683'	Tremblay & Gosselin, 1989a	150, 250, 350 ppm N from N-NO ₃ , N-NH ₄	N-NO ₃ to N-NH ₄ in ratios of 1:1, 2:1, 3:1	shoot & root wt, leaf area, growth ratios, media & shoot tissue nutrient content	no field work	Shoots, but not roots increased with N rate. A minimum 250 ppm N at a NO ₃ :NH ₄ ratio of 2:1 suggested for adequate transplant growth.
celery 'Florida 683'	Tremblay & Gosselin, 1989b	150, 350 ppm N from N-NO ₃ , N-NH ₄	2:1 & 3:1 ratio of NO ₃ :NH ₄ , N-urea of 0% & 50%	shoot & root wt, leaf area, growth ratios, media & shoot tissue nutrient content	total, marketable & side shoot fresh wts	☺ N at 350 ppm & 2:1 ratio of NO ₃ :NH ₄ increased transplant shoot & root wt over low N. 50% urea increased shoot/root dry matter. Marketable yld greatest with 350 ppm N with 50% as N-urea.
celery 'Florida 683'	Tremblay et al., 1989	250 ppm N from NO ₃ :NH ₄ in a 1:1 ratio	pH's of 5.50, 6.36, 7.20; bicarbonate at 0, 500, 1000 ppm.	media & shoot tissue nutrient content	no field work	Studied the effect of high pH or buffering capacity on "curly top" incidence. Could not induce curly top with experimental high pH & buffering capacity.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
celery 'Florida 683'	Tremblay & Senecal, 1988	150, 350 ppm N from 3:2:1 ratio N-urea, N-NO ₃ , N-NH ₄	K from KOH & K ₂ SO ₄ at 50, 200, 350 ppm	shoot & root wt, leaf area, growth ratios	no field work	N at 350 ppm with 200 ppm K promoted transplant shoot growth.
celery 'Florida 683'	Tremblay et al., 1987	200, 400, 600 ppm N from N-urea	P from H ₃ PO ₄ at 100, 150, 200 ppm P; CO ₂ enrichment evaluated	shoot & root wt, leaf area, growth ratios	total, marketable & side shoot fresh wts	☺ CO ₂ enrichment increased transplant shoot & root growth but not yld. N at 400 ppm increased transplant shoot growth, total, marketable & side shoot wts. P did not affect transplant growth, but interacted with N to increase yld.
lettuce 'Empire' 'Florical 50011'	Karchi et al., 1992	32, 58, 175, 292 ppm N from N-NH ₄	P from liquid P ₂ O ₅ at 58, 175, 292, 318 ppm P; in N:P ratios of 1:1, 5:1, 1:5, 1:10	shoot dry wt, leaf area, root wt, growth ratios	no field evaluation	High P & low N extended transplant root growth over longer time periods, increased root dry wt, & enhance greater root to leaf ratios. High N with low P enhanced leaf growth over root growth. Proposed that the low N & high P plants might overcome transplant shock better, resume growth earlier & yld better than high N low P plants.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
lettuce 'Great Lakes R-200'	Kratky & Mishima, 1981	0, 200, 600, 1800 ppm NPK fertilizer (13-11-21) applied as a foliar feed	6 different medias; NPK starter granular fertilizer at 0, 4, 8, 16, 32 g/liter media	shoot fresh wt, media electrical conductivity	% harvestable heads, total salable wt, head wt & firmness	⊗ Transplant fresh wt greatest with 1800 ppm NPK solutions with 0-4g/liter preplant granular fertilizer. Foliar of 600 to 1800 ppm NPK cause excessive succulence. But total salable wt & head wt not affected by any media. Salable heads decreased with 16-32 g/liter preplant fertilizer. Recommended 200-600 ppm foliar 13-11-21 plus 4-8 g 8-14-17/liter preplant in media.
lettuce 'Ithaca'	Masson et al., 1991a	100, 200, 300, 400 ppm N from 2:1:2 ratio N-NO ₃ , N-NH ₄ , N-urea	natural light versus artificial light at 10 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$	shoot & root wt, leaf area, growth ratios	see following citation	400 ppm N increased transplant shoot growth but decreased root growth; supplementary light increased shoot/root growth
lettuce 'Ithaca'	Masson et al., 1991b	100, 200, 300, 400 ppm N from 2:1:2 ratio N-NO ₃ , N-NH ₄ , N-urea	natural light versus artificial light at 10 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$	see previous citation	head wt, density & circumference	☺ Light effect was NS. Head wt & density, not circumference, increased with transplants grown with 400 ppm N.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
lettuce 'Ithaca'	Tremblay & Senecal, 1988	150, 350 ppm N from 3:2:1 ratio N-urea, N-NO ₃ , N-NH ₄	K from KOH & K ₂ SO ₄ at 50, 200, 350 ppm K	shoot & root wt, leaf area, growth ratios	no field work	N at 350 ppm with 350 ppm K promoted shoot growth.
onion 'Sweet Savannah' 'Yula' 'Vega'	Herison et al., 1993	75, 150, 225 ppm N from N-urea with 20N-8.6P-16.6K complete fertilizer	studied 1-3 seed/cell; 8, 10, 12 week-old transplants	shoot & root wt, growth ratio	time to maturity, bulb fresh wt, size, shape & yld	☺ Bulb fresh wt at harvest & bulb yld of bulbs larger than 76 mm in diam were greatest with 10 to 12 week-old transplants fertilized during the transplant production period with urea nitrogen ranging from 150 to 225 ppm N plus a complete fertilizer (20N-8.6P-16.6K).
pepper (bell) 'Maor'	Aloni et al., 1991	0, 30, 100, 200 ppm N from KNO ₃	none	shoot & root wt, growth ratios, leaf no, starch, sugar, flower	transplanted in the greenhouse for 30 day period	N below 100 ppm inhibited shoot growth. Root growth had a negative relation with N supply. Transplants grown with 100 ppm N grew faster post-transplanting & flowered earlier. Carbohydrate status of young pepper transplants influenced their post-transplant recovery. Optimal N supply is essential for full recovery & development of transplants & in this study that was at 100 ppm N.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
pepper (bell) 'Maor'	Bar-Tal et al., 1990a	1.0, 6.0 mM N from $\text{NO}_3:\text{NH}_4$ in a 3:1 ratio	0.01, 0.03 mM P of unknown source	growth rates, root & shoot tissue nutrient content, shoot & root wt	no field work	As N & P rate increased from low to high in each case increased the uptake in transplant of N two-fold & P 5 to 6 fold. Uptake of N & P were found to affect each other's uptake.
pepper (bell) 'Maor'	Bar-Tal et al., 1990b	1, 5, 10, 15 mM N from $\text{NO}_3:\text{NH}_4$ in a 1:1 ratio	0.1, 0.5, 1.0 mM P of unknown source; root volumes of 5, 15, 35, 65, 700 cm^3 per plant	root & shoot tissue nutrient content, shoot & root wt, growth ratios	early & total yld & quality	☺ Largest transplants with >160 mg top dry wt had highest growth rate even after 4 weeks post transplanting. The higher growth rate increased earlier pod yld, but not total yld. The optimal nutrient solution was 5 mM N & 0.5 mM P.
pepper (bell) 'Gatorbelle'	Dufault & Schultheis 1994	25, 75, 225 ppm N from $\text{Ca}(\text{NO}_3)_2$	5, 15, 45 ppm P from $\text{Ca}(\text{H}_2\text{PO}_4)_2$; field planted in South & North Carolina locations	shoot & root wt, ht, leaf area, leaf no	transplant shock, flowering, vigor, marketable yld	☹ N with P affected shoot wt, leaf area, root wt, seedling ht, & leaf no. N & P rates did not affect recovery from transplant shock, earliness, yld or quality. No reported advantage of one nutritional treatment over another. As little as 50 ppm N & 15 ppm P can be used to produce 'Gatorbelle' transplants.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
pepper (bell) 'Yolo Wonder-L'	Knavel, 1977	180, 240, 300, 360 g N/m ³ 1:1 peat:vermiculite media	transplanted in field with 100, 155, 210, 265 kg N/ha	shoot wt, ht, shoot tissue nutrient content	yld, shoot tissue nutrient content	☺ Transplants grown with 240 or 300 g N/m ³ than field grown with 155 kg N/ha ylded more pods than all other treatments. Growing transplants with 360 g N/m ³ were lower ylding those grown with 240 or 300 g N/m ³ . The N level at which pepper transplants are grown has a strong influence on its ylding potential.
pepper (bell) 'Bell Boy'	Tremblay & Senecal, 1988	150, 350 ppm N from 3:2:1 ratio N-urea, N-NO ₃ , N-NH ₄	K from KOH & K ₂ SO ₄ at 50, 200, 350 ppm K	shoot & root wt, leaf area, growth ratios	no field work	N at 350 ppm with 50 ppm K promoted transplant shoot growth.
tomato 'Bonny Best' 'Penn State Earlianna'	Babb, 1940	1030 ppm N from nitrate of soda (calculated by Dufault)	superphosphate at 4150 ppm P, K from muriate of potash at 2249 ppm K. Each NPK source applied separately & all together.	no data given	early & total yld of fruit no & wt	⊗ In this 2 yr study, in first year, transplants grown with N ylded less than those fertilized with P or K or complete fertilizers. Total yld at end of season was NS. In 2nd yr, transplant nutrition was NS.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
tomato 'Camone'	Basoccu & Nicola, 1995	4, 8, 15, 30, 60 mM N of unknown source	Supplemental light & natural light treatments (3 total)	ht, leaf no, area, & wt, & root wt	fruit wt & no, early & total yld	☺ As N rate increased, transplant ht, stem & root dry wts decreased. Leaf area was maximal at 15 mM N. Root: shoot ratio was highest at lowest N rate. Transplants grown at 8 to 15 mM N with natural light ylded more early fruit but total ylds were unaffected by transplant nutrition.
tomato 'Break O'Day'	Brasher, 1941	unknown	10 days prior to planting, plants hardened with 1) strong K soln, 2) left tender 3) weak N soln	no data given	early & total yld	Hardening delayed early growth & reduced early production. Tender plants superior to hardened plants. "Any method used which results in stunting or hardening young tomato plants permanently slows up their field performance, probably decreasing the yld roughly in proportion to the severity of the hardening treatment".

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
tomato (process) 'H 2653'	Garton & Widders, 1990	10, 20 mM N from $\text{NH}_4\text{H}_2\text{PO}_4$, KNO_3 , $\text{Ca}(\text{NO}_3)_2$, or NH_4NO_3 to 4-5 wk-old seedlings for 5 or 10 days before planting in the field	2, 5 mM P from $\text{Na}_4\text{H}_2\text{PO}_4$ or $\text{NH}_4\text{H}_2\text{PO}_4$, applied to 4-5 wk-old seedlings for 5 or 10 days before transplanting	shoot nutrient content	root growth 5 days after transplanting, shoot wt over time, fruit yld	☺ Plants fertilized with low NP ylded equal to or greater than seedlings cultured with higher fertilization regimens. Suggested that initially use lower NP rates to avoid excessive vegetation. Before transplanting, use higher NP rates to increase tissue mineral nutrient status to a higher level. "Lower nutrient status of these seedlings even predisposes the root system to take up NP more rapidly during application of higher NP just before transplanting".
tomato (bareroot transplants) 'H-1350'	Jaworski & Webb, 1966	20, 60 lbs N/acre commercial fertilizer	10 & 90 lbs P/acre; field grown then dug & transplanted	shoot nutrient content	transplant survival, fruit yld	☺ NP levels used to produce bareroot transplants is related to fruit yld. A high N (60 lbs/acre) with low P (10 lbs/acre) reduced ylds in contrast to those grown with 60N-90P lbs/acre.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
tomato (process) 'TH-318'	Liptay & Nicholls, 1993	0, 50, 100, 200, 350 ppm N of unknown source	none	shoot ht, stem diam, shoot tissue nutrient content	root growth, survival, stem strength, early & total ylds	☺ Using high N enhanced transplants capacity for root growth in the field. Higher N in seedling tissues at transplanting may be used immediately for growth than that available in the soil. Suggested that 100 to 200 ppm N be used to grow tomato seedlings. There is also a good correlation between stem strength & survivability in the field.
tomato (process) 'TH-318'	Liptay et al., 1992	100, 200, 350 ppm N of unknown source	ten nutrient soln varying in N, P, K, & Ca; 25, 50, 200 ppm P; 50, 75, 100, 200, 250 ppm K; 100, 200 ppm Ca.	shoot ht, stem strength, root lengths	field survival, root growth, early & total yld	☹ Root growth was greatest with 350 ppm N but these plants survived poorly because of unhardened nature. Increasing K levels decreased root growth, but did not affect ylds. 50 to 100 ppm N depressed early yld & total ylds were the same for all N rates. Recommended 100 to 200 ppm N to improve survival. P at less than 2 ppm affected growth & K at all levels did not have any deleterious effect on plant performance.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
tomato 'Springset'	Masson et al., 1991a	100, 200, 300, 400 ppm N from 2:1:2 ratio N-NO ₃ , N-NH ₄ , N-urea	natural light versus artificial light at 10 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$	shoot & root wt, leaf area, growth ratios	see following citation	400 ppm N increased shoots & roots; supplementary light increased shoot & root growth
tomato 'Springset'	Masson et al., 1991b	100, 200, 300, 400 ppm N from 2:1:2 ratio N-NO ₃ , N-NH ₄ , N-urea	natural light versus light at 10 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$	see previous citation	early & marketable yld & total yld	☺ Earliness, but not total yld, increased with supplementary light. Transplants grown with 300 to 400 ppm N ylded greater early ylds. Total ylds unaffected by N rate.
tomato 'Sunny'	Melton & Dufault, 1991a	25, 75, 225 ppm N from Ca(NO ₃) ₂	5, 15, 45 ppm P from Ca(H ₂ PO ₄) ₂ , 25, 75, 225 ppm K from K ₂ SO ₄	shoot & root wt, ht, stem diam, leaf no., leaf area, chlorophyll	no field work	Transplant shoot root growth increased with increasing N rate. P at 45 ppm affected shoot growth & K have negligible effects. Production of quality transplants requires at least 225 ppm N & 45 ppm P & 25 ppm K.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
tomato 'Sunny'	Melton & Dufault, 1991b	100, 200, 300 ppm N from $\text{Ca}(\text{NO}_3)_2$	10, 40, 70 ppm P from $\text{Ca}(\text{H}_2\text{PO}_4)_2$ & low temperature (36of) before transplanting	shoot & root wt, ht, stem diam, leaf no., leaf area, chlorophyll	early & total ylds & quality	☺ Low temperature stress before transplanting did not effect earliness, yld or quality. N at 50 to 100 ppm is deficient & may reduce yld potential compared to 200 ppm. Earliness improved with transplants grown with 200 ppm versus 50 to 100 ppm. Total yld increased with transplants conditioned with ≥ 100 ppm versus 50 ppm. Suggested 200 ppm N & 10 ppm P be used to grow tomato transplants.
tomato 'Allstar'	Vavrina & Hochmuth, 1994	0, 15, 30, 45, 60, 75 ppm N from NH_4NO_3	nutritionally conditioned transplanted planted in Florida & Pennsylvania	seedling shoot nutrient content	early & total ylds	☺ Earliness & yld of transplants conditioned with ≥ 30 ppm not affected in FL, but in PA, transplants conditioned with 75 ppm ylded greater early ylds, but 45 ppm induced greatest total ylds of all nutritional treatments.
tomato 'Pik-Red'	Weston & Zandstra, 1989	100, 200, 400 ppm N from KNO_3	15, 30, 60 ppm P from superphosphate (20.4%)	shoot & root wt, ht, leaf area, root:shoot ratio	early & total ylds	☺ Largest transplants produced with 400 ppm N-30 ppm P. Transplants fertilized with 400 ppm N & 30 ppm P produced the greatest early & total ylds.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
tomato 'Ohio 7870'	Widders, 1989	4, 16, 28 mM N from $\text{NH}_4\text{H}_2\text{PO}_4$, KNO_3 , $\text{Ca}(\text{NO}_3)_2$, or NH_4NO_3 application began 8 days before planting	0.5, 4.0, 8.0 mM P from $\text{Na}_4\text{H}_2\text{PO}_4$, $\text{NH}_4\text{H}_2\text{PO}_4$, KH_2PO_4 , application began 8 days before transplant	shoot tissue nutrient content, shoot dry wt	shoot NP content & dry wt over time, but no yld data	Relative shoot growth rates declined during first 3 days after transplanting, but increased to a maximum by 10 to 14 days after transplanting. These low relative growth rates need cultural strategies to promote vegetative & root growth to enable the plant to acquire nutrients & water. Internal plant tissue N status is important for improving post-transplanting seedling growth. N from 16 to 28 mM accelerated seedling relative growth during initial 5 days post-transplanting.
watermelon 'Crimson Sweet'	Lamb et al., 1993	$\text{NO}_3:\text{NH}_4$ ratios 100:0, 75:25, 50:50, 25:75, 0:100 from N- NO_3 , N- NH_4	0, 4, 8, 12, 16 mM calcium from CaCl_2	shoot dry wt, leaf area, ht, N content, media nutrient content	no field work	Calcium at 4 to 8 $\text{mmol}\cdot\text{liter}^{-1}$ increased transplant shoot growth & N content, but 12 to 16 $\text{mmol}\cdot\text{liter}^{-1}$ suppressed growth. Either N form at 100 ppm N with 4 to 8 $\text{mmol}\cdot\text{liter}^{-1}$ calcium optimized watermelon transplant growth.

Crop & cultivar	Reference	N rates & source	Other aspects studied	Seedling variables evaluated	Field variables evaluated	Conclusions & recommendations
watermelon 'Crimson Sweet' 'Queen of Hearts'	Schultheis & Dufault, 1994	25, 75, 225 ppm N from $\text{Ca}(\text{NO}_3)_2$	5, 15, 45 ppm P from $\text{Ca}(\text{H}_2\text{PO}_4)_2$; planted in South & North Carolina locations	shoot & root wt, leaf no., leaf area, ht	transplant shock, vining, flowering, yld & internal quality	☹ Transplant shock increased linearly with N transplant fertility. Seedling growth was most vigorous with 225 ppm N - 45 ppm P with 100 ppm K. Yld was unaffected by transplant nutrition in both NC & SC. No advantage for high nutritional regimes & 25 ppm N- 5 ppm P is sufficient for transplant production & acceptable ylds.

TRANSPLANT PRODUCTION AND PERFORMANCE: EFFECT OF CO₂ ENRICHMENT AND LIGHT

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INTRODUCTION

Canham (1966) once stated that future yield of young tomato plants depends on the first weeks of growth and that it is an appropriate time to plan economically for the crop's development. Indeed, the nursery period is a particularly appropriate time for the induction of anatomical or physiological characteristics that would help overcoming the transplant plantation stress and achieving maximal yield. Transplants are grown at very high plant population and under conditions that can be modified almost at will. Even relatively expensive treatments may prove profitable under such greenhouse environments should they show residual effects in the field. Also, every treatment which would result in shorter time spent in the greenhouse would reduce growing costs and increase transplant crop turn-over and translate in lower requirements for greenhouse space.

During spring months in northern latitudes, as little ventilation is necessary, the use of CO₂ enrichment in greenhouse nurseries could be used as a way to reduce propagation time, improve sturdiness and possibly favor growth in the field after planting. The almost entirely juvenile tissues of seedlings are all expanding, utilizing and diluting the enhanced photosynthate production in an enriched CO₂ atmosphere (Lindhout and Pet, 1990). Hence, the greatest advantage of CO₂ enrichment is realized in the vegetative growth of young plants (Kimball, 1983). As leaf tissues formed early in seedling culture begin to mature, starch accumulation begins to slow photosynthetic rates and RGR (Thomas et al. 1975).

High energy costs have induced greenhouse owners into energy saving techniques leading to decreased light transmission through the structure (Bruggink and Heuvelink, 1987). In northern latitudes, the wide variations in growth which occur during the winter season can be explained almost completely by the wide variations in radiation (Klapwijk, 1981). A decrease of 1% in light level will lead to 1% less yield in a greenhouse but this relationship does not hold in the case of young plants (Bruggink, 1987a). Again, manipulating light conditions for vegetable transplant crops could result in benefits not only during the nursery period but also later in the field.

The two major driving forces of photosynthesis are CO₂ and light. For a long time, the greenhouse industry has taken advantage of manipulating these factors to the benefit of crops grown in controlled conditions for extended periods. When plants are young, they grow nearly exponentially while older plants grow more in a linear fashion (Lindhout and Pet, 1990). For crops in their juvenile phase it may therefore be even more profitable to adjust CO₂ and light conditions.

This review will consider research results involving the use of CO₂ enrichment and varying light conditions in greenhouse for growing vegetable transplants aimed mainly to field production.

Transplant growth

Effect of CO₂

The key enzyme for CO₂ fixation is Rubisco. Its activity depends on the ratio of the O₂ and CO₂ concentration in the atmosphere. The major effect of CO₂ enrichment is the shift in balance between the carboxylation and oxygenation activity of Rubisco. This effect is just as important at low as at high light levels as the percentage effect on relative growth rate is about the same over a range of light levels. Kimball (1983) stated that, on average, yields of crops should increase by 33% with a doubling of CO₂ concentration in the earth's atmosphere. Although these estimates have been

developed for plants over their complete life cycles, enhanced growth and dry matter accumulation are correlated with higher net photosynthetic rates in young vegetative tissues under CO₂ enrichment as well.

Optimal CO₂ concentrations in greenhouses lie between 700 and 900 $\mu\text{l l}^{-1}$. Brewer et al. (1986) reported taller tomato transplants as CO₂ concentration changes from 330 to 660 and to 990 ppm. In a study of 96 genotypes of tomato plants, CO₂ enrichment (320 vs 750 ppm) was found to increase young plant growth on average by 2.3 (Lindhout and Pet, 1990). Differences in behavior with regard to CO₂ enrichment among genotypes were relatively few. CO₂ enrichment increased transplant leaf area, shoot and root dry weight and decreased the leaf area ratio of celery transplants (Tremblay et al. 1987).

Woodrow et al. (1987) demonstrated that CO₂ affects both source metabolism and partitioning to sinks (stems, roots and leaf carbohydrate) in tomato plantlets. They found that CO₂ enrichment produced heavier transplants desirable for successful field establishment without elongation growth. Dry matter accumulation in shoot and root was increased as well as leaf dry weight (by 81% over control). Transpiration rates were reduced under CO₂ enrichment conditions by 34%. Increased leaf dry weight accumulation and SLW under CO₂ enrichment suggests that more carbohydrate may be available to the plant for future growth. Apparently, the ratio of total sugars to amino acids in the leaf is shifted in favour of sugar content. In a study with tree seedlings, Luxmoore et al. (1986) suggest that CO₂ enrichment may increase sucrose translocation in roots and facilitate the mobilization of nitrogen and carbon compounds to new root primordia.

Increased net leaf photosynthesis rate and decreased transpiration rate under CO₂ enrichment are well documented (references cited by Woodrow et al. 1987). One of the most important effect of CO₂ enrichment is the increase in water efficiency (Wong 1979) which leads to drought tolerance. Actually, rising CO₂ concentration reduces the transpiration of plants by 20-40% (Mortensen, 1987). Radoglou et al. (1992) reported an increase in water use efficiency of bean leaves as a result of increased assimilation rate and decreased stomatal conductance at higher ambient CO₂ concentrations. This higher efficiency may not be maintained for long periods of time in field conditions. In cotton (Sasek et al. 1985), stomatal conductance after 40 days of CO₂ enrichment took 5 days to reach normal levels in non-enriched conditions.

In short, CO₂ enrichment of vegetable transplants shortens the nursery period and modifies photosynthate allocation to the diverse parts, leading to sturdier, higher quality plant. This, together with the fact that CO₂ enriched plants make a more efficient use of water may impact favorably on the plant's ability to overcome plantation stress.

Effect of light

The use of supplementary lighting makes sense in the case of vegetable transplant production since, the younger the plant, the more its relative growth rate will be affected by light conditions (Bruggink, 1987b). Most northern growers will confirm that plants grown in glasshouses are shorter than those grown under polyethylene, which screens out more light. Increase in stem diameter during the vegetative stage of tomato has been shown to relate proportionally to the amount of light received by the plant (Schoch et al. 1990). For growers, this translates into sturdier, more compact and overall better quality plants, less prone to lodging once transplanted to the field. As with CO₂ enrichment, light integrals not only influence the rate of photosynthesis, but also morphological parameters of the plant (Bruggink, 1992). For example, supplementary lighting increased shoot and root dry weight of celery, tomato, broccoli and lettuce transplants (Masson et al. 1990, Masson et al. 1991a).

Differences among species as to the influence of light conditions do exist. In a comparison of tomato, cucumber and sweet pepper plantlets sown at different times of the year, Bruggink and Heuvelink (1987) found that the NAR of tomato was the most related to light integrals. Generally, NAR was maximum when the mean daily light integral was 400 J cm⁻² day⁻¹ or more and RGR was maximum at 300 J cm⁻² day⁻¹.

In Holland, according to Klapwijk (1981), for every 1 W m⁻² provided by supplementary lighting young tomato plants reduce by 1% the period they need to reach a given growth stage. Lettuce plants took 5 to 9 days less to reach the 5 to 6 leaf stage when grown under 40 W m⁻² supplementary lighting instead of 20 W m⁻² (Poniedzialek et al., 1988). Shorter growing periods of transplant crops translate for the grower in quicker rotations and ultimately in a more efficient use of greenhouses space.

Supplementary lighting may not only shorten crop cycles in nurseries but may be instrumental for crop scheduling and

planning. Krug and Liebig (1989) proposed an equation considering temperature, radiation and transplant period to calculate ideal sowing dates for equal lettuce planting intervals. However, as natural light conditions improve, the benefit of supplementary lighting becomes more marginal. In the Netherlands, intensities of artificial light over 10 W m⁻² had little promoting effect on RGR of young tomato, sweet pepper and cucumber plants and this effect was significant only from the middle of November to the middle of February.

Koontz and Prince (1986) cite studies where weight of young tomato plants were more influenced by photoperiod than by irradiance. Long photoperiods tended to comparatively reduce losses of photosynthates through respiration, in agreement with findings of Riobe and Baubault (1983). Light quality can also be modified as this aspect is known to influence transplant production. Light characteristics in the red and far-red portion of the spectra influenced growth of tomato transplants (Decoteau and Friend, 1991) most probably through an influence on phytochrome. End of day red light treatment or the use of fluorescent light was found to reduce tomato (Decoteau and Friend, 1991) and pepper (Graham and Decoteau 1995) seedling height. However, there was not much effect on subsequent plant growth in the field or fruit production. End of day light manipulation was suggested to be a low-cost and environmentally safe method of transplant height regulation.

Supplementary lighting can be seen as a way to shorten transplant production cycles in greenhouses and make the production planning more predictable and less dependent on natural light conditions. As with CO₂ enrichment, supplementary lighting results in better quality transplants with potential influence on growth performance in the field.

Interaction between CO₂ and light

There is obviously a potential for complementation between CO₂ and light conditions (Hurd and Thornley, 1974). Madsen (1973) showed that young tomato plants grown under high light intensities were able to utilize an increase in CO₂ concentration of the air up to 2200 ppm. In strongly limiting lighting conditions, Canham (1974) rated supplementary lighting as more important than either CO₂ enrichment or temperature management in the production of greenhouse tomato transplants. Hurd (1968) found that effects on young greenhouse tomato plants CO₂ enrichment to 1000 ppm roughly corresponded to an increase of only 30% light. Krug and Liebig (1994 and 1995) produced models integrating, among other aspects, the use of CO₂ enrichment and supplementary lighting for the planning of lettuce transplant production. However, the relationship between CO₂ and light conditions may be relatively loose. The relative increase in net assimilation rate due to an increase in CO₂ concentration from 200 to 1000 ppm was almost as great at the lower as at the higher light level studied. The light compensation point is lowered by increased CO₂ concentration (Mortensen, 1987). Fierro et al. (1993) demonstrated interactive effects of CO₂ and light enrichment on tomato and pepper transplants. Both applied only 3 weeks before transplanting tomato and pepper, they increased accumulation of dry matter in shoots by about 50% as compared to the control. Root dry weight increased 49% for tomato and 62% for pepper. Fierro et al. (1993) results suggest that it is more important of achieving optimal lighting conditions first, and then make use of CO₂ enrichment.

Residual effects on yield

Effect of CO₂

Woodrow et al. (1987) cite inconsistent reports indicating that acclimation to high CO₂ presents either an advantage, a disadvantage or no effect when plant tissues are transferred to low CO₂ levels like in field conditions. According to these authors, the inconsistencies may be due to the age of the leaf tissue under study. They found no effect of CO₂ enrichment on height, total leaf area or number of nodes to harvest of young tomato plants. Bélem (1990) reported early yield increases for tomato and pepper transplants grown under CO₂ enrichment but only on one site out of two. Thomas et al. (1975) reported that vegetative growth of tobacco plants under CO₂ enrichment was accelerated temporarily, but declined to a magnitude close to that of plants raised under ambient CO₂ when transferred under normal CO₂ conditions. As for celery, CO₂ enrichment produced larger transplants but did not affect the total and marketable shoot weight of celery at harvest (Tremblay et al. 1988). In the same study, on the contrary, N fertilization of transplants showed significant residual effects at harvest.

Effect of light

The transfer of young plants from greenhouse to field conditions involves an important change in the quantity of radiation received by the plants. Plants adapted to low light conditions are unable to use efficiently the relatively higher light intensity prevailing in the field (Björkman, 1981). The after-effects on yield of supplementary light level prior to planting are, however, relatively short lived.

Boivin et al. (1987) and McCall (1992) have demonstrated a strong benefit of supplementary lighting applied under limiting light conditions during the nursery period of greenhouse tomato plants. Numbers of leaves formed below the first inflorescence were reduced as well as flower abortion on the first inflorescence, resulting in twice as much early marketable yield for the December 3 sowing. The January 17 sowing showed benefits from lighting treatments but the March 8 sowing was not significantly affected. As natural light conditions improved, the influence of supplementary lighting was reduced.

For lettuce, early plant growth has been shown to influence head weight at maturity and both temperature and solar radiation effects has been reported (Wurr and Fellows, 1991). Supplemental lighting of lettuce transplants for 4 h at 13 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF after dusk increased transplants shoot dry weight but had no effect on head weight at maturity, coefficient of variation of head weight or the timing of maturity (Wurr et al., 1986). According to de Visser and van de Vooren (1975), supplementary lighting of transplants on lettuce yield could not be traced to causes other than higher weight at planting.

In a study on 4 species, Masson et al. (1991b) concluded that celery, lettuce and broccoli yields remained unaffected by supplementary lighting applied during the nursery period. Only tomato early yields were favourably influenced by light treatments.

Fierro et al. (1993) compared control tomato seedlings to ones which had received both CO_2 and supplementary lighting, early yield increases of marketable fruits were 15 or 12% higher, for the early and late sowing, respectively. These yield increases were due to a greater number of fruits.

Need for future research

There may be two reasons for explaining the effect of CO_2 or light treatments on yield. The treatments may "condition" the young plants, determining a better growth balance between root and shoot, a higher water use efficiency or a higher content of reserves which could be used during establishment in the field. Or, the plants may be simply "bigger", ahead in their dry matter accumulation, and so there would be no inherent effect of treatments. Discriminating the causes would help in understanding the mechanism of treatment effects.

CO_2 enrichment of the root zone of seedlings (Yurgalevitch and Janes 1988; Bialczyk et al., 1994) was not discussed in this review. However, since transplants are often irrigated by mists, their rooting could take advantage of CO_2 injection in the nutrient solution as suggested by results of Mortensen (1987) with cuttings.

In a fully productive greenhouse without ventilation and without CO_2 enrichment of the atmosphere, CO_2 concentration may fall below 200 $\mu\text{l l}^{-1}$. CO_2 enrichment even in periods where ventilation is necessary has been found economical and is sometimes used commercially for greenhouse crops (Mortensen, 1987). Whether the same benefit could apply to transplant canopies, which are necessarily much less active in capturing CO_2 in a greenhouse, remains to be seen.

New photosensitive greenhouse films have been recently developed which reflect a part of the green light and the near infrared resulting in higher red/far red and blue/red ratio with significant effects on plant growth (Verlode et al. 1997). Effects of such coatings on growth of vegetable transplants are so far unknown.

These aspects of transplant production have not been fully addressed and would benefit from additional research.

CONCLUSION

CO_2 enrichment and light treatments have been shown to influence transplant growth in greenhouses. They can be used as tools to achieve high quality transplants, mostly when conditions are otherwise limiting and therefore offer an alternative to other approaches such as mechanically-induced stress, DIF temperature management or the use of growth regulators. Whether CO_2 and supplementary lighting have any inherent effect on final yield, other than through the production of heavier, more developed plants at the moment of planting, remains to be determined. Commercially, CO_2 enrichment for vegetable transplants probably has an economical potential only in northern areas where greenhouses can be kept close for a significant part of the day. As to supplementary lighting, profitability lies in the following factors: 1) cheap electricity rates; 2) growers who can take advantage of lighting installations for other crops than transplants only;

3) recuperation of the heating power of lamp fixtures to decrease heating costs.

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TRANSPLANT PRODUCTION AND PERFORMANCE: MECHANICAL CONDITIONING FOR HEIGHT CONTROL

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Mechanical stress inflicted by wind, rain, hail, animal movements, and many agricultural and horticultural practices, is a powerful force in plant form and development in outdoor or natural settings (Mitchell, 1996). Mechanical conditioning is physical stimulation or stress deliberately applied in order to manage plant growth and quality (Latimer, 1991). Mechanical conditioning reduces plant growth and improves plant strength and stature. It may be applied by rubbing stems, brushing shoots, shaking potted plants or whole flats, vibrating pots or plants, as mechanical impedance, or by perturbing plants with water, forced air or wind. The theory behind the application of mechanical stress for regulation of plant growth and processes in protected environments has been reviewed recently (Latimer, 1991; Latimer and Beverly, 1993; Mitchell, 1996), but much has been accomplished in evaluating methods of application and the application details of interest to transplant producers. In addition, we have made great progress in identifying a wide range of crops responsive to mechanical conditioning and in identifying benefits other than height control.

In transplant production, the goal is to produce plants which will: (1) withstand the physical stress of handling, shipping, and transplanting, (2) adapt rapidly to the move from the protected greenhouse to the field environment, (3) become established and resume active growth soon after transplanting, and (4) produce acceptable yields without reduction or delay compared to alternative stand establishment methods. Ideally, the method of growth control should produce a short, stocky transplant with thick, strong stems, and a deep green color, and subsequently improve the post-transplant performance, field establishment and early or total yield. Since chemical plant growth regulators (PGRs) are no longer labeled for vegetable transplants, growers are limited to cultural or nonchemical methods of growth regulation. Mechanical conditioning can substitute for PGRs to produce many of the desirable characteristics for a vegetable transplant.

APPLICATION METHODS

The application procedures most studied for transplants have been wind, shaking, brushing, and more recently impedance; all of which result in physical displacement of the growing points. Wind applied with a oscillating or unidirectional fan provided growth regulation of tomato (*Lycopersicon esculentum*) (Adler and Wilcox, 1987) and underbench aeration (wind) provided a vibration treatment which also effectively reduced the height of tomato transplants grown under high density (Liptay, 1985). Wind obviously causes changes in the microclimate surrounding the plant, and wind stress from fans has been reported to cause desiccation damage to broccoli (*Brassica oleracea*) transplants (Latimer, 1990). Although very effective in mechanical conditioning, shaking of individual pots (Mitchell et al., 1975) is not applicable to transplant production. However, the automated, mechanical oscillatory shaking (AMOS) device developed to provide a combination shaking and rubbing treatment across an entire greenhouse bench (Beyl and Mitchell, 1977a) may provide a model for transplant treatment.

Brushing transplant shoots has probably received the most attention from researchers working with vegetable transplants and ornamental bedding plants. Brushing provides a tactile or thigmic stimulation of the plant growing points. Generally, brushing reduces plant height, and commonly leaf area and dry weight, but increases stem and petiole strength. Methods of application have included brushing plant shoots with a small broom (Takaki et al., 1977), a dusting brush (Hiraki and Ota, 1975), a folded sheet of typing paper (Biddington and Dearman, 1985a), a piece of cardboard (Latimer, 1990), a suspended aluminum bar (Nakaseko, 1988) or PVC pipe (Sanders, 1994), a steel bar suspended in a cloth sling (Latimer et al., 1991), a wooden dowel rod (Baden and Latimer, 1992; Schnelle et al., 1994), a PVC pipe (Latimer and Thomas, 1991), single or multiple layers of burlap (Autio et al., 1994), and a sheet of polystyrene foam (Garner and Björkman, 1996). The brushing material or mechanism must be sufficiently strong and durable to manipulate the shoots in a high

density planting, especially after the treatment has caused the typical increase in stem stiffness and strength and the plants have attained some height. Pöntinen and Voipio (1992) found brushing to be much more effective at reducing transplant height than either wind or shaking.

Mechanical impedance is also a studied method of applying a tactile stimulation to transplants without the abrasion associated with brushing. Using a vinyl net covering as a static counterforce provided growth reduction of chrysanthemum (*Dendranthema grandiflorum*) (Beyl and Mitchell, 1977b) and lily (*Lilium longiflorum*) (Hiraki and Ota, 1975). More recently, tomato transplants grown under a sheet of Plexiglas for 15 h per night for 12 consecutive nights were 21% shorter than unimpeded controls and had a 20% increase in stem diameter without affecting leaf dry weight (Samimy, 1993). To address concerns of the length of treatment time, and the expense and impermeability of the Plexiglas, Garner and Björkman (1997) compared rectangular frames covered with fiberglass screening or mylar film to a Plexiglas sheet and used morning (1 h) vs. night (15 h) treatment times. Permeability of the impedance material had no effect on growth regulation and only the overnight treatment gave a commercially useful degree of growth reduction. The authors concluded that impedance is more laborious, requires more equipment, and is less effective than brushing (Garner and Björkman, 1997).

BENEFITS OF MECHANICAL CONDITIONING

Effective height regulation. Mechanical conditioning can reduce plant height by 20% to more than 50% when compared to untreated plants. These reductions in growth are similar to those attained with chemical plant growth regulators. Adler and Wilcox (1987) reported a 48% reduction in height of tomato plants treated with either chlormequat chloride or thigmic stress (stem rubbing).

Although tomato has probably been studied more than any other crop with respect to mechanical conditioning, brushing is quite effective on a large number of other vegetable crops including eggplant (*Solanum melongena*), cucumber (*Cucumis sativus*), squash (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), and some cultivars of broccoli and cabbage (*Brassica oleracea*) (Latimer and Baden, 1991), Jalapeno and bell peppers (*Capsicum annum*) (B.A. Galloway and J.R. Schultheis, personal communications), as well as lettuce (*Lactuca sativa*) and celery (*Apium graveolens*) (Biddington and Dearman, 1985a). However, significant cultivar differences also have been identified (Johjima et al., 1992).

In addition, height of several ornamentals, aster (*Callistephus chinensis*), dusty miller (*Senecio bicolor*), and petunia (*Petunia* spp.), has been effectively reduced with brushing with burlap (Autio et al., 1994). Plant height of columbine (*Aquilegia X hybrida*), New Guinea impatiens (*Impatiens X hybrida*), and marigold (*Tagetes erecta*) was reduced 20% to 35% in response to brushing with a wooden pole, but ageratum (*Ageratum Houstonianum*) was not responsive to brushing (Latimer and Oetting, 1997). Plant growth habit appears to affect plant response to mechanical conditioning by brushing, i.e., more brittle or stiff plants receive less bending action than more flexible plants, and thereby exhibit less reduction in plant growth.

Improved plant appearance. In addition to growth control, mechanical conditioning improves plant appearance. Specific chlorophyll content is higher in mechanically conditioned tomato (Mitchell et al., 1975), eggplant (Latimer and Mitchell, 1988), lettuce and celery (Biddington and Dearman, 1985a). In addition, mechanical conditioning increases specific leaf weight of tomato (Heuchert and Mitchell, 1983), eggplant (Latimer et al., 1986), lettuce, celery and cauliflower (*Brassica oleracea* L.) (Biddington and Dearman, 1985a). The darker green, thicker leaves combined with shorter stems enhanced the healthy, vigorous appearance of brushed transplants. Mechanical conditioning greatly improves plant uniformity in the flat and the subsequent appearance of the plants during handling (Garner and Björkman, 1996; Latimer and Beverly, 1993). Growers using the procedure estimated a 2-wk increase in shelf life of the treated plants (Schnelle et al., 1994).

Improved plant strength. Mechanical conditioning also increases stem and petiole strength. Although shaking reduced tomato stem diameter, both ultimate shear strength and the modulus of rupture of stems and petioles were increased in shaken plants (Heuchert et al., 1983). Further analysis of stem structural

components indicated an increase in percent cellulose in the fiber component of the shaken tomato stems. Rubbed bean (*Phaseolus vulgaris* L.) stems were stronger than unbrushed stems (Jaffe et al., 1984), and stem strength increased with increasing duration of treatment up to 14 days. The value of increased stem strength in stand establishment is apparent. We have observed clear differences in the "toughness" of treated plants and recorded less breakage during transplanting. In addition, plants maintained a more upright habit when planted or transferred to outdoor conditions (Latimer and Mitchell, 1988; Samimy, 1993). Increased stem strength should also prove important to plant maintenance during shipping and subsequent handling.

Improved stress tolerance? The value of mechanical conditioning in improving tolerance to stress has been less consistent. Although brushing increased drought tolerance of soybean [*Glycine max*] (Suge, 1980), brushing slightly decreased the drought tolerance of celery, cauliflower and lettuce (Biddington and Dearman, 1985b). In related work, brushed and untreated cucurbit transplants, subsequently planted in pots of sand, were subjected to cyclic drought stress. Cuticular water loss was higher from detached leaves of the brushed transplants, but transplant growth rate under drought-stressed conditions was the same as that of control plants (Latimer and Beverly, 1994). A recent analysis of water loss from brushed tomato plants (van Iersel, 1997) supports earlier observations that brushed plants use more water than untreated plants (Latimer, 1991). However, due to the reduction in leaf area and other morphological changes, the plants do not appear to suffer greater drought stress under field conditions (Latimer, 1991; van Iersel, 1997).

Improved stand establishment. Regardless of the lack of direct effects on stress tolerance, mechanical conditioning can improve field establishment of transplants. One of the best characteristics of brushing as a means of growth reduction is the lack of persistence of the effect after treatment ceases. Plants generally resume normal or accelerated growth within 3 days after treatment is stopped (Mitchell et al., 1975). Liptay (1985) noted that while vibrated tomato plants had 34% less shoot dry weight at the time of transplanting, there was no difference in shoot dry weight after 3 weeks in the field. In two of three broccoli tests, brushed transplants increased in shoot dry weight faster than untreated plants during the first 14 to 21 days after field transplanting (Latimer, 1990). This may be due to the net effect of an accumulation of other growth responses including increased specific leaf weight and specific chlorophyll content, more upright habit with stronger stems and petioles, and less leaf area which could reduce water loss (Latimer, 1991). Gartner (1994) evaluated root biomechanics of tomato plants subjected to flexing (to simulate wind). She found that flexed plants had higher root : shoot dry weight ratios and a wider stem at the root/shoot junction, and concluded that flexed plants should be more resistant to forces affecting stems and could potentially withstand more force under windy situations than could an untreated plant. Mechanical conditioning also reduces the incidence of stem pithiness in transplants. In tomato plants subjected to severe drought stress, prior conditioning by brushing reduced the occurrence of "pithy" or "hollow" stems from 95% among unbrushed plants to only 5% (Pressman et al., 1983).

Improved insect resistance. In addition to growth responses, brushing of tomato, eggplant, and watermelon transplants generally reduced the number of thrips, and of aphids on tomatoes, relative to the untreated controls (Latimer and Oetting, 1994). Similar reductions in populations of two-spotted spider mites were seen in brushed marigold, ageratum and New Guinea impatiens plants (Latimer and Oetting, unpublished data). Brushing for height control may be advantageous in an integrated pest management program in the greenhouse.

Limited effects on crop yield. Although cultivars vary in their response to mechanical conditioning, generally, little or no effect on yield occurs with plants treated only during the transplant production stage. Brushing greenhouse-grown seedlings did not affect subsequent head weight of lettuce (Wurr et al., 1986) or broccoli (Latimer, 1990), or fruit yield of four cultivars of tomato (Johjima et al., 1992) or of three of four cultivars of cucumber (Latimer et al., 1991). However, pretransplant brushing did delay or reduce fruit production in 'Sunrise' tomato in one experiment (Beverly et al., 1992), and mechanical conditioning **during** crop production has reduced yield of tomato (Buitelaar, 1989) and potato (*Solanum tuberosum*) (Akers and Mitchell, 1985).

POTENTIAL COMMERCIALIZATION OF MECHANICAL CONDITIONING

Currently, large-scale commercial adoption of brushing in transplant production is constrained by both logistical and physiological considerations. Many of the grower concerns of when or how much to treat have been addressed in recent research reports. While some of the current constraints to adoption of mechanical conditioning are researchable problems, some are simply matters of engineering or economics. Brushing by hand is too labor intensive to be economical for commercial application. Some grower initiatives have developed brushing systems for use with the irrigation booms (Sanders, 1994). Additional engineering work in this area could have excellent returns.

Management of growth reduction. The degree of growth reduction attained depends on the duration or intensity of the mechanical conditioning treatment. Adler and Wilcox (1987) found that the longer a wind or a rubbing treatment was applied, the more plant height of tomato was reduced. In addition, the timing of treatment initiation which affects treatment duration, affected the degree of growth reduction. For example, as the initiation of brushing tomato was delayed from the cotyledonary stage to the third true-leaf-stage (resulting in a range of 8 to 18 days treatment), stem length reductions decreased from 43 to 29% for 'Sunny' and from 37 to 17% for 'Wolfpack' compared to untreated plants (Latimer, unpublished data). Similarly, Garner and Björkman (1996) found that growth reduction of tomato depended on the number of days of treatment, i.e., when brushing was initiated at a canopy height of 6 cm (first true leaf stage), growth reduction was greater than when treatment was initiated at 8 or 10 cm. As these authors point out, the ability to delay treatment initiation reduces the grower's labor investment in the treatment, increases the flexibility of the treatment, and reduces the potential for disease spread in the treated plants. (See next section on plant damage.) However, brushing must be initiated before the plants become spindly or tall enough to tangle in the flat when treatment is initiated.

Recent research in the dose response of tomato transplants to brushing found that a range of 10 to 40 brushing strokes per day gave similar growth reductions (Garner and Björkman, 1996). Furthermore, a time period between the strokes of up to 10 min resulted in the same growth reduction as continuous brushing. Sanders (1994) cited effective growth reduction of tomato, eggplant and pepper transplants with brushing with 8 cycles (back and forth) applied three to six times per day. Although measurements of stem elongation rates found maximum elongation occurred at the end of the light period and the beginning of the dark period, brushing treatments applied in the morning were more effective in reducing tomato transplant height (Garner and Björkman, 1996). Similar morning sensitivity was identified in shaken chrysanthemums (Beyl and Mitchell, 1977b). Thus, mechanical conditioning provides flexibility in growth control, i.e., the treatment can be managed to reduce plant growth as much or as little as desired, within the maximum range of response for the selected species.

Management of plant damage. Two cultivars of bell pepper exhibited excessive plant damage for the small amount of growth regulation provided by a brushing treatment (Latimer, 1994). In addition, transplant quality decreased as treatment was initiated at later stages of development. However, peppers grown under subirrigation methods showed no damage to a brushing treatment (B. Galloway and J. Schultheis, personal communications). Initiation of brushing treatments after leaves have fully expanded and become tender results in more damage than for leaves that develop during the treatment period. In all cases, plants should not be subjected to a tactile type of mechanical conditioning when the leaves or growing points are wet as this may increase plant damage as well as the potential for disease spread.

The growth habit or texture of some plants makes them more susceptible to damage than others. Garner et al. (1997) found that although pansies (*Viola X Wittrockiana*) were responsive to mechanical conditioning, impatiens (*Impatiens Wallerana*) and geraniums (*Pelargonium X hortorum*) were too easily damaged by the treatment. Damage of flowers is also a concern, especially for ornamental bedding plants. Although the leaves of New Guinea impatiens were not particularly damaged by brushing, the damage to the flowers was excessive and unsightly (Latimer and Oetting, 1997).

Application technologies. Most of the brushing systems tested to date require that the tops of all brushed plants be uniform to receive similar treatment. This is complicated by growing different species, or cultivars, or plants of different seeding dates, in the same house. This is the primary limitation to commercial use of

brushing in transplant production. Sanders (1994) reported successful application of brushing by attaching a bar to the boom irrigation system. A sliding, or rolling, apparatus supporting a brushing bar was also commercially successful on a small scale (Latimer and Thomas, 1991; Schnelle et al., 1994). Growers using mechanical conditioning are very pleased with the treated plants, but agree that the process must be automated to be commercially successful.

Development of alternative methods that reduce physical contact with young plant tissue, such as air blasts (Beyl and Mitchell, 1977a) or shaking of entire benches, may be more acceptable for a wider variety of crops. These methods also would obviate the need to maintain crops at a uniform height as is required for brushing.

FUTURE NEEDS IN MECHANICAL CONDITIONING

Mechanical conditioning is an excellent means of regulating the growth of vegetable transplants and some ornamental bedding plants. It improves the stature, appearance, handling characteristics, and overall quality of treated plants. However, mechanical conditioning must be automated to make it commercially feasible. Growers prefer applying treatments during non-work hours and desire exact "recipes" for growth control of individual crops. With the differences in cultivar and species responses, this request will be difficult to fulfill as researchers must further define cultivar differences in response to mechanical conditioning. However, increasing verification of the flexibility of the treatment for growth regulation gives us more latitude in suggesting "recipes". We must identify factors affecting plant damage in response to mechanical conditioning treatments and develop methods that reduce damage to sensitive cultivars or species. We should also continue to examine ways that mechanical conditioning can interact with other cultural practices to improve plant growth regulation and final plant performance. For example, Newport and Carlson (1991) combined drought and negative temperature differential (-DIF) with shaking to attain a 41% height reduction of tomato seedlings grown in flats.

Although mechanical conditioning alone may not attain the ultimate goal of conditioning, to control transplant growth during production and enhance posttransplant productivity, it generally does provide good to excellent growth control with no detrimental effects on transplant establishment or crop productivity.

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TRANSPLANT PRODUCTION AND PERFORMANCE: CONTROLLING HEIGHT WITH TEMPERATURE

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ABSTRACT

Temperature management has emerged as an important tool for plant height control in greenhouse production systems. This is particularly important in vegetable transplant production where chemical controls for plant height are limited. Plant height is a function of the number of nodes and the length of each internode, and both are strongly influenced by greenhouse temperatures. Node number, or formation rate, is primarily a function of the average greenhouse temperature, increasing as the average temperature increases. Internode length is strongly influenced by the relationship between the day and night temperature, commonly referred to as DIF (day temperature - night temperature). As DIF increases, so does internode length in many plants. Although the nature and magnitude of temperature effects vary with species, cultivar, and environmental conditions, these two basic responses can be used to modify plant growth in transplant production. Although data are limited, controlling transplant height with temperature does not appear to adversely influence plant establishment or subsequent yield.

INTRODUCTION

Controlled-environment production of transplants, including vegetables, bedding plants, ornamentals and forest trees is one of the major commercial uses of greenhouse facilities in the United States. Transplant production systems have become increasingly automated as new equipment has been developed in the latter half of the 21st century, but the successful application of this automation is largely dependent on growing high quality uniform transplants. Development and widespread use of computer-controlled environmental management systems has allowed the investigation and implementation of new control strategies where the environment is modified to regulate both the rate of crop development and the morphology of the plant to improve transplant quality.

Temperature is the one of the most easily and frequently modified environmental factors influencing plant growth. One can find many early references to the profound effects of temperature on plant developmental rates, and morphology. Interactions between thermoperiod and photoperiod were well described as early as the 1940's and 50's. (Went, 1944; 1957). This basic information remained a scientific curiosity, until the development of automated environmental control equipment made it possible to manage greenhouse temperature with a precision that was impractical before. In the 1980's, a number of research groups in the United States and Europe began to re-examine the effects of temperature on the growth of plants in the greenhouse. The primary objective of these studies was to provide commercial producers with the information needed to take full advantage of the new control technologies (i.e. Karlsson et al., 1983). During one of these studies, while examining the effects of day and night temperatures between 14 and 30C on growth of Easter Lily, John Erwin made an interesting observation. He found an interaction between day and night temperature that affected stem length similar to that described by Went (1944; 1957). In attempting to describe this response with a mathematical model, he discovered that it could be described with a single term made up of the day temperature minus the night temperature. This approach was then applied to a number of other crops and it quickly became apparent that this relationship, coined DIF, could be used to describe much of the stem elongation response to diurnal thermoperiod and photoperiod interactions (Erwin et al., 1989; Karlsson et al., 1989, Berghage and Heins, 1991). Subsequent research has shown near universality of the general response (Erwin and Heins, 1995), but it has also demonstrated that the magnitude, and nature of the thermomorphogenic effects varies between plant species, and among cultivars within a species, as well as with timing and duration of the temperature fluctuations (Erwin and Heins, 1995; Myster and Moe, 1995).

The manipulation of thermomorphogenic stem elongation responses has been broadly applied in commercial horticulture to reduce, or in some cases eliminate the use of chemical growth regulators (Roberts, 1991). This has been particularly important in the production of transplants where concerns about chemical residues may make their use undesirable, or in fact in many cases illegal.

Managing plant growth with temperature

Plant height or stem length is simply the sum of the lengths of each of the internodes. It follows then, that to manage stem elongation, it is necessary to control or manage internode number, internode length, or both. Many environmental or genetic factors, and endogenous, and exogenous chemicals can influence either node number or internode length. The purpose of this report is to review the effects of temperature on transplant height and quality. Other factors will be considered only as they relate to, or interact with temperature, responses.

Node number

The rate of node development is, like most other development rates (i.e. Karlsson et al., 1989a), driven primarily by short and long term average temperature. Leaf unfolding rate (a measure of node, and internode formation) shows a curvilinear response to temperature, increasing as temperature increases to an optimum, then decreasing if temperature becomes too high. For many plants optimum temperatures for leaf unfolding fall between 22 and 30C. For example, with sweet pepper seedlings, Yaping and Heins (1996) reported maximum leaf count at an average temperature of about 26C. Other greenhouse crops with optima in this range include poinsettia (Berghage, 1989), dahlia (Brondum and Heins, 1993), and Boston fern (Erwin et al., 1993). Some species have optima above or below this range. For example, optimum temperature for leaf unfolding exceeds 30C in Easter lily (Karlsson et al., 1988), and 30-35C in vinca, *Catharanthus roseus* (Pietsch et al., 1995). In each of the studies cited above, average daily temperature was highly correlated with leaf unfolding while the relationship between day and night temperature (DIF) was not. While the rate of leaf unfolding may not be influenced by DIF, the total node number often is. This is frequently reported in determinant species where flower initiation is influenced by night temperature (poinsettia, Berghage, 1989), or temperature fluctuations, (chrysanthemum, Jensen, 1993).

The rate of temperature-driven leaf unfolding in many plants is influenced by light. Leaf unfolding rate increases as irradiance increases, (Pietsch et al., 1995) or photoperiod lengthens (Kozai et al., 1995), although much of this response is often attributed to increased leaf temperature. Other factors may also influence the rate of node formation. For example, McCall and Atherton (1995) report a lower leaf number for tomato plants grown with high salinity compared with low salinity in a positive DIF environment.

Internode length

Response to DIF.

Internode length in many plants is greatly influenced by diurnal temperature fluctuations. Day/night temperature fluctuations (DIF) have been used to describe some, or in a few cases, nearly all of the temperature-driven internode, or stem elongation responses in plants. For example, Easter Lily internode elongation (length) increases as DIF increases between -16 and +16C (Erwin et al., 1989). The response in lily is curvilinear with the magnitude of the response increasing as DIF increases. The effect of DIF on internode length is due to increased cell elongation rather than an increase in cell number (Erwin et al., 1994). Stem and internode length responses to DIF have been reported for many other ornamental and vegetable crops including: *Dendranthema grandiflora* (Karlsson et al., 1989b), *Euphorbia pulcherrima* (Berghage and Heins, 1991), *Xanthium pensylvanicum* (Erwin, 1991), *Nephrolepis exaltata* (Erwin et al., 1993), *Streptocarpus nobilis* (Erwin, 1991), *Campanula* (Moe et al., 1991), *Kalanchoe blossfeldiana* (Mortensen, 1994), *Begonia x hiemalis* (Grindal and Moe, 1994), *Begonia x cheimantha* (Bakken and Moe, 1995), *Catharanthus roseus* (Piersch et al., 1995), *Brassica* (Bakken and Flones, 1995), *Solanum tuberosum* (Kozai et al., 1995), *Cucumis sativus* (Grimstad and Frimanslund, 1993), *Lycopersicon esculentum* (Grimstad, 1993), and *Capsicum annum* (Yaping and Heins, 1996). For a more complete list see Myster and Moe (1995).

Plants respond rapidly to a change in DIF; altered growth rates can often be observed in as few as 24 hours (Erwin and Heins, 1995). The response does not appear to have much of a residual effect; that is, plants respond to the current environmental regime with little lag or long term carry over (Berghage, 1989). Although a response to DIF has been described for nearly all plants examined, exceptions include spring bulbs or ephemerals (Erwin and Heins, 1995), the magnitude and nature of the response is affected by other environmental conditions, timing of the temperature fluctuations during the photo/skotoperiods, genetics, and plant maturity, or growth stage.

Environmental factors that confound or modify the DIF response.

Daily average temperature influences internode length and thus the response to DIF in many plants. Sweet pepper seedling internode length was correlated with average temperature as well as with DIF (Yaping and Heins, 1996). Poinsettia has an optimum average temperature for internode elongation of about 24C, as well as a correlation with DIF (Berghage, 1989). Similarly, Grimstad and Frimanslund (1993) report independent effects of average temperature, and DIF on

cucumber internode length. Internode length increased with increasing average temperature and increasing DIF, except at low day temperature (15C) where there was little response to DIF.

Internode elongation response to DIF is affected by light and photoperiod. Light quality has been shown to influence the response to DIF, presumably through effects related to phytochrome photoequilibria (Moe and Heins, 1990; Myster and Moe, 1995; Erwin and Heins, 1995). Incandescent lighting used for day length extension can eliminate a plant's response to negative DIF while fluorescent lighting can enhance the response (Moe et al., 1991). Increasing irradiance decreases DIF response (Myster and Moe, 1995). For example, increasing irradiance from 100 to 200 $\text{mmol m}^{-2} \text{s}^{-1}$ decreased the stem elongation of petunia in response to a positive DIF compared with a negative DIF (Kaczperski, et al., 1991). Kozai et al. (1995) report that with *Solanum tuberosum*, the shortest internodes were produced with plants grown with higher irradiance (140 $\text{mmol m}^{-2} \text{s}^{-1}$) longer photoperiods (16hrs), and a negative DIF, while the longest internodes were produced with low irradiance (70 $\text{mmol m}^{-2} \text{s}^{-1}$) and positive DIF. In general, the response to DIF has been found to decrease as photoperiod increases (Erwin and Heins, 1995; Myster and Moe, 1995).

Nutrient solution salinity effects the magnitude of the response to DIF in tomato seedling stem elongation (McCall and Atherton, 1995). Reduced internode length in response to a negative DIF was greater with lower nutrient solution salinity (EC 3) than high salinity (EC 15). Plants had reduced y_p when grown under a positive DIF environment, however, since there were no significant interactions between water relations and DIF, the authors conclude that the mechanisms for inhibitory effects may be different.

Genetics. The magnitude and nature of the DIF response is influenced by plant species and cultivar. Easter lily response is fairly large and straight-forward (Erwin et al., 1989). In contrast, plant height and internode length in *Kalanchoe blossfeldiana* increases with either positive or negative DIF (Mortensen, 1994; Jensen, 1994). In a trial of 20 seed geranium cultivars, Strefeler (1995) found that, although the potential for elongation varied among cultivars, the response to DIF was the same. *Euphorbia pulcherrima* cultivars however, vary in their response to DIF (Moe et al., 1992a; 1992b). Tomato, corn, and cucumber have strong responses to DIF, while squash, watermelon, pea, and bean are less responsive (Erwin and Heins, 1995).

Timing of temperature changes. The timing and length of temperature changes influences the response to photo/skotoperiod temperature responses. Although there are a number of conflicting reports in the literature (Erwin and Heins, 1995), there is a general consensus that many plants are sensitive to a temperature drop, or low temperature pulse, during the first 2 to 4 hours of the photoperiod (Myster and Moe, 1995). For example, a low temperature pulse given during the first 2 hours of the day reduced internode length of cucumber and tomato seedlings (Grimstad, 1993). Plant height was reduced in direct response to the magnitude of the low temperature pulse (between -2 and -10C). In contrast to the relatively consistent response to temperature pulse immediately after dawn, pulses at other times of the day or night have provided inconsistent results. A temperature pulse before dawn was not effective in salvia or petunia (Erwin, 1991) but has been effective with *Begonia x hiemalis* (Grindal and Moe, 1994). A temperature pulse provided during the middle of the dark period has generally proved to be least effective in reducing stem elongation (Jensen, 1994; Grindal and Moe, 1994; Erwin and Heins, 1995).

Daily stem elongation in a number of greenhouse crops follows a pattern similar to the response to a low temperature pulse. Stem elongation is greatest during the end of the night and beginning of the day, decreasing during the day and increasing again during the night (Erwin and Heins, 1995; Lecharyn et al., 1985). However, this pattern does not appear to be consistent for all plants and the relationship does not seem correlated with response to temperature DIF or pulses. For example, Bertram and Karlsen (1994) report that there was no clear pattern in elongation in the DIF-sensitive plants poinsettia or pelargonium, and that in petunia, also a DIF-sensitive species, elongation occurred mostly during the day.

Effects of DIF on other quality factors for transplants. Leaf expansion and orientation can be affected by DIF (Erwin and Heins, 1995). Leaf area of *Solanum tuberosum* plantlets (Kozii et al., 1995), and *Brassica* transplants (Bakken and Flones, 1995) was reduced when grown with a negative DIF. Likewise, Yaping and Heins (1996) report that leaf area of sweet pepper seedlings was highly correlated with DIF, but in this case, only when average daily temperature was considered. In contrast, leaf area of Easter lily was correlated with night temperature and not DIF (Erwin et al., 1989). Leaves on plants grown with a negative DIF tend to point downward, while those grown with a positive DIF point upward (Erwin et al., 1989).

Leaf chlorophyll content is reduced in plants grown with a negative DIF (Berghage et al., 1991). Total

leaf chlorophyll increases as DIF increases in *Fuchsia* and *Dendranthema* (Erwin and Heins, 1995). Reduced leaf chlorophyll results in visibly chlorotic plants in negative DIF environments. For example, sweet pepper leaf reflectance at 550 nm decreased as DIF increased (Yaping and Heins, 1996). DIF-induced leaf chlorosis is often reversible, with plants greening rapidly after removal from the negative DIF environment (Erwin and Heins, 1995).

Lower day temperatures and reduced leaf chlorophyll have been considered responsible for the frequently reported reduction in dry weight of plants grown in negative DIF environments. Grimstad (1993) reported reduced plant dry weight in both cucumber and tomato in response to a low temperature pulse. Likewise, dry matter production in chrysanthemum is reduced in negative DIF grown plants (Jensen, 1993). Negative DIF has been shown to reduce total soluble carbohydrate content of *Lilium* (Miller et al., 1993), and affects carbohydrate partitioning in many plants (Erwin and Heins, 1995). In contrast, there are a number of reports where DIF did not influence plant dry weight (Kozii et al., 1995; Bakken and Fiones, 1995), or where plants grown with a negative DIF had greater dry weight than those grown with a positive DIF (Bakken and Fiones, 1995).

Effects of DIF on transplant establishment and yield. Although little research has been reported on the effects of DIF on transplant establishment and yield, work to date suggests that using a negative DIF to control transplant height does not adversely affect establishment or crop yields, and may in some cases improve yield or crop quality. Bakken and Fiones (1995) report that swede (*Brassica napus* L. *rapifera* Metzg.) dry weight at harvest was not affected by transplant production temperature regime, but that harvested root quality was better from transplants grown with a negative DIF. They also reported that yield of cabbage and Brussels sprouts was increased using negative DIF-grown transplants. Similarly, early yield and fruit quality of greenhouse cucumber was unaffected by a low temperature pulse (Grimstad, 1993). Early fruiting in greenhouse tomato was delayed by a low temperature pulse, but only when given for long time periods (4 or 5 weeks) and large temperature differences (9 and 6C) (Grimstad, 1993). Adequate height reduction could be obtained with a shorter treatment period or smaller pulse (Grimstad, 1993).

Summary

Managing plant height in the greenhouse is an important quality consideration for transplant producers. Alternatives to the use of chemical growth regulators are needed because of environmental, or health concerns, and the unavailability of chemical controls for many plants. Height control through temperature management is achieved by two basic mechanisms, reduced node and internode number, primarily a function of average temperature, and reduced internode elongation, primarily through increased night and or decreased day temperatures, or low temperature pulses at, or near the beginning of the day (negative or zero DIF). Although negative DIF has been shown to reduce leaf chlorophyll, and plant dry weights, this has not translated into adverse effects on transplant establishment or yield. The most effective management strategies will take both number and internode length into account to produce compact transplants using greenhouse temperature as one of a number of height management tools.

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TRANSPLANT GROWTH CONTROL THROUGH WATER STRESS - A REVIEW

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ABSTRACT

The theme of this review is modulation of extension growth in transplant production through restraint of watering of the seedlings. The purpose of the modulation is to produce transplants of 1) appropriate height for ease of field setting and 2) adequate stress tolerance to withstand outdoor environmental forces. Physiological responses of the plant are discussed in relation to the degree of watering restraint and are related to the degree of hardening or stress tolerance development in the transplants. Optimal stress tolerance or techniques for measuring same have not been fully defined in the literature. However, stress tolerance in seedlings is necessary in order to withstand environmental forces such as wind and sandblasting after the seedlings are transplanted in the field. It is also imperative that the seedlings undertake a rapid and sustained rate of growth after outdoor transplanting. Water stress applied to plants elicits many different physiological responses. For example, as leaf water potential begins to decrease, leaf enlargement is inhibited before photosynthesis or respiration is affected, with the result of a higher rate of dry matter accumulation per unit leaf area. The cause of the reduced leaf area may be a result of reduced K uptake by the roots with a concomitant reduction in cell expansion. Severe water deficits however, result in over-stressed seedlings with stunted growth and poor establishment when transplanted into the field. In transplant production systems appropriate levels of water stress can be used as a management tool to produce seedlings conducive to the transplanting process.

INTRODUCTION

The scope of this review encompasses seedling water management practices and the effects of varying degrees of water stress on the physical, physiological and mechanical stem strength attributes of transplants. It also relates the implications of slight or severe water stress on "hardening" or stress tolerance development in transplants. The overall objective of water management in transplant production is to modulate growth in order that the seedlings withstand the temporal physical conditions in which they are transplanted and also undertake a rapid and sustained rate of new root and shoot growth.

Stress Tolerant Transplants

Transplant producers growing seedlings in the greenhouse intuitively regulate watering level to restrain excessive growth. The restraint is necessary for survival of the seedlings in the field after outdoor transplanting (Latimer, 1990; Riviere et al., 1990) and for easy and proper seedling placement during the transplanting procedure. Water restraint can result in "hardened" or stress tolerant seedlings. The stress tolerance is important for holding transplants when field establishment cannot be done immediately (Marr and Jirak, 1990), for a high percentage survival in the field (Liptay, 1987), and a more rapid rate of establishment (Liptay, A. and S. Nicholls, 1993). The goal in transplant production is to achieve an optimal seedling size with the appropriate level of stress tolerance to withstand environmental or other stresses when the plugs are transplanted into the field.

Varying Water Stress

Water stress can be applied to seedlings or plants in varying degrees. One effect of water stress at moderate levels is a reduction in plant leaf area. Frensch and Hsiao (1994) reported that the rate of solute flow into plants can become rate limiting for cell expansion under conditions of mild water stress. This was also shown to occur in soybeans and sunflower at about -400 KPa (Boyer, 1970). However, when plants were rewatered following drought stress the leaf growth rate did not recover to the well-watered rate of enlargement. Photosynthesis and respiration were not inhibited until more severe water stress was imposed.

Muchow et al. (1986) also reported similar results for soybean. In addition, at very low water potential, where biomass production was reduced, the decrease was associated with lower stomatal conductance but was not associated with specific leaf N content. Schulze and Bloom (1984) reported similar results for radish and tomato. In white clover, short drought periods reduced leaf area and the number of stolons but had no effect on leaf number. With longer drought periods the number of leaves was also decreased (Belaygue et al., 1996). The decrease in shoot growth was linear with decreasing water content of soils for onions (Kuchenbuch et al., 1986b).

Sharkey and Seemann (1989), reported that reductions in whole leaf photosynthesis was primarily the result of stomatal closure rather than damage to chloroplasts. Ben et al. (1987) reporting on varying levels of water stress, indicated that the CO₂ saturated rate of photosynthesis under high light conditions was the most sensitive stress parameter. Furthermore, mild water stress did not affect quantum yield of photosynthesis but, acute water stress damaged chloroplasts. Thus there appear to be several degrees of water stress; the less severe being desirable for transplant production while the more severe being too restrictive on growth.

Water Stress and Morphology

The primary root of maize continued slow growth at low water potentials that completely inhibited shoot growth (Sharp et al., 1988; Saab et al., 1990). Root growth in tomato seedlings behaved in a similar manner (Liptay and Tan, 1985). However, in both cases the roots had lower rates of volume expansion and were very thin. Wu et al. (1994) reported that an increase in cell wall loosening contributed to the maintenance of primary root elongation at these low water potentials. Moreover low water potentials had no effect on osmoticum deposition close to the apex but did decrease deposition in the more mature tissues (Sharp et al., 1990). Wu et al. (1996) reported that growth in the root apical region at low water potentials involved an increase in cell wall extension properties such as an accumulation of expansin. Sharp (1996) indicated that ABA accumulation may help maintain primary root growth and inhibit shoot growth under low water potentials. Mulholland et al. (1996) demonstrated that leaf expansion can be enhanced by exogenous application of synthetic ABA either to the rooting environment or directly to the xylem sap. Moreover, Sharp (1996) recommended caution when interpreting the effects of hormones applied to well-watered plants because hormonal sensitivity or response of tissues varies with water status.

Plasma membranes of living cells have been shown to be freely permeable to water while creating a barrier to other molecules (Chrispeels and Maurel, 1994). The water "channels" were proteins called aquaporins which allowed water to pass freely while excluding ions and metabolites. In plants, aquaporins are in the vacuolar membranes (tonoplast) and may be present in the plasma membrane. The driving forces of water, which cause irreversible expansion of cells, are both hydraulic and osmotic. Maize plants have been shown to respond to water deficits by hydraulic signals from the roots and by hardening of the walls (Chazen and Neumann, 1994). There is a different response between the differentiating tissue and the more mature tissue and also among organs (e.g. the root vs. the shoot).

Mechanical Properties

Water stress has an effect on the mechanical strength of a seedling or plant stem and this response varies with plant species (Niklas, 1991). Niklas and Moon (1988) used elastic modulus as a measure of the bending strength of a stem that can support a weight on the stem tip or continue to grow vertically. The elastic modulus of a stem depended on stem water content (Niklas, 1989b) and also on the ratio of cell wall to protoplasm (Niklas, 1989a). Buckling of the stem in chives occurred at -1300 KPa (Niklas and O'Rourke, 1987). Nonami and Boyer (1990) discussed elastic and plastic properties of cell walls. They found elastic deformation was instantaneous, reversible, independent of time, and was present only when the force applied to the plant tissue changed and followed Hooke's law. Plastic deformation was not instantaneous, not reversible, occurred continuously at a rate proportional to the force applied and was Newtonian in nature. At low water potentials the plastic properties and conductance of water of the cell walls decreased, but, there was little effect on the elastic properties. Thus specific water stresses could result in desirable improvements to the strength of the transplant and the seedlings' stress tolerance.

Growing Medium and Water Stress

Decreasing the water content in a soil decreased K uptake by onion roots; the drought resulted in an increasingly steep K gradient around the root (Kuchenbuch et al., 1986b). In soybean, the K content of the xylem sap decreased with

decreasing water potential (McQuate et al., 1986). This osmotic adjustment was different in different plants. Kuchenbuch et al. also found similar results for onion plants (1986a). Less K uptake under lower water potentials may explain reduced leaf area (Boyer, 1970), as a result of reduced cell expansion at reduced K levels in the cells (Marschner, 1986).

Transpiration

Increased field survival in "hardened" or stress tolerant transplants may result from their altered stomatal regulation. For example, Spence et al. (1986) reported that plant stomata adapted to drought stress maintain stomatal opening at lower plant water potentials than non-adapted plants. Stomata from water-stressed plants were smaller, had a different shape and had a mechanical advantage over non-stressed plants in opening. The pores of stomata of water-stressed plants required only 1.9 times the turgor pressure of the surrounding epidermis to initiate opening vs 2.4 times for pores of the guard cells from well-watered plants.

Plants may use several mechanisms in response to drought stress. Sorghum plants delayed the onset of plant water deficit by producing more xylem vessels (Fernandez and McCree, 1991). Tomato plants have more and larger stomata than black locust and are more prone to water stress (Hinckley, 1973). Gu et al. (1996) reported that upon rewatering, after a water stress, tomato plant transpiration returned to normal but growth rate did not.

Physiology

Water deficit did not appear to promote ethylene synthesis in cotton, bean or rose (Morgan et al., 1990). Though leaf area was less in plants experiencing drought stress, the dry weight of the seedlings was greater per unit area than for non water-stressed plants (Latimer, 1990). The greater weight may be part of the development of stress tolerance in plants. Solute concentration in tissue increased after growth rate had fallen. Glutathione reductase, an indicator enzyme of drought stress, increased under stress conditions (Burke and Hatfield, 1987). Guralnick and Ting (1987) reported the time required to restore various physiological processes after a prolonged drought for portulaca as follows: water potential and CO₂ uptake were normal after 24 h; RuBP carboxylase was normal after 3 days; the CAM pathway was normal after 5 days; chlorophyll levels were higher than normal after 5 days; PEP carboxylase and PEP carboxykinase activity were normal after 6 days; the chlorophyll a/b ratios returned to normal levels only after 27 days. Sells and Koeppel (1981) reported that proline oxidase activity decreased in mitochondria after only a slight water stress while proline increased in water-stressed plants (Stewart et al., 1977). Another factor associated with water stress may be anti-oxidant concentrations. Gogorcena et al. (1995) reported a decline in antioxidant levels associated with water stress in peas. However, Castillo and Layzell (1995) reported that oxygen limitation plays only a minor role during drought stress. There are a number of scientists experimenting with the role of anti-oxidants in relation to development of stress tolerance in plants.

Incident Radiation and Water Stress

The volume of water used by plants in the greenhouse is generally related to incoming radiation (Hesse, 1985; Musard and Dupuy, 1972; Stanhill and Albers, 1974). There appears to be a linear relationship between the amount of water consumed by the plants and the amount of incoming solar radiation plants received. Wiertz and Richter (1987) have developed an irrigation model which includes this relationship.

CONCLUSIONS

Seedling growth control in transplant production in greenhouses can be achieved by regulating the amount of water available to the plants. The severity of water restriction is critical. A desirable level of water restriction results in stocky, stress resistant seedlings able to withstand environmental stresses after transplanting outdoors. If water restriction is too severe seedlings die or are overhardened slowing new shoot and root growth. The difference in the severity of stress tolerance is related to various levels of physiological restraint on growth, the more severe being disadvantageous. In conclusion, more studies are needed to quantify and understand the physiological responses of the plants as these are related to water stress and the physical properties of the growing media.

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TRANSPLANT PRODUCTION AND PERFORMANCE: UNDERSTANDING ROOT SYSTEMS TO IMPROVE SEEDLING QUALITY

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ABSTRACT

Root architecture can be very important in plant productivity. Importance of studies on root morphology and development is discussed to improve seedling growth. Root systems of dicotyledonous species are reviewed, with emphasis on differences between growth of basal and lateral roots. The presence of different types of roots in plant species suggests possible differences in function as well. The architecture of a root system related to its functions is considered. Classical methods for studying root systems comprise excavation of root system, direct observation and indirect analyses. While the first method can only be destructive and the third effective in understanding root architecture only on a relatively gross scale, observation methods allow the scientist a complete and non-destructive architectural study of a root system. The three groups are reviewed related to their potential to give valuable information related to the root architecture and development of seedling, with emphasis on the availability of a medium-transparent plant-growing system, enabling the non-destructive daily observations and plant measurements under controlled environmental conditions. Effects of CO₂-enrichment on seedling growth is reviewed, emphasizing the effects of CO₂ on root growth.

INTRODUCTION

Researchers generally concentrate on the physiology of the shoot and neglect the roots of plants, partly because roots are out of sight and are more difficult to study. However, their functions indicate that vigorous root systems are as essential as vigorous shoots for growth and development of healthy plants. In fact, the growth of roots and shoots is completely interdependent, and one plant part cannot develop effectively if the other fails (Kramer, 1983).

Early seedling root growth and development determine the optimum root system throughout the entire life of a plant, consequently affecting growth during this period, and potentially leading to optimization of yields (Leskovar and Stoffella, 1995; Lynch, 1995). The spatial distribution in the soil of the root system can determine the potential of a plant to exploit the soil's resources, which are unevenly distributed on the earth's surface, or subjected to localized depletion by the roots (Lynch, 1995). The production of a primary root system, i.e. the primary branching from the radicle, is an important phenomenon in growth and survival of a plant (MacIsaac et al., 1989). A primary root system increases the surface area available for the uptake of water and mineral elements. In addition, with its architecture, a primary root system provides physical support to the developing shoot.

MORPHOLOGY AND DEVELOPMENT OF YOUNG ROOTS IN DICOTYLEDONOUS SPECIES

Seedling root morphology has been described as differing between monocotyledonous and dicotyledonous species (Fahn, 1982; Leskovar and Stoffella, 1995; Sutton and Tinus, 1983; Zobel, 1975, 1986, 1995, 1996). Dicotyledonous species were described as presenting three types of roots: radicle, adventitious and lateral roots (Esau, 1965, 1977). The radicle will form the taproot (primary root), adventitious roots are initiated from the hypocotyl, lateral roots from the taproot. Beginning with Zobel (1975), several researchers have identified a fourth type of roots, called basal roots, arising from the lower-hypocotyl - upper taproot. Zobel (1986) indicated that initiation of basal roots is under different genetic control from initiation of lateral and adventitious roots. In fact, a double homozygote from a lateralles tomato mutant (recessive mutant called *diageotropica*, *dgt*) and an adventitiousless tomato mutant (recessive mutant called *rosette*, *ro*) originated roots in the hypocotyl and upper portion of the taproot. Assuming only 3 types of root, the double homozygote should have had only a taproot. Because these roots were genetically not lateral, nor were they adventitious, Zobel (1975) classified these roots as

'basal' roots. Basal roots have been reported to be produced by mungbean (*Vigna radiata* L.) (Leskovar and Stoffella, 1995), bell pepper (*Capsicum annuum* L.) (Stoffella et al., 1988), tomato (*Lycopersicon esculentum* Mill.) (Zobel, 1975), beans (*Phaseolus vulgaris* L.) (Stoffella et al., 1979) and lettuce (*Lactuca sativa* L.) (Nicola, 1997).

Evidence that basal and lateral roots differ in terms of morphology, point of origin from the taproot and development was reported by Nicola (1997) in lettuce (*Lactuca sativa* L.) seedlings grown in transparent medium (Fig. 1). Basal and lateral roots in lettuce seedlings originated from two distinct regions of the taproot and developed differently. A thicker, short upper radicle was visibly distinguished from a smaller, long, lower radicle 2 days after seeding (DAS). This distinction was even more evident 3 and 4 DAS. A restriction area separated the two portions of the taproot. Basal roots originated at two opposite sides of the short upper portion of the taproot and located close to the hypocotyl, and did not produce secondary branches during the first 18 DAS. Lateral roots originated at about 120° apart along the longer portion of the taproot and produced secondary branches. Basal roots presented a horizontal extension in the medium surface, while lateral roots presented a growth extension toward the deep part of the medium. The number of lateral roots 18 DAS was two- to threefold the number of basal roots.

Adventitious roots cannot be confounded with lateral roots for two main reasons. First, adventitious roots originate from the stem, while lateral roots originate from the taproot. Second, the former type originates from tissues other than the pericycle, while the latter type originates from the pericycle. Conversely, basal roots are not clearly classified with respect to their point of initiation. Zobel (1986) stated that he demonstrated that basal roots initiate from the pericycle of the lower hypocotyl and upper taproot. Consequently, basal roots were not adventitious in anatomical origin, nor lateral or adventitious in genetic control of their initiation (see above).

Detailed information of the sequence of events occurring in root branch formation of young seedlings could be valuable to further determine the root development of field-grown plants. In bell pepper seedlings, basal roots emerged prior to lateral roots (Stoffella et al., 1988) and only after full cotyledon expansion. A similar development was found in lettuce seedlings (Nicola, 1997). In tomato seedlings, lateral roots emerged prior to basal roots (Aung, 1982). Weinhold (1967) described basal roots as arising acropetally (toward the shoot apex) from the germinating seedling, while lateral roots arose basipetally (toward the radicle apex). Nicola (1997) reported in lettuce a similar development only for the first 8-10 DAS, after then new basal and lateral roots emerged also between older primary branches, contemporary to emergence of basal and lateral roots that followed the respectively original acropetal and basipetal patterns. Charlton (1996) did not distinguish between lateral and basal roots, but he defined lateral roots as "roots derived from lateral endogenous primordia formed in preexisting roots" (p.149). The author said that lateral roots appeared at a relatively constant distance behind the tip of a growing root, that lateral roots initiated in rows or ranks, and that within each rank they appeared to initiate and emerge in acropetal sequence under normal conditions. Charlton (1996) reported that between the basipetally emerged lateral roots, particularly in dicots, additional laterals may arise for a long period in roots with secondary growth. Esau (1965) referred to these additional lateral roots as adventitious.

THE ARCHITECTURE A OF ROOT SYSTEM RELATED TO ITS FUNCTIONS

Fitter and Stickland (1991) and Lynch and van Beem (1993) suggested that the architecture of a root system may have ecological implications for uptake of water and nutrients from soil. Fitter (1986, 1987, 1996) suggested that, in general, plants with a more herringbone-like distribution of roots, that is, with branches mainly along the central root axis, may occur under low soil resource availability, whereas secondary branches increase when resources are present in abundant supply, thereby increasing acquisition of water and nutrients. Variation in root growth between species was indicated by Seiler (1994) to be an important factor determining differences in drought tolerance among genotypes. Early, rapid root growth and branch root development were suggested to confer an adaptive advantage in more efficient utilization of soil water. Root elongation can be advantageous to plants in drying soil, and may be particularly important for seedling establishment. Growth of new plants is restricted largely to surface soil layers which are vulnerable to drying (Sharp et al., 1988).

Basal roots differ from the three more classic types in terms of morphology, gene expression, and development (Zobel, 1996; Nicola, 1997). This suggests possible differences in function as well. Differentiation in primary

branch formation between lateral and basal roots could be valuable for increased survival adaptation of seedlings to different soil conditions which can affect root resource acquisition potentiality. The differentiated functions of basal and lateral roots have not yet been fully understood, but several authors have hypothesized that basal roots would provide a mean for plants to uptake surface-applied nutrients and water during crop production, and they may also play a role in supporting the plant (Bole, 1977; Eshel and Waisel, 1996; Jackson, 1995). Finding in lettuce that lateral roots originated in three directions with respect to the taproot is an indication that lateral roots can explore more soil volume for resources than basal roots, thus lateral roots may be able to reach and exploit localized patches of nutrients in the soil (Lynch, 1995). Conversely, basal root bi-directional and superficial formation gives the root system a horizontal extension in the soil surface, assuring the basal roots the capacity to exploit the most fertile portions of agricultural soils (Bole, 1977; Eshel and Waisel, 1996).

Zobel (1996) reported that species that demonstrated the most drought tolerance had the most deeply penetrating root system, implying that plants with an extensive lateral root system would be favored in these instances. Eshel and Waisel (1996) suggested that the major function of basal roots was to exploit the most fertile portions of agricultural soils more efficiently than lateral roots. In addition, Bole (1977) found that basal roots of rape (*Brassica campestris* L.) were capable to absorb phosphorus more efficiently than lateral roots. According to results of Brekle (1991), excess of heavy metals stimulated growth of lateral roots while decreased growth of basal roots. Eshel and Waisel (1996) reported that basal roots are less sensitive to gravity than lateral roots, and therefore can extend the root system horizontally.

STUDYING ROOT SYSTEMS OF SEEDLINGS

Research on vegetable crops has focused mainly on shoot growth, possibly since shoots are considered the main source for maximization of the edible part of the plant (even when the edible part of the plant is below ground, i.e. tubers, roots or stolons). Much is now known about early seedling shoot growth and development, unfortunately little is understood about root growth and development.

Studies on root morphology and architecture have attracted much research in the field of forestry and fruit crops, since these species have a long life span and have a high dependence on below-ground conditions, e.g. water supply, fertility, soil physical property, aeration. As early as 1927, Weaver and Bruner (1927) lamented on the lack of studies on how a root system functions. During the 1980s an increase in interest of studying roots occurred, particularly related to the regulation of root growth and development (Feldman, 1984).

Measurements of root biomass and root length are typically used to describe root distribution in the soil profile, but analysis of root morphology and architecture can improve the understanding of water and nutrient extraction from soil (Fitter, 1985). However, field methods to study root growth enabling repeated direct observations of undisrupted root growth have been extremely limited (Mackie-Dawson and Atkinson, 1991).

Many methods to study the root system have been developed in recent years. Mackie-Dawson and Atkinson (1991) reviewed many of these methods. There are 3 major groups of methods that can be used to assess root growth in the field. The methods comprise: 1) Excavation of the root system; 2) Direct observation; and 3) Indirect analyses. Methods from the first group require the removal of the root system from the soil, usually by washing, and can cause a major loss of root material when dealing with young seedlings. Early in development, much of the root system is very fragile, making full excavation and architectural analysis extremely difficult (Jackson, 1995). In studying root systems actively growing in soil, Jackson (1995) felt that a major difficulty was the extraction of fine roots and the measurement of root architectural variables.

Observation methods require a viewing surface that is inserted into the soil (Mackie-Dawson and Atkinson, 1991). The development of the root system *in situ* can then be seen through a window into the soil, allowing the same area of volume of soil and root system to be observed continuously. The major constraint in using these methods is that only a portion of the root can be observed, but extrapolation of the root measurements to the entire root system may be not representative. If a whole root viewing surface could be developed, observation methods could represent a major advance for study of the root system. Although hydroponic liquid culture allows direct observation of the

entire root system, present growing conditions prevent normal root hair development and may also alter other aspects of root morphology.

The extent and activity of the root system have been indirectly measured by relating the root function to supply of water and mineral nutrients to the plant (Mackie-Dawson and Atkinson, 1991). For example, a relationship between root length and maximum soil moisture depletion was observed in pasture species by Evans (1978). Consequently, variation in soil moisture with depth as determined by measurement of soil water potential has been used for estimating root activity through mathematical models. Similarly, the absorption of ^{32}P was found to be correlated with root length by Atkinson (1989). This relationship was studied using a combination of an observation window in a laboratory study and the injection of the radio-isotope in the soil. These indirect methods can be effective in understanding root architecture, however, only on a relatively gross scale.

Another approach to the study of early seedling growth is that of micropropagation in tissue culture, particularly where all external carbon is derived from CO_2 (Kozai, 1991). In this type of autotrophic culture, growth and development are largely influenced by physical environmental factors which include light, CO_2 , humidity, air flow speed, temperature, and O_2 . However, use of photoautotrophic micropropagation of whole seedlings is fairly new, moreover there is little research on the effects of the physical environment on photoautotrophic growth and development of whole seedlings.

Seedling growth *in vitro* does not require a supply of growth substances as does an explant or a cutting. In fact, the seed from which the seedling originates provides all the substances necessary for early growth. Seedlings can grow autotrophically if provided sufficient CO_2 . When using airtight vessels for growing photoautotrophic seedlings, the CO_2 concentration was often measured to be as low as the CO_2 compensation point (less than $100 \mu\text{mol mol}^{-1}$) during most of the photoperiod (Fujiwara et al., 1987; Kozai et al., 1992). Using loose caps or gas permeable film for capping the growth vessels resulted in CO_2 concentrations often lower than $200 \mu\text{mol mol}^{-1}$ (Kozai, 1991) (which is lower than the atmospheric concentration of $350 \mu\text{mol mol}^{-1}$). During the dark period, CO_2 concentration rose to $3000\text{-}9000 \mu\text{mol mol}^{-1}$, but remained low ($100\text{-}200 \mu\text{mol mol}^{-1}$) for the entire light period.

Little research has been conducted *in vitro* using seeds and even fewer studies have been done using *in vitro* culture to study root architecture and morphology. Stoffella et al. (1988) used test tubes filled with gelled medium (gelrite) supplemented with mineral nutrients and sucrose as a carbon source (photo-mixotrophic growth), to characterize the early root morphology of bell pepper seedlings. A whole-plant observation method was implemented to study the early root growth and development of lettuce seedlings (Nicola, 1997; Nicola et al., 1996). Nicola et al. (1996) and Nicola (1997) used test tubes filled with Phytigel gelled medium supplied with mineral nutrients and sucrose to grow lettuce seedlings and study root morphology and architecture (Photo-Mixotrophic Whole Plant Culture, PMWPC). Plants were grown 9 days in the tubes and then removed. The lack of recyclable air and depth for root extension did not permit further growth. Consequently, a Photosynthetic Whole Plant Culture (PWPC) system was designed and built by Nicola (1997) to study root seedling growth, with the attempt of removing the exogenous sucrose supply as a carbon source and the introduction and circulation of fresh air into the plant container to avoid the lowering of CO_2 concentrations during the light period. Glass one-liter bottles were used to accommodate a large quantity of transparent nutrient medium, thus permitting the growth of seedlings for up to 18 DAS without supplying sucrose as a carbon source. Filtered and calibrated air was allowed to flow in and out the bottles, thus permitting the seedlings to use their photosynthetic capacity. Environmental conditions of the growth room such as temperature, relative humidity and light were preconditioned and controlled throughout the duration of the experiments. The growing system developed was easily constructed, calibrated and monitored during lettuce seedling root growth studies, and enabled direct non-destructive plant observations and measurements, under controlled environmental conditions. During the studies pregerminated seeds were transferred one into each bottle and grown until plant harvest. Number of lateral and basal roots, number of secondary root branches and of leaves could be daily counted, by visual observation through the bottle. The root growth observation method developed could represent a valuable tool to investigate root morphology and development in different species, to improve selection and breed new varieties based on their root architecture potential.

INCREASING ROOT GROWTH WITH CO₂

Increased concentration of carbon dioxide in the atmosphere has received increased interest in recent years (Eshel and Waisel, 1996). Plant adaptation to rising CO₂ concentrations in the atmosphere is a paramount priority these days, because it is estimated that CO₂ in the atmosphere will double from the 1978 average concentration of 338 $\mu\text{mol mol}^{-1}$ by the middle or later part of the 21st century (Murray, 1995).

Enrichment of atmospheric CO₂ has been reported to increase growth rate and yield of a wide variety of plants, and it is widely used in greenhouse crop production. Sonnewald et al. (1996) reported that the initial response to elevated CO₂ in the atmosphere can lead to an approximate increase of 50% in leaf photosynthesis. The increase in atmospheric CO₂ increases photosynthetic efficiency in terrestrial C₃ plants because the present CO₂ concentration is insufficient to saturate the ribulose-1,5-bisphosphate carboxylase/oxygenase enzyme system (Rubisco) and the increased CO₂ competitively inhibits ribulose-1,5-bisphosphate oxygenation and photorespiration (Long et al., 1996). However, an acclimation effect occurs, and the rate of photosynthesis again declines in many species, mainly due to a decline of Rubisco activity (Evans, 1983, 1987; Geiger and Servaites, 1991; Kramer, 1981; Sage et al., 1989; Sonnewald et al., 1996; Torisky and Servaites, 1984).

Tremblay et al. (1987) increased growth rates of shoot and roots of celery transplants raised in greenhouse. Lettuce yield in a tunnel was enhanced by enrichment of CO₂ in the air (Enoch et al., 1970; Wittwer and Robb, 1963). Del Castillo et al. (1989) found that soybean plants grown under elevated CO₂ concentration had greater root mass than those grown under normal atmospheric CO₂ concentration. However, CO₂ concentration did not affect the rate of elongation of individual roots. The number of branches increased because of CO₂ enrichment, resulting in an increased total root length, without an increase of the volume of soil explored by the roots. Aguirrezabal et al. (1993) studied root systems in hydroponically grown sunflowers as affected by carbon nutrition. The results they obtained indicated that the control of carbon partitioning among various components of a single root system was determined by a combination of the distance of each sink from the source and its level of branching. Studies of Nicola (1997) on lettuce seedlings grown in a controlled environment have indicated that enriching the atmosphere up to 2000 $\mu\text{mol mol}^{-1}$ CO₂ enhanced root branch formation in the seedlings, particularly basal roots, compared to the control of 350 $\mu\text{mol mol}^{-1}$ CO₂, without affecting plant biomass 18 DAS. The ecological and physiological implications of such altered root morphology and architecture are related to water and nutrient acquisition capacity from soil of the root system (Fitter, 1985; Fitter and Stickland, 1991; Lynch and van Beem, 1993). Using CO₂ enrichment in controlled environment would be a valuable approach to increase early root branching in seedlings during greenhouse transplant production.

CONCLUSIONS

Root growth, development and architecture are important aspects of seedling growth. Stresses that reduce root growth may injure the plant by reducing the volume and extent of soil exploration. If root growth declines due to stress, the supply of water and nutrients to the shoot may be reduced, with a subsequent reduction in shoot growth. However, conditions that limit photosynthesis can reduce shoot growth, limit assimilate translocation to the roots, and in turn, can limit root growth. Therefore, stress originating in either the root or shoot affects whole plant growth (Brown and Scott, 1984; Miller, 1986).

Studies relating the effects of the environment immediately after radicle protrusion can provide valuable insights into implications of root perturbation on early seedling growth and subsequent plant development. Understanding root architecture is important to improve transplant quality and production. Lack of methods to study root growth, which enable direct observation of the roots without their disruption, generally limits study on root architecture. A medium-transparent whole-plant method for studying early root growth is available and might be used for precise and accurate non-destructive plant measurements under controlled environmental conditions. It could be a valuable tool to investigate root morphology and development in different species, to better study, select and breed new varieties based on their root architecture potential. Although architectural changes of root systems caused by environmental conditions may occur without noticeable biomass changes in the whole seedling, they might affect the transplant stand establishment and subsequent yield of vegetable crops.

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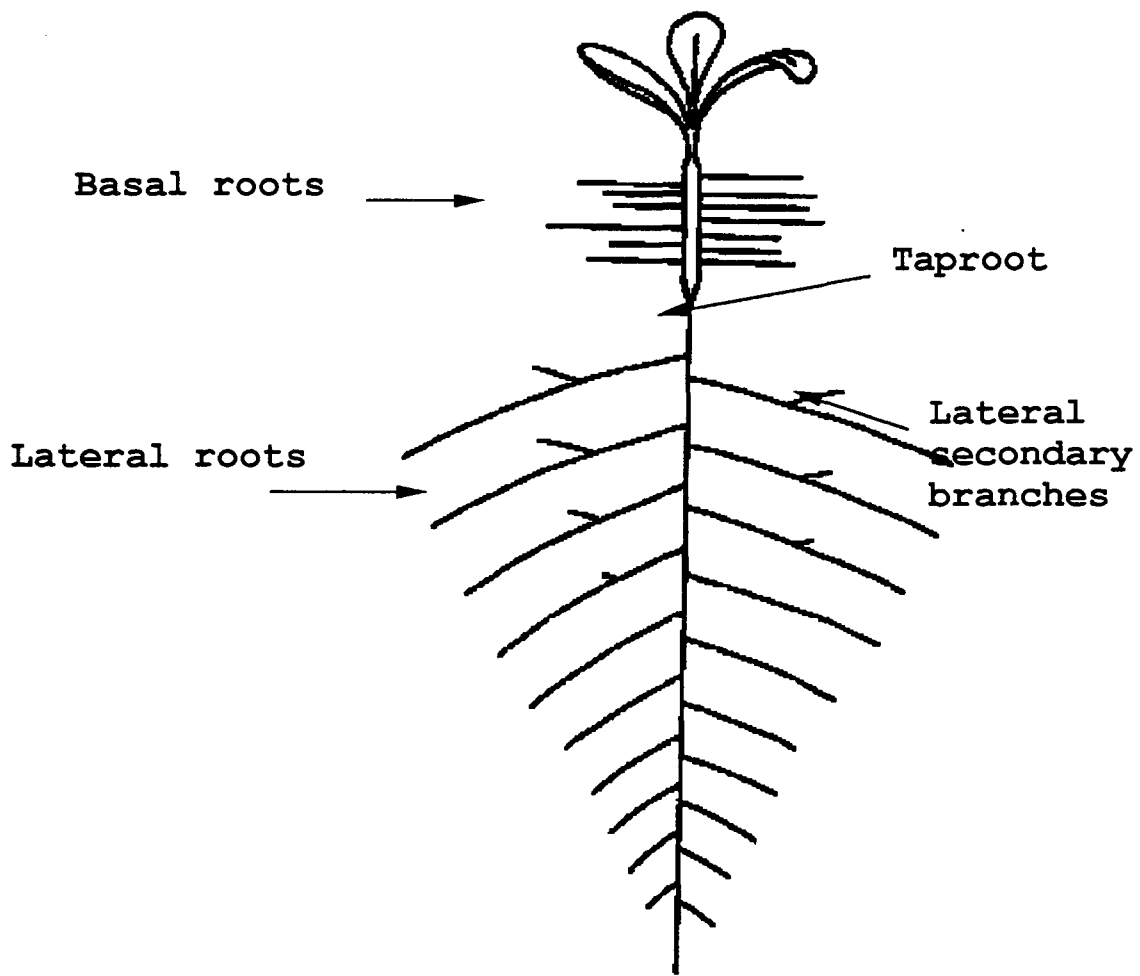


Figure 1. Diagram of lettuce seedlings 18 days after seeding. The different types of roots present in lettuce seedlings are shown: taproot, basal and lateral roots and lateral secondary branches.

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RELATIONSHIP BETWEEN SEED COMPANIES AND PUBLIC UNIVERSITIES IN THE CONDUCT OF RESEARCH ON STAND ESTABLISHMENT

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ABSTRACT

Agricultural trends causing change in the corn seed industry are reviewed. Among these are increase in farm size, earlier planting, adoption of conservation tillage, and better educated farmers who have more sophisticated information needs and requests. Steps taken by the authors' company to develop and assure new corn hybrids with excellent germination and ability to establish acceptable final stand are presented and illustrated with examples. The rapid pace of change in technology development and the greater sophistication of the customer represent opportunities for collaboration between industry and university scientists. Examples of researchable topics relevant to the theme of this conference are discussed.

INTRODUCTION

This paper will use corn (*Zea mays* L.) to provide examples of the important role of stand establishment in attainment of high crop yield as well as the opportunities for collaborative research efforts between public universities and the seed industry. Other crops could serve equally well, but the authors have drawn upon their work experience, which is the evaluation of corn inbred lines for acceptability as parent lines in production of hybrid corn seed.

The corn seed industry has undergone tremendous change in the past decade, and that trend is likely to continue into the 21st Century. Agricultural trends underway in the USA, and indeed in many other major corn-growing regions of the world, are leading to increased customer expectations of suppliers of crop seeds. Among these trends are: (1) increasing average farm size; (2) increasing equipment size, allowing farmers to plant more acres more quickly; (3) planting the majority of the corn crop earlier, weather permitting; (4) increasing use of conservation tillage, resulting in cooler soil temperatures at planting.

From 1989 to 1996, no-till corn acreage in the USA increased 2.5-fold to 13.2 million acres (Conservation Technology Information Center, 1996). Taken together, these factors mean that early planting happens on more acres, increasing the probability that any climatic event(s) or cultural practice(s) that cause problems of poor stand establishment will occur on more acreage than in the past. With more surface residue, plus the advent of new herbicides and herbicide tank mixes, today's seedbeds and the soil physical-chemical environments in which corn seed germinates are quite different than they were ten years ago.

Today's farmers are better educated with more sophisticated information needs and higher performance expectations. They are expressing interest in new technological advances such as precision farming, "on-the-go" adjustments in crop inputs, yield monitoring, and new genetics containing value-added traits. Farmers pay more for these new technologies and have greatly increased performance expectations. Most seed companies have entered the race to deliver the new technological advances available via biotechnology. Seed companies have expectations of recouping their investments by assigning premium prices to their newly developed products. It is only fair that our customers expect more value in the form of product performance, service, and information for the higher prices they are being charged.

In today's business environment, technology is changing rapidly. The pace of change in technology development and in adoption of new technologies has increased, and the overall competitive climate is changing. Governmental regulations that affect our business and intellectual property protection relative to our business also present a new "landscape". As mentioned above, customer needs and expectations are changing. No single organization can any longer afford to do everything (with respect to technology development) internally. Many industry scientists and managers recognize that in the rush to be "first to market" with new technology introduction, knowledge of new products is incomplete. These knowledge gaps, coupled with the greater sophistication and information requirement of our customers, represent opportunities for collaboration between industry and university scientists. Examples of researchable topics relevant to the theme of this conference will be discussed in the final section of the presentation.

DISCUSSION

Development of Corn Hybrids with Superior Stand Establishment.

Before a new corn hybrid is released as a commercial product, it undergoes extensive testing, during which numerous traits are evaluated, including the ability to establish acceptable stands under a variety of conditions. Though germination ability and consistency of stand establishment are critical traits, they are only two of many traits that each experimental hybrid must possess. Hybrids that "survive" a typical seed company's hybrid testing program have surpassed threshold levels of grain and/or silage yield, standability (stalk strength), ear retention, maturity, grain drydown, test weight, insect and disease tolerance, and drought tolerance.

At Pioneer we start the process of developing hybrids with good germinability in our inbred evaluation program (the "Corn Parent Test"). All newly developed inbreds are tested *per se* for characteristics that enable us to judge their suitability as female and/or male parents in hybrid seed production. Cold test germination and early stand count (stand establishment in inbred experiments) are two of the traits we consider when determining acceptability of new inbreds as female parents. If initial parent testing indicates that an inbred does not have acceptable cold test germination, the breeder will discontinue further testing of hybrids involving that inbred unless it can be used as a male. In that case, the other inbred parent would have to be an acceptable female. Some example data from the Pioneer Parent Test are shown in Table 1. Inbred no. 1 had unacceptable cold test germination in its initial parent test. Thereafter, it could only be considered a male parent. Note that it was also below average for early stand count. Inbred no. 2 would have been considered marginal, and only its above-average early stand count value would offer a slim chance that further testing might render it an acceptable female parent. Unless the hybrid(s) it was in was(were) phenomenal for yield, or superior for one or more defensive traits, it is unlikely that the breeder would invest more resources on further pre-commercial testing of hybrids in which this inbred had to be the female. In contrast, inbred no. 3 had excellent cold germination and stand establishment in its first year of testing. The lowest cold test value was 95%, which was 107% of the location average.

What is typical after inbreds have undergone extensive testing? Inbred no. 4 in Table 1 is the female of a hybrid that Pioneer has sold for many years. It is notable for the consistency of its cold test germination and stand establishment across years and environments.

Inbred no. 5 has been through three years of parent testing and appears to be solid with respect to cold test germination and early stand establishment. This inbred will be discussed in more detail in the next section. In summary, our experience is that cold test germination is highly heritable. Test results from the first one or two years of evaluation are usually quite predictive of the inbred's performance over time as a seed parent.

As each hybrid advances through the stages of pre-commercial testing, we monitor the female parent's average cold test percentage over a period of years. We also evaluate stability of cold test and early stand count values across locations and years. Only hybrids with consistently superior performance advance to the next year of testing. Figure 1 shows the average Parent Test cold test germination percentage over the period 1993-1996 for all female parents of the hybrids in a recent "class" of Pioneer pre-commercial corn hybrids. These hybrids were in first year pre-commercial stage in 1993, and would have had initial sales (limited volume) in 1995. All females had a cold test of $\geq 80\%$ in all years. In the R3 year, 77% of the females had average cold test of $\geq 85\%$, and the proportion of females that met this criterion increased to 90% in the R4 year and 96% by the R5 (commercialization) year. Females with cold test of $\geq 90\%$ increased from 33% in the R3 year to 56% in the R5 year. These data show the important role in our hybrid advancement process of good germinability and stand establishment under adverse cold spring planting conditions.

Impact of Sub-Optimal Stands During Hybrid Development

At Pioneer we also monitor stands established in hybrid yield trials, which allows us to evaluate consistency of seed quality on seed produced the previous summer or winter on the seed parent in question. A poor germinating seed lot of a pre-commercial corn hybrid can be quite detrimental to the hybrid advancement process. If stands are sub-optimal in hybrid yield trials, it is difficult to evaluate genetic advantage for grain yield independent of stand differences. Recently we learned that a particular seed lot of one pre-commercial hybrid that has Inbred No. 5 (from Table 1) as its female had

a cold test germination level that was below acceptability standards for use in research testing. This caused a round of consternation within the organization due to the prospect of not being able to yield test (small- and macro-plots) that hybrid in 1997. The only source of seed available in quantity sufficient for the requisite testing level was produced this past winter in South America. The data in Table 2 were collected for hybrids with Inbred No. 5 as the female parent in hybrid yield trials conducted in 1994-96. It seems clear from these data that across all 14 male parents, hybrids involving Inbred No. 5 had early stand counts and seedling vigor scores that were consistently at or above average. Likewise, final stands (data not shown) were all above average, and grain yield was at or above average for 13 of the 14 hybrids. The situation is somewhat perplexing, because this inbred has no previous history of problematic cold test or stand establishment (Figure 2). This example illustrates an area of investigation in which a seed company could likely benefit from collaboration with the public sector.

Since breeders want to maximize the rate of genetic gain, any delay in introduction of a new hybrid due to failure to establish adequate stands during yield testing is viewed as a serious set-back. At Pioneer, one area of focus in recent years has been to understand the field and dryer management practices required to produce research seed of excellent quality at our off-season nursery and production locations. Several of these nurseries are located in sub-tropical environments, and most rely on irrigation. We still have much to learn about the behavior of temperate corn inbreds during the grain-filling and seed maturation phases of growth in these environments, in relation to achievement of high seed quality. This is another area where industry could benefit from assistance from university scientists interested in genetic and environmental control of seed maturation.

Impact of Sub-Optimal Stands on Corn Yield

There can be both biological and mechanical reasons for poor stands in commercial corn fields. Although mechanical factors that affect planting accuracy, such as planter maintenance, planter calibration, accuracy of seed sizing, and planting speed, are important, they will not be addressed in this paper.

Carter and Nafziger (1989) summarized the impact of uneven emergence and uneven plant spacing on corn grain yield. Nielsen (1993) quantified the effects of uneven plant spacing; his data showed a yield reduction of 2.5 bushels/acre for every one-inch increase in standard deviation of plant-to-plant spacing. Both uneven emergence rate and uneven plant spacing likely have a biological component, related to genetics and/or seed quality.

Percent emergence obviously influences final plant population. Perfect stands rarely occur since warm germination percentages of commercial seed lots typically range from 95% to 98%. However, farmers want stands that come close to the kernel drop rate they planted minus the allowance for warm germination percentage. Exceptions may include situations where extremely early planting dates are practiced. Farmers are advised to increase their seeding rate by 15-20% if corn is planted in early April in the Central Corn Belt of the USA (Wickham, D.A. 1997. Walking your fields. Pioneer Hi-Bred International, Inc. Customer Newsletter. April 9).

There is a large body of literature describing the optimal final plant population needed for maximum grain yield of corn. Results from most recent studies indicate that highest corn yield is obtained with final populations between 26,000 and 30,000 plants per acre (Carter, 1996; Nafziger, 1994 and references therein). The optimum population is largely independent of yield level of the field or environment, with the exception of extremely low yield environments. The data shown in Figure 3 are typical. Duvick (1984) has shown that modern corn hybrids are more tolerant of higher plant population than older hybrids. A key trait that has changed through breeding effort is resistance to barrenness. Additionally, newer hybrids require higher plant population to fully express their genetic potential. Obviously, superior stand establishment is vital to consistent realization of maximum corn yield.

Obstacles to Industry-University Cooperation

The University and Industry Consortium recently surveyed its members on obstacles to research cooperation. The current situation is well summarized in the summary statement appearing in the consortium's most recent report (University & Industry Consortium, 1966 Fall Meeting Minutes, University of Nebraska, October 22-23, 1996):

"The high survey response rate indicates the interest in and criticality of this issue to both universities and businesses. There was agreement among virtually all respondents that the most significant and important

issues concern intellectual property, confidentiality, and timeliness of university projects."

"The lack of cooperation due to these issues has cost universities funding, as industry has needed to obtain research assistance from non-university organizations. Furthermore, these barriers have resulted in project delays, increased internal training costs for business and low morale among faculty members who have become frustrated with the limitations on the conduct of research that are caused by these barriers."

The data from the survey and ideas from discussion sessions at recent Consortium meetings are being used to develop solutions to overcome the obstacles to industry-university cooperation.

Researchable Areas for Potential Collaboration

Why is collaborative research in the field of stand establishment so minimal? Possible reasons include:

- Stand establishment is not a glamorous topic.
- Stand establishment is not considered biotechnology.
- Research in this field may not be considered basic enough to be publishable in prestigious journals.
- Funds for this kind of research are limited and/or unavailable within the university system.

What topics does the seed industry suggest as cost-beneficial and worthy of collaboration? The following areas are just a few that the authors would suggest:

- What environmental events cause failures in stand establishment?
- What biochemical markers could be used to predict problems with stand establishment in a seed lot? Can rapid assay methods be developed for detection of seed lots that may give unacceptable seedling emergence?
- Are the genetic parameters that define superior stand establishment identified?
- What molecular markers are associated with superior stand establishment?
- Can superior stand establishment traits be transferred across species?
- Is the baseline temperature (10 C) below which corn seed germination is said not to occur still valid for today's genetics?
- Are the threshold temperatures of 10 and 30 C (50 and 86 F) that are in current widespread use to calculate the heat units governing corn growth and development valid for modern genetics, across corn relative maturities and all corn-growing regions?
- What are the mechanisms responsible for a seed parent's sensitivity to physiological injury during ear corn drying? What are the genetic controls operative during kernel development and maturation that predispose a seed parent to injury during drying? Are there biochemical or molecular markers that could be used to detect injury after it has occurred in a seed lot, or to facilitate a breeding approach to select seed parents more tolerant of prevalent drying temperatures?
- When were current cold test germination test standards set, and are the temperatures used still relevant to soil temperature at planting time in today's agricultural practices?
- What are the genetic and environmental factors that cause split pericarps ("popped kernels") in developing kernels of corn during late grain-fill? What are the cause-and-effect relationships between split pericarps, fungal disease development, and germinability of affected and adjacent seeds?

Favorable Outcomes of Greater Collaboration

What do industry scientists want from a collaborative research effort? We believe they want: (1) involvement in the creative process, from experimental conceptualization and design to summarization and interpretation of results; (2) results obtained in a timely manner; (3) ready access to the information generated; (4) publication (although not usually a requirement for promotion, publications help an industrial scientist become recognized by peers for scientific contributions).

Enhanced collaboration between universities and the seed industry would also achieve the following desirable outcomes:

- Training of graduate students, who represent the pool of future plant scientists and seed analysts to fill both public and private positions.
- Basis for continuing collaboration on other topics after the first collaborative project is completed.
- Improved communication between public and private sectors, and hence improved understanding of each others' needs, perspectives, strengths, and potential for future contributions.
- Better understanding of the biology underlying germination, seed quality, seedling vigor, and the edaphic, climatic, biological, and cultural practice factors that influence stand establishment.
- Ultimately --- better products that help our customer, the farmer, and help feed the growing world population.

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Table 1. Data from Pioneer Parent Test for Cold Test Germination and Early Stand

INBRED	YEARS TESTED	STATISTIC	CLD	CLD	EST
			TST ABS	TST %MN	CNT %MN
1	1	Average	65	77	93
		Reps	4	4	13
		Locs	4	4	13
		High	90	102	112
		Low	35	42	65
2	1	Average	80	95	109
		Reps	4	4	17
		Locs	4	4	17
		High	86	105	135
		Low	72	82	93
3	1	Average	97	114	108
		Reps	4	4	18
		Locs	4	4	13
		High	98	119	131
		Low	95	107	86
4	10	Average	92	104	97
		Reps	191	191	999
		Locs	110	110	478
		High	98	121	136
		Low	76	87	31
5	3	Average	93	107	108
		Reps	20	20	120
		Locs	20	20	89
		High	98	121	130
		Low	79	96	57

CLDTST = Cold test germination.
 ESTCNT = Early stand count.
 ABS = Absolute percentage value.
 %MN = Percent of experiment mean.

Table 2. Stand Establishment (ESTCNT), Seedling Vigor (SDGVGR), and Grain Yield (BU ACR) for 14 Hybrids with a Common Female Parent

Female Inbred	Male Inbred	# REPS	# YEARS	ESTCNT %MN	SDGVGR %MN	BU ACR %MN
No. 5	M - 1	108	2	105	117	106
No. 5	M - 2	120	3	101	108	100
No. 5	M - 3	90	2	102	99	101
No. 5	M - 4	75	2	102	99	104
No. 5	M - 5	125	2	104	126	102
No. 5	M - 6	132	2	102	106	100
No. 5	M - 7	83	2	103	118	103
No. 5	M - 8	82	2	103	121	101
No. 5	M - 9	144	2	104	115	105
No. 5	M - 10	43	2	102	104	102
No. 5	M - 11	283	3	98	104	102
No. 5	M - 12	55	2	101	106	102
No. 5	M - 13	108	2	104	101	95
No. 5	M - 14	61	2	103	110	104

%MN = Percent of experiment mean.

Figure 1. Cold Test of Females of 1993 R3 Hybrids over Years

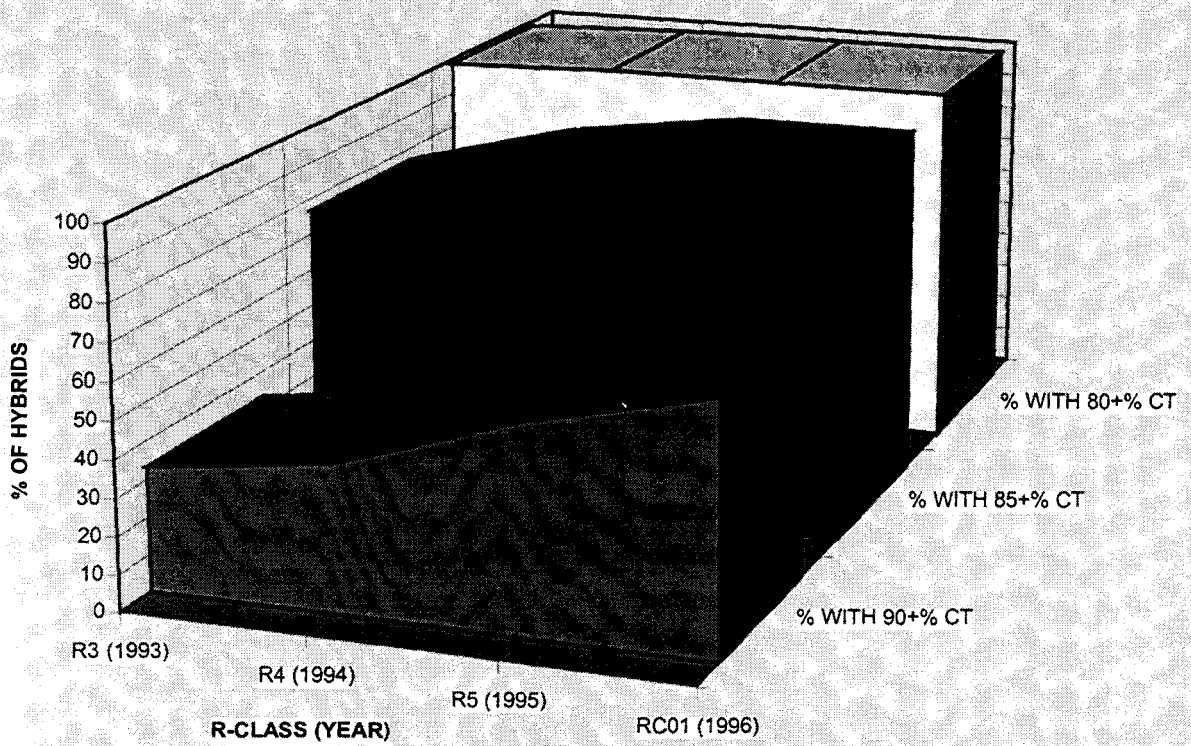


Figure 2. Cold Test (%MN) for Inbred No. 5

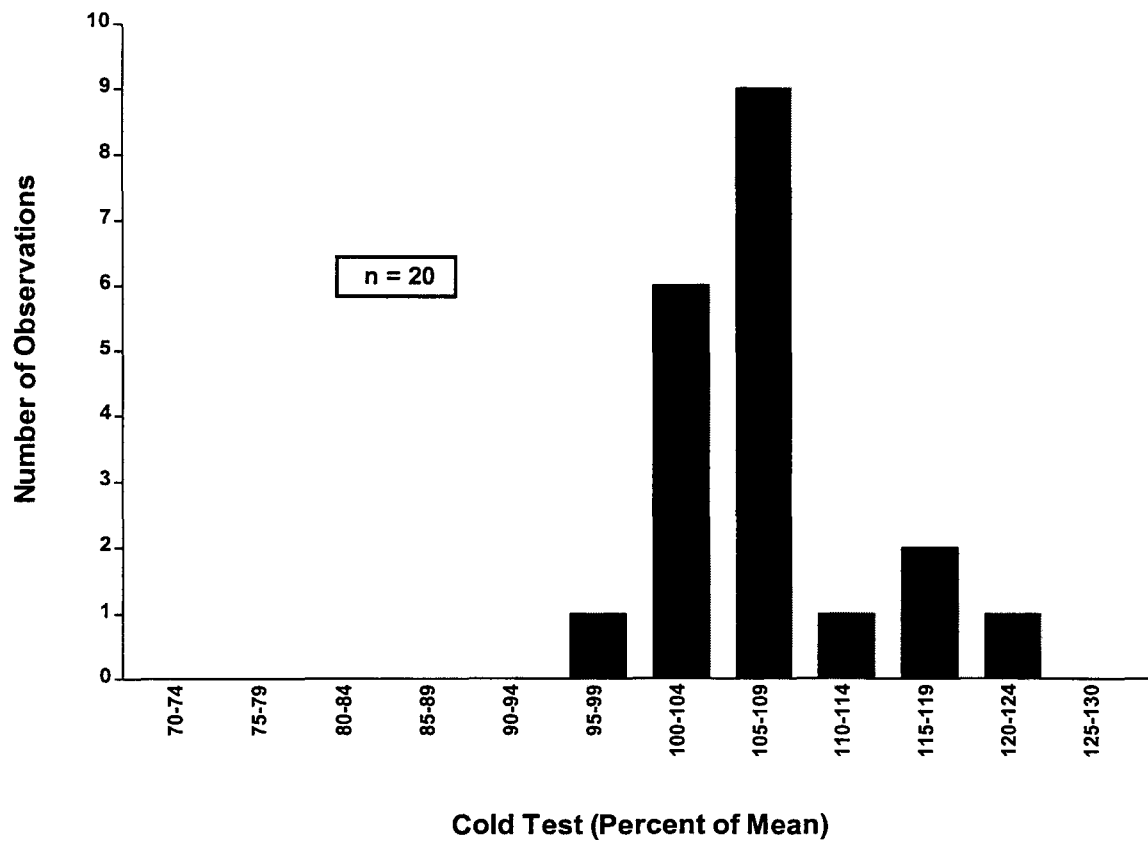
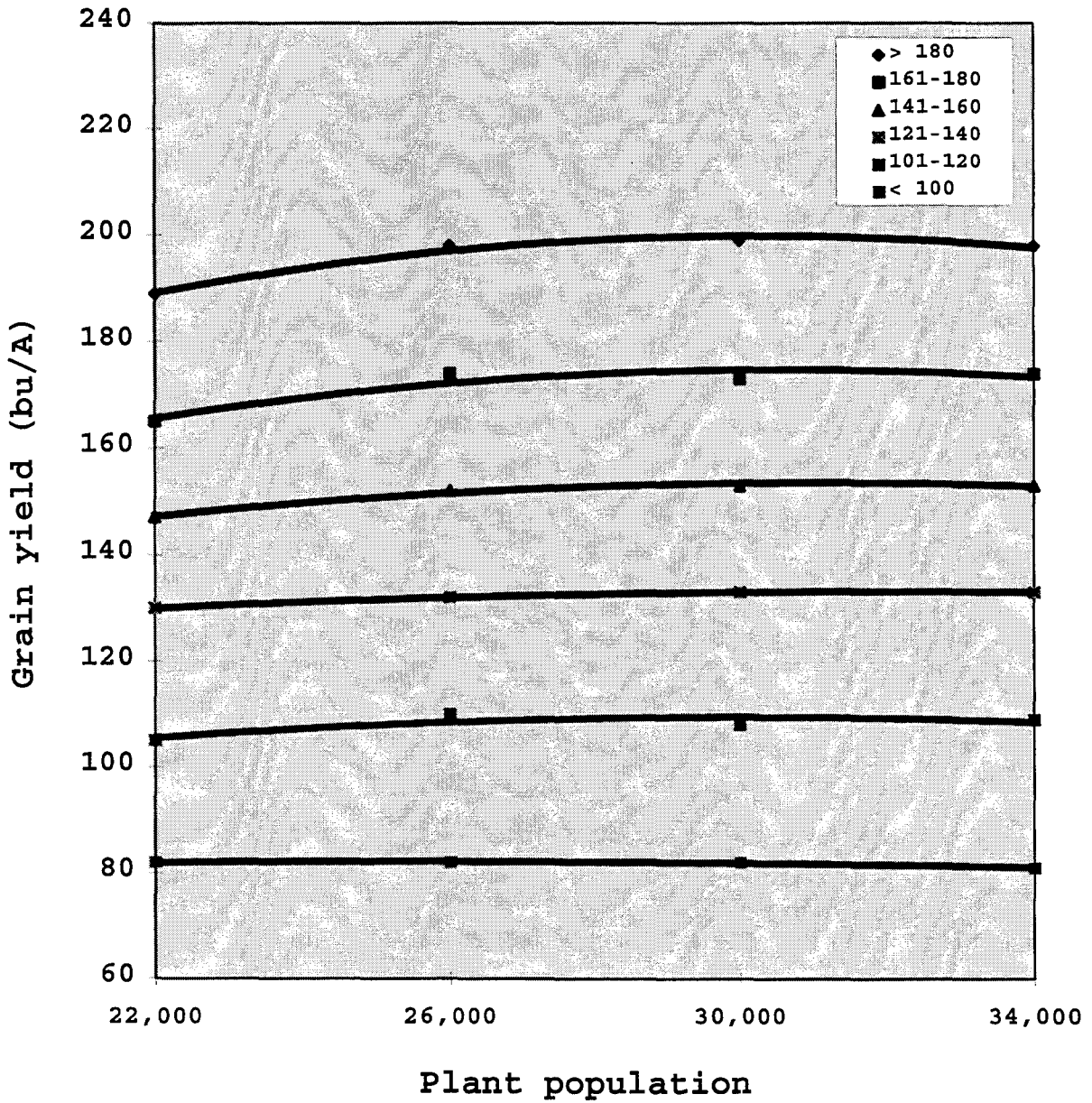


Figure 3. Influence of Corn Population on Yield in Different Yield Environments (from Carter, 1996)



THE ROLE OF THE NATIONAL PLANT GERMPLASM SYSTEM IN STAND ESTABLISHMENT

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ABSTRACT

The United States operates an efficient and effective program of introduction, evaluation, utilization, and preservation of genetic resources which is accomplished through the various research units comprising the National Plant Germplasm System (NPGS). NPGS is managed by the Department of Agriculture's Agricultural Research Service (USDA-ARS). Active participants include federal, state, and private-industry scientists working cooperatively to ensure that we have an adequate and safe food supply for future generations. Since 1898, more than 595,000 seed and plant accessions have received PI numbers. Of these, about 450,000 accessions, representing over 8250 species, are available from the NPGS. The role of the NPGS in stand establishment is to provide the genetic diversity and genes useful for overcoming such environmental problems as high/low temperatures, flooding/drought, soil salinity, microorganisms, etc. to achieve rapid, uniform and complete germination.

THE NATIONAL PLANT GEMPLASM SYSTEM

The United States has one of the most advanced programs for preservation of genetic resources, the National Plant Germplasm System (NPGS). The United States is described as a "germplasm-poor" country (for primary food crops); most of the crops we utilize in our vast agricultural industry have been introduced from other countries. This has necessitated a strong program of introduction, evaluation, utilization, and preservation of genetic resources. Management of the NPGS is through the U.S. Department of Agriculture's Agricultural Research Service (USDA-ARS). Active participants include federal, state, and private-industry scientists working cooperatively to ensure that we have an adequate and safe food supply for future generations. The reader is referred to volume 7 of Plant Breeding Reviews, which contains a thorough review of all aspects of NPGS (Janick 1989).

The NPGS evolved over a period of about 100 years, beginning with the establishment of the Section of Seed and Plant Introduction in 1898 as part of the USDA (Hyland, 1977, 1984; White et al., 1989). This office began the formal collection and introduction of seeds and plants beginning with Plant Introduction (PI) number 1, a cabbage (*Brassica oleracea*, unverified name 'Bronka') originally collected near Moscow, Russia and donated in February, 1898 by N. Hanson of the Agricultural College of South Dakota. Unfortunately, this PI was lost long ago and is not available in the NPGS. Hyland (1984) estimated that of the first 160,000 PI accessions, only 5 to 10% survive today. It was to guard against such losses that the National Research Council identified the need to preserve this germplasm. The resulting legislation, the Research and Marketing Act of 1946, established the four Regional Plant Introduction Stations and the National Potato Introduction Station (White et al., 1989).

Many of the accessions that found their way into the NPGS were the germplasm and genetic stock collections of individual public and private plant breeders, geneticists and taxonomists, which had been collected from all over the world during the careers of these scientists. As these individuals retired or left the field, these valuable collections of a lifetime were either passed on to their successors in a particular institution, or they lay dormant until discarded or until all viability was lost (in general, facilities and knowledge about long-term storage at most institutions were lacking). The establishment of the Regional Stations gave this material a stable and safe home, until the National Seed Storage Laboratory was established in 1958 to provide long-term storage for these genetic resources.

MISSION. NPGS is a cooperative effort by public (State and Federal) and private organizations to preserve the genetic diversity of plants. The world's food supply is based on intensive agriculture, which relies on genetic uniformity. But this uniformity increases crop vulnerability to pests and stresses. Scientists must have access to genetic diversity to help bring forth new varieties that can resist pests, diseases, and environmental stresses. The NPGS aids the scientists and the need for genetic diversity by: acquiring, preserving, evaluating, documenting, and distributing crop germplasm. Through these efforts, NPGS assists in improving the quality and productivity of crops (excerpted from the NPGS web site at

<http://www.ars-grin.gov/npgs/>.

NPGS SEED AND CLONAL REPOSITORIES. In order to carry out the mission of the NPGS, the U.S. Department of Agriculture, in cooperation with agricultural experiment stations of several State universities, has established research and preservation centers at numerous locations around the United States (Table 1). For seed propagated plants, the majority of the germplasm is held at one of five stations which are: the four Regional Plant Introduction Stations located at Ames, Iowa; Geneva, New York; Griffin, Georgia; and Pullman, Washington; and the National Small Grains Collection located at Aberdeen, Idaho. Genetic stock collections of selected species are held at various Genetic Stock Centers, primarily located on university campuses. Clonally propagated species of fruit and nut crops are held at the National Clonal Germplasm Repositories (Table 1). Each location has specific crops assigned as priority species; however, some species may be assigned to more than one location. Funding for all of the locations is provided through the USDA-ARS and from various State Agricultural Experiment Stations. Private industry has provided additional financial support in the form of special grants and has assisted in the regeneration of seed accessions.

OBTAINING GERmplasm. Access to germplasm is now a major international issue, since the adoption of the Convention on Biological Diversity. In the past, germplasm (for agriculture) was freely available and exchanged among breeders and countries. Now the potential for huge royalties (mostly an unrealistic expectation) on germplasm has led to the placing of restraints on international exchange. In the United States, however, the policy of free exchange is still in effect and bona fide users (breeders, taxonomists, molecular biologists, etc.) can readily obtain access to germplasm within the NPGS. Accessing the Germplasm Resources Information Network (GRIN) via computer telephone links and/or the internet, will provide information on availability of materials. Placing of orders can also be accomplished on-line (see above web site for NPGS).

THE ROLE OF NPGS IN STAND ESTABLISHMENT

The primary role of the NPGS with regard to stand establishment is through the use of the genetic diversity, present within the various collections, to provide genes for overcoming genetic deficiencies or the many problems encountered in the field such as high/low temperature, flooding, drought, salinity, etc. The following examples, taken from the recent literature, illustrate how use of genetic diversity within a species has helped to overcome these deficiencies and problems.

TEMPERATURE STRESS. Many kinds of seeds experience reduced germination when imbibed at temperatures below optimum. Bean seeds (*Phaseolus vulgaris*) are particularly sensitive to chilling injury when the seed moisture content is low (10% or less) (Pollock and Toole, 1966; Pollock et al., 1969; Roos et al. 1976). In other crops, similar temperature sensitivities have been identified; for example in cotton (Christiansen, 1963); maize (Cal and Obendorf, 1972), soybean (Obendorf and Hobbs, 1970; Bramlage et al., 1978, 1979; Orr et al., 1983), and sorghum (Phillips and Youngman, 1971). Beans are an example of how breeders have addressed this problem. Superior cultivars were identified for tolerance to low temperature (Dickson, 1971; Dickson and Boettger, 1984; Zaiter et al., 1994) and genetic studies have been done on the inheritance of genes linked to this cold tolerance (Otubo et al., 1996).

For high soil temperatures the classical example of sensitivity is lettuce (*Lactuca sativa*). Many studies have reported the beneficial effects of seed treatments to overcome this 'thermodormancy' including priming (Guedes and Cantliffe, 1980; Weges et al., 1991; Bradford and Somasco, 1994), use of hormones (Braun and Khan, 1976; Gray and Steckel, 1977; Saini et al., 1986; Huang and Kahn, 1992) and imbibition in osmotic solutions (Small and Gutterman, 1992). Although breeders and geneticists may not have identified specific genes to overcome thermodormancy, cultivar differences do exist (Gray, 1975) and specific metabolic pathways involved in dormancy have been identified (Kahn and Prusinski, 1989). Future progress in breeding is expected as we learn more about pathways, enzymes and the proteins involved in controlling physiological responses to temperature.

WATER STRESS. Seeds can experience either too much water (flooding) or too little water (wetting/drying) during the germination stage, resulting in poor or erratic stands. Subjecting seeds to an oversupply of water (submergence) is well known in seed laboratories to result in poor germination. Oxygen deprivation is usually thought to be the explanation as germination is generally considered to be an aerobic process. In the soil, flooding is not uncommon in spring plantings and is of concern to farmers. Maize is one crop that is readily damaged by flooding (Fausey and McDonald, 1985). Martin et al. (1991) examined the physiological basis for inhibition of maize seed germination by flooding and concluded that an uncharacterized, extremely volatile inhibitor (carbon dioxide?) accumulated during seed soaking. The fact that maize

cultivars differing in their susceptibility to flooding damage (VanTaoi et al., 1985) exist, as is true with rice (Adkins et al., 1990; Setter et al., 1994) and soybeans (Hou and Thseng, 1991); and that genes associated with flooding tolerance have been identified (Sachs et al., 1996) shows the value of germplasm as sources for these genes. However, it is necessary to understand the mechanism of damage in order to exploit genetic diversity in improving crop species tolerance to flooding (Crawford and Braendle, 1996).

Agricultural seeds that experience wetting and drying in the field usually result in lower stands. Native or wild species often overcome these difficulties through wide adaptation amongst ecotypes including different dormancy patterns. However, domesticated species can be screened for adaptation to dry sowing. Maiti and Moreno (1995) reported significant differences among sorghum genotypes for tolerance to wetting and drying. The genotypes resistant to imbibition injury and drying had a specific protein which was absent from susceptible lines.

SALINITY STRESS. In many areas where irrigation is needed, salinity stress can greatly reduce stand establishment and plant growth (Epstein et al., 1980). Flowers et al. (1986) estimated that 30 to 40% of the world's irrigated acreage is affected by salinity. There has been some success in breeding for salt tolerance in some crops. Foolad (1996) found excellent response to selection in a tomato cross between PI174263 (salt-tolerant) and salt-sensitive cultivar UCT5. Similar or identical genes having additive genetic effects were indicated. The results showed that selection can be based on individual seed performance and early segregating generations. Other crops where genetic variability for salt tolerance has been identified through screening cultivars or germplasm accessions include: alfalfa (Allen et al., 1985; Al-Niemi et al., 1992), crested wheatgrass (Johnson, 1990), tall fescue (Horst and Beadle, 1984), triticale (Norlyn and Epstein, 1984), and wheat (Kingsbury and Epstein, 1984).

MECHANICAL DAMAGE. Some crops, e.g. *Phaseolus vulgaris*, have poor stands because of their susceptibility to mechanical damage. In the case of snap beans this susceptibility varies among cultivars and has been linked with the white seed coat color (Atkin, 1958). In a study of mechanical damage resistance, Bay et al. (1995b), found that protection of the embryonic axis by the cotyledons was key to reducing the amount of damage. Another factor influencing damage was the lignin content in the seed coats (Bay et al., 1995a). Thus selection for lesser exposure of the embryo axis and/or greater lignin content in the seed coat would result in greater resistance to mechanical damage.

OTHER SEED COAT PROBLEMS. Seed coat color has also been associated with rate of water uptake in peanuts (Singh, et al., 1992) and the colonization of seeds with *Aspergillus* fungi. Peanuts with colored testa showed greater resistance to seed invasion (Carter, 1973). Pigmented seed coats in chickpea and cowpea were found to have slower rates of imbibition than unpigmented types, and thus experienced less imbibition damage (Legesse and Powell, 1996). In soybean, hard seeded lines were found to be more resistant to soaking injury (Ragus, 1987). Similarly, seed coat permeability in snap beans can influence the response to imbibitional chilling injury (Taylor and Dickson, 1987).

CONCLUSIONS

While I have not been able to cover all aspects of stand establishment (for example the interaction with microorganisms), it is very obvious that improvements in seed germination and stand establishment can be made through the efforts of plant breeders. It is also quite evident that physiologists, molecular biologists and seed technologists must play an important role in identifying genetic traits, metabolic pathways and the interactions with cultural practices to achieve this objective. The National Plant Germplasm System stands ready to provide the genetic material for all scientists to utilize in their efforts.

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Table 1. Location of and brief list of crops at germplasm repositories in the National Plant Germplasm System.

REGIONAL PLANT INTRODUCTION STATIONS

Ames, IA - amaranth, artichoke (Jerusalem), asparagus, beet, bentgrass, buckwheat, cantaloupe, carrot, chicory, collard, coriander, corn, crambe, cucumber, dill, dogwood, endive, gourd, honeydew melon, horseradish, kale, kohlrabi, muskmelon, mustard, ornamentals, parsley, parsnips, pawpaw, pumpkin, rutabaga, spinach, squash, sugarbeet, sunflower, sweetclover, turnip, zucchini

Geneva, NY - artichoke, birdsfoot trefoil, broccoli, brussels-sprouts, cabbage, cauliflower, celery, Chinese cabbage, grasses, ornamentals, legumes (forage), onion, pumpkin, radish, shallot, squash, tomato

Griffin, GA - Bermuda grass, blackeyed pea, castor bean, clover, eggplant, forage legumes, gourds, grasses, guar, kenaf, lespezeza, luffa, mungbean, okra, peanut, pearl millet, Pennisetum, pepper, pigeonpea, pumpkin, Serradella, sesame, sorghum, squash, sweet potato, vetch, water chestnut, watermelon, wingbean, zoysia grass

Pullman, WA - alfalfa, beans, bluegrass, bromegrass, canarygrass, chickpea, chive, clover, fescue, garlic, leek, lentil, lettuce, Lupine, milkvetch, onion, orchardgrass, pak choi, pea and pea genetic stocks, ryegrass, safflower, sainfoin, teff, vetch, wheatgrass, wildrye

NATIONAL SMALL GRAINS COLLECTION

Aberdeen, ID - Aegilops, barley and barley genetic stocks, oats, rice, rye, Triticale, wheat

NATIONAL CLONAL GERMPASM REPOSITORIES

Brownwood, TX - chestnut, hickory, pecan

Corvallis, OR - blackberry, blueberry, boysenberry, cranberry, currant, filbert, gooseberry, hazelnut, hops, ornamentals, mint, pear, raspberry, strawberry

Davis, CA - almond, apricot, cherry, fig, grape, kiwifruit, nectarine, olive, peach, persimmon, pistachio, plum, plumcot, pomegranate, tomato genetic stocks, walnut

Geneva, NY - apple, grape

Hilo, HI - carambola, guava, lychee, macadamia, papaya, passionfruit, pineapple, rambutan, tropical plants

Mayaguez, PR - bamboo, banana, Brazilnut, cashew, cassava, cocoa, plantain, sweetpotato, tanager, tropical plants, yam

Miami, FL - avocado, cassava, mango, ornamentals, passion fruit, sugarcane, Tripsacum, tropical plants (note: materials are to be moved to Mayaguez and/or Hilo)

Riverside, CA - date, grapefruit, lemon, lime, orange, tangerine

Sturgeon Bay, WI - potato

Washington, DC (National Arboretum) - dogwood, holly, ornamental plants, magnolia, maple, oak, rhododendron

GENETIC STOCK AND SPECIAL COLLECTIONS

Brookings, SD - native grasses

College Station, TX - cotton, cotton and sorghum genetic stocks

Columbia, MO - wheat genetic stocks

Fargo, ND - flax, wheat (durum) genetic stocks

Lexington, KY - clover

Logan, UT - grasses (forage, range)

Oxford, NC - tobacco

Raleigh, NC - gamagrass (note: to be moved to Mayaguez)

Salinas, CA - endive, lettuce

Tifton, GA - grasses (wild), pearl millet

Urbana, IL - maize genetic stocks, soybean, soybean genetic stocks

NATIONAL SEED STORAGE LABORATORY

Fort Collins, CO - base collection of all seed crops, and apple buds, genetic stocks (barley, tomato, and wheat)

CONCERNS AND GOALS IN STAND ESTABLISHMENT IN EUROPE

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Additional index words: Sugar beet, maize, cold test, precision sowing, seed quality, imidocloprid, vegetable transplants, carrots, onions, priming.

ABSTRACT

The focus in this brief review is on those crops in the European Union like sugarbeet and maize in which stand precision is economically important. In sugar beet, seed is of excellent quality and mostly achieves a 90% emergence but the variable and increasingly dry conditions at the time of crop establishment is cause for concern. The northerly spread of silage maize can lead to seeds facing harsh (below 10°C and wet) conditions to the point where some lots do not reach the intended 90% emergence. A cold test as a means of discerning differences in emergence potential at the top end (75-100%) in such conditions is widely used but is of uncertain value. However, cold tests are being used to select high vigour lots for seed treatment with the phytotoxic but extremely effective insecticide imidocloprid. This is a specific example of a general area of concern, the interaction of chemical seed treatments with seed quality. In vegetables established by transplants (*Brassica*, lettuce, leeks, celery) highly selected and vigorous seed ensures the required standards for complete and uniform seedlings in modular trays but at a price to growers who have great expectations. Uniformly rapid emergence to a high and predictable level is the goal in stand establishment in directly sown vegetable crops (carrots, onions, parsnips, leeks). Priming (osmotic or drum) is used along with improved control of soil moisture by effectively timed irrigation. The aim in any one crop is to minimise the spread of emergence over time and thereby reduce variation in seedling and ultimately product size so that growers can meet the marketing requirements of size and continuity with minimal discard.

Concerns and goals in Europe are the occupation of many, mostly in politics and economics. Any views on such topics are thoroughly disputed and differ sharply according to the standpoint and interests of the commentator. The task of discussing stand establishment in Europe is much less controversial although there are different points of view. Most of what is written refers to Western Europe, which is now almost entirely made up of the 15 countries (Table 1) of the European Union (E.U.). *Arable cropping in the E.U.* Before embarking upon a resumé of crop establishment an outline of the crops of the E.U. will set the scene. France is the premier cropping country of the E.U. with 25% of the arable area on which are grown crops typical of both Northern Europe such as rye and oil seed rape and Southern Europe, sunflower and grain maize (Table 1). This spread of crops is indicative of the climatic range to be found in the country with the largest landmass in the E.U. The top five countries as far as crops are concerned (France, Spain, Germany, Italy and the UK) together account for 87% of the total arable area of which 57% is sown with the 9 crops in Table 1. However, at 76M hectares the total arable area in the 15 countries of the E.U. is only 41% of that of the USA (Table 1) and 58% of that of Russia. *Agronomic significance of plant establishment.* For the purpose of this review it is appropriate to group crops according to the agronomic significance of plant establishment. There are the precision sown wide-spaced field crops (sugar beet, maize and sunflower) and the close-spaced crops (small grain cereals, oilseed rape and pulses). In vegetables grown outside, two groups can be distinguished: crops established as transplants such as *Brassica* and those established mostly by direct sowing, for which some (e.g. carrots) spacing is crucial, for others (peas and beans) precision in plant establishment matters less. Both groups of vegetables are well represented in the E.U. particularly cauliflower (44k Ha) and green peas (31k Ha) in France, onions (28k Ha) and green beans (25k Ha) in Spain, green peas (45k Ha), cabbage (25k Ha) and carrots (17k Ha) in the UK and most field vegetables in Italy.

There are two discernible themes in relation to the agronomic significance of plant establishment for the above groupings. First, the concerns and goals associated with the need for precision and reliability in establishment in

some crops and, second, the added demands that arise with the extension of crops into new areas and sowing times. There are, quite rightly, high aspirations for the achievement of goals in crop establishment but what is technically feasible has to be tempered by cost considerations for the seed suppliers, growers and consumers.

In addition, two apparently countervailing trends in the use of chemical seed treatments can be identified both of which would claim to be aimed at addressing environmental concerns. The first is the introduction of new, sometimes multiple, chemical treatments of seeds which reduce the need for later field applications, most recently seen in the successful introduction of the insecticide imidacloprid as a seed treatment for sugarbeet (9). The second is the search for ways to eliminate the use of chemicals on seeds by testing for damaging levels of seed-borne fungi as practised on wheat and barley in Scandinavia (4) or, more ambitiously, by the use of biological seed treatments (18). Both the increase and the elimination of the use of chemical seed treatments can influence crop establishment.

These general remarks would not be complete without a reminder of the intensive approach to crop production in many parts of Europe. High inputs are used and high yields are achieved and for many crops, not for the most part vegetables, there is the financial support of the Common Agricultural Policy which is certainly the case for sugar beet whose production is both costly and controlled.

Plant establishment in sugar beet. In the E.U. sugar production is directed by a relatively few processors. In the U.K., the extreme example, there is only one company, British Sugar, which influences all aspects of production starting with the seed, both variety and quality. Genetically monogerm seed (using the term seed in the agronomic sense which is the case throughout this review) enabling drilling to a stand, came into general use in the UK and many parts of Europe in the 1970s. The challenge then was to ensure that a stand of at least 70,000 plants per hectare was reliably achieved. Such populations especially when sown early (mid to late March in South East England) can develop a full crop canopy for near complete light interception by early summer (2). Since then research and development taken through to practise has led to reliable methods of achieving populations, certainly in England, of over 90,000 plants per hectare

(2), a key factor being improvements in seed quality.

The minimum laboratory germination required by British Sugar for supply to growers was increased from 80 to 85% in 1981, and then to 90% in 1987. In the 1990s all seed supplied to English growers averaged 95% germination after pelleting and chemical treatment. These improvements, which were seen throughout Europe, were achieved through limiting the selection of seed production areas to S.W. France and Northern Italy, close control of production methods and intensive grading based on frequent sampling for tests of germination and emergence potential during seed processing. There may also have been a genetic component to overall improvement in emergence since the cultivars being grown in England in the 1990's have been largely diploids which consistently emerge to a higher level than triploids. In the National Institute of Agricultural Botany (Cambridge) trials the average emergence of the diploid varieties recommended for 1997 was 81% (range 77 to 83%) whereas for the triploids it was 77% (range 75 to 79%) (16).

All seed in Europe is pelleted, chemically treated and precision drilled. In the US the term "European System" is sometimes used to describe this method of establishing a sugarbeet crop with a target population of around 90,000 plants per hectare with between row spacings of 45 or 50 cm and between plant spacings in the row of 18 to 20 cm (2). Present concerns about establishment are about reliability in the face of variable spring temperatures and rainfall. Both too much moisture leading to unworkable soils and too little can delay establishment into April. The anxiety in S.E. England these days is more about lack of moisture both at establishment, and later in the season when irrigation is required of what is a diminishing resource in England, water. Improved cultivation techniques that retain moisture can reduce the adverse effects of dry springs on establishment as was the case this year.

The two fungicides incorporated into seed pellets in Europe are thiram, also used as a seed soak against *Phoma betae* in England, and hymexazol. Both fungicides control soilborne seedling disease caused by *Pythium* spp and hymexazol also controls *Aphanomyces colloides* (1). The milestone chemical seed treatment of the 1990's has been, without doubt, the insecticide imidacloprid. Now used on sugarbeet throughout Europe applied to the outside of the pellet to control both insects that can reduce establishment and the virus yellows transmitting aphid *Myzus*

persicae. The chemical is systemic and can provide protection for at least 10 weeks after drilling (10) and at 90g active ingredient per Unit (100,000 pelleted seed for sowing on 1 Ha) The seed treatment is much more environmentally acceptable than applications of eight times more active ingredient of a granular insecticide later in the season. There have been reports of slower emergence of treated seed but no reduction in final counts (10). Any phytotoxic effects may be minimised by application to the outside of the pellet.

Advancing seeds by a limited hydration treatment lasting up to 4 days followed by drying back has been shown to reduce the time between sowing and emergence (by 9 days in some early March sowings), and to increase the percentage ground cover earlier in the season and, consequently in some instances, sugar yield (11). Although the treatment has only come into commercial use to a limited extent the fact that such a prolonged and technically demanding treatment was contemplated on a large scale crop is indicative of both its profitability and the willingness of the industry to innovate and incur cost. At present prices seed costs are around \$160 per hectare and gaining returns on such an investment largely depends on maintaining the sheltered economic environment afforded by the Common Agricultural Policy.

Grain and Forage Maize. The expansion of maize growing in Europe is a triumph of the genetic potential of the species and the endeavours of plant breeders. From an area in 1960 of 3M hectares of grain maize the countries of the E.U. now grow 4 M hectares. Even more impressive is the rapid recent expansion of forage maize into more North and Westerly parts of Europe. There are now available fast maturing cultivars capable of reaching milky ripe cob stage and a whole crop dry matter of around 30% more than halfway up England and even in S.W. Scotland. The result has been an increase in the English forage maize crop by more than 50% in the last five years to over 100,000 hectares and in France as much forage maize is grown as grain maize at 1.6M hectares.

As is often the case for crops extended to new more marginal areas establishment can be problematic, certainly the challenge to seed quality is not diminished. The advice to growers in England and N.W. France is not to sow until the soil temperature has reached 9-10°C for several days which can be at the end of April and into May. However, growers are tempted to sow earlier, when soil temperatures are erratic, in an attempt to maximise dry matter production. The result is a challenge to seed quality and the seed supplier.

The precision sowing of both maize for grain and silage makes establishment a crucial stage. The target populations range from 75,000-90,000 plants per Ha for grain maize to 100,000-110,000 per Ha for forage maize and sowing rates are adjusted for an estimated establishment of 90% (21). Warm laboratory germinations are expected to be over 95% and all seeds are put through some version of a cold test. In work on official variety trials in Belgium little correlation between the routine severe cold test and field emergence was seen in three years but in 1991 when temperatures were low between sowing and emergence (7.2°C daily average at 5 cm depth) and the time between sowing and emergence was as long as 29 days cold test germination was significantly correlated with field emergence. The conclusion reached was that to attain 90% field emergence a cold test germination of at least 95% normal seedlings was needed (21). In all years a proportion of lots, and in the case of 1991 the majority, failed to achieve 90% field emergence. Since these were lots submitted by companies for official trialling there is the possibility of under-achievement of target populations in commercial fields where a 90% establishment is assumed. Furthermore, although some of the poorer emerging lots were picked out by the cold test many were not. There has always been anxiety about the repeatability of cold tests, especially when soil was used, and the value of the test to predict field emergence might also be a cause for concern especially for seeds sown in marginal growing areas.

One such area is the North and Midlands of England where the need for a high level of emergence is increased by the lowering of population densities to produce an earlier harvest. The adverse soil conditions the germinating seed meets in the field are not just cold but also often wet and although the term cold test is used, most versions of the test involve high moisture in the substrate, with the routine test used in the Netherlands being particularly severe (5). Even for grain maize production wet conditions after sowing can cause emergence problems as was the case in South West France in May of 1996.

Tests of emergence potential only allow the seed supplier to reject, or market judiciously, the questionable lots. The goal is always to produce consistently only high quality seed. Most of the seed production for Europe is done in Southern France with some out- of-season S. Hemisphere production in, for example, Chile. Heated drying down to 12-14% of the cob before shelling is the norm. Seed quality is thought by producers to be most affected by

climate prior to harvest. A particular problem for some hybrids is what is referred to sometimes as silk cut, a thin break in the pericarp across the grain. The suggestion is made that this can lead to imbibition damage in wet cold conditions (7) and thus reduce cold test germination and field emergence.

Size grading and sorting is intense during processing with several tests for germination in the warm and the cold, the aim being to achieve a seed product capable of achieving a high uniform level of seedling establishment even in the marginal areas for production. The ultimate goal of developments in production and processing is levels of reliable emergence close to 100%, as is seen in some vegetables, which at sowing costs of more than \$200 per hectare is a demand some growers feel is reasonable to expect of maize.

On mainland Europe the insecticidal seed treatment imidochloriprid is used on maize to control aphids and consequently virus diseases. This has become a cause for concern for the seed suppliers. The effectiveness of the chemical has created a demand from growers but the hint of phytotoxicity seen in sugarbeet is a more serious problem in maize to the extent that only the most vigorous lots as indicated by the cold test are treated. As is often the case phytotoxicity is seen more in laboratory tests than the field. This is an example of a more general concern in the European seed industry, the interaction between new chemical seed treatments, including multiple applications, and seed quality. Apart from the pressure to produce only high quality vigorous seeds there may be a case in the future for modifying seed testing methods so that new crop protection products with highly desirable properties are not rejected because of adverse effects in seed quality tests not seen in the field.

Other major field crops in Europe. All seed sold of the major field crops in the E.U. is certified, mostly as C1 and C2, first and second generation from basic seed. The quality in terms of the laboratory germination test is uniformly and consistently high for the most part. The International Rules for Seed Testing are applied (14) and 'minimum standard' legislation contained in seven E.C. directives are the basis for national Seed Regulations. This conformity within the E.U. is intended to facilitate trade between countries of the Union, in other words to create a single market. Many of the minimum germination standards are below what is achieved by seed suppliers or expected by growers, for example, 90% for maize, 85% for wheat or 80% for peas. This European approach contrasts with the truth-in-labelling law of the USA.

As well as the relative ease with which highly germinable seeds can be produced agronomic features of these crops (wheat, barley, oats, oilseed rape and combining peas) do not place a premium on the need for precision in emergence and populations. The seeds are close-spaced and plants can compensate with the result that there is a long optimum plateau of densities for maximum yield, added to which the seeds are relatively inexpensive and can be sown at insurance sowing rates.

Vegetables established as transplants. In Europe the vast majority of commercial *Brassica* crops (broccoli, cauliflower, cabbage, sprouts) as well as lettuce and some leeks and celery are established in the field as modular transplants produced in controlled conditions in glasshouses. The momentum to move away from bare root transplants or direct sowing and thinning has its origin in the marketing of fresh produce, most of which in the UK, for example, is in the hands of five supermarket chains. Their demands for uniformity, consistency and continuity in size and quality have pushed growers into expensive methods of crop establishment of F_1 varieties in which the specialist plant raiser is the first stage of production. The range of times in emergence in any one directly drilled field crop, for example in lettuce, leads to a range of head sizes unsuitable for once-over harvesting (13) and hence the move to modular transplants.

Plant raisers are the most demanding of all growers when it comes to seed quality. Their costs of production are such that close to 100% establishment in modular trays is their expectation and a full tray of uniform seedlings is expected by their customers, the vegetable grower. In a survey of seed being used by plant raisers conducted by our laboratory in 1988 differences were revealed in several crops (15) not only in laboratory germination but also in the level of deterioration (or ageing) as determined by the controlled deterioration vigour test. A range in laboratory germination of seed lots of cauliflower from 81 to 98% differed in mean time to emerge, final emergence, mean plant height and its variation (as indicated by coefficient of variation) all of which were associated with the level of deterioration as indicated by germination after a period of controlled deterioration (Table 2). Such data and practical experience have set high standards for the seed supplier who has responded but at a price.

The expectation is that the seed of F₁ *Brassica* seed for transplants has a laboratory germination of at least 95%. Seed is produced and stored with extreme care and then graded and tested intensively to produce for the market F₁ hybrid seed selling at up to \$32 per 1,000 for precision seed compared to what is referred to as natural seed at \$20 per 1,000. Many are coated to include fungicide. Lettuce in the form of pills for mechanised sowing into modular trays can cost up to \$15 per 1,000 pills. Not unexpectedly the concern of raisers and eventually of growers is cost which is a reflection of the expense of highly refined seed production methods, involving rejection and upgrading. A goal for the future are production methods of quality seeds at less cost. A thought for the future might be the restoration of vigour in germinable but deteriorated seeds by inducing repair. This has been achieved in our laboratory and elsewhere by methods of limited hydration (20).

The interaction of seed quality and chemical seed treatment with imidochlopirid has been a recent problem in Europe for lettuce. As a result of grower demand treated seed is available for transplant production for later sowings prone to aphid attack but only high vigour seed are used to minimise emergence delay especially in stress conditions of high temperature and low moisture availability (8).

One crop, winter cauliflower, the speciality product of the Brittany area of N.W. France, which supplies the rest of Europe is established in the field as bare root transplants larger than can be produced in modules. The seed of F₁ varieties bred at the breeding station supported by several cooperatives (Organisation Bretonne de Sélection) is produced locally under glass or plastic and sown in phased sowings into nursery beds to produce transplants. This is a specialist job and, just as for modules, the target is uniformity and highly germinable vigorous seed lots are required (Lunn, personal communication).

Directly sown field vegetables. The directly sown field vegetables in which the marketed produce is a vegetative organ, well exemplified by carrots, onions and leeks, present a particular stand establishment challenge in Europe as elsewhere. The requirements in vegetable production for today's fresh market is for continuity of supply of product of a specified and uniform size. As much as 60% of the carrot crop can be rejected for the intended market at grading after the costs of growing, lifting and washing have all been incurred. The grower's objective is to maximise the target size at a timing which is of the market outlet's choosing.

Size is determined by sowing density and spatial arrangement. This has been the subject of much mathematical modelling to describe quantitatively the effects of plant competition (3) one aim of which is to forecast marketable yields of, for example, carrot roots. A significant variable leading to lack of produce uniformity is initial seedling size which arises from variation in time of seedling emergence (12). Thus the goal in emergence is not just high and predictable field emergence but uniformity in the rate of emergence which minimises the number of days between the first and last seedling to emerge. To this end research and development efforts in Europe have been focussed on seed priming and the quantitative description of the influence of temperature and soil moisture on the pattern of emergence.

Two methods of priming to achieve more rapid and uniform emergence that are used commercially are osmotic priming in some way, and so-called drum priming (19). In drum priming, seeds are held at limited levels of hydration in a rotating drum for several days and then dried back. The method is in use, certainly in the U.K., for relatively slow germinating field vegetables like carrots, leeks and parsnips.

Seedling emergence patterns have been quantitatively related to the effects of thermal time, day degrees above a base temperature, and the germination (protrusion of the radicle) over time in carrots and onions. In the absence of water stress germination and emergence patterns can be predicted from the thermal time above a base temperature for which 1.4°C has been used (12). The normal field situation, however, is one of variable soil moisture and the thermal time relationship only holds provided soil water potential remains above a minimum (-1.5MPa is suggested) that allows germination to proceed. Against this background a more rational basis to the timing of post-sowing irrigation has been advocated (12). If seeds are sown, as is good practise, into a moist seed bed allowing imbibition, then a timing of a single irrigation to coincide with the thermal time in °C days that leads to between 50 and 75% germination (radicle protrusion) gave a high emergence level with a small spread of emergence times. Osmotically primed leek seed showed the fastest and least spread of emergence without further irrigation beyond a moist seed bed. Complete germination of the primed seed was achieved after so few day degrees that most seed had germinated in the seed bed before the soil water potential had fallen below -1.5MPa (12). Such

work underlines the value of priming and the significance of maintaining minimal soil water potential in order to achieve the target of high and uniformly rapid emergence in direct sowings.

Precision in stand establishment, both numbers and rate, is not crucial in such vegetable crops as peas and beans grown to immature green seed stages for freezing or canning. There is though the problem in peas of sowings as early as February in the UK to achieve a more prolonged throughput in freezing plants of seeds at the desired stage of maturity. To avoid failures in such sowings only the most vigorous seed lots are used and in the UK and Sweden measurements of the leakage of electrolytes into seed soak water are used routinely as a vigour test to select lots with a low propensity to leak for use in early sowings.

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Table 1. Distribution in 1,000 hectares of the main arable crop established by seed in the European countries in 1994. Data also presented for Russia and USA for comparison

Country Total	Wheat Arable	Barley	Rye	Oats	Grain	Sugar Maize	Sun- Beet	Oil Flower	Dry Seed Rape	Peas
France	18316	4580	1408	45	166	1637	437	986	671	664
Spain	15609	1970	3589	154	345	340	183	1355	69	8
Germany	11805	2435	2070	723	392	346	503	189	1058	10
Italy	8329	2371	392	7	144	909	282	235	14	7
U.K.	5942	1811	1106	8	109	0	169	0	496	79
Sweden	2780	252	449	39	341	0	53	0	129	5
Finland 2593	89	505	9	333	0	34	0	67	6	
Greece	2421	908	163	19	44	212	42	21	0	0
Denmark	2372	574	704	89	39	0	66	0	170	101
Portugal 2200	241	58	66	75	178	1	133	0	0	
Austria 1420	241	253	77	49	179	52	37	71	39	
Ireland 1314	74	170	0	21	0	35	0	6	1	
Netherlands	920	122	44	6	6	12	115	2	0	2
Belgium-Lux	777	215	72	3	14	26	95	0	17	4
Total	76798	15883	10983	1245	2078	3839	2067	2958	2768	926
Russia	130302	22191	16404	3888	8333	524	1104	3133	147	1673
USA	185742	24997	2698	165	1623	29509	584	1388	140	54

Source: F.A.O Production Year Book (1995) Vol. 49.

Table 2. Effect of cauliflower seed quality on emergence and seedling growth in module trays (mean of 50 cells)

Cultivar Lot	Laboratory [†] Germination (%)	Germination after Controlled Deterioration (%)	Mean time to emergence (days)	Final Emergence (%)	Plant height (mm) at first leaf	CV of plant height
A	98	99	4.4 (4) [†]	92	26.7	21.5
B	88	69	4.9 (11)	87	27.4	28.7
C	81	55	4.9 (11)	83	20.6	24.7
D	90	37	6.2 (14)	88	19.8	25.9

- 400 seeds; ** 100 seeds, [†]Figures in brackets indicate number of days over which emergence Source: Reference (15)

Priming and Synthetic Seed Applications to Stand Establishment Problems

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ABSTRACT

Essential prerequisites to optimize stand establishment are rapid and uniform field emergence under all environmental conditions. Over the past two decades seed enhancement through seed priming has led to great improvements in a growers ability to routinely achieve this goal in both the field and greenhouse. Numerous vegetable and ornamental crop species have been primed successfully. In order to maintain a superior product, seed companies have to maintain seed quality and longevity in the primed seed. Although many of our crop species are propagated via zygotic seeds, many other crop species would benefit from improved stand establishment via a synthetic or somatic seed system. Such crops as sweet potato, potato, cassava, strawberry, and various ornamental and tree species are candidates to be propagated through synthetic seeds. Other crops would include expensive seed crops such as seedless watermelon, parthenocarpic cucumber, asparagus, geranium, various tomatoes, and difficult crops to hybridize such as rice, soybean, cotton and various tree species. In order for a synthetic seed system to be economically viable, it must be able to produce large numbers of singulated synthetic seeds synchronously which could be planted via conventional techniques.

INTRODUCTION

Rapid and uniform field emergence are two essential prerequisites to increase yield, quality, and ultimately profits in crops. Uniformity and percentage of seedling emergence of direct-seeded crops have a major impact on final yield and quality (Wurr and Fellows, 1983). Rate and uniformity of emergence are inherent to seed quality and environmental conditions during seedling emergence. Slow emergence results in smaller plants (Ellis 1989) and seedlings, which are more vulnerable to soil-borne diseases (Gubels 1975; Osburn and Schroth 1989). Extended emergence periods predispose the planting bed to deterioration and increased soil compaction (Heydecker 1978), particularly under adverse environmental conditions.

In the last two decades, seed priming has become a common seed treatment to increase the rate and uniformity of emergence in many vegetable and flower species. Heydecker (1973) acknowledged the use of the term "priming" of seeds by Malnassy (1971) to describe a presowing seed treatment to enhance germination and increase seedling emergence uniformity under adverse environmental conditions. In the same report, terms such as halopriming (soaking in salt solutions) or osmopriming (soaking in other osmotic solutions) were proposed as alternatives to priming (Heydecker 1973). The use of a salt as an osmoticum can lead to an increase in fresh weight (water) of a seed. In this case, germination is delayed through increased solute potential of the embryo. Osmoconditioning or osmotic conditioning are also used to describe the same treatment when generally uses PEG as the osmoticum (Khan et al. 1978, 1992a,b). Kubik et al. (1988) and Taylor et al. (1988) introduced the term "solid matrix priming" (SMP) for a presowing seed treatment in which a solid-matrix instead of an osmotic solution was used to enhance germination. Dr. John Eastin was awarded U.S. Patent 4,912,874 for the process. He described it as a process that uses solid matrix materials, water, and seed in combinations to control water, oxygen, and temperature effects on germination (Whitmore 1991). The process controls the hydration of seed to a level that allows "pre-germination" activity but that prevents radicle emergence. Matricconditioning was proposed by Khan et al. (1990) as an alternative term to SMP, to distinguish seed conditioning by matric and osmotic forces. Callan et al. (1990) coined the word "biopriming," a treatment where sweet corn seeds were coated with a bacteria and soaked in warm water until the seed moisture content increased to 35-40%. In the discussion that follows, the terms priming and solid matrix priming will be used. The cadre of other terms has actually led to confusion of what seed priming is and what is needed to obtain consistent positive effects from priming.

SEED PRIMING

Heydecker (1973) defined seed priming as a presowing treatment in which seeds are soaked in an osmotic solution that allows them to imbibe water and go through the first stages of germination, but does not permit radicle protrusion through the seed coat. The seeds then can be dried to their original moisture contents and stored or planted via conventional techniques. This definition can be partially extended to SMP. The main difference between the two treatments is that in SMP, matric forces regulate water uptake by the seed instead of the osmotic potential of a liquid soak solution.

Seed priming treatments modify embryonic axis growth and subsequent seedling development. The response varies according to the species and priming conditions. Embryo volume and cell number per embryo of leek and onion were not modified by priming (Gray et al. 1990). In the same experiment and under the same conditions, the carrot embryo volume increased almost 50% and the number of cells increased by two-fold. Progressive rupture of the endosperm after 9 h of priming was one of the potential factors leading to increased germination of 'Minetto' lettuce seeds at high temperature (Guedes et al. 1981).

Generally, the major effects of seed priming on growth has been observed as earlier more-uniform emergence and not accelerated growth, *per se*, of the species. Root growth of primed pepper seeds was analyzed by Stoffella et al. (1992). They concluded that the number of basal and lateral roots and taproot length 14 days after seeding were not modified by seed priming. Root length of primed lettuce seeds germinated at 35° was greater than that of nonprimed seeds after 96 h (Wurr and Fellows 1984). Root dry weights of primed and nonprimed bell peppers, grown with sprinkler or drip irrigation, were similar 50, 70, and 90 days after planting (Leskovar and Cantliffe, 1993). Seed priming did not affect the rate of radicle growth and degree of root branching in tomato seeds after germination (Odell and Cantliffe, 1986). Primed parsley seeds yielded 52% more shoot fresh weight compared to nonprimed seeds 24 days after sowing (Pill 1986). The differences in root or shoot growth between primed seeds and nonprimed seeds is more evident under stressful conditions. Root growth from perennial ryegrass seeds germinated at low temperature (5°, 10° and 15°) was greater in primed seeds than in nonprimed seeds, but no differences were observed at 25° (Danneberger, et al. 1992). Shoot dry weight was higher for primed tomato seeds at stressful temperature conditions than for nonprimed seeds (Odell et al. 1992a). Jett and Welbaum (1992) increased germination and seedling emergence rate of broccoli by priming, but did not increase growth rate of the root at temperatures from 25° or 35° compared to a control.

Synchronization and rapid seed emergence are the commonly reported benefits of seed priming. Particular advantages of seed priming are augmented under adverse conditions (Knypl and Khan 1981; Wiebe and Muhyaddin 1987). However, the effects of priming on yield and plant quality characters were more elusive.

Seed priming promoted early growth of eggplant, pepper, cucumber, and muskmelon plants, but no differences were detected in early and final yield, and fruit size between primed and nonprimed seeds (Passam et al. 1989). Flowering of processing tomato was earlier for plants established from primed seeds than from nonprimed seeds, but fruit maturation, yield, or fruit soluble solid content were unaffected (Alvarado et al. 1987). Similar results for the same species were reported by Argerich and Bradford (1990). Early seedling growth of tomato was improved by seed priming, but priming increased marketable yield only under more stressful conditions (Odell et al. 1992b). Seed priming did not increase final yield of celery (Rennick and Tiernan 1978). Early yield of lettuce was greater from primed seeds than from nonprimed seeds (Cantliffe et al. 1981). However, the number of marketable lettuce heads was not improved by the priming treatment (Seale and Cantliffe, 1986). Soybean growth or maturation dates were unaffected by seed priming (Helsel et al. 1986). Beneficial effects of priming on yield and quality in a similar fashion to seedling growth and development have been reported only in crops growing under stressful environmental conditions.

There is no doubt about the beneficial effects of priming on the rate and synchronization of seed germination. The success or failure of priming treatments are influenced by a complex interaction of factors including plant species, osmoticum, water potential of the priming agent, duration of priming, temperature, seed vigor, and dehydration and storage conditions following priming.

During priming, seeds are brought to the "brink" of cell division and after dehydration and reimbibition the seed more or less resumes regrowth processes at the point prior to dehydration. Seed priming (Khan 1992)

under controlled temperature conditions, allows seeds to imbibe water and go through the initial stages of germination (I and II) without radicle protrusion through the seed coat. Priming has been referred to by such terms as advancing, invigoration, hardening, wet-dry, and vigorization. In essence, the process allows “slow” and “fast” germinating seeds of a single lot to attain the same stage of germination readiness (Heydecker 1973).

Basically, the process of priming is not unique. Any factor that controls germination rate and/or delays cell division can be incorporated into the process. The greater the factor controls germination rate, the more benefit it potentially will have when used in the priming process. For example, Hegarty (1970) “hardened” sweet corn seeds by soaking them in water or carrot by soaking and drying three times in water or 0.01 M K_2HPO_4 . The results in water were positive, thus priming can be done by using water as the soak solution.

The use of an osmoticum in place of water is commonly employed to prime seeds today. The question of which osmoticum is next to be answered. Commonly, PEG or inorganic salts are used. Benefits of each have been discussed and generally PEG is preferred when responses to it as the osmoticum are at least equal to those received from the use of salt. If salts accumulate in or around the embryo, damage can result. PEG is a colloidal suspension, which has been shown to reduce water uptake rate and accumulation by seeds during priming when compared to water or salt solutions.

The use of a matricum for solid matrix priming follows the same principals as described above for solution-osmotic priming. The choice is mainly based on choosing a material wherein water uptake by the seed can be more closely regulated. Concentration of the matricum or osmoticum can be altered to fine-tune water relations.

The other factors that affect the results from seed priming, aeration, temperature, duration, light, dehydration, and storage (Cantliffe 1983) are dependent on the species being primed. All crop seeds need air to germinate, thus optimizing aeration during priming is essential. Research results have shown that where light is needed for germination, it too is essential to optimize priming. With respect to temperature, many species have responded well to priming near the lower cut-off temperature for germination of the species in question to occur. This usually maintains better control of the priming process by slowing the germination processes to a minimum. An exception to this generalization is tomato (Ells 1963), where a priming temperature of 25°C has proven to optimize the process.

The dehydration process after priming has long been overlooked as having a major influence on the results derived from priming. The method of dehydration has been shown to be extremely important to the ability of solution primed lettuce seeds to store well and overcome thermodormancy (Weges and Karssen 1990), and to solid matrix primed *sh2* sweet corn to germinate well, especially under adverse soil temperature conditions (Parera and Cantliffe 1994). The entire area of dry-back after priming demands further attention.

Storage of primed seeds should consider at least two main points: (1) primed seeds must be stored under optimal storage conditions for the species in question, and (2) primed seeds will not store as long as nonprimed seeds. In many cases, to maximize results, primed seeds should not be stored more than 3 months.

During his early work, Heydecker (1975) asked whether “to recommend a few (prime) treatments, useful between them for the majority of seeds, or shall have to find separate best treatments even for individual seed lots.” Such questions were the results of greatly varied results from priming between different species, among cultivars of the same species, and even between lots of the same cultivar. Later, it was felt that this variation was primarily due to differences in seed quality (Perkins-Veazie and Cantliffe 1984) and not to minute unknown differences among lots, cultivars, or species. To achieve consistent and beneficial results from priming, only the highest quality seed should be used.

The use of solution-osmoticum or solid-matricum for the priming process has shown benefits for improving seedling emergence percentage and rate under a wide variety of environmental conditions. Heydecker et al. (1975) proposed that for the priming process to be commercialized depends on the development of a reliable, cheap, large-scale, aerobic method that suppresses pathogen growth on the seed. The solution-based priming process has given excellent results with tomato, pepper, parsley, lettuce, carrot, onion, and watermelon to name but a few species. The same procedures have not been beneficial when used on seeds of *sh2* sweet corn wherein seed-borne pathogens become a problem (D. J. Cantliffe, unpublished). For this species and its

numerous cultivars, SMP has been extremely effective (Parera and Cantliffe 1992). The SMP process has also been shown to be effective with a large assortment of seeds including grasses, ornamentals, and vegetable species. Various seed additives can be effectively incorporated with the seed during SMP (Eastin 1992). Thus, the continued commercialization of this process will remain dependent on those factors so mentioned by Heydecker et al. (1975) as well as patent rights to the process (since the SMP process has a patent).

To further refine priming and obtain greater more consistent benefits from the process, it would be extremely useful to understand the physiological/molecular basis of priming. Even with the quality and quantity of studies reporting the molecular and physiological effects of seed priming, the biochemical mechanisms of priming remain largely unelucidated. Bradford (1986) and Ni and Bradford (1992) postulated that during the priming treatment there is solute accumulation in the embryo and no germination occurs since embryo water potential is below the threshold required for growth. During dehydration of primed seeds the level of solutes in the embryo is maintained, so that during reimbibition the higher turgor potential of the embryo resulting from the steepened water potential gradient may induce fast germination. Gray et al. (1990) concluded that the production of osmotic solutes in the embryo was not the main reason for improving germination rate in primed seeds. They suggested that during priming the seeds are artificially maintained in phase II of imbibition, and the substances generated in this latent period may increase cell wall extensibility or remove restrictions for radicle growth. Karssen et al. (1989) arrived at the same conclusion after studying the effect of priming in tomato, celery, and lettuce seeds. After priming, the seeds contained increased levels of certain products such as protein (enzymes). Generally, priming of the 3 species permitted rapid and uniform germination by stimulating extensibility of the cell wall in the radicle and by weakening endosperm cell walls in front of the radicle tip. Ni and Bradford (1992) contend that, whereas the above might occur, it occurs during imbibition in the osmotic solution and that a second phase of wall-weakening has to occur prior to radicle emergence. Embryo water potential changes are more related to the weakening and radicle protrusion during this second phase.

Although there is active participation of membranes in seed hydration and dehydration mechanisms, the role of changes in membrane structure during and after priming have not been studied extensively. Basra et al. (1988) reported changes in quantity and quality of membrane phospholipids during and after priming. Parera and Cantliffe (1991) demonstrated that sweet corn seeds primed via SMP had less solute leakage and reduced water uptake rates during early imbibition than nonprimed seeds. Similar results were reported by Zuo et al. (1988) in pea seeds after priming. The role of alterations in membranes during and after priming needs further study.

The numbers of different species commercially primed and available to producers is small. The reasons for this might include the high cost to the grower for primed seeds, lack of knowledge and/or experience by growers of the benefits derived from using primed seed, and the inconsistency in results obtained from priming. The former two reasons are marketing problems. The latter continues to warrant further scientific investigations as to why inconsistencies are noted. The priming process has evolved to a highly technological procedure and with the addition of SMP has permitted enhancement of species such as *sh2* sweet corn, which could not be accomplished effectively by other processes. In the future, the use of seed priming will be incorporated with beneficial biologicals such as fungi and bacteria, or with the additions of certain fungicide, seed sterilants, growth promoting, and/or dormancy breaking procedures. The magic of priming may not be in what it does with regard to germination enhancement, but more in determining how the seed retains its ability to continue germination without harm after the germination process was initiated, then subsequently stopped via dehydration. Finally, more practical and essential subjects such as seed vigor and priming effectiveness, effects of dehydration after priming, storage conditions, and the ideal combination of matrix-solid, water, and seeds in SMP treatments are in need of further studies.

SYNTHETIC SEEDS

Most crop species are propagated via seeds. As a propagule, the seed usually can be readily mass produced, can be planted rapidly with mechanical equipment, and has various tissues which provide nutrition to the growing seedling and protection to the stored seed. Several species, however, are not easily propagated through zygotic seeds (Cantliffe, et al. 1987). These include sugar cane, sweet potato, potato, cassava, Napiergrass, various ornamental and tree species, and strawberry. In other cases, several species are impossible or difficult to hybridize (i.e. rice, soybean, cotton, forest trees, fruit trees), suffer poor seed quality (conifers), are

expensive as seeds, especially as hybrids (tomato, greenhouse cucumber, seedless watermelon, asparagus, geranium), or are in need of specialized propagation procedures such as transgenic clonal plants. These specific cases would immediately benefit from a synthetic seed system.

In order for the system to be economically viable, the synthetic seed systems would have to produce large numbers of competent somatic embryos inexpensively and synchronously that could be planted via conventional techniques directly to a transplant container or the field. The system should be mechanized wherever possible and should not be dependent on costly equipment and extremely demanding of aseptic conditions.

Gray et al. (1987,1990) defined synthetic seeds as somatic embryos engineered to be of use in commercial plant production. Somatic embryogenesis can be readily induced in many species. Somatic embryos develop from somatic cells and can be used to duplicate a genotype (Raghavan, 1986). Somatic embryos, like zygotic embryos, are bipolar, thus, develop into complete plants similar to a germinating seed. Structurally, somatic embryos are similar to zygotic embryos but as synthetic seeds somatic embryos do not process a protective seed coat nor storage tissue.

Both types of embryos exhibit similar ontogenies; i.e. for dicots globular, heart, torpedo, and cotyledonary stages (Ammirato, 1987). Gray and Purohit (1991) provided a thorough description of the comparative aspects of somatic and zygotic embryo development. A limitation of using somatic embryos for mass clonal propagation resides in their propensity to produce clusters of embryos from a mass of embryonic tissue known as a proembryonal cell complex (Haccius, 1978). Such embryos tend to develop asynchronously causing the somatic embryos to mature non-uniformly. The somatic embryos can become disorganized, form new embryogenic cells, or precociously germinate. Another disadvantage of somatic embryos is their inability to germinate and form plants readily (Schultheis et al. 1990). The reasons for this phenomenon are unknown and few efforts have been given to this as a research topic.

The heterogeneity of embryo maturity typically found in somatic embryogenic cultures has impeded commercialization of synthetic seed systems (Harrell et al. 1993). Asynchrony during somatic embryo formation may rest primarily on the culture conditions during development. Further, commercialization of a synthetic seed system would require mass production of synchronous embryos that could be handled in extremely large volumes. This would place a need for batch production in bioreactors into the system.

DELIVERY METHODS FOR SOMATIC EMBRYOS

To date, most embryo-to-plant systems require several intermediate steps prior to successful plant establishment in the field. For example, Haydu and Vasil (1981) germinated napiergrass (*Pennisetum purpureum* Schum.) somatic embryos in tissue cultures until they grew into plants 1-5cm long. These small plants were then transferred to culture tubes to establish a more vigorous root system. The next step was potting the plantlets into soil-vermiculite in a growth chamber, then acclimating the plants to lower relative humidities. Finally, plants were transplanted in the greenhouse and/or the field.

For some species, plantlet formation is a very slow process. Celery somatic embryos required 5 weeks before forming roots and leaves (Dunstan et al. 1982). After adequate growth was obtained the plants were transferred to soil in propagation boxes in the greenhouse, then to individual pots. Spiegel-Roy and Vardi (1984) developed a procedure for plant regeneration of citrus that required several transfers from solid and liquid media. After plants reached a certain size, they were cultured in tubes on paper bridges for further development prior to transfer to soil. Sixteen to 18 weeks were required before plants obtained from somatic embryos were growing in the greenhouse.

Extensive transfer steps were also required to establish plants from papaya (*Carica papaya* L.) somatic embryos (Litz and Conover 1982). Embryos were germinated and formed plants on White's medium supplemented with 0.1-2 mg/l NAA and 0.05-0.2 mg/l BAP. Plants were then moved to a soilless potting mix and hardened off under intermittent mist for 2-2.5 weeks. These few examples illustrate the laborious and time-consuming task before plants from somatic embryos could be transplanted in the field.

Several delivery methods for propagating somatic embryos directly from the vitro conditions to the field or greenhouse have been proposed. They include: (1) simultaneous dehydration of somatic embryos using a water-soluble resin and planting in a wafer or seed tape (Kitto and Janick 1985a,b); (2) dehydration, then planting dried somatic embryos with a conventional drill (Gray et al. 1987); (3) encapsulation of singulated somatic embryos in an alginate gel capsule, then planting using a conventional drill (Redenbaugh et al. 1984, 1986; Jeon et al. 1986); and (4) gel seeding somatic embryos with fluid drilling equipment (Drew 1979; Baker 1985; Schultheis et al. 1986a,b; 1990; Cantliffe et al. 1987).

STRUCTURAL ASPECTS OF SYNTHETIC SEED

Synthetic seeds consist of either a quiescent or nonquiescent somatic embryo with or without a protective encapsulation. The exact form of synthetic seed that is required will depend upon its specific applications. Naked, nonquiescent somatic embryos, germinated in soil plugs, could be used to propagate certain ornamental crops that are now laboriously micropropagated by tissue culture. Manpower reduction achieved by producing plants by somatic embryogenesis, when compared to existing micropropagation, would confer a cost advantage. Dehydrated, quiescent somatic embryos without encapsulation would be useful for germplasm storage since they can be hand manipulated and carefully stored in protective containers. Cost of manipulating somatic embryos for germplasm storage would be similar to that of seed. Nonquiescent encapsulated embryos could be useful for crops that are first grown in greenhouses before transplanting to the field, such as carrot and celery (Fujii et al. 1987). However, for mass propagation of field crops, a protective encapsulation will be necessary.

Somatic embryo encapsulations are analogous to the seed coat and endosperm of normal seed. Encapsulations may provide physical protection to the somatic embryo and carry nutrients, growth regulators, antibiotics, fungicides, etc. to assist in germination and plant survival (Kitto and Janick 1985b,c; Redenbaugh et al. 1986, 1988; Janick et al. 1989). Encapsulated somatic embryos could conceivably be handled as seed using conventional planting equipment. Both hydrated and dry encapsulations have been envisioned.

The commercial application of somatic embryogenesis for many crops requires high volume, low cost production, which invariably translates to a requirement for automated production and harvest. Relative need for automation will vary depending on crop application. Synthetic seed production can be separated into several discrete steps, each of which are amenable to automation: (1) production of cell cultures and somatic embryos; (2) sorting and harvesting of somatic embryos; and (3) encapsulation and dehydration.

Production unit operations vary considerably depending on the culture system used. While mass production of somatic embryos is probably best accomplished in liquid medium, which can be scaled up in bioreactors (Redenbaugh et al. 1988), a number of possible alternatives have been explored. For Petri plate-based protocols, production involves subculturing callus from plate to plate under aseptic conditions. Subculturing involves medium preparation, plate lid removal, harvesting calli, sieving and dispensing calli on new plates. Kurata and Futaya (1992) developed an automatic sieving system applicable to many of these operations. The goal of this system was to select appropriately sized calli for the production of carrot somatic embryos. However, the system was not as effective as manual sieving, but did demonstrate the validity of the concept.

For a liquid-based production protocol (primarily bioreactors), unit operations are typical of chemical process tasks. Durzan and Durzan (1991) list the process control objectives as: (1) suppression of the influence of external disturbances on the somatic embryogenesis process; (2) assurance of the stability of embryo development; and (3) optimization of the overall, long-term performance of somatic cells and embryos. Process control requires identifying the outputs to be controlled (e.g., embryo production rate and maturity), how these outputs are to be quantified, and the inputs to be used to manipulate the outputs. In some instances production objectives may be met by controlling elemental physical and chemical parameters such as temperature and pO_2 without the need to measure the output variables. A successful example of this type of process control is the model system for the bioreactor production of poinsettia globular somatic embryos (Preil 1991). The manipulated variables (inputs) for this system included temperature, agitation, pH, pO_2 , redox potential, and pCO_2 . The controlled variable (output) was embryo production rate. Relationships between the controlled and manipulated variables were established by off-line experimentation which avoided the need for on-line monitoring of embryo production.

On-line monitoring of the callus production of the somatic embryogenesis process was demonstrated by Harrell et al. (1991). A machine vision system was used to nondestructively monitor the growth of sweet potato callus during a 10-day culture period in an airlift bioreactor. Growth data obtained with the system included overall reactor population and population estimates for the 200-1200 μm fractions at 200- μm intervals. A model of callus growth was developed to explain the mechanics of callus enlargement. The model was based on the assumptions that (1) the calli could not shrink or subdivide, (2) there was a fixed percentage of the initial population within each fraction that was nonviable, and (3) growth rates did not vary with time during the culture period. It was determined with this system that growth rates and nonviable ratios decreased as fraction size increased.

Obstacles to automated production lie primarily in the biological systems. While well-developed somatic embryos capable of surviving direct field planting have been produced in liquid flask culture (e.g., Gray et al. 1992), there have been difficulties involved with scale-up of cultures in larger bioreactors. The main obstacle is that somatic embryos generally do not develop as well in liquid culture medium when compared to solid medium. For example, plant recovery from suspension culture-derived somatic embryos of alfalfa was less than from callus and decreased still more when scale-up to a larger bioreactor was attempted (Stuart et al. 1987). Improvements in bioreactor design are needed as well as a better understanding of somatic embryo development under liquid culture conditions to achieve progress in large-scale production.

Although somatic embryos are similar to zygotic embryos, the former lack many of the stored reserves zygotic embryos have available for the early seedling growth. Thus, it is important to find which "growth factors" are necessary to achieve rapid and complete plant formation from somatic embryos. It is also necessary to use a synthetic seedling method which could be easily adapted for field use. The incorporation of additives around the embryos to sustain and improve their growth and development into plants would be required for the successful implementation of synthetic seeding. Research indicates that the use of a fluid-drilling system using a gel carrier may overcome many of these drawbacks.

The use of synthetic seeds as propagules for direct-field planting offers exciting possibilities for species which are traditionally propagated via asexual means, difficult or impossible to hybridize, or which are expensive to produce. A prerequisite for a synthetic seed system to succeed is the need for the production of synchronously singulated somatic embryos that germinate and grow rapidly at maturity. To this end, the system is lacking.

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EFFECT OF CROP ADDITIVE ON HARD RED SPRING WHEAT SEED PERFORMANCE

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ABSTRACT

Acetates are frequently applied to soil in close proximity to seed prior to or during planting to stimulate early plant growth. Little research has been conducted with acetates applied to seed. Laboratory and field trials were conducted in 1994-96 to evaluate the effect of Agricultural Crop Additive (ACA), containing 15% ammonical nitrogen and 17% zinc oxide, applied at 0, 1.8, 3.7 and 7.4 ml kg⁻¹ on hard red spring wheat (*Triticum aestivum* L.) seed. Replicated laboratory tests conducted in growth chambers on paper towels indicated that germination percentage and seedling dry weight were increased significantly with each of the three ACA treatment levels. Similarly, treated seed planted in soil at 20 °C had increased emergence percentage, while seedling top growth was greater at 7 and 20 °C. Field trials located at Casselton and Prosper, ND indicated all ACA treatments had significantly higher grain yield and test weight than the check. These data indicate seed viability and vigor were improved with applications of acetate and zinc to wheat seed.

INTRODUCTION

Spring wheat management is continuing to change in response to world demand for more grain and declining monetary margins associated with production practices. Fertilizers are routinely applied to soil at or before planting (Oplinger et. al., 1985), topdressed (Shah et. al., 1994) or foliar applied (Altman et. al., 1983; Finney et. al., 1957) to enhance wheat yield, protein content and protein quality. Thasanasongchan (1981) evaluated the effects of acetates on corn (*Zea mays* L.) and soybean (*Glycine max* L.) under growth chamber and field conditions and found that soil- and foliar-applied acetates increased vegetative growth and grain yield.

ACA Concentrate (ACA) or ammonia zinc acetate is formulated by combining acetic acid, water, anhydrous ammonia and zinc oxide to provide a product containing 15% ammonical nitrogen and 17% zinc by weight. ACA has been tested for several years as a soil additive (Oplinger, 1990), but has not been reported as having been tested as a seed additive. The objective of this research was to evaluate the effect of ACA on seed germination, plant emergence, plant growth rate, grain yield and grain test weight under laboratory and field conditions when applied to seed.

MATERIALS AND METHODS

Laboratory and field experiments were conducted in 1994, 1995 and 1996. ACA was applied to '2375' hard red spring wheat (HRSW) seed at 0, 1.8, 3.7 and 7.4 ml kg⁻¹. ACA was applied in water solution with a laboratory model seed treater utilizing a total volume of 9.2 ml kg⁻¹. Vitavax (carboxin, 2,3-dihydro-5-carboxanilido-6-methy-1,4-oxathiin) seed treatment was also applied to seed for planting in the field at 1.9 ml kg⁻¹ in water solution utilizing a volume of 3.7 ml kg⁻¹.

Laboratory experiments were conducted in a growth chamber to evaluate for germination percentage, seedling growth rate and emergence percentage. Experimental design was a Completely Randomized Design (CRB) with four replications. Standard germination tests followed Association of Official Seed Analysts (AOSA) procedures. Seedling dry weight (SDW) was recorded following germination on paper and drying 48 h at 54 °C. Seedling emergence percentage, growth rate and emergence index were from soil moistened to 70% of water holding capacity in one quart plastic containers exposed to 7 and 20 °C temperatures with 24 h fluorescent light. Dry weight was recorded from plant material clipped at the soil surface and dried. An emergence index was calculated following the procedure proposed by Maguire (1962):

$$X = \frac{\text{number of seedlings}}{\text{days of count}} + \frac{\text{number of seedlings}}{\text{days of count}} .$$

Field experiments were planted at Casselton and Prosper, ND to evaluate field performance. Soil type at Casselton was Bearden silty clay and Prosper was Perella fine silty-Bearden silty clay complex. Experimental design was a Randomized Complete Block Design (RCBD) with six replicates. Plots were planted at 280 seed m⁻² with a 6-row plot drill in 20 cm row spacing. Planting dates at Casselton were April 22, 1994; April 28, 1995; and May 13, 1996. Planting dates at Prosper were May 6, 1994; May 19, 1995; and May 30, 1996. Soybean was the previous crop in all environments.

Agronomic traits measured were plant stand, grain yield and test weight. Plant stand was measured at two-leaf stage as an average of two randomly selected 0.06 m² sections of each plot. Plots were harvested with a plot combine after plots were trimmed to a uniform length. Harvested plot area was 2.4 by 1.2 m. The grain was dried 2 d at 38 °C in a forced air drier and cleaned to measure grain yield and test weight. Conversion to yield was based on a plot width of 2 m to compensate for border effect.

Laboratory data were subjected to analysis of variance (ANOVA) using a CRD with treatments considered fixed effects and runs random effects. Field data were analyzed as a RCBD with treatments considered fixed effects and blocks random effects. Differences between individual means were evaluated using Fisher's Protected Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Laboratory Trials. Germination percentage increased with ACA applications of 3.7 and 7.4 ml kg⁻¹ (Figure 1). Similarly, seedling dry weight of all ACA treatments levels following germination test were significantly greater than the check with all ACA treatment levels.

The above data were confirmed by plant emergence tests in warm soil where all treatments with ACA had increased emergence percentage and SDW when compared to the check (Figure 2). Average germination percentages for untreated seed were substantially reduced from normal to 81.5 and 77.5% in 1994 and 1996, respectively, due to high infection levels with *Fusarium spp.* in the field (data not shown). These data appear to indicate that application of ACA was able to overcome the negative effect of disease on seed to a limited extent. The increased SDW corresponds with Thasanasongchan (1981) who found corn and soybeans treated with acetates enhanced early plant growth.

Emergence percentage of treatments with ACA did not differ from the check when planted under cold conditions (Figure 3). *Fusarium* is a more effective disease against wheat under warm conditions. Dickson (1923) reported that wheat germinated in a cool soil resists blight but when germinated in a warm soil succumbs to the attack with the critical soil temperature about 54°F. Seedling dry weight agrees with data from the previous experiment, in that, treatments with ACA applied at 1.8 and 3.7 ml kg⁻¹ had significantly more growth than the check. However, the 7.4 ml kg⁻¹ treatment was not significantly different from the check for SDW.

Field Trials. There were no significant treatment differences for plant stand (Figure 4). These results compare closely with emergence percentages in the laboratory when tested under cold soil conditions (Figure 3). Soil temperatures in these trials averaged 10.5 °C at a 10 cm depth on the day of planting. By contrast, the results from the germination and warm soil emergence tests conducted at 20 °C did not correspond with plant stand in the field. Vitavax seed treatment utilized for field trials may also have served to mitigate treatment differences.

Analyses for treatment effects indicated that HRSW containing ACA seed treatments were all significantly higher than the untreated check for grain yield and test weight (Figure 5). The highest numerical yield was associated with the 1.8 ml kg⁻¹ treatment, indicating that an optimum treatment level may be lower than those tested in this study. These data also coincide with results on corn (Thasanasongchan, 1981) when ear weight progressively decreased with delay in application of ACA from planting time, which may ultimately prove to favor a seed application over the current commercial method of applying ACA to the soil.

These data indicate that additional research is required to delineate whether ACA application on HRSW seed will enhance seedling emergence with other cultivars and environmental conditions. Rate of ACA application had no linear effect on seedling emergence percentage in the laboratory or grain yield in field trials. Therefore, these data imply that future research is needed to determine the minimum effective level for ACA application on seed. In addition, research is suggested to determine the effectiveness of ACA on other crop species.

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Figure 1. Hard red spring wheat germination and seedling growth rate on paper with ACA application

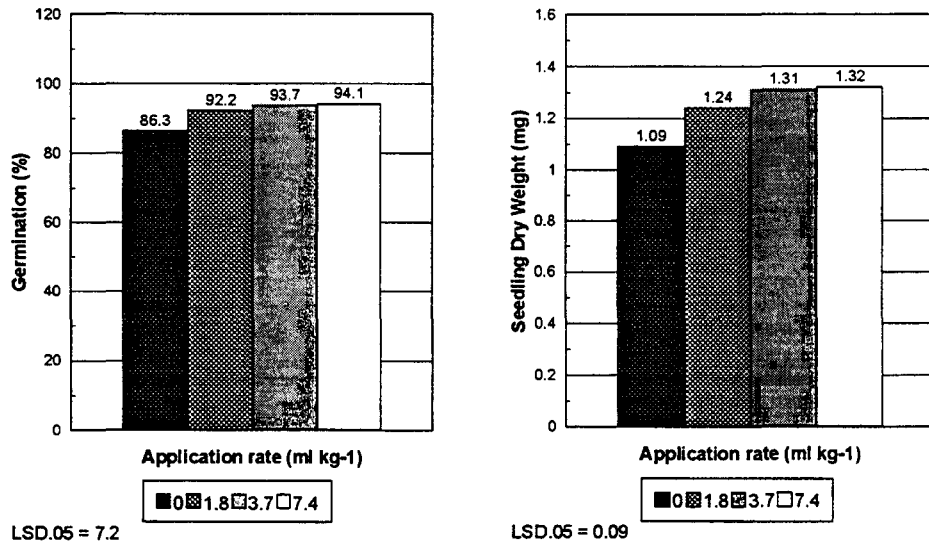


Figure 2. Hard red spring wheat seedling performance in cold soil conditions with ACA application

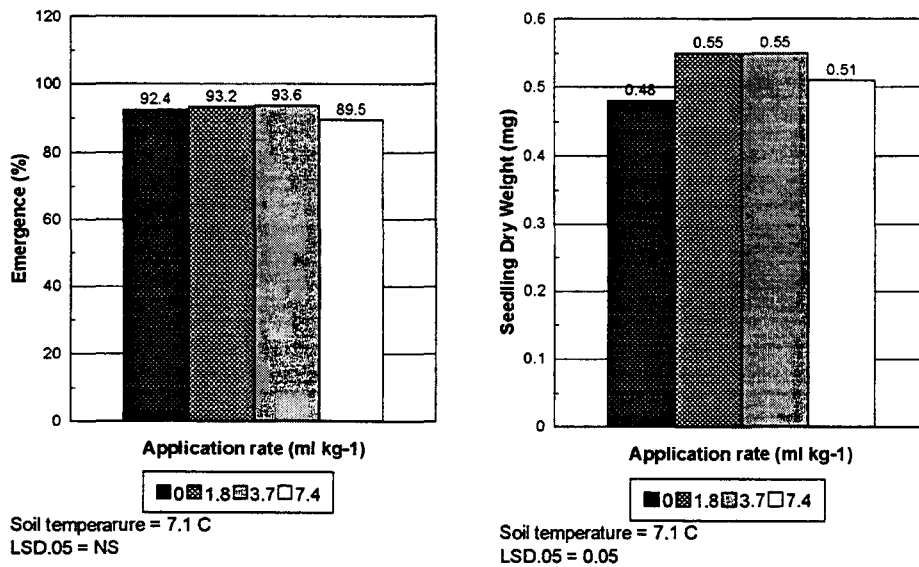


Figure 3. Hard red spring wheat seedling performance in warm soil with ACA application

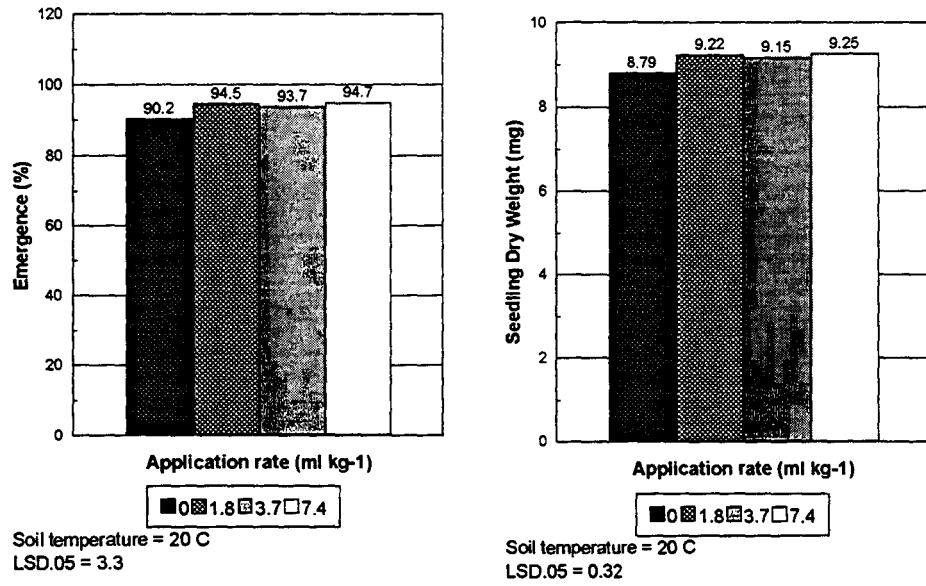


Figure 4. Hard red spring wheat plant stand under field condition

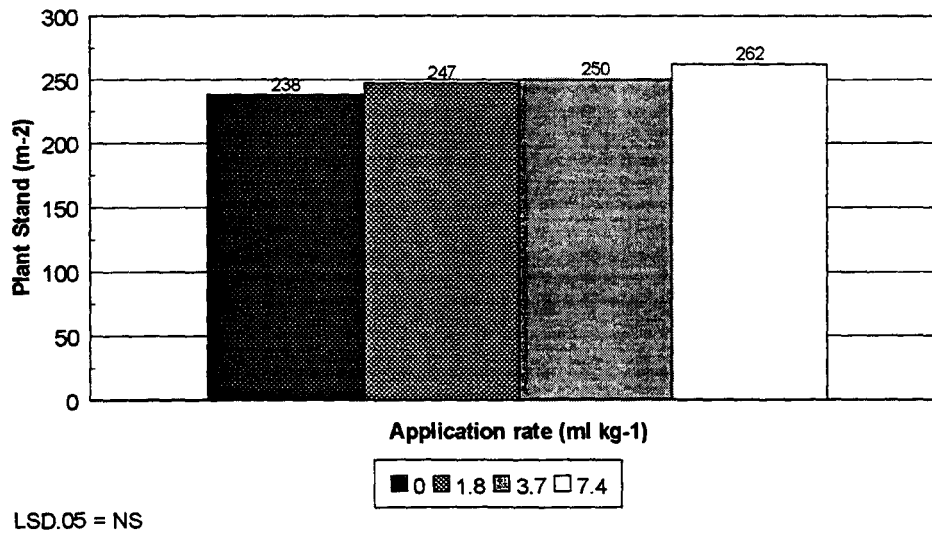
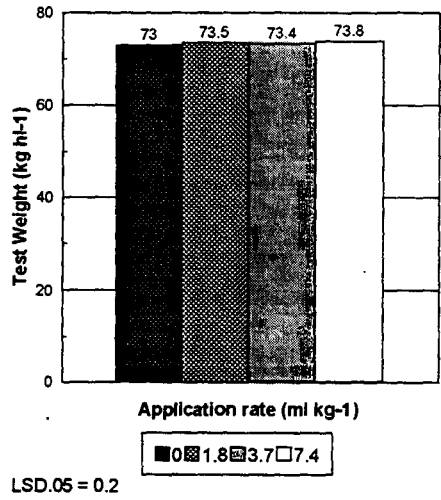
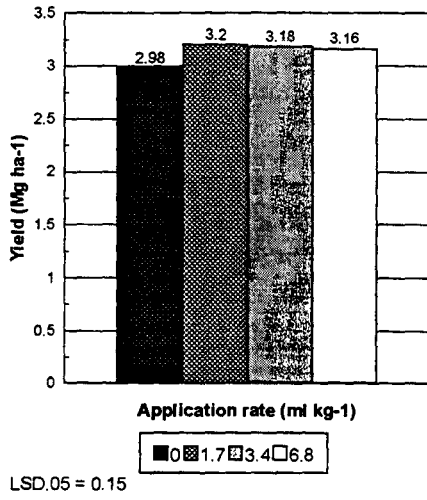


Figure 5. Field performance of HRSW following ACA application



DEVELOPMENT OF *SHRUNKEN-2* SWEET CORN SEED AND ITS RESPONSE TO GROWTH ADDITIVES ADDED AS FILM COATINGS

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ABSTRACT

The study investigated the impacts of the *shrunken-2*(*sh2*) endosperm mutation on sweet corn (*Zea mays* L.) embryogenesis and germination acquisition during seed development. Comparison between *sugary*(*su*) and *sh2* isogenic lines (two pairs) showed that *sh2* not only reduced the endosperm reserve accumulation, but also significantly retarded embryo development, especially during early seed development, resulting in a smaller embryo after maturation. Microscopic observations indicated that *sh2* seed exhibited impaired starch grain deposition in scutellar and axis tissue. During the seed development stage, *sh2* seeds acquired the same germinability at least 10 days later than *su* and dent corn seeds. During the late maturation stage, the germinability of *sh2* seeds tended to decline but *su* and field corn inbred seeds remained unchanged. We also investigated the possibility of using "growth additives" in a film coating to improve *sh2* seed germination and seedling growth. Five different chemicals were added in the polymer binder polyvinyl pyrrolidone and then coated onto the seeds of the two *sh2* inbred lines. Among the treatments, 0.1 ppm epiBR significantly increased cold test germination percentage and seedling growth of the two inbred lines tested.

INTRODUCTION

The use of sweet corn cultivars with the mutant endosperm gene *shrunken-2* (*sh2*) has increased dramatically in recent years. *Sh2* corn often exhibits poor germination and inconsistent stand establishment, especially in cold and wet soils (Styer et al, 1980; Andrew, 1982). The poor seed vigor of *sh2* mutant has been attributed to various factors including high susceptibility to seed and soil-borne diseases (Berger and Wolf, 1974), insufficient mobilization of limited endosperm reserves (Wann, 1980; Sanwo and DeManson, 1992), imbibitional damage (Perera and Cantliffe, 1991) and solute leakage (Waters and Blancette, 1983; Wann, 1986). It has been accepted that the *sh2* gene is only expressed in the endosperm. However, there are conflicting reports on the impacts of *sh2* endosperm mutation on the functioning of embryo. Styer and Cantliffe (1984) suggested that the embryo of *sh2* may be dysfunctional, but Wann (1980) found no significant embryo dysfunction. He and Burris (1993) found no difference in the embryo respiration rate between *sh2* and dent cultivars.

In order to improve seed vigor in *sh2* corn, enormous research efforts have been devoted to breeding (Tracy, 1994; Juvik, 1995), seed production refinements (Scheleppi and Burris, 1989; Tracy, 1994; Wilson et al, 1994; Fritz, 1995) and developing various seed treatment methods. The following seed treatments were reported to be effective in improving *sh2* corn seed germination and seedling growth: fungicide treatment (Berger and Wolf, 1974; Cantliffe et al, 1975), hydration (Bennett and Waters, 1987), bio-priming (Callan et al, 1990), osmotic priming (Murray, 1990), solid matrix priming (Perera and Cantliffe, 1991). However, little information is available about incorporating plant growth regulators and other beneficial chemicals into a coating on the *sh2* corn seed.

The objectives of this study were to (i)investigate the impact of the *sh2* endosperm mutation on seed development and germination acquisition by using pairs of isogenic lines of *sh2* and *su*; and (ii)incorporate plant growth substances and other potentially beneficial chemicals into a seed coating as an attempt to improve *sh2* seed germination and seedling growth especially under adverse conditions.

MATERIALS AND METHODS

Two pairs of near-isogenic lines, Ia453 *sh2* and Ia453 *su*, Ia5125 *sh2* and Ia 5125 *su*, along with two field corn inbred lines A632 and B73, were used to study the impacts of endosperm mutations on seed development. Seeds used were from self-pollinated ears of the near-isogenic lines grown in four-row plots on a Clarion loam at Ames, Iowa in 1996. Selfed ears were harvested at intervals of 4 to 6 days beginning 16 days after pollination and continuing to 60 days after

pollination. Ears were husked, and fresh seeds removed immediately after harvesting. Three replicates of 20 fresh seeds were used to determine fresh and dry weight of whole seed. Another three replicates of 20 fresh seeds were used to determine embryo and endosperm fresh and dry weight after embryo and endosperm (including pericarp and testa) were separated. Other seeds were dried at 30C in thin layers until seed moisture was reduced to 8-9%. After drying, the seeds were treated with fungicides. The fungicide combination treatment consisted of vortexing 40 grams of seeds with 4 ml of 20% polyvinyl pyrrolidone (PVP) solution in a plastic beaker. PVP solution contained 1.00% Captan (a.i.), 0.50% Apron (a.i.) and 3.00% Thiram (a.i.). After coating, the seeds were dried back to their initial moisture content (8-9%) at ventilated ambient air (25C). Three replicates of 50 seeds were then planted on rolled towels and germinated at 25C for 7 days under light.

Two *sh2* inbred lines, WH9261 and WH2, were used to evaluate growth additives incorporated into a film coating. Naphthaleneacetic acid (NAA), gibberellic acid (GA3), 24-epibrassinolide (epiBR), malate and citrate were added into 20% PVP solution respectively at different concentrations (Table 1 and 2). Three replicates of 40 grams seeds from each inbred were coated with PVP solution containing three fungicides by the aforementioned method. Seeds coated only with PVP and fungicides were used as the control. After coating, warm germination and cold test were performed in light (Wilson and Lawson, 1994). 50 seeds from each replicate were planted on rolled towel in the test. At the end of the test, normal seedlings were counted and seedling shoot and root dry weights were measured.

RESULTS AND DISCUSSION

Impacts of sh2 Endosperm Mutation on Embryo and Seed Development

Sh2 lines showed a much slower and longer increase in embryo dry weight than *su* lines during seed development and maturation in the two pairs of isogenic lines (Fig. 1 and Fig.2). At the final harvest (60 days after pollination), the *sh2* embryo had a lower dry weight than *su* embryos. The *sh2* lines failed to increase the endosperm dry weight as early as 28 days after pollination, while *su* lines continued to increase their endosperm dry weight until 38 days (Ia5125 *su*) to 44 days (Ia453 *su*) days after pollination.

Sh2 lines acquired normal germinability much slower than *su* and dent lines (Fig 3). Developing seeds of *su* and dent lines had a similar pace in obtaining normal germinability. However, the developing seeds of *sh2* lines took at least 10 days more to reach similar germination level. Moreover, *sh2* lines maintained their highest germination capacity for a shorter period during late maturation stage.

In another study, microscopic observations revealed that starch grains were not well developed in the embryonic tissues of Ia453 *sh2*, especially in scutellum. In *sh2* scutellum, the starch grains were not filled with starch reserves leaving large empty vacuole, which was opposite the well filled starch grains in Ia453 *su* scutellum. In our study the *sh2* endosperm mutation had at least two negatives effects on embryogenesis: first it delayed embryo development and growth, resulting in the formation of a smaller embryo thus delaying acquisition of germinability; second, starch deposition in embryonic tissues was impaired, especially in scutellum.

Growth Additive Incorporated Coating for Improving Germination and Seedling Growth

Three growth regulators showed different effects on seed germination and seedling growth. 0.5 ppm NAA significantly increased germination percentages and seedling root dry weight of WH9261 in warm germination test (Table 1). It increased the shoot and root dry weight but not the germination percentage of WH2 under the same conditions (Table 2). But in the cold test, NAA had no beneficial effect for the two lines. GA3 significantly and visibly stimulated coleoptile elongation of the two inbred lines both in warm germination and cold test (data not shown), which may be very favorable for seedling emergence under soil conditions because weak coleoptile extension in many *sh2* cultivars may be a factor causing their reduced stand establishment. Higher dosages of GA3 (3 100 ppm) significantly reduced the germination percentage of WH2 both in warm germination and the cold test (Table 2). A new regulator epiBR has been reported to have promising effects on cereal seedling growth under cold conditions (Mandava, 1988; Sakurai and Fujioka, 1993; Hooley, 1996). We found that 0.1 ppm BR increased the germination percentage and seedling growth of the two inbred lines significantly in the cold test, but not under the favorable warm germination conditions. When TCA cycle

intermediates were added in the coating formulation, they seemed to increase the early germination growth of coated seeds (data not shown), but after seven days germination in light, the promotive effects tended to diminish.

In conclusion, *sh2* gene has the tendency to retard embryogenesis and germination acquisition during seed development. Addition of certain growth regulators as a film coat may improve germination of *sh2* seeds in the cold test.

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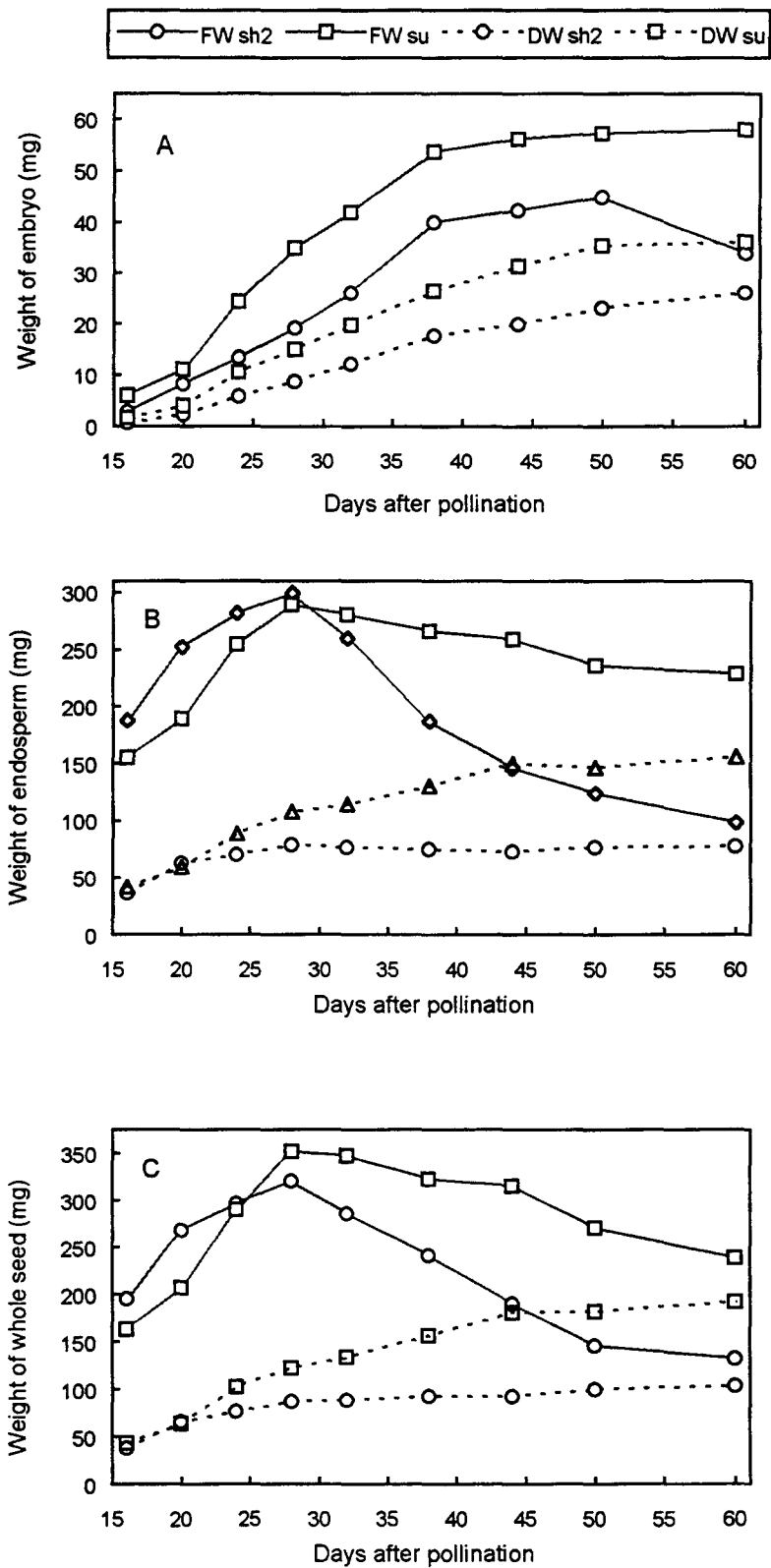


Figure 1. Changes in embryo (A), endosperm (B) and whole-seed (C) fresh weight (FW), dry weight (DW) of isogenic lines Ia453 *sh2* and Ia453 *su* during development.

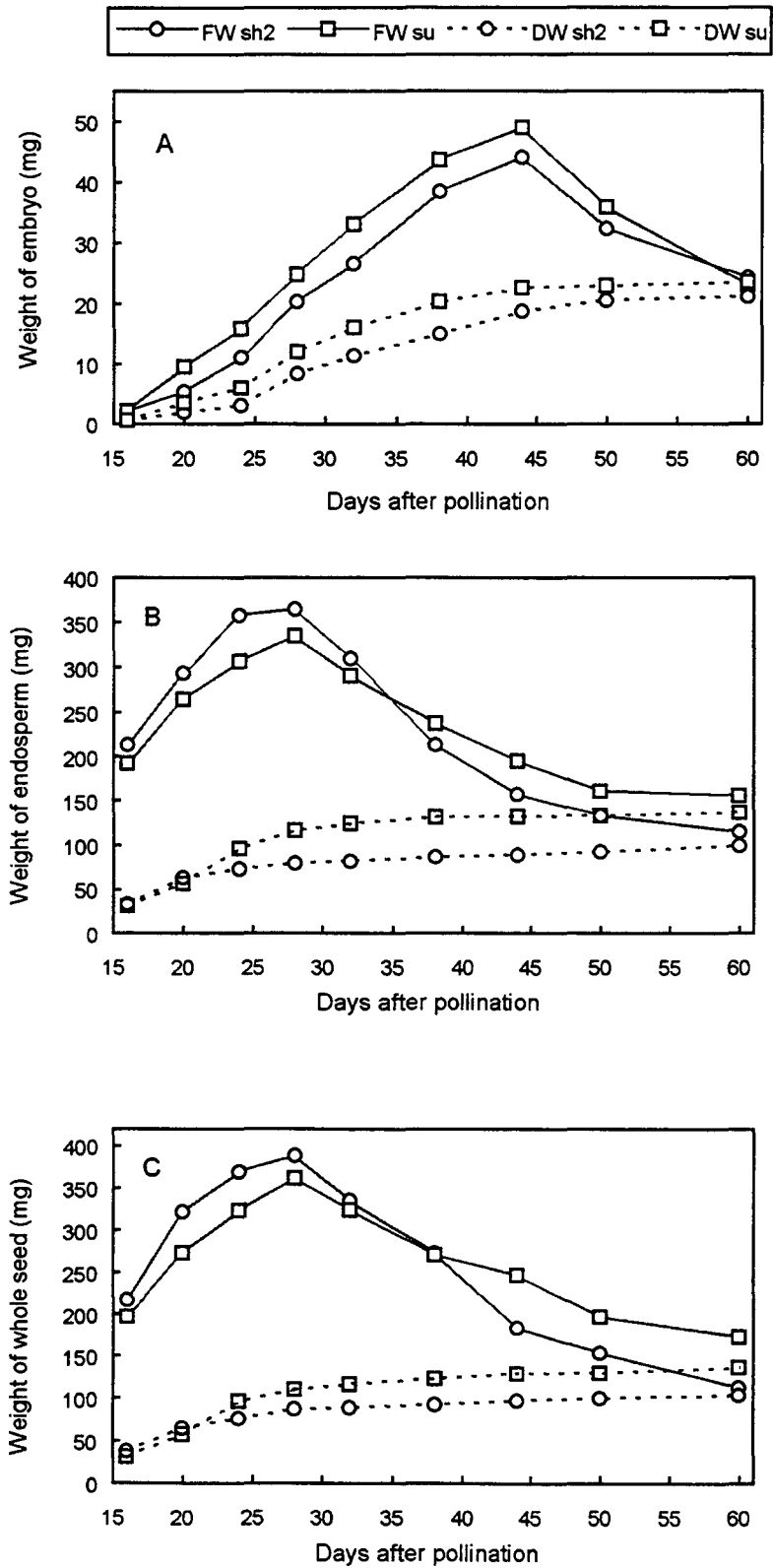


Figure 2. Changes in embryo (A), endosperm (B) and whole-seed (C) fresh weight (FW), dry weight (DW) of isogenic lines Ia5125 *sh2* and Ia5125 *su* during development.

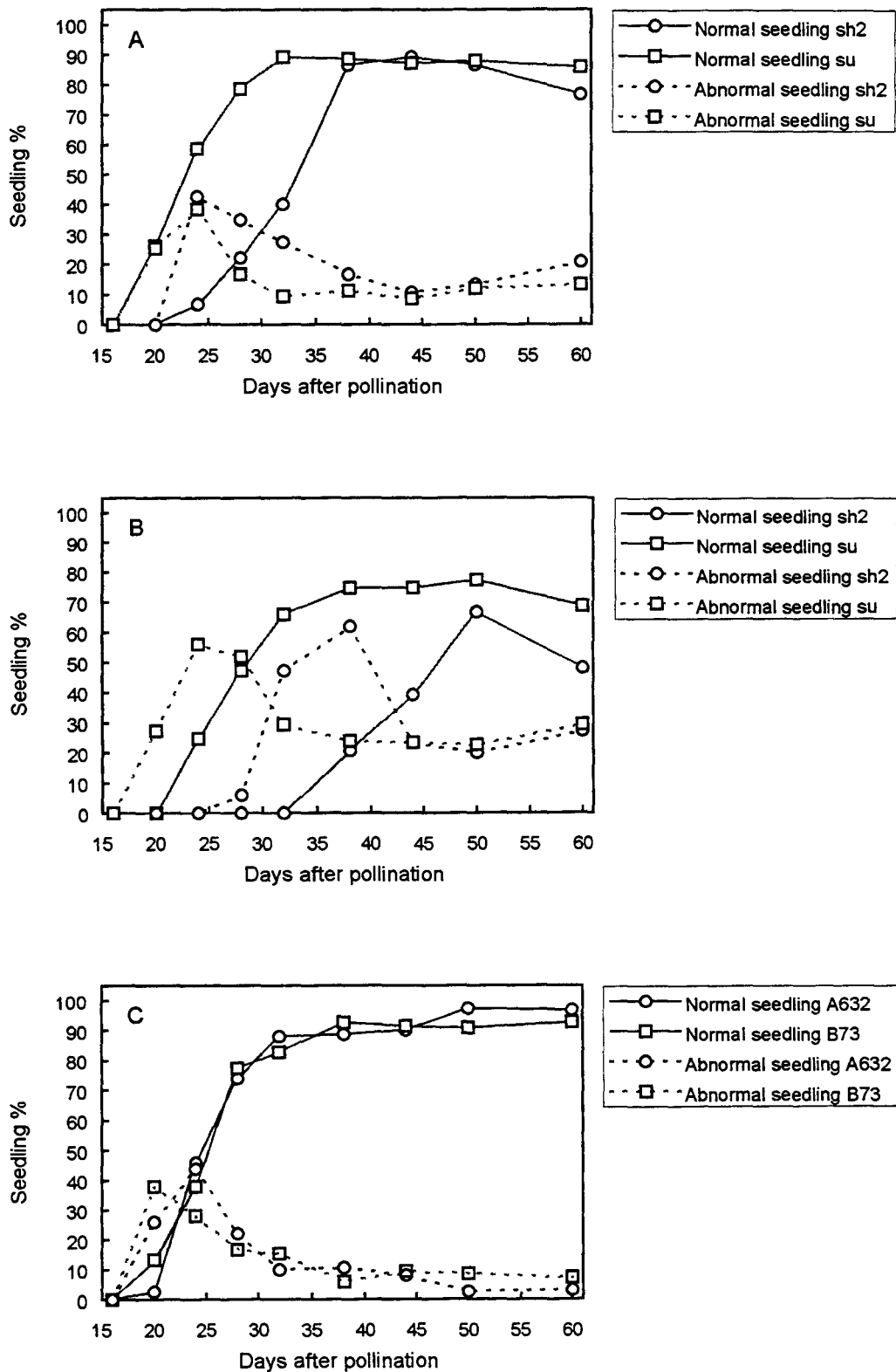


Figure 3. Germinability of seeds during their development. A. Isogenic lines: IA453 *sh2* and Ia5125 *su*, B. Isogenic lines IA5125 *sh2* and Ia5125 *su*, C. Field corn lines: A632 and B73.

Table 1. Effects of coating treatment on seed germination and seedling growth of inbred “WH9261”

Treatment	Warm Germination			Cold Test		
	Normal Seedling%	Shoot Dry Weight mg/seedling	Root Dry Weight mg/seedling	Normal Seedling%	Shoot Dry Weight mg/seedling	Root Dry Weight mg/seedling
Control	83.3	25.9	8.7	54.7	25.4	5.5
NAA 0.1 ppm	88.7	28.6**	9.2	56.6	26.2	5.4
NAA 0.5 ppm	90.0*	26.5	9.5*	51.3	24.5	4.7
GAs 25 ppm	84.0	26.5	8.6	58.7	24.8	5.0
GAs 50 ppm	88.0	29.2***	8.4	57.3	26.2	6.5***
GAs 100 ppm	83.3	29.8***	8.1	60.7*	26.2	6.0*
GAs 250 ppm	86.7	25.7	8.1	56.7	27.3***	5.7
epiBR 0.01 ppm	83.3	27.0	8.6	48.0*	27.5***	6.1*
epiBR 0.1 ppm	86.7	26.0	8.7	65.3***	26.4*	6.3**
Malate 500 ppm	84.0	26.4	8.9	54.0	25.5	5.5
Malate 1000 ppm	87.3	27.2	8.9	54.0	25.3	5.8
Malate 2500 ppm	84.7	26.4	8.6	44.7**	25.7	5.9
Malate 5000 ppm	83.3	26.2	8.1	46.7**	27.6***	5.5
Citrate 500 ppm	75.3**	26.8	8.2	43.3***	25.4	5.2
Citrate 1000 ppm	81.3	26.1	8.5	56.7	24.9	5.6
Citrate 2500 ppm	83.3	26.9	8.3	62.0*	25.4	5.6
Citrate 5000 ppm	81.3	27.3	8.2	59.3	24.4	5.3

*, ** and *** Treatment showed significant difference from control after coating at P < 0.05, P < 0.01 and P < 0.001 respectively, by the LSD test.

Table 2. Effects of coating treatment on seed germination and seedling growth of inbred “WH2”

Treatment	Warm Germination			Soil Free Cold Test		
	Normal Seedling %	Shoot Dry Weight mg/seedling	Root Dry Weight mg/seedling	Normal Seedling %	Shoot Dry Weight mg/seedling	Root Dry Weight mg/seedling
Control	70.7	17.2	5.6	64.7	20.0	6.2
NAA 0.1 ppm	66.0	17.9	5.6	66.0	20.7	6.3
NAA 0.5 ppm	72.0	18.3*	6.4**	66.7	19.7	6.7
GAs 25 ppm	71.3	17.3	5.6	62.0	20.9	6.4
GAs 50 ppm	74.0	17.4	5.5	58.0*	20.5	5.0***
GAs 100 ppm	64.3**	17.2	5.4	47.3***	19.6	4.8***
GAs 250 ppm	61.3***	16.0**	5.2	37.3***	21.4*	4.6***
epiBR 0.01 ppm	72.0	17.1	5.4	67.3	21.8**	6.0
epiBR 0.1 ppm	75.3	17.0	5.8	74.0**	21.5*	6.2
Malate 500 ppm	70.7	17.4	5.5	62.0	20.4	6.3
Malate 1000 ppm	73.3	18.5**	6.1	67.3	20.4	6.4
Malate 2500 ppm	70.7	16.7	5.9	62.7	20.3	6.1
Malate 5000 ppm	70.7	16.6	5.9	71.3*	20.5	6.1
Citrate 500 ppm	72.0	16.9	6.7***	64.0	20.9	6.3
Citrate 1000 ppm	71.3	17.4	5.6	67.3	21.5*	6.3
Citrate 2500 ppm	71.3	17.1	5.7	62.0	20.3	5.9
Citrate 5000 ppm	70.0	17.4	5.1*	62.0	20.6	5.8

*, ** and *** Treatment showed significant difference from control after coating at P < 0.05, P < 0.01 and P < 0.001 respectively, by the LSD test.

INFLUENCE OF THE AERATED HYDRATION SEED INVIGORATION TREATMENT ON THE RESPONSE OF BRASSICA SEED TO STORAGE

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ABSTRACT

The effect of periods of aerated hydration (AH) at 20°C on Brussels sprouts and cauliflower seeds differing in seed quality due to ageing and on their subsequent storage potential was examined during both rapid ageing and more conventional storage conditions. An 8h AH treatment of unaged and aged seeds of both species had little effect on laboratory germination, but an increase in K_1 indicated improved seed quality. Improved seed longevity after AH treatment, particularly for aged seeds, was observed during storage at 20% seed mc and 45°C. AH treatment of two further Brussels sprout cultivars resulted in reduced MGT of both unaged and aged seeds and this increase in germination rate was retained during 8 weeks storage at 20°C and 4.8-6.2% mc. The vigour of aged seeds, revealed by the controlled deterioration (CD) test, was also enhanced by AH with this improvement maintained during storage. The influence of the length of AH was examined for unaged and aged cauliflower seeds stored at 10°C and either 5.3% or 12% mc for 3 months. Both 12 and 28h AH increased the rate of germination of aged and unaged seeds, and the retention of this improvement during storage was particularly evident in the aged seeds. The low vigour of untreated aged seeds was improved by 12 and 28h AH to a level similar to that of the high vigour unaged seeds and this increased vigour was retained after storage at 5.3% mc. Both the aged untreated and treated seeds showed a reduction in vigour when stored at 12% mc, but the AH treated seeds retained a higher CD germination. Prolonged hydration (28h AH) of high vigour unaged seeds resulted in reduced vigour which fell further during storage.

INTRODUCTION

The use and potential of physiological seed treatments which involve seed hydration is well recognised and a range of methodologies have been developed for these so-called invigoration treatments. These include osmoconditioning eg. priming in polyethylene glycol (PEG) or salt solutions, matricconditioning and aerated hydration (3,10,12,15). All treatments aim to allow controlled hydration of the seed to a moisture content close to, but below, that at which radicle emergence occurs, before drying the seed to a moisture content whereby it can be easily handled and/or stored. Such seed treatments result in more rapid and uniform germination, and increased seed vigour and seedling growth (3,10,12,15) with greater improvements commonly seen in medium or low vigour seeds, that is, seeds which are partially deteriorated, than in high vigour seeds (4,9,13,17,22).

There are however conflicting reports of the response of seeds to storage following an invigoration treatment. The response of carrot and tomato seeds to storage after a hydration treatment depended on the severity of the storage conditions and therefore rate of deterioration (1,2,6,7,13) and the type of hydration treatment influenced the response of the same species to poor storage conditions (1,6,17,21). The length of the hydration period also appears to be important in determining response to storage. Primed leek seed stored in conventional conditions showed an increase in the percentage of abnormal seedlings after 100d storage (16), with a greater increase in abnormal following longer priming treatments. Similarly wheat seeds given a 20h PEG treatment deteriorated more rapidly than seeds given a 2h hydration treatment (18). Work on tomato (20) has suggested that there is also an interaction between the length of the hydration period and initial seed quality in determining seed response to storage.

Increased rates of germination, root growth and vigour after the aerated hydration treatment have been reported for *Brassica* seed (22,23) but the response of treated seeds to storage has not been investigated. The aim of this work was to examine the response of *Brassica* seed to storage after aerated hydration. A number of comparisons were made, namely the response of treated seeds to rapid ageing compared with more conventional storage conditions, the effect of initial seed quality on response to hydration treatment and subsequent storage, and the effect of a short and a prolonged aerated hydration on seed longevity in store.

MATERIALS AND METHODS

Seeds of Brussels sprouts (*Brassica oleracea* var *gemmifera*, cv. Asmer Aries) and cauliflower (*Brassica oleracea* var *botrytis*, cv Hipop) were obtained from Asmer Seeds, Leicester. Two further cultivars of Brussels sprouts (Content, Talent) and one of cauliflower (Alpha 7 Jubro) were obtained from Nickersons Seeds, Rothwell, Lincolnshire. Seeds of each cultivar that retained high germination but had reduced vigour were produced by a period of ageing at 20% seed moisture content (mc) and 45°C using the controlled deterioration method (14). The ageing periods, determined in preliminary experiments, were 12h (Content and Talent), 24h (Asmer Aries), 30h (Hipop) and 32h (Alpha 7 Jubro).

Seed moisture content (mc) was determined by drying 4 replicates of 25 seeds at 130°C for 1h(11) and expressing the weight loss as a percentage of seed air dry weight.

Seed germination was tested on 4 replicates of 25 seeds set to germinate in petri dishes containing germination papers (Papier fabriek Schut bv, Heelsum, Holland) to which 4ml water were added. The petri dishes were placed on a tray, enclosed in a polythene bag and held at $20 \pm 1^\circ\text{C}$ for 10d. Germination, defined as the appearance of the radicle, was counted daily and at the final count, the proportion of normal and abnormal seedlings (11) was assessed. The mean germination time (MGT) was calculated using the method of Nichols and Heydecker (19) as follows:

$$MGT = \frac{\sum (fx)}{\sum f}$$

where f is the number of seeds newly germinated after x days and x is the number of days after the seeds were set to germinate.

Seed vigour was assessed by the controlled deterioration (CD) vigour test (14) carried out at 20% mc and 45°C. Seed moisture content was raised by placing a weighed sample of seed of known moisture content onto a germination paper to which 4ml water was added. Seeds were allowed to imbibe until they reached the desired moisture content. The weight of seeds at the desired moisture content was calculated as:

$$\text{Weight at desired mc} = \frac{(100 - \text{initial mc}) \times \text{initial seed weight}}{(100 - \text{desired mc})}$$

Frequent weighing determined when the raised moisture content was reached. Seeds were then immediately sealed into an aluminium foil packet (W. Coles and Co., London) and placed in a refrigerator (<10°C) overnight to allow moisture equilibration through the seed. The seeds were then placed in a water bath at 45°C for 24h, after which a germination test was set up as described above. In this case, germination was counted after 10d and any seed that produced a radicle was counted as having germinated.

Aerated hydration treatment was given to both unaged and aged seeds at 20°C in 50cm long perspex columns (5cm diameter) holding 500ml distilled water, each column being aerated by a continuous supply of air from an aquarium pump (Rena 'Pixie', RenaFrance, Annecy, France) at a rate of 1.51 min^{-1} (22,23). The hydration columns were held in a growth cabinet (Fisons, model 600G/3TTL, Fisons Scientific Apparatus, Loughborough, UK) both before treatment, to ensure that the water was at 20°C before addition of the seeds, and throughout the treatment period. Seeds were allowed to dry back after hydration to within 1% of their initial moisture content by leaving them overnight in open petri dishes at 20°C. The attainment of the dried back moisture content was in

most cases determined by reweighing the seed. Previous work comparing the moisture contents calculated after reweighing with those determined by the oven drying method has found these to be within 0.5% of each other. Unaged and aged seeds, both untreated and after an aerated hydration treatment were stored in foil packets either at their initial air dry moisture contents or at a raised moisture content, achieved as described in the CD test. After the period of moisture equilibration, the packets of seeds for storage were placed at their storage temperature; a further packet was used as the unstored (control) sample.

Three storage experiments were conducted. Asmer Aries (Brussels sprouts) and Hipop (cauliflower) were stored in conditions that would achieve very rapid deterioration, namely 20% mc at 45°C in a water bath for 32h. The Brussels sprout cultivars Content and Talent were stored at their initial moisture contents (4.8-5.5%; 5.5-6.2% respectively) in an incubator at 20°C for 8 weeks. Alpha 7 Jubro (cauliflower) seeds were held in an incubator at both their initial moisture content (5.3%) and 12% mc at 10°C for 3 months. Samples were taken at different times during storage. Each sample of seeds having a raised moisture content, including the control, was allowed to dry back to close to its initial moisture content before any further assessments were carried out.

RESULTS AND DISCUSSION

Storage of unaged and aged untreated seeds of Brussels sprout cv Asmer Aries and cauliflower cv Hipop at 20% mc and 45°C for 32h resulted in a decline in germination (Table 1). Germination of the untreated, aged seeds of Hipop decreased the most rapidly, probably because their lower initial germination indicated that the unstored seeds were at a more advanced stage of deterioration than other seeds. During storage of Aries, the unaged seeds given an 8h aerated hydration (AH) treatment before storage retained a similar germination to the untreated seeds (Table 1), whereas the germination of unaged Hipop seeds given 8h AH treatment was about 10% higher than that of the untreated seeds at each sampling time. In contrast the AH treated aged seeds of both Aries and Hipop deteriorated more slowly than the untreated seeds. Thus for Aries, after 32h storage, AH treated seeds had a germination of 62% compared with 1% for the untreated seeds. Aged seeds of Hipop showed comparable improvements although after 32h storage, germination was lower than that for Aries. Thus an aerated hydration treatment increased the longevity of seeds during a period of rapid ageing in poor storage conditions, particularly in seeds that had previously undergone a period of ageing.

The germination of Aries and Hipop was also converted to probits to allow calculation of the regression of probit germination against time and derivation of the K_i or probit initial germination (8). This revealed that K_i increased after AH in both varieties (Table 2) indicating that the initial seed quality had been increased by the AH treatment. The failure of the laboratory germination to reveal this improvement emphasises that K_i gives a more accurate prediction of initial germination than does the laboratory germination test (8). The increase in K_i also suggested that the repair of accumulated damage had occurred during the hydration treatment. The similar rates of deterioration (ie decline in probit germination per unit of time) of the untreated and AH treated unaged seeds of each variety (Table 2) agreed with the prediction that under the same storage conditions, all seeds of one species will deteriorate at the same rate (8). In contrast the aged untreated seeds deteriorated far more rapidly, although after AH treatment the rates of deterioration were similar to those of the unaged seeds.

A second experiment examined whether improved storage potential was also evident at earlier stages of deterioration, ie. before germination declined, and whether storage potential was improved under more conventional storage conditions. Two cultivars of Brussels sprouts, Content and Talent, were stored with and without 8h AH treatment at their initial moisture content for 8 weeks. The germination of all seeds remained above 90% (Content) and 85% (Talent) after 8 weeks storage (data not presented), but there was a clear effect of AH treatment on MGT and vigour both before and after storage (Table 3).

The MGT of unaged seeds of both cultivars was reduced by AH treatment before storage (Table 3) and this improvement was maintained throughout the storage period. Similar improvements and their retention during storage were evident in aged seeds, although the higher MGT values reflected their more deteriorated status.

The unaged seeds of Consort had high vigour before storage (Table 3), as revealed by their high germination after CD and there was little effect of AH either before or during storage. In contrast the low vigour of the unstored

aged seeds of Content increased after AH treatment and the improvement in vigour was maintained during storage even though the vigour of untreated seeds declined. The vigour of both the unaged and aged seeds of the cultivar Talent was much lower than that of Content (Table 3). Aerated hydration treatment gave a small increase in vigour of all Talent seeds which was retained during storage, although the vigour of both the untreated and treated aged seeds remained low.

Improvements seen in the performance of unstored seeds following aerated hydration were therefore retained during subsequent storage resulting in improved longevity both in conditions that induced rapid ageing (Table 1) and in more conventional storage conditions (Table 3). There was no evidence of reduced storage potential following aerated hydration. This contrasts with carrot and tomato where although improvements in rate of germination after priming were retained during storage at 10°C and 20°C (2,7), primed seeds deteriorated more rapidly during accelerated ageing (6) or controlled deterioration (2). The greatest response to AH treatment was in medium vigour seed confirming previous work that has suggested that improvements can only occur in partially deteriorated seed (4,9,13,17,22) in which there is capacity for improvement. Little improvement was achieved in the present work if seed had very high vigour or where extensive deterioration had produced very low vigour seeds, an observation also made by Goldsworthy et al (9) in wheat.

The influence of the length of the hydration period on seed storage potential was examined using unaged and aged seeds of cauliflower (cv Alpha 7 Jubro) given either 12 or 28h AH before storage. Aerated hydration had little effect on the laboratory germination of seeds before storage and seed retained >85% germination after 3 months storage at 10°C either at their initial moisture content or at 12% mc (data not presented). The MGT of both unaged and aged seeds was reduced by AH treatment before storage (Table 4). In unaged seeds this improvement was evident only after 12h AH whereas aged seeds showed a further reduction in MGT after 28h AH. There was little change in the MGT of either unaged or aged seeds following storage at the initial seed mc for 3 months (Table 4). The same was true for unaged seeds stored at 12% mc. The marked increase in MGT seen in untreated aged seeds stored at 12% mc did not occur if the aged seeds had received 12h or 28h AH before storage. Differences in the response of these cauliflower seeds to AH and storage were more clearly evident in the vigour of the seeds measured as germination after CD. The initial seed vigour of unaged seeds was high, whereas the aged seeds had medium to low vigour (Table 4). Aerated hydration of the high vigour unaged seeds for 12h had little effect, but after 28h AH seed vigour had fallen. In contrast, both 12h and 28h AH increased the initially lower vigour of the aged seeds to a level comparable with that of unaged seeds. Similar differences in response to prolonged hydration have been observed in Brussels sprouts (23) where the vigour of unaged seeds declined after more than 16h AH whereas the vigour of aged seeds was still increased after 32h treatment.

The unaged, untreated seeds retained high vigour during storage at both moisture contents (Table 4). Unaged seeds given 12h AH also showed little deterioration, whereas after 28h AH vigour declined during storage, a greater fall occurring at 12% mc. High vigour tomato seeds were also found to deteriorate rapidly after prolonged hydration for 48h (20), whereas improvements achieved after 24h hydration were retained during storage. The medium to low vigour of aged, untreated seeds decreased further during storage at their initial mc, whereas the treated seeds retained the high vigour achieved after AH treatment (Table 4). There was a decline in the vigour of the treated aged seeds after storage at 12% mc, but even so, the AH treated seeds retained higher vigour than untreated seeds.

Thus in a second species we have seen that seeds given aerated hydration exhibit better storage potential than untreated seeds, provided that a prolonged treatment has not been given to high vigour seeds. The consistent observation in both Brussels sprouts and cauliflower that improvements in MGT and vigour occurred only in partially deteriorated seeds supports the hypothesis that the metabolic repair of previously sustained deterioration contributes to the improvements achieved by hydration treatments (5,22,23).

The deleterious effect of prolonged aerated hydration on high vigour seeds both before and during storage may result from the embryo axis advancing during treatment to a stage that is closer to germination than the more slowly germinating low vigour seeds. The more rapid deterioration of seeds primed in KNO₃ compared with PEG-primed seeds was also explained by their more advanced germination (1). It may be that at this advanced stage the seeds cannot tolerate desiccation and are damaged. This damage may not influence their ability to germinate, but may increase their sensitivity to the stress of storage, particularly in conditions that induce rapid ageing.

The interaction of metabolic repair and advancement of germination during prolonged hydration treatments may therefore help to explain the conflicting reports of the storage potential of seeds given invigoration treatments. If a treatment allows advancement beyond a certain point of embryo development during germination, even if the radicle has not emerged, these seeds may show reduced storage potential.

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Table 1. Changes in the laboratory germination (total germination) of unaged and aged seeds of Brussels sprouts (cv Asmer Aries) and cauliflower (Hipop) during storage at 20% mc, 45°C with (AH) or without (UT) an 8h pre-storage aerated hydration treatment

Storage time (h)	Asmer Aries				Hipop			
	Unaged		Aged		Unaged		Aged	
	UT	AH	UT	AH	UT	AH	UT	AH
0	96	96	91	92	96	96	74	77
12	89	92	48	92	89	98	29	66
24	72	90	14	65	79	89	10	47
32	58	62	1	62	62	79	1	25

Table 2. Effect of 8h aerated hydration of unaged and aged seeds on the K_i and on $1/\sigma$ during subsequent storage at 20% seed mc and 45°C.

	Asmer Aries				Hipop			
	Unaged		Aged		Unaged		Aged	
	UT	AH	UT	AH	UT	AH	UT	AH
K_i	6.75	7.25	6.2	6.67	6.75	7.28	5.67	5.99
$1/\sigma$	0.050	0.051	0.095	0.047	0.043	0.049	0.095	0.043

Table 3. Response of unaged and aged seed from two cultivars of Brussels sprouts to storage at their initial seed moisture content and 20°C for 8 weeks. Seeds were stored either untreated (UT) or after 8h aerated hydration (AH)

Storage time (weeks)	Content				Talent			
	<u>Unaged</u>		<u>Aged</u>		<u>Unaged</u>		<u>Aged</u>	
	UT	AH	UT	AH	UT	AH	UT	AH
<i>Mean germination time (d)</i>								
0	2.00	1.59	2.84	2.39	2.12	2.03	2.99	2.25
2	2.03	1.81	3.28	2.62	1.90	1.48	2.52	2.13
4	-	2.02	3.45	3.15	1.90	1.69	2.73	2.17
6	2.45	2.21	3.89	3.10	2.25	2.09	3.35	2.40
8	2.17	1.52	3.66	2.90	2.39	2.19	3.25	2.80
<i>Germination (%) after Controlled Deterioration</i>								
0	88	83	44	68	65	72	28	41
2	89	90	27	67	-	-	-	-
4	84	93	34	76	57	70	21	36
6	81	89	19	58	27	36	1	22
8	87	91	34	61	44	58	8	17

Table 4. Effect of storage of unaged and aged seeds of cauliflower cv Alpha 7 Jubro at two seed moisture contents at 10°C, following aerated hydration for 12 (12 AH) or 28 (28 AH) hours.

Seed mc	Storage time (mo.)	Unaged			Aged		
		UT	12AH	28AH	UT	12AH	28AH
<i>Mean germination time (d)</i>							
Initial	0	2.22	2.27	2.02	3.44	2.92	2.27
	3	2.02	2.02	1.72	3.15	2.85	2.18
12%	0	2.12	2.12	2.10	3.37	2.70	2.29
	3	2.09	1.98	1.96	5.53	3.01	2.18
<i>Germination (%) after Controlled Deterioration</i>							
Initial	0	87	90	66	44	77	81
	3	93	92	48	32	88	78
12%	0	92	94	52	42	81	82
	3	94	88	14	15	66	57

HYDROTHERMAL TIME AND CELL CYCLE ACTIVITY DURING PRIMING OF TOMATO SEEDS

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ABSTRACT

During seed priming, metabolic processes are activated that subsequently result in more rapid completion of germination. The temperature, water potential, and duration of treatment are the key factors manipulated during priming. We have developed a hydrothermal priming time model to integrate these factors and rationalize the selection of priming treatments. Five tomato seed lots were primed at two temperatures, three water potentials, and six durations. Water potential and duration effects on germination rates following priming could be accounted for in all lots by a hydropriming time model. For most lots, the effect of temperature could also be included as thermal time, but some lots showed relatively little response to priming temperature. Overall, the accumulation of hydrothermal priming time during the treatment was linearly related to the subsequent germination rate, and a general model accounting for over half of the variation in germination rate responses to priming was developed. We also examined whether cell cycle activity in the embryonic meristem increased in proportion to accumulated hydrothermal priming time. While cell cycle activity and an increase in nuclei having a 4C DNA content occurred in some seed lots during priming, an increase in nuclear DNA content was not consistently associated with more rapid germination in all lots. Thus, initiation of the cell cycle may occur during priming, but is not required for advancement of germination rates in all genotypes.

INTRODUCTION

Seed priming is a technique of hydrating seeds sufficiently to allow activation of pre-germinative metabolism, but preventing radicle emergence. After holding the seeds under these conditions for a period of time, they can be dried and will subsequently exhibit more rapid and uniform germination when planted. The critical parameters controlled and manipulated during priming treatments are the water potential (ψ), temperature (T) and duration. We have previously developed a simple model to relate the duration, ψ and T of seed priming to subsequent germination rates (Tarquis and Bradford, 1992; Haigh and Bradford, 1994). The model is similar to a thermal time or degree-days model, where the amount by which the ψ or T exceeds the minimum ψ (termed ψ_{min}) or T (termed T_{min}) at which advancement will occur is multiplied by the duration of treatment:

$$\text{Hydrothermal priming time (MPa}^\circ\text{h)} = (\psi - \psi_{min})(T - T_{min}) t_p, \quad (1)$$

where t_p is the duration of priming treatment. This equation should normalize priming treatments across different T and ψ values and express them in terms of the accumulated hydrothermal priming time that the seeds have experienced. The hypothesis is that median germination rates (GR_{50} , or the inverse of the median time to germination) are directly related to the accumulated hydrothermal priming time. That is, regardless of the specific ψ or T (so long as they are above ψ_{min} and T_{min}), GR_{50} should increase in direct proportion to the accumulation of hydrothermal priming time calculated according to Equation 1. This would allow treatments at different ψ or T to be compared on the same time scale and predictions to be made about how seeds would respond to different conditions. We report here results from investigations on five tomato seed lots to test whether the hydrothermal priming time model accounts for the effects of a range of priming treatments on subsequent germination rates.

Cell cycle advancement of radicle meristem cells has been reported to be one of the physiological effects of seed priming possibly related to the germination advancement effect (Bino et al., 1992, 1993; Lanteri et al., 1993, 1994; Saracco et al., 1995). Meristematic cells progress through the cell cycle to replicate their nuclear DNA and create new daughter cells (Deltour, 1985). In the cell cycle, an initial quiescent, or "gap" phase (G_1) is followed by a DNA synthetic phase (S phase) during which the genome is duplicated. This in turn is followed by a second gap phase (G_2) prior to M phase, where mitosis and the separation into two daughter cells takes place. The proportions of cells within the G_1 , S and G_2 phases of the cell division cycle can be empirically defined through measurement of the nuclear DNA content and its relationship to the haploid genome size.

Flow cytometry is a convenient method to determine nuclear DNA contents or cell ploidy. In a flow cytometer, isolated nuclei are passed through a capillary to create a fine stream of individual particles. The DNA is stained with a dye that absorbs incident ultraviolet light and fluoresces at a visible wavelength. As the nuclei pass through the incident beam, the fluorescence signal intensity is measured as the nuclei are counted, giving a frequency diagram of the number of nuclei having specific DNA contents. The quantity of DNA corresponding to the diploid complement is represented as 2C. During the cell cycle, nuclear DNA content in a fraction of the cells will increase during S phase to the 4C DNA amount. During seed development, the cell cycle arrests in either G₁ or G₂, with 2C or 4C chromosome complements, respectively. The seeds of most species arrest cell division in the G₁ phase of the cell cycle, and thus most cells of the embryonic axis contain the 2C chromosome complement at maturity, although cells having 4C or higher DNA contents may also occur (Bino et al., 1993). The cell cycle must resume for seedling growth to occur, but species vary with respect to when the cell cycle is activated following imbibition (S. Gurusinghe and K. Bradford, unpublished results). During imbibition or priming of tomato and pepper seeds, DNA synthesis is initiated and the percentage of 4C nuclei in meristematic tissues increases (Bino et al., 1992; Lanteri et al., 1993, 1994; Baker and Bradford, 1995; Saracco et al., 1995; van Pijlen et al., 1996). Here we report experiments conducted to clarify the relationships among hydrothermal priming time, germination rates, and cell cycle activity in radicle meristem cells of primed tomato seeds.

MATERIALS AND METHODS

Priming treatments and hydrothermal priming time. Tomato seeds of cultivars Spectrum 579 (four lots), Nema 1435 (four lots) and Hypack 2409 (three lots) were generously provided by Dr. Sharon Kurtz of Petoseed. After initial characterization of germination time courses at a range of ρ , seeds of five selected lots were primed on filter paper in Petri dishes at either 15 or 20°C at -1.0, -1.5 or -2.0 MPa PEG 8000 for varying durations. The times of treatment were selected to give approximately equivalent accumulated hydrothermal priming times under each condition, assuming values of $T_{min} = 7^\circ\text{C}$ and $\rho_{min} = -2.5$ MPa in Equation 1. After treatment, rinsing and drying, the seeds were imbibed on water at 20°C and GR_{50} was determined for each lot from the complete germination time course. GR_{50} values were then plotted as a function of hydrothermal priming time (Eqn. 1), and the values of ρ_{min} and T_{min} were varied until the optimal regressions were obtained. For the five lots, similar values of $T_{min} = 9.1^\circ\text{C}$ and $\rho_{min} = -2.4$ MPa were obtained. These values were then used to calculate a common hydrothermal priming time scale. To account for inherent differences in germination rates among lots, the relative priming response was calculated as the GR_{50} after priming divided by the GR_{50} before priming, or the relative increase in germination rate due to priming.

Nuclear isolation and flow cytometry. For each sample, embryo radicle tips were excised and finely chopped with a new razor blade in nuclear isolation buffer [50 mM Tris-HCl, 1 mM MgCl₂, 0.1% Triton X-100, 2 mgL⁻¹ 4',6-diamidino-2-phenylindole (DAPI), pH 7.5]. After addition of 1.2 mL of DAPI buffer, the sample was passed through a nylon filter of 30 μm pore size and the DAPI-stained nuclei were immediately analyzed with a Partec CA II flow cytometer (Partec GmbH, Otto-Hahn Strasse 32, D-48161 Munster, Germany) equipped with an HBO 100 W/2 mercury arc lamp. KG-1, BG-38, and UG-1 filters were used for excitation, Tk-420 as dichroic mirror, and GG435 as a barrier filter. In each sample, approximately 5000 nuclei were counted at a rate of 20-50 nuclei/s. Data were electronically processed and displayed as pulse amplitude frequency distribution histograms and were analyzed using Multicycle software (Version 2.53, Phoenix Flow Systems, Inc., San Diego, Calif., USA). Debris such as cell clumps or other fluorescent particles were excluded from the peak analysis using an exponential model. Peaks of fluorescent particles (nuclei) showing Gaussian distribution were fitted by non-linear regression using a least squares method. The percentages of nuclei in the G₁, S and G₂ phases were calculated from the areas under the fitted curves. The relative change in percent nuclei in G₂ phase (those containing twice the normal amount of DNA, or 4C) was calculated as the percentage of nuclei in G₂ after priming divided by the percentage of nuclei in G₂ before priming.

RESULTS AND DISCUSSION

Detailed results for only one of the five lots will be presented, as similar relationships were found for all lots, although some lots responded more to priming temperature than others. As expected, a priming response (i.e., an increase in GR_{50}) occurred more rapidly at higher T and ρ (Fig. 1A,B). It took only about half as long to achieve a given GR_{50} at 20°C as it did at 15°C. Similarly, it took considerably longer for equivalent advancement

to occur as the ψ decreased. Using hydropriming time (Eqn. 1), the data at all ψ within each temperature could be combined on a common scale (Fig. 1C,D). GR_{50} increased linearly at all three ψ 's as hydrothermal priming time increased within each T . The estimated ψ_{min} was -2.4 MPa at 15°C and -2.15 MPa at 20°C. The slope of the regression line is greater at 20 than at 15°C, as would be expected at the warmer temperature. Data from both temperatures were then be combined on a hydrothermal priming time scale, using a T_{min} of 11°C (Fig. 1E). There was a highly significant relationship between accumulated hydrothermal priming time and subsequent seed germination rates ($R^2 = 0.77$), and the overall ψ_{min} for this lot was estimated to be -2.25 MPa. The average ψ_{min} and T_{min} over all five seed lots tested were -2.39 MPa and 9.1°C, respectively.

Using these mean values in Equation 1, data for germination rates of all five lots and all priming treatments were plotted on a common hydrothermal priming time scale (Fig. 2). As there were inherent differences in germination rates among lots even before priming, relative GR_{50} values (the increase in GR_{50} due to priming) are presented to emphasize only the advancement due to priming. The accumulation of hydrothermal priming time accounted for over half of the variation in relative priming response across all lots and priming treatments (Fig. 2). Thus, a reasonable estimate of expected germination rate response to priming for tomato seeds across a range of temperatures and water potentials can be obtained from Equation 1 using the common values of approximately 9°C for T_{min} and -2.4 MPa for ψ_{min} .

We had hypothesized that there might be a consistent relationship between the hydrothermal time for completion of germination before priming (at ψ 's where radicle emergence can occur) and the optimal conditions for priming at lower ψ 's where radicle emergence is prevented. Unfortunately, we found little correlation between the hydrothermal time parameters before priming and the hydrothermal priming requirements (data not shown). Germination time courses before priming were described well by a hydrothermal model, and the hydrothermal priming time model accounted well for priming responses, but there was little correlation between them (Cheng, 1996).

Previous experiments with two genotypes of tomato, PI341988 (a rapidly germinating, stress tolerant line) and T5 (a fresh market type), indicated that advancement of radicle meristem nuclei into the DNA synthesis cycle can occur during priming, although cell cycle activity alone was not the basis of reduced storage life of primed seeds (Baker, 1995; Baker and Bradford, 1995). Based on those two genotypes, an increase in 4C nuclei seemed to be consistently associated with an increase in germination rates following priming. We wished to test this relationship further by determining nuclear DNA contents in a number of cultivars under a wider range of priming conditions. We therefore measured nuclear DNA contents of radicle meristem cells in all of the priming treatments described above.

While priming resulted in increased germination rates in all cultivars (Fig. 2), not all cultivars exhibited nuclear DNA synthesis (Fig. 3). Both lots shown exhibited an increase in GR_{50} with increasing hydrothermal priming time (Fig. 3A,B). However, one lot also exhibited an increase in nuclear DNA content while the other did not (Fig. 3C,D). In the Spectrum 579-1 lot, the percentage of G_2 nuclei increased 2- to 4-fold over its initial percentage during priming (Fig. 3C). In Nema 1435-2 seeds, no increase in percentage of nuclei in G_2 occurred (Fig. 3D), even though germination rate increased during priming (Fig. 3B).

In conclusion, hydrothermal priming time is a useful model for understanding the influence of T and ψ on seed responses to priming conditions. For tomato seeds, minimum values of T_{min} (9°C) and ψ_{min} (-2.4 MPa) were defined as being the lowest at which germination advancement would occur. Germination rates following priming generally increased as the hydrothermal priming time accumulated according to the equation $(\psi + 2.4)(T - 9)t_p$, where ψ is the water potential of the priming solution, T is the temperature, and t_p is the duration of priming. This equation can be used to normalize treatments at different T or ψ , or to predict priming response to a specific treatment. A priming effect on germination rate can occur without initiation of DNA synthesis in the radicle meristem cells during the priming treatment. On the other hand, a high percentage of meristem cells in G_2 phase, when it occurs, is associated with rapid germination. Since genetic and/or physiological variation exists for entry into the cell cycle, it may be useful to determine whether particular aspects of seed vigor are associated with the tendency to initiate DNA synthesis in the radicle meristem prior to emergence.

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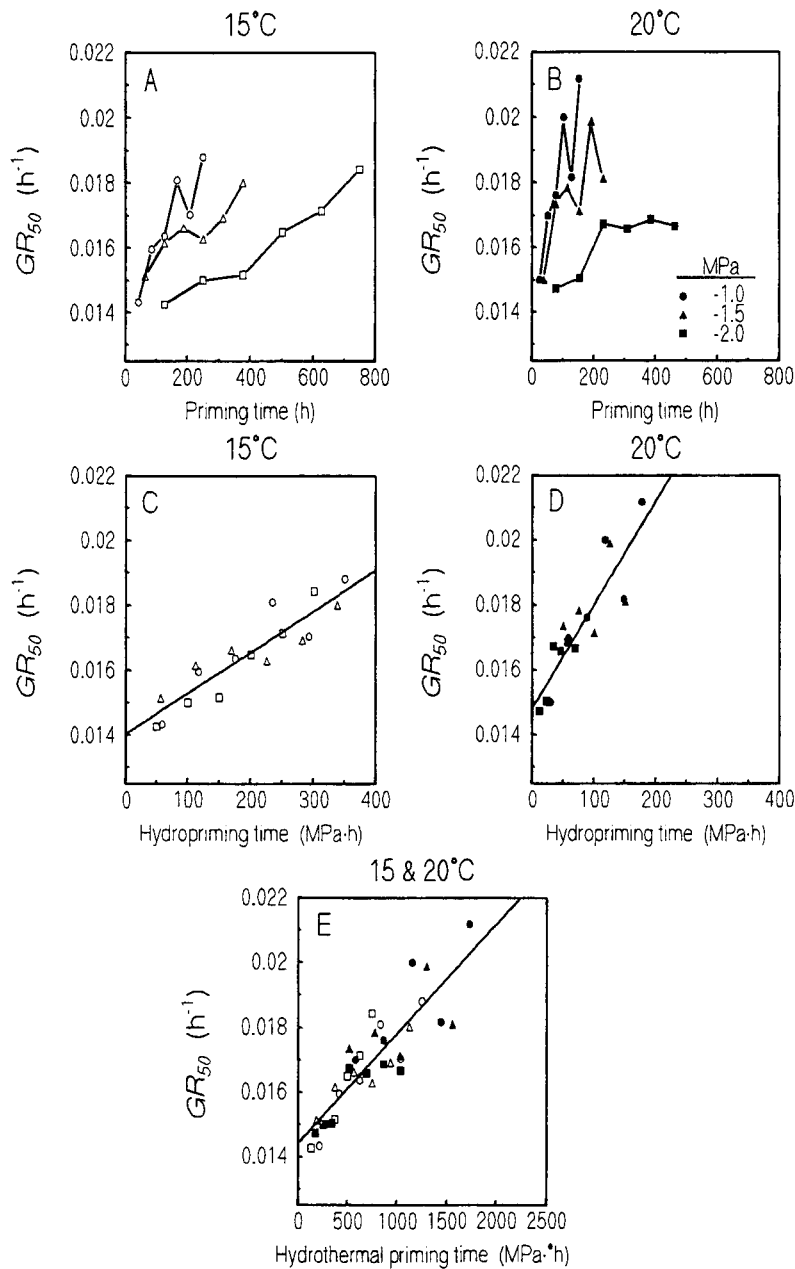


Figure 1.

Germination rates in response to priming treatments at three water potentials (ψ) and two temperatures. Tomato seeds (cv. Nema 1435 lot 2) were primed at -1.0, -1.5 and -2.0 MPa at either 15°C (A) or 20°C (B) and median germination rates (GR_{50}) were determined after drying and imbibing in water at 20°C. Within each temperature, ψ treatments were then combined on a common hydropriming time scale (C, D) using $\psi_{min} = -2.40$ MPa at 15°C ($R^2 = 0.83^{**}$) and $\psi_{min} = -2.15$ MPa at 20°C ($R^2 = 0.78^{**}$). Data from both T and all ψ were then combined on a common hydrothermal priming time scale (E), using $\psi_{min} = -2.25$ MPa and $T_{min} = 11^\circ\text{C}$ ($R^2 = 0.77^{**}$).

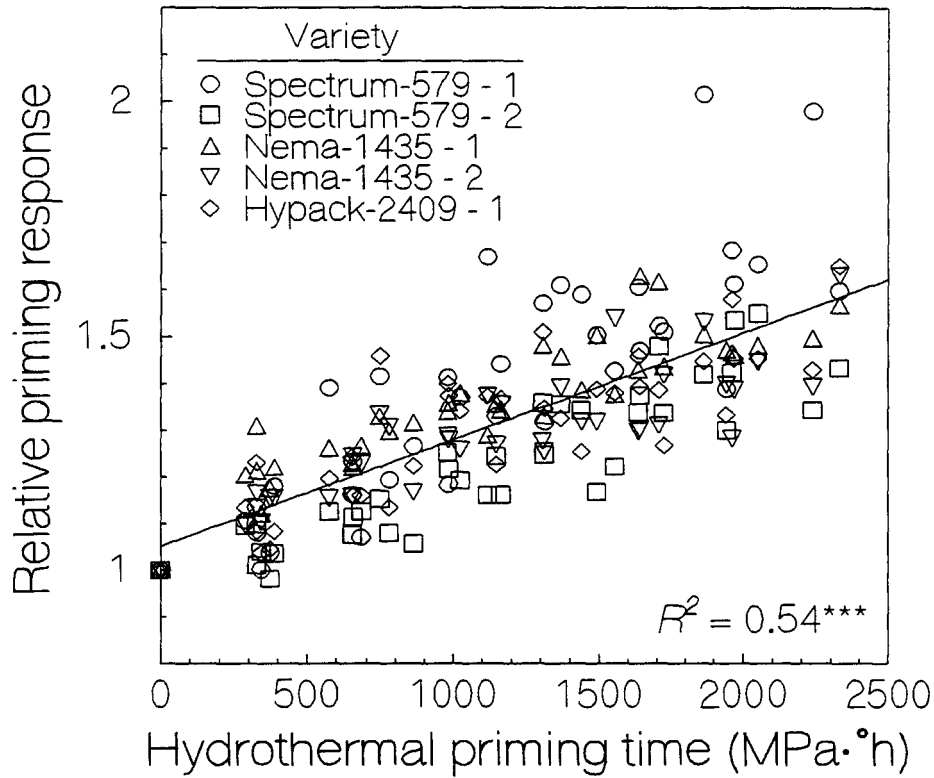


Figure 2.

Relationship between hydrothermal priming time and relative priming response for five tomato seed lots. Hydrothermal priming time for all lots was calculated according to Equation 1 using values of $T_{min} = 9.1^{\circ}\text{C}$ and $\psi_{min} = -2.39 \text{ MPa}$. Relative priming response is the median germination rate (GR_{50}) after priming divided by the GR_{50} before priming. For all five lots, relative priming response increased linearly with accumulated hydrothermal priming time at two temperatures (15 and 20°C) and three water potentials (-1.0, -1.5 and -2.0 MPa).

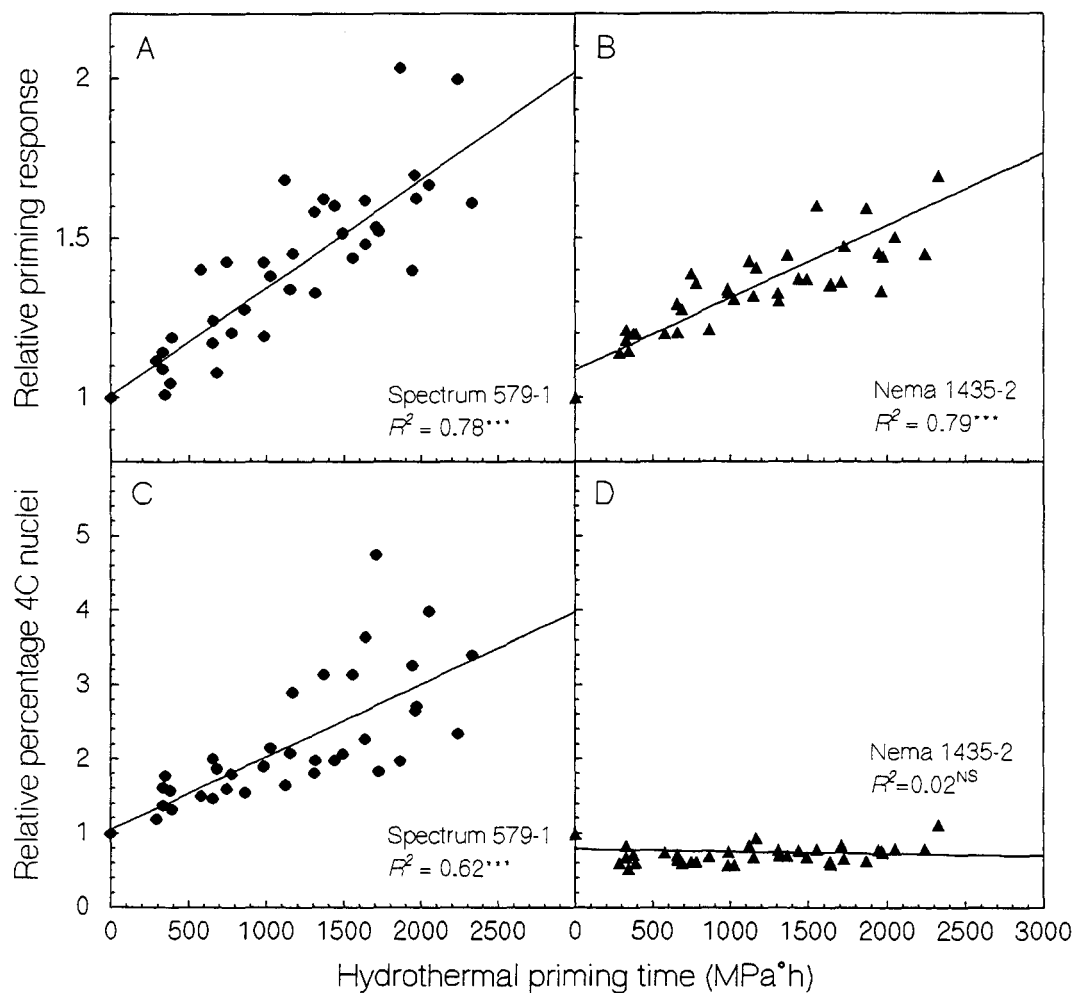


Figure 3.

Relative germination rate responses (A, B) and relative percentages of 4C nuclei in radicle meristem cells (C, D) in lots of Spectrum 579-1 and Nema 1435-2 tomato seeds. Relative priming responses are calculated as the median germination rate (GR_{50}) after priming divided by the GR_{50} before priming. Similarly, the relative percentage of 4C nuclei is the percentage after priming divided by the percentage before priming. The hydrothermal priming time is calculated according to Equation 1 using values of $T_{min} = 9.1^{\circ}\text{C}$ and $\psi_{min} = -2.4 \text{ MPa}$. The time scale normalizes priming treatments from two temperatures, three water potentials, and six durations for each lot. While both seed lots showed an increase in relative GR_{50} with increasing hydrothermal priming time, the relative percentage of 4C nuclei in the radicle meristem increased only in the Spectrum 579 lot.

DEHYDRATION OF PRIMING SEEDS CAN ALTER RATE OF SUBSEQUENT RADICLE EMERGENCE

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ABSTRACT

In seed priming, considerable attention has been paid to specific conditions present during imbibition. In contrast, drying procedures are rarely even reported. This research was conducted to evaluate the importance of drying rate following priming for several species. Seeds of carrot, pepper, tomato, and perennial ryegrass were imbibed in contact with water at 26°C for various periods. Seeds were next dried at -4, -40, -100, and/or -150 MPa for 4-168 h, then rehydrated. Germination (radicles emerged to at least 1 mm), solute leakage, and seedling growth were measured. Treatment differences in final germination percentage and seed vigor were generally not significant. Drying rate did not affect subsequent rate of radicle emergence for tomato seeds. For other seeds, the onset of radicle emergence was delayed and the rate of germination slowed when drying at -150 MPa occurred shortly before radicles would have emerged. However, slowed germination rates were not observed when drying was initiated earlier during germination. Delayed germination was also minimized when drying occurred at -4 MPa or when drying at -150 MPa was preceded by a sufficient period at -4MPa. When primed, dried seeds were sown into a 50:50 peat:perlite mixture in a greenhouse, drying rate affected emergence of carrot, pepper, and tomato seeds. Shoot growth was greater for seeds that had been dried at -4 MPa. These results indicate that drying procedures should be an important consideration in seed priming treatments.

INTRODUCTION

Seed priming, wherein germination is partially completed under controlled conditions prior to sowing, has received much attention both in the scientific literature as well as in attempted field applications. The vast majority of technical papers on this subject contain considerable detail on the specific conditions present during seed imbibition (e.g., temperature, water potential, nutrient and/or hormone additions). Rarely are drying procedures even reported, except to note that seeds were dried under "ambient" conditions. However, some recent studies dealing with desiccation tolerance of seeds indicate that the rate of drying can be a critical factor in determining the extent to which seeds tolerate desiccation (reviewed by Vertucci and Farrant, 1995).

The research reported here was conducted to evaluate the importance of drying rate for primed seeds. Data from both laboratory germination and greenhouse emergence experiments suggest that attention to drying procedures may be an important consideration in enhancing effectiveness of seed priming efforts.

MATERIALS AND METHODS

Seeds of 'DelRay' perennial ryegrass were donated by Northrup King Co., Minneapolis, Minnesota. Seeds of 'Floradade' and 'Sunny' tomato, 'Carospike' and 'Carobrite' carrot, and 'Marengo' and 'California Wonder' pepper were donated by Asgrow Seed, Gilroy, California.

For all priming treatments, three replicates of 50 seeds each were incubated on a single blue germination blotter (Anchor Paper, St. Paul, Minnesota) in a dark 25°C chamber. Blotters were saturated with distilled water by immersion. An additional 3 ml water was added and dishes were inclined at an angle of 8° to provide a continuous water reservoir in each dish (Allen et al., 1992).

Using this incubation method, preliminary experiments were conducted to determine the required priming duration for seeds of each seedlot. Four to eight hours was subtracted from the time radicle emergence first

occurred, which led to the following priming durations: 40 h for perennial ryegrass, 36 h for both tomato cultivars, 72 h for both carrot cultivars, 36 h for 'California Wonder' pepper, and 72 h for 'Marengo' pepper. Primed seeds were dried above saturated salt solutions at water potentials ranging from -4 MPa (mild dehydration) to -150 MPa (harsh dehydration) as described by Allen et al. (1992, 1993) and Debaene-Gill et al. (1994). Unless indicated, dehydration treatments lasted 72 h. In some treatments, seeds were initially dried at -4 MPa for 24 h, then dried at -150 MPa for 48 h. Based on the results, additional experiments were conducted with perennial ryegrass seeds to examine the effects of the following: 1) drying following shorter priming durations, 2) variable durations of mild drying preceding harsh drying, and 3) intermediate drying treatments. In addition, seed vigor determinations (electrolyte leakage, shoot and root growth of germinated seeds) of treated seeds were conducted. These results for perennial ryegrass seeds have been published previously (Debaene-Gill et al., 1994), but will be briefly summarized here.

Primed and dehydrated seeds (6 replications of 20 seeds each) of tomato, carrot, and pepper were sown at a depth of 0.5 cm into greenhouse flats containing a 50:50 peat:perlite mixture. Flats were watered as needed for four weeks following sowing. Greenhouse temperatures ranged from 19 to 36°C. Seedling emergence was measured daily for four weeks, at which time seedling heights were measured.

RESULTS AND DISCUSSION

For seeds subjected to priming treatments, specific conditions present during drying influenced subsequent seed performance. For example, when perennial ryegrass seeds were subjected to slow drying at high water potentials, germination typically required a total of 48 hours in contact with water, even when priming occurred over a series of hydration-dehydration cycles (Table 1). However, when primed seeds were dried at lower water potentials (-40 and -150 MPa), radicle emergence was delayed considerably (Debaene-Gill et al., 1994). For example, the hydration hours required for first radicle emergence increased from 40 hours for seeds dried at -4 MPa to 110 hydration hours for seeds dried at -150 MPa. Delayed germination following drying at low water potentials was negated by either of the following treatments: 1) drying initiated earlier during the germination process, or 2) drying at -4 MPa for 24 h prior to drying at -150 MPa. Slower germination due to rapid drying was more pronounced with increased dehydration durations. However, seeds exposed to -4 MPa for 24 h retained the ability to germinate rapidly following rehydration when stored for up to one week. Storage durations longer than one month led to variable results, probably due the shorter storage life of primed seeds (Tarquis and Bradford 1992).

Similar results were obtained with carrot (Carobrite) and pepper (both cultivars). Rapid dehydration of primed seeds resulted in delayed germination upon rehydration, which often completely eliminated the germination advancement associated with priming (Table 2).

In contrast, tomato seeds appeared to be largely unaffected by drying rate. Note that initially drying seeds at -4 MPa for 24 h did not consistently overcome the germination delay resulting from dehydration at -150 MPa. The choice of 24 hours at -4 MPa was based on data for perennial ryegrass seeds, and was not determined empirically for each seedlot in Table 2. Thus, more optimum drying treatments could probably be developed for these seedlots.

In general, greenhouse emergence of primed seeds was faster than controls if seeds were dried at -4 MPa prior to planting (Table 3). While dehydration at -4 MPa removed considerable water, it is important to note that this drying treatment did not return seeds to the water content of air dry seeds (see Figure 1 in Debaene et al., 1994). This water potential is associated with a relative humidity of approximately 97%. Seeds exposed to -150 MPa (relative humidity approximately 32%) return to air dry water contents in less than 24 hours. For seeds that require drying back to the original water content prior to sowing, exposure to lower water potentials would be required even if initial drying occurred at a slow rate.

Because germinating seeds lose desiccation tolerance approximately coincident with radicle emergence, we were interested in knowing whether vigor was being reduced by drying at -150 MPa. Measurements of electrolyte leakage and seedling growth failed to detect vigor loss in primed and dehydrated perennial ryegrass seeds (Debaene et al., 1994); however, vigor may have been affected in ways these indices do not detect. Several studies have shown that exposure to mild water stress can induce mechanisms that render plant

tissues more tolerant of heightened stress levels. A number of proteins induced by water stress (presumably with a protective function) have been found when germinated cereal seeds were exposed to slow drying (reviewed by Kermodé, 1990). The drying rate of primed seeds could determine the extent to which such protective mechanisms are able to function. For example, primed seeds dried under "ambient" Idaho conditions may show a significantly different response than the same seeds dried in the "ambient" Florida climate. The data presented here indicate that drying conditions should not be overlooked in developing seed priming treatments.

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Table 1. Hydration hours required for 90% of perennial ryegrass seeds to germinate when exposed to hydration-dehydration cycles.*

Hydration phase duration	Dehydration phase duration	Dehydration water potential	
• 4 MPa -10 MPa		h	
8	8	40	48
8	16	32	56
8	24	32	48
16	8	48	48
16	16	48	48
16	24	48	48
24	8	48	48
24	16	48	48
24	24	48	48

*Adapted from Allen et al., 1993

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Table 2. Hours to 90% germination for primed carrot, pepper, and tomato seeds following drying at various water potentials.

Treatment	Carrot		Pepper		Tomato	
	Carobrite	Carospike	California Wonder	Marengo	Floradade	Sunny
Control (unprimed)	156 (7.9)*	118 (0.6)	137 (12)	162 (5.0)	98 (2.8)	91 (2.5)
72h @ -4MPa	94 (1.9)	86 (8.1)	51 (1.6)	86 (8.6)	44 (0.2)	47 (0.1)
72h @ -150MPa	120 (8.4)	89 (0.5)	114 (3.7)	333 (54)	52 (3.8)	66 (1.3)
24h @ -4 MPa->						
48h @ -150MPa	118 (9.9)	72 (1.0)	70 (7.5)	204 (6.0)	47 (0.8)	47 (0.6)

*The standard error for each mean is indicated in parentheses

Table 3. Days to 90% greenhouse emergence for primed carrot, pepper, and tomato seeds following drying at various water potentials.

Treatment	<u>Carrot</u>		<u>Pepper</u>		<u>Tomato</u>	
	Carobrite	Carospike	California Wonder	Marengo	Floradade	Sunny
Control (unprimed)	11 (0.7)*	9 (0.2)	16 (1.3)	16 (0.8)	9 (0.6)	10 (0.4)
72h @ -4Mpa	10 (1.0)	10 (1.4)	10 (0.3)	11 (0.5)	5 (0)	5 (0.2)
72h @-150Mpa	14 (1.2)	9 (1.3)	11 (0.2)	13 (0.2)	10 (0.2)	10 (0.3)
24h @ -4 Mpa->						
48h @-150 Mpa	10 (0.2)	8 (0.2)	11 (0.7)	13 (0.3)	9 (0.5)	8 (0.2)

*The standard error for each mean is indicated in parentheses

Seeds dried at -150 MPa emerged before untreated controls for pepper, and for Sunny tomato if this treatment was preceded by 24h at -4 MPa.

Treatments that resulted in faster greenhouse emergence generally resulted in greater shoot growth as well (Table 4). This is most evident with the -4 MPa drying treatment.

Table 4. Height of greenhouse-grown seedlings (in mm) four weeks following priming and sowing. Treatments refer to water potential conditions during drying.

Treatment	<u>Carrot</u>		<u>Pepper</u>		<u>Tomato</u>	
	Carobrite	Carospike	California Wonder	Marengo	Floradade	Sunny
Control (unprimed)	47 (1.4)*	49 (0.9)	51 (0.9)	56 (2.0)	68 (2.2)	60 (1.7)
72h @ -4MPa	53 (1.3)	52 (1.5)	58 (1.3)	67 (2.1)	92 (2.8)	83 (1.9)
72h @ -150MPa	46 (1.8)	47 (0.5)	59 (1.4)	60 (1.3)	68 (2.2)	62 (2.1)
24 @ -4 Mpa-> 48h @ -150 Mpa	47 (1.1)	51 (1.1)	57 (1.6)	61 (1.6)	68 (1.1)	72 (2.3)

*The standard error for each mean is indicated in parentheses

EFFECTIVENESS OF *PSEUDOMONAS AUREOFACIENS* AB254 IN CONTROLLING *PYTHIUM ULTIMUM* ON TOMATO SEED

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ABSTRACT

In a world of increasing emphasis on IPM techniques, biologicals have the ability to show similar levels of control as their chemical counterparts and are becoming a viable alternative for disease control. In this experiment, processing tomato seeds (cv. 8245) were inoculated with *Pseudomonas aureofaciens* AB254 using two different techniques; coating and bio-osmopriming. Coatings were applied by harvesting the bacteria from plates, mixing them with methyl cellulose and coating the seeds with the resultant slurry. In bio-osmopriming the seeds are suspended in a salt-bacterial broth mixture for 1 week. At the end of this time the seeds are both osmoprimed and colonized by bacteria. These two techniques applied vastly different numbers of colony forming units/seed (cfu's/seed). Bio-osmopriming applies 10^5 cfu's/seed while coatings provide on the order of 10^8 cfu's/seed. Osmoprimed, bio-osmoprimed, osmoprimed coated, and osmoprimed and metylaxyl treated seeds were sown in 200 count flats infected with *P. ultimum* at 1.9×10^4 propagules/gram and kept at 5 C for 36 hrs to allow infection to take place and then moved to 25 C. At the end of 1 week, emerged seedlings were counted. Coated seeds germinated as well as metylaxyl treated seeds (77% and 80% respectively, however there was a slight reduction in the emergence of bio-osmoprimed seeds (73%). This is still significantly higher than the control seeds which had a 56% emergence. This small difference in the level of control compared to the huge difference in bacterial numbers suggests other mechanisms may be at work. Possible explanations for this are that 1) coatings provide excessive numbers of bacteria compared to those needed for control, or 2) that the placement of the bacteria applied during bio-osmopriming is significantly different making the smaller populations more effective, either by the bacteria entering the seed or through a more strategic colonization of the seed surface.

INTRODUCTION

Osmoprimed seed has been shown to provide earlier and more uniform germination as well as improve performance under environmental stresses such as salinity (Wiebe and Muhyaddin, 1987), excessively high or low temperatures (Valdes et al. 1985; Bradford, 1986; Pill and Finch Savage, 1988) and reduced water availability (Frett and Pill, 1989). These attributes combined with the reduced rates of damping-off associated with *Pseudomonas aureofaciens* AB254 creates a bio-osmopriming treatment that promotes rapid and more uniform germination under a wider range of soil temperatures while exhibiting the disease resistance and improved growth associated with bacterial coatings. Control of *Pythium spp.* on sweet corn seed was equal to that of the fungicide metalaxyl (Callan et al., 1991).

There are three primary goals of this project. The first is to determine the effectiveness of *P. aureofaciens* AB254 in controlling damping-off of tomato seedlings caused by *Pythium ultimum* and to compare differences in effectiveness between various application techniques such as coatings and bio-osmopriming. This bacterial strain has been used to successfully control *Pythium* on beets, corn, summer squash, winter squash, cucumber, muskmelon, watermelon, pumpkin, pea, and bean, but as yet has not been tested on tomato. The second goal of this project was to try to combine osmopriming and bioprimering into a single procedure accomplishing the essential elements of both enhancement techniques. The third objective was to determine how these two forms of application affect the storage life of the coating. Seeds of each treatment will be removed from storage every 4 months to assess which application technique retains the highest percentage of the original bacterial population. Seeds will also be examined using Scanning Electron

Microscopy (SEM) to look for physical differences in colony morphology between these two application techniques.

MATERIALS AND METHODS

The bio-osmoprimering apparatus consists of a lab air source, fiberglass filter, moisturizing flask, valve bank, priming flask, and sterilization beaker. Air flows from the air source through a fiberglass filter which removes any debris and continues on to the moisturizing flask. The moisturizing flask is a 1000 ml Erlenmeyer flask fitted with a two hole stopper. Air flows in through one hose down to the bottom of the flask and is bubbled through approximately 500 ml of distilled water. The moist air is then exhausted out through the other hose. This moisturizing flask increases the humidity of the air flowing into the priming flask to reduce evaporation of the water from the priming flask which would change the osmotic potential of the solution. The priming flask consists of a 500 ml Erlenmeyer flask containing 325 ml of priming solution and fitted with a two hole stopper. This solution is aerated by an aquarium air hose bent into a circle around the inside perimeter of the flask. It is important to have the air hose touch the priming flask at as few points as possible to reduce the number of seeds which get trapped between the hose and the glass. Air flow into the priming flask is regulated by two valves in tandem. Air comes into the valve bank from the moisturizing flask and then can either flow into the priming flask or be vented. Air flow is regulated by adjusting the amount of air which vents out of the system. Two valves are needed here so that pressure can be released and does not build up in the system and cause an air line to fail. Air from the priming flask is then exhausted into a sterilization beaker. This small beaker contains 250 ml of a mixture of approximately 60% distilled water, 30% ethyl alcohol, and 10% vanilla extract, which is refreshed on a regular basis. This is done to prevent large numbers of airborne bacteria and to counteract any unpleasant odors created by the bacteria's digestion of the nutrient broth. The priming flask rests inside a 1000 ml beaker filled with water to the level of the priming solution. This is done to create a water jacket which will provide constant temperatures during the treatment period. The priming flask is kept in a chamber at 20C to control temperature. A slow mixing of the solution is provided by a magnetic stirrer located beneath the beaker.

This study will consist of 4 different treatments with 5 g of seed per treatment; 1) untreated, 2) osmoprimered, 3) AB254 coated, 4) bio-osmoprimered. Prior to use, all apparatus from the moisturizing flask on is sterilized in 10% ethyl alcohol for 24 hrs. Seeds in all treatments are sterilized using 10% ethyl alcohol for 5 min and then dried between sheets of germination paper under ambient conditions for 24 hrs. These sterilized seeds are essentially free of microbes at this point. In treatment 2, seeds are osmoprimered in a dark, aerated, -0.8 MPa NaNO₃ solution for 1 week at 20C. The seeds are then rinsed in distilled water to remove any remaining salts and dried by placing them on a mesh screen and left under ambient conditions for 24 hrs. Once the wet seeds are placed on the screens, the screens are tipped so that any excess moisture runs off one corner.

Seeds in treatment 3 are coated by inoculating them with *Pseudomonas aureofaciens* AB254. Original stock cultures were supplied by Dr. Nancy Callan at the Montana State University Western Agricultural Research Center. The bacteria is cultured on trypticase soy agar and then harvested using a bent glass rod and approx. 5 ml of distilled water per petri dish. One plate per 5g seedlot is needed. From the harvested bacteria a 1.5% methyl cellulose suspension of AB254 is made, poured onto the seeds, and stirred. The pubescence on the surface of tomato seeds absorbs a great deal of water and makes even coverage difficult without this additional water. After all the seeds are thoroughly coated they are placed between sheets of germination paper (Lightweight brown toweling, Anchor Paper) and left to dry for 24 hrs under ambient conditions. Placing the seeds between sheets of paper reduces the possibility of airborne contamination.

In treatment 4, bio-osmoprimering, the seeds are placed in the priming flask containing 325 ml. of aerated, -0.8MPa NaNO₃ solution. The seeds are left in this solution at 20C for 4 days. At that time, 42 ml of 800% nutrient broth (64g/L, Difco), 0.1ml polyalkylene glycol, and 0.2 ml bacterial stock are added to the flask and left for an additional 3 days to allow for bacterial proliferation. The mixture of all of these added compounds is of approximately the same

osmotic potential as the existing NaNO_3 solution so as to maintain the integrity of the osmotic solution. On the seventh day, the seeds are removed from the priming flask and placed on mesh screens under ambient conditions and allowed to dry for 24 hrs. When the bio-osmoprime seeds are removed from the system they hold more moisture than inoculated treatments so screens, rather than germination paper, are used to allow for better air circulation. For treatments 2, 3, and 4, it is important to stir the seeds several times during the drying process. Otherwise the seeds will clump causing uneven drying and handling problems.

The bacterial stock used in the coating and bio-osmoprime treatments contains King's B broth and 80% glycerol at a ratio of 3.5:2. Using the techniques of Callan (personal communication), storage vials are prepared by pipetting 2 ml of King's B broth into 1 dram vials. These vials are then autoclaved with the caps loosened. After cooling, the bacteria is added using sterile technique and allowed to multiply for two days at room temperature. Each day, the caps are loosened slightly to allow air exchange and then tightened and shaken. Then 1.15 ml 80% glycerol is added. The vials are then shaken a final time to homogenize the mixture and are stored below -20 C . When retrieving the slurry from storage, time the vials spend out of the freezer should be minimized. Temperature fluctuations of this magnitude can kill bacteria.

On treatments 3 and 4, bacterial colony forming units (cfu's)/ seed are counted to evaluate the quality of the coating using the techniques of Callan, 1990. Samples are diluted in a phosphate buffer solution consisting of 8.5g/L NaCl, 11.4g/L $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, and 6.8g/L KH_2PO_4 . Under sterile conditions, 3 samples of 5 seeds each are placed in 5 ml. of phosphate buffer solution and vortexed for 3 seconds every 7 minutes for 30 minutes. The resulting solution is then diluted down to 10^{-3} to 10^{-6} of the original solution and plated out on trypticase soy agar (45g/L, Difco). After 1 ½ days colonies resembling AB254 are counted.

This research had several component projects and each is outlined below:

Experiment 1. *Colony forming units /seed applied using bio-osmoprime vs. inoculation.* Four tomato seedlots were bio-osmoprime, osmoprime and then AB254 coated, or simply AB254 coated using the techniques described above. Samples were then taken from each of these seedlots and cfu's/seed counted. Cfus reported are the average of three 5-seed samples. Data is given in Table 4. Other species were also bio-osmoprime using this technique, results are shown in Table 2.

Experiment 2. *Evaluations of AB254's ability to control Pythium ultimum compared to the fungicide metalaxyl when applied using various techniques.* In this experiment there were four treatments; control, AB254 coated, bio-osmoprime, and metalaxyl treated. Control, AB254 coated, and metalaxyl treated seeds were osmoprime prior to further treatment to eliminate the effects of priming so that the effects of the biological could be evaluated. AB254 inoculated and bio-osmoprime treatments were performed as described above. Metalaxyl (Apron 25W, Ciba-Geigy) treated seeds were treated with .0003g a.i./g of seed. The fungicide was combined with 8 ml distilled water to make even coating easier. The seeds were then dried between sheets of germination paper for 24 hrs. 200-cell trays were filled with sterilized soilless mix which had been inoculated with corn meal agar containing *P. ultimum*. Seeds of each of these 4 treatments were then sown in randomized blocks, watered and the flats placed in a germinator at 5C for 24 hrs to allow for infection to take place. At the end of this time they were removed and placed in growth chambers at 25C for germination and emerged seedlings counted after 7 days. The soilless mix/agar blend at the time of sowing contained 1.45×10^4 or 2.4×10^4 pythium propagules per gram. This number is so high because of the low density of this media compared to field soil. Propagules/gram were determined by plating 3 samples of .001g inoculated soilless mix on PDA (39g/L, Difco). Pythiums were counted after 24 hrs and confirmed by locating apersporia characteristic of *P. ultimum*. Koch's postulates were followed to determine that *P. ultimum* was the pathogen responsible for the death of the seeds.

Experiment 3. Storage characteristics of bacteria on bio-osmoprime vs. inoculated seeds.

Seeds of each treatment were placed in storage at 40F and 50 % R.H. After 6 months, due to a mechanical failure the seeds were placed in a sealed container along with a pouch of Lithium chloride which provided very low humidity. Cfu/seed were counted after 4 and 8 months.

RESULTS AND DISCUSSION

Experiment 1. Colony forming units 's/seed applied using bio-osmoprime and inoculation. The tomato plant has an ideal seed for applying beneficials with a high surface area to volume ratio, and a pubescence which provides an abundance of binding sites as well as protection for the bacteria. While both these factors contribute to these high numbers of colony forming units (cfu's) on a seed this size, reasonable numbers can also be applied to much smoother coated seeds such as those in the curcubit family (Table 2). Colony forming units/seed were counted using the techniques described above. There was little difference between bio-osmoprime seedlots with cfu's/seed being between 3 and 8×10^5 . Inoculating tomato seeds with AB254 yields 4 to 8×10^8 cfu's/seed (Table 4). These numbers are the same regardless of whether the seed was untreated or osmoprime prior to inoculation. The reason for the difference in bacterial concentration between bio-osmoprime and coatings is that the bio-osmoprime solution is too dilute to contain any higher populations of bacteria. If it were to have a higher bacterial concentration, it would become too thick and begin binding the seeds to the apparatus and to themselves. While bio-osmoprime yields far less cfu's/seed the following experiment proves that a coating applied in that manner can be nearly as effective.

Experiment 2. Evaluations of AB254's ability to control *Pythium ultimum* compared to the fungicide metalaxyl when applied using various techniques. In this experiment, AB254 has proven itself to be as effective as the fungicide metalaxyl in controlling damping-off caused by *P. ultimum*. Germination tests were performed to determine how seeds treated in these 2 ways stood up to pathogen pressure. Sterile soilless media was inoculated with *Pythium* and sown with seeds that were either bio-osmoprime or were osmoprime and then treated with one of the following 4 additional treatments; AB254 coated, metalaxyl treated, and untreated. The experiment was performed on two separate occasions, each with one replication. For both occasions and replications, inoculated seeds germinated as well as metalaxyl treated seeds (77% and 80% respectively), however there was a slight reduction in the emergence of bio-osmoprime seed (73%) (Table 5). This was still significantly higher than the control untreated seeds which had 56% germination. A small difference was noted in the level of control compared to the huge difference in bacterial numbers. Possible explanations of this may be; 1) 10^8 bacteria/seed is excessive and the number of bacteria required for adequate control on tomato is much smaller, and/or 2) that the placement of the bacteria applied during bio-osmoprime is significantly different making the smaller populations more effective, either by the bacteria entering the seed or through a more strategic colonization of the surface. Most likely, both of these statements are true to some degree. There are limits to the amount of microbial life that a seed can support (Osburn et al. 1989). Inoculum beyond that amount dies and can become a nutrient source for beneficials and pathogens alike (Dandurand, 1993). In an effort to discover more both AB254 coated and bio-osmoprime seeds were examined under an electron microscope looking for differences in colony morphology and location.

Experiment 3. Storage characteristics of bacteria on bio-osmoprime vs. inoculated seeds. Low humidity from months 6 thru 8 reduced bacterial numbers in both treatments. However, bio-osmoprime seeds retained a higher percentage after 8 months than inoculated treatments. Again this may be due to a more strategic colonization of the seed. Optimal storage of bio-osmoprime seed most likely presses the envelope of optimal seed storage by being more humid than would normally be desired. Similarly with bacteria cold and dry conditions associated with good seed storage are not beneficial for bacteria.

SUMMARY

Biologicals present a unique opportunity for preventing soil-borne diseases by providing organic control of pathogens.

Unfortunately at this time, formulations and delivery systems have not developed to the degree where they can compete with chemicals under all situations. Bio-osmopriming is a step toward improving the effectiveness of biologicals by incorporating physiological improvements which improve germination and contribute to disease prevention. Bio-osmopriming has been found to both successfully prime the seed and inoculate it with beneficial bacteria with an improved storage life compared to inoculated treatments. While applying only a fraction of the cfu's of coatings, bio-osmopriming still controls damping-off caused by *P. ultimum*.

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Table 1. Seedling emergence of 11 cucurbit species and cultivars in the Western Agriculture Research Center growth room.

Crop	Nontreated	AB254 Coat	AB254 Bio-Prime	Metalaxyl
Buttercup Squash	75.0 a ^z	93.1 b	97.7 bc	98.1 c
Yellow Crookneck Squash	56.5 a	87.5 b	89.4 b	88.4 b
Table King Acorn Squash	62.5 a	86.1 b	94.9 c	96.8 c
Spaghetti Squash	88.4 a	96.8 b	97.2 b	95.8 b
White Wonder Cucumber	34.3 a	67.1 c	57.9 b	72.2 c
Lemon Cucumber	84.3 a	100.0 c	93.1 b	97.2 b
Marketmore Cucumber	70.4 a	84.7 b	62.5 a	99.1 c
Hearts of Gold Muskmelon	44.9 a	84.7 bc	82.0 b	90.7 c
Minnesota Midget Muskmelon	57.9 a	85.2 b	94.9 c	96.3 c
Sugarbaby Watermelon	41.2 a	88.9 b	88.0 b	95.8 b
Conneticut Field Pumpkin	66.2 a	93.5 b	100.0 c	99.1 c

^z Mean separation within rows on the arcsin of the square root of the proportion, LSD (5% level). Reprinted in part by permission of Nancy Callan

Table 2. Colony forming units (cfu's)/seed achieved by bio-osmopriming seed of various species.

Species	Cfu's/seed
Cucumber	6.03 x 10 ⁵
Muskmelon	4.00 x 10 ⁷
Zucchini	8.20 x 10 ⁷

Table 3. Thermogradient table results for bio-osmoprimed and conventionally osmoprimed OH 8245 tomato seeds after 3, 5, and 7 days at all temperatures.

Seed treatment	<u>Seedling emergence (%)</u>			
	3 Days	5 Days	7 Days	Ungerminated
Unprimed	24.0	42.5	11.0	22.3
Osmoconditioned	65.0	4.5	4.0	26.3
Bio-osmoprimed	62.3	6.3	6.0	25.5
LSD	2.50	3.09	2.83	NS

Table 3b. Thermogradient table results for bio-osmoprimed and conventionally osmoprimed OH 8245 tomato seeds at each temperature after 7 days.

Treatment	<u>Temperature settings</u>									
	1	2	3	4	5	6	7	8	9	10
	<u>Ave. temperature</u>									
Untreated	95.5	96.8	96.3	95.5	96.0	94.5	94.3	78.8	15.0	5.3
Osmoconditioned	93.8	94.3	93.3	90.8	93.0	91.3	91.8	73.8	24.3	11.0
Bio-osmoprimed	92.8	91.5	90.8	88.5	89.0	89.3	87.3	73.5	33.8	17.3
LSD	NS	5.17	NS	NS	NS	NS	6.94	NS	10.07	NS

Table 4. Colony forming units (cfu's)/seed achieved by bio-osmopriming OH 8245 tomato seed.

Treatment	Cfu's/seed
Bioprimed (with methyl cellulose)	6.60×10^8
Bioprimed (without methyl cellulose)	5.00×10^6
Bio-osmoprimed #1	7.70×10^5
#2	6.05×10^5
#3	3.08×10^5

Table 5. Percent final seedling emergence of OH 8245 tomato seeds following application of various seed treatments when sown in soilless mix inoculated with *Pythium ultimum*.

Seed treatment	Final seedling emergence (%)
	<u>%</u>
Untreated	56
Metalaxyl treated	80
Bioprimed	77
Bio-osmoprimed	73

Table 6. Bacterial populations after 4 and 8 months of storage.

	Colony forming units (cfu)/seed		
<u>Bio-osmoprimered lots</u>	<u>Initial</u>	<u>4 mo.</u>	<u>8 mo.</u>
1	7.7×10^5	6.6×10^5	3.3×10^4
2	6.1×10^5	4.7×10^5	1.4×10^4
<u>Inoculated lots</u>	<u>Initial</u>	<u>4 mo.</u>	<u>8 mo.</u>
1	7.5×10^8	1.9×10^8	8.7×10^6
2	5.6×10^8	1.2×10^8	8.4×10^6

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HYDROTIME ANALYSIS AS A VIGOR TEST OF PRIMED AND PELLETTED LETTUCE SEED

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ABSTRACT

Vigor tests are generally more sensitive measures of seed quality than viability tests, but the seed industry has been reluctant to use vigor tests because they are relatively technical and perhaps less reproducible among laboratories. With proper standardization of the physical and chemical environment in which the seed is germinated, certain vigor tests are reproducible. A hydrottime analysis of germination provides information relevant to assessing seed quality. For any seed lot, the sensitivity to reduced water potential, rate of germination, uniformity of germination, and viability are quantified from parameters derived from the hydrottime model. This approach was used to assess seed quality of five lettuce varieties each of which were either pelleted or primed by four commercial seed enhancement companies. A vigor index was created which clearly detected priming or pelleting treatments that reduced seed quality. The index was sufficiently sensitive to identify varieties that did not perform well, and these results were validated by field emergence studies.

INTRODUCTION

Defining and assessing seed quality continues to challenge the seed industry. The standard germination test for viability is the most widely used index of seed quality, but does not give any indication of seed vigor. Vigor has been shown to be a more sensitive indicator of seed quality than viability in a number of species (Naylor and Gurmu, 1990; Powell and Matthews, 1984; Ram and Wiesner, 1988). Seed lots which meet industry standards for percent germination may vary widely in seed quality because a difference of just a few percentage points relates to a very large difference in seed deterioration (Ellis and Roberts, 1980).

A hydrottime analysis of germination provides information relevant to assessing seed quality. Germination rate, the uniformity of germination, viability, and the sensitivity of germination to water potential (ψ) are quantified by the hydrottime model (Bradford, 1990, 1995; Gummerson, 1986). The analysis is driven by the rate of germination, which is related to the sensitivity of germination to reduced ψ (see Bradford, 1995 for discussion). A given seed has a base or threshold water potential (ψ_b) which reflects its sensitivity to reduced ψ . Imbibition in solutions which are below (more negative) a seeds base threshold will prevent germination from occurring; imbibition in solutions above the threshold allows germination (radicle emergence) to proceed. The rate of germination is inversely proportional to the difference between the ψ of the imbibition solution and the individual seeds ψ_b . The distribution of ψ_b values is normally distributed, and the median ψ_b value ($\psi_b(50)$) characterizes the sensitivity of the seed lot to reduced ψ . The uniformity of germination is quantified by the variation in the ψ_b values (s_{ψ_b}). A high quality seed lot will have a low $\psi_b(50)$, a low s_{ψ_b} value, and high germination percentages at reduced ψ .

Vigor tests which lends itself to standardization of the physical and chemical environment in which the seed is imbibed, should be reproducible between laboratories. A hydrottime analysis of germination depends upon controlling only two variables - the incubation temperature and the ψ of the imbibition solution, and both are easily measured and controlled. Most incubators operate with an accuracy of ± 1 °C so only temperature calibration is necessary. The hydrottime analysis of germination is performed by imbibing seeds at three different ψ , using polyethylene glycol (PEG) to make solutions of reduced ψ . The PEG solutions are prepared using the equations of Michel (1983) as an approximation, but the actual ψ needs to be verified with a vapor pressure osmometer, as it may vary widely from that predicted by the equations. The ψ of the PEG solutions changes during imbibition of the seeds due to evaporation of the solution, water uptake during imbibition, and exclusion of PEG from the filter paper matrix (Hardegee and Emmerich, 1990). Therefore, to maintain a constant ψ , solutions must be changed, the frequency of which can be determined by experimentation. Standardization of the germination substrate is easily accomplished as blotter paper of specific standards is commercially available.

Yuma county, in southwestern Arizona, produces about 20,000 ha of lettuce (head, leaf, and Romaine)

worth \$465 million in gross receipts (1995 ADA figures). Pelleted seed is used in virtually all plantings, and much of this is primed. Planting of head lettuce begins September 1, when soil temperatures average 34°C, and are still above 30°C by October 1. Most lettuce cultivars are inhibited by soil temperatures greater than 25 to 32°C (Borthwick and Robbins, 1928; Gray, 1975; Thompson et al., 1979). Various seed priming treatments have been employed to alleviate thermoinhibition which, coupled with sprinkler irrigation to reduce the soil temperatures, allow lettuce seed to germinate under these conditions. However, priming treatments make lettuce seed more susceptible to deterioration in storage, and increase the incidence of abnormal seedlings when seeds are germinated at high temperatures (Cantliffe, 1981; Guedes and Canliffe, 1980; Tarquis and Bradford, 1992).

We used the hydrotime analysis of germination to assess seed quality of commonly used lettuce cultivars, priming and pelleting treatments. Field germination was performed to test the model's effectiveness in assessing the potential of the seed to produce a propagule in the field. A vigor index was constructed from parameters derived from the hydrotime model which clearly identified less vigorous varieties, and pelleting and priming treatments which reduced seed quality.

MATERIALS AND METHODS

Seeds of five lettuce cultivars (Desert Queen, Marvel, and Seeker from Genecorp Seeds; Diplomat from Seminis Vegetable Seeds; and Fall Green from Ferry Morse) were obtained in July 1996. Approximately 0.908 kg seed of each variety was sent to four commercial seed enhancement companies (Incotec; International Seed Technology, Seed Dynamics, Seed Systems). Half of the seed received by the seed enhancement companies was pelleted, while the other half received both a priming and pelleting treatment. The seed was returned and planted in September for field emergence studies. Germination counts were conducted 10 days after planting (DAP). Data for the seed pellet and priming treatments are coded so as not to endorse one proprietary product over another.

To determine the sensitivity of germination to water potential, seeds were imbibed in distilled water or polyethylene glycol solutions (PEG). Seeds were placed on blotter paper moistened with 4.0 mL distilled water (0 MPa) or PEG (-0.25 or -0.5 MPa) in covered 5.0 cm diameter Petri dishes and incubated at 20 °C in the dark. Benlate fungicide was added to the solutions at a concentration of 0.2% (w:v). Time to 50% germination was calculated from probit-transformed germination time courses. To maintain constant y , seeds which had not germinated were transferred to fresh solutions after the first 24 h of imbibition and every 48 h thereafter. Germination rates were averaged from four replicates of 50 seed per replicate, and was scored at frequent intervals to obtain complete germination time courses.

Data were analyzed using a hydrotime model as described by Bradford (1990, 1995) by repeated probit analysis using the SAS statistical package (SAS Institute Inc., Cary, NC).

Hydrotime model: The water potential (y) that just prevents a particular fraction of the seed population from germinating is known as the base water potential (y_b), or threshold water potential. Germination rate (inverse of time to radicle emergence) is linearly related to the difference between the y and y_b of a given seed. Times to germination of fraction g of the seed population (t_g) in solutions of different y can be related to the hydrotime constant (qH) with units of MPa·h:

$$qH = (y - y_b(g)) t_g, \quad (1)$$

where y is the water potential of the imbibition medium, $y_b(g)$ is the base water potential allowing germination of percentage g , and t_g is the time to germination of percentage g . For a given $y_b(g)$, the value of qH indicates the progress toward germination (per unit of hydrotime); higher values indicate lower germination rates.

To estimate the distribution of $y_b(g)$ within the seed population, a probit regression method was employed. This distribution was estimated by solving equation 1 for $y_b(g)$.

$$y_b(g) = y - (qH / t_g), \quad (2)$$

The optimal qH value was obtained by varying the value of qH in repeated probit regressions of probit g versus $y - (qH / t_g)$ until the optimal fit was obtained.

Using the repeated probit analysis technique, the median threshold ($y_b(50)$) and standard deviation (s_{y_b}) of this distribution was estimated for each species. These parameters indicate the median water potential threshold for germination of the seeds, and the uniformity of threshold values among individual seeds. Lower

values of $\psi_b(50)$ indicate a greater tolerance of stress and a more rapid germination rate. Smaller values of σ_ψ indicate a more uniform population, or a shorter time between the first and last seeds to germinate.

Vigor index: Parameters from the hydrotime analysis were ranked (Wilcoxon rank scores) according to its relative performance within that category. In each category, the treatment which had the highest final germination at -0.5 MPa, lowest t_{50} , lowest $\psi_b(50)$, and the lowest σ_ψ were defined as having the best performance. The rankings from each category were summed to produce a single number, the vigor index. Vigor index -1 (V.I.-1) = summation of rankings of final germination @ -0.5 MPa, t_{50} , $\psi_b(50)$, and σ_ψ . This summed value of rankings was again ranked using the Wilcoxon rank summed procedure (SAS Institute, Inc.) and subjected to a non-parametric analysis of variance test (NPARWAY 1, SAS Institute, Inc.) and mean treatment differences were determined using the Kruskal-Wallis test statistic.

RESULTS AND DISCUSSION

Priming, or osmopriming, of seed is performed to reduce germination time, synchronize germination, improve germination percentage and stand establishment (Kahn, 1992). The most consistently observed priming effect in these experiments was the reduction of germination time. Priming reduced the germination rate in 70% of the seed treatments, as shown by the probit-estimated t_{50} values (Table 1). The uniformity of germination is reflected by the σ_ψ values, which decreased in 50% of the seed treatments (Table 1). Bradford and Somasco (1994) reported an increased sensitivity of lettuce seeds to reduced ψ at 20°C, while at 31°C priming decreased the sensitivity to reduced ψ . Our data show median ψ_b values decreased in 50% of the seed treatments (Table 1). Priming also increased germination of seeds imbibed at -0.5 MPa in 55% of the seed treatments (Table 1). However, there was no consistent relationship among the different parameters in response to priming treatments. For example, a reduction in t_{50} was not associated with a corresponding reduction in σ_ψ values. Likewise, priming treatments which decreased sensitivity to reduced ψ_b (lower, or more negative $\psi_b(50)$ values) did not result in higher germination at -0.5 MPa (Table 1). The modeled germination time courses for these data fit the actual time courses well, and coefficients of determination were all highly significant (data not shown).

The overall performance of the enhanced seed was determined using the vigor index from the hydrotime analysis. Field emergence of seeds enhanced by companies 'A' and 'C' was significantly higher than seed from companies 'B' and 'D' (Prob>F, 0.0038; Table 2) and occupied nine of the top ten slots (data not presented). Predicted performance of the pellet (seed enhancement company) based on the hydrotime analysis matched exactly the order of the field emergence data (Table 2). Variety differences were observed in field emergence (Table 2), but the analysis of variance of the predicted performance using the ranked vigor index was not highly significant (Prob > F, 0.1092). The predicted performance based on Wilcoxon rank sum scores did not match perfectly the top performing varieties, but did accurately pick the bottom two varieties (Table 2). Based on the vigor index rankings, nine of the top ten treatments were primed (data not presented). Field germination of primed seed was lower than non-primed seed, but the Wilcoxon ranking predicted just the opposite, although the F test was highly insignificant (Prob>F, 0.7454; Table 2). We are continuing to evaluate the model using a number of other species, and refining the method to increase efficiency and sensitivity.

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Table 1. Summary of indices associated with quality of lettuce seeds.

Pelleting	Variety	Treatment	Germination @ -0.5 MPa (%)	t_{50} (h)	θ_H (MPa·h)	$\psi_b(50)$ (MPa)	σ_{ψ_b}
A	Desert Queen	non-primed	94.0	22.2	19	-0.82	0.210
		primed	94.6	17.8	22	-1.06	0.232
	Diplomat	non-primed	95.3	22.9	28	-1.12	0.214
		primed	98.0	19.5	19	-0.91	0.130
	Fall Green	non-primed	96.0	20.1	22	-1.01	0.219
		primed	99.3	19.1	22	-1.07	0.165
	Marvel	non-primed	85.9	21.1	14	-0.67	0.101
		primed	91.3	18.5	16	-0.83	0.176
	Seeker	non-primed	78.7	34.6	28	-0.85	0.278
		primed	94.7	26.5	28	-1.01	0.309
B	Desert Queen	non-primed	85.3	21.2	19	-0.93	0.215
		primed	96.0	19.5	29	-1.37	0.400
	Diplomat	non-primed	74.9	21.1	13	-0.71	0.155
		primed	63.3	20.6	13	-0.66	0.139
	Fall Green	non-primed	89.9	18.6	13	-0.86	0.268
		primed	61.3	87.9	71	-1.33	1.687
	Marvel	non-primed	80.7	20.9	11	-0.57	0.091
		primed	88.0	24.1	19	-0.79	0.189
	Seeker	non-primed	38.7	39.5	22	-0.67	0.196
		primed	53.3	41.3	26	-0.77	0.252

Table 6. Summary of indices associated with quality of lettuce seeds - continued.

Coating	Variety	Treatment	Germination @ -0.5 MPa (%)	t_{50} (h)	θ_H (MPa·h)	$\psi_b(50)$ (MPa)	σ_{ψ_b}
C	Desert Queen	non-primed	99.3	24.9	28	-1.10	0.203
		primed	99.3	20.6	23	-1.07	0.201
	Diplomat	non-primed	100	24.6	23	-0.94	0.109
		primed	99.3	22.8	19	-0.86	0.097
	Fall Green	non-primed	98.0	21.9	19	-0.84	0.119
		primed	99.3	19.4	19	-0.95	0.181
	Marvel	non-primed	100	23.2	16	-0.68	0.112
		primed	100	21.1	14	-0.69	0.058
	Seeker*						
	D	Desert Queen	non-primed	65.3	36.3	21	-0.66
primed			98.0	19.4	17	-0.73	0.183
Diplomat		non-primed	84.7	29.0	21	-0.71	0.176
		primed	93.3	22.3	17	-0.68	0.148
Fall Green		non-primed	44.7	45.7	19	-0.51	0.186
		primed	82.7	45.9	31	-0.69	0.115
Marvel		non-primed	74.0	36.0	19	-0.58	0.148
		primed	56.7	57.7	26	-0.54	0.141
Seeker		non-primed	68.7	41.4	26	-0.63	0.203
		primed	71.3	29.2	17	-0.59	0.148

*Seeker was not included by this company.

Table 2. Comparison of field emergence of primed and pelleted lettuce with predicted performance based on the Wilcoxon rank values of the vigor index created from the hydrotime analysis model. Lower predicted scores represent better performance.

Pellet	Emergence (%)	Predicted	Variety	Emergence (%)	Predicted	Treatment	Emergence (%)	Predicted
A	97	2	Desert Queen	95	1	non-primed	100	2
B	88	3	Diplomat	100	2	primed	94	1
C	100	1	Fall Green	97	3			
D	93	4	Marvel	91	4			
			Seeker	87	5			

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EVALUATION OF A BULK PLANTING SYSTEM FOR LOW-COST SEEDING OF CABBAGE

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ABSTRACT

Cabbage (*Brassica oleracea* L. Capitata) was direct-seeded with a precision seeder and with a relatively inexpensive bulk seeder. Some of the treatments with the bulk seeder consisted of blending good hybrid seed with non-viable inexpensive seed at several ratios to reduce the seed cost.

The study demonstrated that small farmers can obtain equivalent stands and yields with bulk seeders if adequate hoe labor for thinning is available, thus avoiding the capital expense of a precision vegetable seeder. Low percentages of hybrid seed in the bulk seeder were not practical. Precision seeding to a stand reduced the need for hoe labor and gave equivalent yields in all but one of the four tests.

Keywords Seeders, planters, precision seeding, cabbage, cultural practices

INTRODUCTION

Larger commercial vegetable growers have the option of transplanting or using one of several precision seeders to direct-seed vegetable crops. Many small vegetable growers in the Southeastern United States feel that they cannot afford a precision seeder, and are thus limited to transplanting many of their small-seeded crops. There are some relatively inexpensive bulk seeders available that will meter small-seeded vegetable crops, but they do not provide the uniform seed spacing or the controlled metering rate of the precision seeders.

The objective of this project was to evaluate a bulk seeder using blends of high-quality hybrid seed and inexpensive non-viable seed (obtained by killing the germination of open-pollinated seed). It was hypothesized that such a blend could provide a stand that would be acceptable (after thinning) at a lower cost than bulk seeding or precision seeding pure hybrid seed.

REVIEW OF LITERATURE

Precision seeding has been a major research thrust for many years; however, most of the research and development work has dealt with planters for agronomic crops. Work on precision seeders for vegetables and other horticultural crops has been very limited. Much of the research on precision seeding has been based on vacuum metering, although Parish (1972), using a vertical plate concept, developed a mechanical precision seeder that provided good seeding uniformity and was suitable for use with vegetable crops. Hassan (1981) developed a precision drum seeder for tree seeds that used a vacuum system to singulate the seeds. Short and Huber (1970) developed a vacuum metering system for cucumber seeds and demonstrated its effectiveness in the laboratory but did not experiment with a field machine. Snyder and Hummel (1985) and Sial and Persson (1984) studied nozzle configurations for vacuum metering of seed.

Giannini, Chancellor, and Garrett (1967) published a thorough discussion of the need for precision seeding in vegetable culture and discussed the development of a very successful precision lettuce seeder that used vacuum principles for singulation. Compared with the then-standard "bulk-metering planter," their vacuum seeder used 90% less seed, thus reducing thinning time and resulting in improved yields. Inman (1968) discussed the development and adoption of precision seeders in the California vegetable industry, and compared precision

seeding with bulk seeding. Parish et al. (1991) compared vacuum and belt-type precision seeders, and found no differences when planting uniform spherical seeds.

Sterrett et al. (1991) studied management techniques for broccoli using a systems approach. Using economic analyses, they compared transplant and direct seeding techniques for broccoli. Their results showed that maximum profits could be obtained with direct seeding for an early crop and with transplanting for the main season crop. Kahn (1990) evaluated the possibility of reducing seed requirements through reduced plant populations of turnip greens. Kahn's study concluded that the plant populations of turnip greens could be decreased to approximately half of the recommended rate without a significant reduction in yield or quality. This reduced seeding rate translated into a seed cost savings. Orzolek (1991) noted that improved technologies of precision planters enable growers to avoid the costs of seeding at high densities and later thinning to a final expected plant stand.

Bergeron et al. (1992) and Bracy et al. (1993, 1995) reported on field studies and an economic analysis of precision direct seeding cabbage to a stand without thinning. They demonstrated that seeding directly to a stand was feasible, and found that significant cost savings were possible. All of the above studies were based on the assumption of large acreages where the cost per hectare (acre) of a precision planter would be reasonable.

MATERIALS AND METHODS

Field tests were conducted at the Hammond Research Station on Cahaba fine sandy loam soil. Two cultivars of cabbage (*Brassica oleracea* L. Capitata) were used for this study in 1993: hybrid 'Headstart' and open-pollinated 'Flat Dutch'. A second hybrid, 'SolidBlue 770', was added for the 1994 tests. The germination of the hybrid seed used in 1993 was 97%. In 1994, the germination of both hybrids was 98%. The open-pollinated seed was heated in an oven to completely destroy the germination. Seed sizes for the first two cultivars were approximately the same. The 'SolidBlue 770' seed was somewhat smaller. The open-pollinated seed was not size-graded for uniformity.

Two vegetable seeders were used: a Stanhay model S870 precision seeder (Hestair Farm Equipment, Suffolk, England) and a "Planet Jr." bulk-metering seeder (Powell Manufacturing Co., Bennettsville, SC). The Stanhay seeder used a punched rubber seed belt to singulate the seeds. The Planet Jr. seeder metered seeds gravimetrically through an orifice hole.

Eight treatments were planted, as follows:

- T1 □ 100% hybrid seed in precision seeder - 89 mm (3.5 in) seed spacing
- T2 □ 10% hybrid seed in bulk-metering seeder
- T3 □ 20% hybrid seed in bulk-metering seeder
- T4 □ 30% hybrid seed in bulk-metering seeder
- T5 □ 40% hybrid seed in bulk-metering seeder
- T6 □ 50% hybrid seed in bulk-metering seeder
- T7 □ 100% hybrid seed in bulk-metering seeder
- T8 □ 100% hybrid seed in precision seeder - 287 mm (11.3 in) seed spacing

Seed percentages are by weight. Four randomized replications were used.

The basic cultural system used was described by Bracy et al. (1991) and Parish et al (1992). After bedding with a disk bedder, 8-24-24 fertilizer was applied at a rate of 560 kg/ha (500 lb/a). The fertilizer was knifed into the center of the planting beds approximately 50 mm (2 in) below planting depth. After fertilization and rebedding with a disk bedder, the beds were shaped with a pan-type bed shaper prior to planting. The cabbage was seeded approximately 0.6 cm deep on 3/9/93, 9/14/93, and 3/15/94 in two rows spaced 30.5 cm apart on beds 1 m wide. The cabbage plants were thinned to a spacing of 30 cm, 3 - 4 weeks after emergence. After planting, Metolachlor was broadcast at 1.76 l/ha (1.5 pt/a) of formulation. The plots were sidedressed twice with 112 kg/ha (100 lb/a) of ammonium nitrate each time, banded next to the row.

Selective harvests were made over the plots so heads were harvested individually when ultimate maturity was obtained, regardless of plant population. Head maturity was determined according to the criteria described by Boudreaux (1991) and involved a manual physical assessment of head firmness.

RESULTS

Stand counts, plant spacing uniformity, and yield data are shown in tables 1-4. There were no differences in plant spacing uniformity before thinning, expressed as coefficient of variation (CV), except for the spring 1993 planting. In spring 1993 the high percentages of hybrid seed in the bulk seeder resulted in the least uniform stand. In the spring 1994 'SolidBlue 770' planting, there were differences in plant

spacing uniformity after thinning. This was due to very non-uniform stands as planted that could not be corrected by thinning in all cases.

In the spring 1993 planting, marketable yields were similar for all hybrid seed ratios except the 10% ratio (Table 1). There was no difference in average head weight.

In the fall planting, plant stand was lower than in the spring crop due to poor weather conditions (Table 2). Plant stand for all treatments was less than a desired stand of 64,500 plants/ha. Yields from 30, 50, and 100% hybrid seed were similar to the precision-seeded plots. Yield from the precision-seeded, 287 mm treatment was greater than some of the bulk seeder-planted treatments even though fewer seed were planted. This may have been due to better seed placement and covering by the precision seeder. There was no difference in average head weight.

In the spring 1994 'Headstart' planting, yields were greatest for the 100% bulk and both precision-seeded treatments, although the yield of the precision-seeded 287 mm treatment was not significantly different from the yield of the some of the bulk seeder treatments (Table 3).

In the spring 1994 'SolidBlue 770' planting, the 89 mm precision treatment outyielded all the other treatments (Table 4). The 100% hybrid bulk-seeded treatment outyielded the 287 mm precision treatment.

There were no differences in average head size among treatments except for the 'SolidBlue 770' in spring, 1994 (Tables 1-4).

CONCLUSIONS

This study demonstrated that small farmers can obtain equivalent stands and yields with bulk seeders if adequate hoe labor is available for thinning, thus avoiding the capital expense of a precision vegetable seeder. Low percentages of hybrid seed in the bulk seeder were not practical. Precision seeding to a stand reduced the need for hoe labor and resulted in equivalent yields in all but one planting.

Any inexpensive brassica seed of the same diameter (such as collard) could have been made non-viable and used for the bulk blending with the hybrid cabbage.

Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 97-07-____. Use of trade names does not imply endorsement by the Louisiana Agricultural Experiment Station of products named nor criticism of similar ones not named.

Table 1. Seed counts, coefficients of variation (CV) for seed spacing, and yield - 1993 spring planting, 'Headstart'.

Treatment	Plants/ha		CV		Yield kg/ha	Head size kg/head
	before thinning	after thinning	before thinning	after thinning		
100% hybrid, prec., 89 mm	69,400b ²	33,900a	66b	44	28,800a	1.4
10% hybrid, bulk	11,400c	12,800d	24d	41	7,600b	1.4
20% hybrid, bulk	25,900c	18,500cd	46c	61	18,200ab	1.5
30% hybrid, bulk	29,200c	23,500bc	46c	67	19,600ab	1.4
40% hybrid, bulk	62,300b	32,400a	78ab	54	29,300a	1.3
50% hybrid, bulk	74,400b	30,600ab	86ab	54	24,700a	1.3
100% hybrid, bulk	125,300a	38,100a	92a	46	32,900a	1.3
100% hybrid, prec., 287 mm	57,600b	32,400a	70b	50	20,600ab	1.2
				NS		NS

²Means followed by same letter are not significantly different at the 0.05 level, DMRT.

Table 2. Seed counts, coefficients of variation (CV) for seed spacing, and yield - 1993 fall planting, 'Headstart'.

Treatment	Plants/ha		CV		Yield kg/ha	Head size kg/head
	before thinning	after thinning	before thinning	after thinning		
100% hybrid, prec., 89 mm	22,500c ^z	15,300b	66	58	16,000b	1.4
10% hybrid, bulk	8,200d	7,200c	28	54	3,500d	1.0
20% hybrid, bulk	11,400cd	9,600bc	44	55	8,500cd	1.1
30% hybrid, bulk	19,300cd	14,600b	125	65	16,700b	1.4
40% hybrid, bulk	12,800cd	11,400bc	49	56	10,500c	1.3
50% hybrid, bulk	16,100cd	10,400bc	67	58	13,000bc	1.3
100% hybrid, bulk	50,200a	24,200a	118	66	23,200a	1.2
100% hybrid, prec., 287 mm	37,100b	21,700a	98	62	18,300ab	1.2
			NS	NS		NS

^zMeans followed by same letter are not significantly different at 0.05 level, DMRT.

Table 3. Seed counts, coefficients of variation (CV) for seed spacing, and yield - 1994 spring planting, 'Headstart'.

Treatment	Plants/ha		CV		Yield kg/ha	Head size kg/head
	before thinning	after thinning	before thinning	after thinning		
100% hybrid, prec., 89 mm	71,200a ^z	37,100a	73	35	33,200a	1.3
10% hybrid, bulk	7,200d	7,200e	38	37	10,800c	1.5
20% hybrid, bulk	16,100cd	12,100de	88	70	18,800bc	1.6
30% hybrid, bulk	23,500bc	18,500cde	87	63	17,200bc	1.6
40% hybrid, bulk	25,900bc	22,500bc	64	49	20,700b	1.5
50% hybrid, bulk	30,600b	26,700b	61	50	20,900b	1.5
100% hybrid, bulk	66,200a	34,600a	87	39	31,300a	1.3
100% hybrid, prec., 287 mm	19,300c	19,300bcd	50	62	23,900ab	1.5
			NS	NS		NS

^zMeans followed by same letter are not significantly different at 0.05 level, DMRT.

Table 4. Seed counts, coefficients of variation (CV) for seed spacing, and yield - 1994 spring planting, 'SolidBlue 770'.

Treatment	Plants/ha		CV		Yield kg/ha	Head size kg/head
	before thinning	after thinning	before thinning	after thinning		
100% hybrid, prec., 89 mm	85,500b ²	34,600a	77	36c	37,000a	1.3bc
10% hybrid, bulk	11,400d	8,900c	67	65ab	17,400c	1.9a
20% hybrid, bulk	20,300cd	14,600c	89	64ab	20,600c	1.4b
30% hybrid, bulk	43,500c	25,000b	103	55abc	23,300bc	1.4b
40% hybrid, bulk	34,600cd	25,000b	86	71a	18,800c	1.1bc
50% hybrid, bulk	38,100cd	24,200b	108	57abc	23,600bc	1.4b
100% hybrid, bulk	132,400a	37,100a	108	35c	28,900b	1.1bc
100% hybrid, prec., 287 mm	29,200cd	23,500b	67	43bc	20,700c	1.0c
			NS			

²Means followed by same letter are not significantly different at 0.05 level, DMRT.

EVALUATION OF SEED COVERING DEVICES AND PRESSWHEELS FOR DIRECT SEEDING OF MUSTARD AND CABBAGE

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ABSTRACT

Stands of brassica crops obtained with precision seeders are sometimes inadequate and/or nonuniform. Although several types of covering devices and presswheels are available from precision seeder manufacturers, the effects of covering devices and presswheels on plant emergence of direct seeded Brassica crops have not been determined. In Spring and Fall 1996, six crops of mustard (*Brassica juncea* [L.] Czerniak) and four crops of cabbage (*Brassica oleracea* L. Capitata group) were direct seeded with a precision belt seeder using four covering devices and four rear presswheels. All of the covering devices and presswheels evaluated were adequate for direct seeding mustard and cabbage under the soil moisture conditions and soil type (silt loam or fine sandy loam) found in these experiments. Although poor stands were obtained with all seed covering devices and presswheels when 199 mm of rain occurred within three days of planting, plant stand of cabbage was greater when the paired arm device was used than with drag-type or no covering devices.

Key Words. *Brassica oleracea*, *Brassica juncea*, emergence, precision seeding

INTRODUCTION AND REVIEW OF LITERATURE

A uniform stand is often difficult to obtain when direct seeding *Brassica* crops. Perkins-Veazie et al. (1989) noted that problems with soil crusting, high humidity, temperature extremes, and pathogen attack limit the use of direct seeding of cabbage in Florida. Johnson and Wilcox (1977) noted that emergence problems limited the adoption of direct seeding for some vegetable crops. Parish et al. (1992, 1995) reported on several years of research using precision seeding for commercial vegetable crops. Many of the research plots in those experiments had to be replanted due to insufficient stands that were associated with equipment insufficiency or weather conditions following planting (personal communication).

Akyurt and Taub (1966) developed a precision seeder for sugar beets (*Beta vulgaris* L.) that provided two-point seed contact with the undisturbed capillary system of the soil and a seed cover consisting of a porous and dry top soil layer. Inman (1968) reported on precision planting equipment and aids for vegetable crops. His report dealt primarily with precision metering and did not discuss seed covering or seed-to-soil contact.

Abernathy and Porterfield (1969) evaluated the effect of planter opener shape on furrow characteristics and found that the planter openers reduced soil density to a depth greater than the operating depth of the opener. They did not evaluate covering devices, presswheels, or their effects on seed emergence. Cochran et al. (1974) conducted soil bin studies on planter furrow openers and depth control devices to establish quantitative relationships for design of depth-gauging devices on planters but did not relate their work to seed emergence.

Hudspeth and Wanjura (1970) developed a planter to improve emergence of cotton seed (*Gossypium hirsutum*). The planter was not outfitted with a presswheel but had a seed firming wheel in the furrow ahead of knife coverers. They reported increased emergence compared with a commercial cotton planter that used presswheels.

Wurr and Fellows (1985) evaluated the effects of different seeding depths and presswheel pressures on emergence and growth of lettuce (*Lactuca sativa* L.). Presswheel pressure had no effect on emergence or growth. Shallow seeding depths were most effective when seeding was followed by a rain and soil moisture

was kept near optimum. Under drier soil conditions, deeper seeding was more effective.

Morrison and Gerik (1985a, 1985b) developed simulation models to predict emergence of wheat (*Triticum aestivum* [vulgare]), soybeans (*Glycine max*), alfalfa (*Medicago sativa*), and grain sorghum (*Sorghum vulgare*) with different presswheel designs. They determined that depth-control wheels beside the openers were most effective, but the width of the depth-control wheels precluded the narrow row planting configurations needed for most vegetable crops. They also conducted field experiments to evaluate depth-gauging wheels on planters. The field tests confirmed that wide, dual side wheels worked well, as did experimental narrow linked front-rear gauge wheels. Morrison (1989) determined that improved emergence of crops was dependent upon good planting depth control, limited in-row starter fertilizer rates, and proper selection of rear presswheels and downforce on those presswheels.

Richey (1981) discussed the development of a commercial agronomic planter for precision metering by vacuum. The development of furrow-forming shoes and ribbed presswheels reduced emergence time for corn (*Zea mays*) by one-third in many cases.

Parish et al. (1991) compared belt and vacuum precision seeders in the laboratory and in the field. Plant-spacing uniformity with non-spherical seeds was better with vacuum seeders. Poor emergence of cabbage, broccoli, onion, and spinach was noted with all seeders in some plantings.

Price and Taylor (1994) evaluated the precision spacing of snapbean seed (*Phaseolus vulgaris* L.) by vacuum planters and found poor seeding uniformity with all four models tested. They correlated emergence with yield and found those plants with early emergence yielded twice as much as those with late emergence.

Bracy et al. (1993, 1995) and Bergeron et al. (1992) measured plant spacings of cabbage and cauliflower directly seeded with different nominal seed spacings and found that seeding directly to the desired stand, rather than over-planting and thinning, was possible and practical. They reported that when plantings did not result in an adequate stand, all treatments were affected with no advantage to the heavier seeding rate (Bergeron et al., 1992).

Hegarty (1979) reported that preemergence losses of broccoli in the field were due in part to the inability of seedlings to emerge from the soil (soil impedance) and in part to biotic factors. Factors over which the grower has some control (soil tilth, fertilizer level, and seeder performance) have more effect on plant stands than soil temperature or soil moisture (Hegarty, 1976). He noted that "the more extreme reductions in emergence levels in all crops seem to be associated with soil structure problems associated with the sowing operation itself, or with rainfall after sowing".

MATERIALS AND METHODS

Ten experiments were conducted at the Hammond Research Station, Hammond, La., on a soil classified as a Cahaba fine sandy loam soil (thermic Typic Hapludult) during Spring and Fall 1996. Plantings were made in four locations on the station on Mar. 15, Apr. 3, Aug. 20, Oct. 24, and Nov. 5 with mustard and on the same dates, with the exception of Apr. 3, with cabbage. Planting times and field locations were varied in an attempt to assess planter component performance under different soil moisture conditions and texture. Fields were prepared and planted in the same manner for all plantings using the precision system developed by Parish et al. (1992). The crops were planted on precision-shaped raised beds spaced 1.2 m on center. The beds were nominally 152-mm high with a flat top. As the crops were not grown to maturity, no fertilizer, herbicide, or supplemental irrigation was applied to the test area.

A Stanhay Model S870 precision seeder (Hestair Farm Equipment, Suffolk, England) was equipped with combinations of four covering devices and four rear presswheels for a total of 16 treatments. Covering devices included standard drag (15.9-mm sq. steel bar), light drag (9.5-mm sq. steel bar), paired arms (6.4-mm x 30.2-mm steel paddles), and no covering device. Presswheels included standard smooth steel banded (114-mm wide x 229-mm dia.), concave split (89-mm wide x 273-mm overall dia. with 13-mm space between conical wheels), flat split (114-mm wide x 229-mm dia. with 25-mm space between wheels), and cage (114-mm wide x 229-mm dia., covered with expanded steel mesh). All presswheels and covering devices (with the exception of the light drag) are stock parts available with the Stanhay seeder. The light drag covering device (a replica of the standard drag with a smaller drag bar) was fabricated in the university's shop.

'Savannah' (Sakata Seed America, Morgan Hill, Ca.) or 'Florida Broadleaf' (local source) mustard was

planted using a single-line belt and single-line opener with a nominal seed spacing of 68 or 84 mm. The March planting date also included a planting of mustard using a two-line belt and two-line opener at a 39-mm nominal seed spacing. The two-line configuration is the preferred method for growing mustard, but in later plantings this configuration was not included to reduce the number of component and seed hopper changes. At all planting dates except the April date, 'XPH 595' cabbage (Asgrow Seed Co., Kalamazoo, Mi.) was planted using a single-line belt and single-line opener with an 84-mm nominal seed spacing. Cabbage was not planted at the April planting date due to timeliness in obtaining seed. All treatments were replicated four times in a randomized block design.

Soil samples (8-cm deep in the center of the bed top) were taken at planting to determine soil moisture. Additional soil samples (15-cm deep) were taken in each field for particle size analysis and verification of texture class. Precipitation for the first fifteen days following planting was recorded. Plants were counted when emergence appeared complete, approximately 2-3 weeks after planting. Data were analyzed using an ANOVA procedure in CoStat Statistical Software (CoStat, 1995).

RESULTS

At the March, April, August, and October planting dates, soil moisture was optimum, ranging from 8.0 - 11.2%, at or slightly below field capacity (Table 1). Soil moisture at the November planting was more than 19%, which was not conducive for optimum seed covering.

Plantings in March, April, and November were made in same soil type (silt loam) as indicated by particle size and textural class (Table 1). The August and October plantings were made in the same field, and the soil (fine sandy loam) contained more sand than the other three fields utilized in these experiments.

Precipitation at all plantings was adequate for germination and growth (Table 2). All plantings received at least 14 mm of precipitation by three days following planting. Heavy rainfall (199 mm) occurred within three days of the October planting.

Mustard plant populations were not significantly different with any row configuration, covering device, or presswheel used in the planting operation at any of the five planting dates (Table 3). No interaction between covering device and presswheel were noted, so data for each planter component is presented separately. Low plant populations at the October planting reflect the heavy precipitation that occurred shortly after the planting operation was complete.

No differences in cabbage plant population due to covering device and presswheel interaction or presswheel alone were noted at any of the four planting dates (Table 4). Cabbage populations were affected by covering device at the October planting; when plots planted with the paired arms covering device had significantly greater plant population than plots planted with the other covering devices. The greater population at the October planting was probably due to more soil being placed over the seed furrow, resulting in more soil remaining to cover the seed after the heavy rainfall. The paired arms was the most aggressive covering device, since the arms directed the soil into the seed furrow as opposed to the leveling action of the drag-type covering devices.

CONCLUSIONS

All of the covering devices and presswheels evaluated were adequate for direct-seeding of mustard and cabbage under soil moisture conditions and soil type found in these plantings. Strong visual differences in the appearance of the drill area among the treatments were apparent immediately after planting, prior to any rainfall. Uniform plant emergence with all covering devices and presswheels was probably due to adequate rainfall within three days of planting that effectively closed the seed furrows after planting. Different results may be reported if planting in heavier soil type, under drier or wetter soil conditions, or if precipitation does not occur within a few days after planting.

Visual observations indicated that operating the seeder without a covering device, regardless of presswheel attached, left the seed furrow open and seed exposed. Although plant populations from the November planting did not support this, paired arm covering device appeared to do a better job of covering the seed furrow when soil conditions were wet. The paired arms device may also offer an advantage with some crops when heavy rainfall occurs soon after planting.

Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 97-07-0008. Use of trade names does not imply endorsement by the Louisiana Agricultural Experiment Station of products named or criticism of similar ones not named.

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Table 1. Soil moisture at planting and particle size analysis by planting date and field location.

Planting date	Field number	Soil Moisture (%)	Particle size (%)			
			sand	silt	clay	Textural class
March	21	11.2	36.2	53.3	10.5	silt loam
April	44	8.0	33.9	50.5	15.6	silt loam
August	17	9.3	67.6	22.5	9.9	fine sandy loam
October	17	9.3	67.6	22.5	9.9	fine sandy loam
November	20	19.1	23.6	66.3	10.1	silt loam

Table 2. Daily precipitation (mm) following planting.

Day after planting	Planting dates				
	Mar.	Apr.	Aug.	Oct.	Nov.
1	0	1	14	22	0
2	6	0	1	173	0
3	15	51	0	4	14
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	1	0
8	0	0	13	0	0
9	0	0	3	4	0
10	0	27	23	0	0
11	1	0	6	0	0
12	10	98	0	0	0
13	1	0	0	0	2
14	0	0	0	0	12
15	71	0	0	14	2
Total, day 1-5	21	52	15	199	14
Total, day 1-15	104	177	60	218	30

Table 3. Effects of covering devices and presswheels on plant population of mustard at five planting dates, Hammond, LA, 1996.

Seeder component	Plants/ha (1000's)					
	Mar. ^z	Mar. ^y	Apr. ^y	Aug. ^y	Oct. ^y	Nov. ^y
Covering device (CD)						
Standard drag	2812	784	1070	1618	276	1732
Light drag	2802	834	952	1450	313	1342
Paired arms	2671	827	1063	1308	225	1339
None	2916	801	955	1473	235	1894
Significance	NS	NS	NS	NS	NS	NS
Presswheel (PW)						
Standard smooth	3014	827	975	1450	296	1342
Concave split	2741	838	935	1329	326	1810
Flat split	2671	774	1070	1413	202	1598
Cage	2775	807	1060	1658	225	1557
Significance	NS	NS	NS	NS	NS	NS
Interaction CD*PW	NS	NS	NS	NS	NS	NS

^zMustard planted with two-line belt and two-line opener.

^yMustard planted with one-line belt and one-line opener.

^{NS}Nonsignificant

Table 4. Effects of covering devices and presswheels on plant population of cabbage at four planting dates, Hammond, LA, 1996.

Seeder component	Plants/ha (1000's)			
	Mar.	Aug.	Oct.	Nov.
Covering device (CD)				
Standard drag	554	249	183b	193
Light drag	624	202	167b	170
Paired arms	567	206	336a	177
None	478	236	108b	147
Significance	NS ^z	NS	*	NS
Presswheel (PW)				
Standard smooth	532	178	191	209
Concave split	642	214	260	168
Flat split	512	254	180	147
Cage	537	247	163	163
Significance	NS ^z	NS	NS	NS
Interaction CD*PW	NS	NS	NS	NS

^zNS,*Nonsignificant or significant at $P \leq 0.05$, respectively.

Banded Phosphorus Effects on Alfalfa (*Medicago sativa* L.) Seedling Growth and Subsequent Productivity After Temporary Waterlogging

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ABSTRACT

Banded P has been shown to increase alfalfa (*Medicago sativa* L.) seedling growth in fall seedings, resulting in larger seedlings which in some cases have been better able to withstand environmental stresses. This field study was conducted to evaluate the effect of banded P on alfalfa seedling growth in the fall and subsequent productivity after temporary flooding stress. Field experiments were established in late August at Wooster and Columbus, OH in 1994 and at Wooster and S. Charleston, OH in 1995. A split-plot randomization of a randomized complete block design was used with 4 replications. Whole plot treatments were flooded and unflooded. Subplot treatments were no P and 62 kg ha⁻¹ P₂O₅ banded under the seed. Flooding was initiated 21-26 d after seeding and continued for 11-18 d. Root and shoot dry wt. were recorded at the start of flooding, directly following flooding, and mid to late November. Total forage yield was recorded for the year following establishment. Banded-P increased seedling dry wt. before flooding by an average of 60%. Flooding reduced root and shoot dry wt. regardless of P treatment. Flooded seedlings established with banded-P were larger at the termination of flooding at three of the four locations. In November, flooded seedlings seeded with banded-P had greater root dry wt. at S. Charleston and Wooster in 1995 and larger shoot dry wt. at S. Charleston in 1995 only. Heaving was observed at all locations except Columbus. Severe winter heaving resulted in total stand death at Wooster in 1996. Although banded-P reduced heaving at Wooster in 1995, its ability to decrease heaving is variable and appears to have little effect under severe heaving conditions like those observed at Wooster in 1996. Flooding reduced subsequent yield at Wooster in 1995 and S. Charleston in 1996. Banded-P increased total yield approximately 15% in the unflooded treatment at S. Charleston in 1996 and 15% in the flooded treatment at Wooster in 1995. Although banded-P increased seedling growth in the fall, its effect on heaving, stand density, and yield the year after establishment was minimal.

INTRODUCTION

Alfalfa is best adapted to well-drained fertile soils. This adaptation limits its growth potential on a relatively large number of soils that do not meet those criteria (Lowe et al., 1972). While a low fertility status can often be corrected through the use of lime and fertilizer, excess soil moisture is much more difficult to rectify (Alva et al., 1985). In addition, a large proportion of annual rainfall in the midwestern U.S. occurs in the spring and fall, precisely when new alfalfa stands are being established. This often results in temporarily waterlogged soils, especially in low lying fields or areas within a field. Excess soil moisture can profoundly affect the establishment and maintenance of alfalfa stands.

Waterlogging reduces the growth of alfalfa seedlings (Fick et al., 1988). This reduction in growth is a direct result of anaerobic conditions in the rhizosphere (Noble and Rogers, 1994). Barta (1980) found that 7 d of flooding reduced both root and shoot dry wt. of alfalfa by approximately 60% in comparison with an unflooded control. Similarly, Thompson and Fick (1981) showed that 20 d of flooding reduced root dry wt. by 80% and shoot dry wt. by 35%. A reduction in the seedling growth rate during establishment can lead to less vigorous stands with a higher incidence of seedling mortality (Sheard et al., 1971).

Phosphorus, which diffuses through the soil at an extremely slow rate, has been shown to be a major nutrient limiting alfalfa seedling growth (Haynes and Thatcher, 1951). A band of fertilizer directly below the seed is an efficient method of providing plant available P to developing seedlings, resulting in increased growth rates (Brown, 1959; Haynes and Thatcher, 1950, 1951, 1953; Henderlong, 1961; Parsons, 1958; Robinson et al., 1959; Sheard et al., 1971; Tesar et al., 1954). Brown (1959) found that banding superphosphate under alfalfa seed approximately doubled the seedling height, shoot dry wt., and tissue phosphorus content during the first month after planting. Henderlong (1961) showed that band-fertilizing with P increased alfalfa seedling wt.

250 to 300% over unfertilized control plants at the end of the fall growing season. Sheard et al. (1971) concluded that P uptake from a band located 5 cm directly beneath the seed resulted in increased dry wt. and vigor for both legume and grass seedlings.

Banded-P has had inconsistent effects on alfalfa yield following establishment. Brown (1959) found that while banded-P increased early seedling growth, it did not significantly affect first harvest yield. In contrast, Haynes and Thatcher (1951) found that alfalfa band-seeded with P yielded 50% more than alfalfa seeded where the same rate of P was broadcast. The yield advantage observed by Haynes and Thatcher (1951) was due mostly to increased seedling survival in plots receiving banded-P. Carmer and Jackobs (1963) concluded that yield increases from banded-P were dependent upon environmental conditions following seeding. For example, under hot and dry conditions a yield advantage from banded-P was observed. However, no increase in yield was observed under conditions favoring good seedling growth.

Banded-P has been shown to increase seedling survival (Duell, 1974; Haynes and Thatcher, 1951, 1953; Henderlong, 1961; Sheard et al., 1971; Tesar et al., 1954). Placing alfalfa seed directly over a band of P fertilizer resulted in significantly more seedlings than broadcasting the same seed over banded-P fertilizer (Tesar et al., 1954). Henderlong (1961) concluded that banding P at the time of seeding resulted in higher plant populations, with an average of 50% over check plots (no banded fertilizer). The use of banded-P in the fall increases seedling growth and vigor, resulting in larger plants which are more winterhardy (Tesar and Marble, 1988). The variable effect of banded-P on yield and seedling survival seen in past research may have been the result of differing soil fertility levels. However, because soil fertility was not adequately documented in many past studies, concrete conclusions can not be drawn.

Smaller, less developed seedlings are normally more susceptible to environmental stresses such as drought or flooding. Sheard et al. (1971) concluded that slow development of forage species during establishment makes seedlings more vulnerable to death when environmental stresses are present. Therefore any practice that will speed up early development should be employed and band-seeding with P is one such practice. The increased growth rate of alfalfa seedlings due to banded-P results in larger more developed seedlings which may be better able to withstand excess soil moisture commonly present during establishment especially when seeding in the fall. Banded P may also improve the recovery of young seedlings after temporary flooding stress. To date, the potential benefits of banded-P for alfalfa seedlings subjected to temporary waterlogging stress has not been determined. This study was designed to evaluate the effect of banded-P on alfalfa seedling growth in the fall and subsequent productivity after temporary flooding stress.

MATERIALS AND METHODS

Field experiments were established in late August 1994 at The Ohio State University in Columbus (40° 00' N, 83° 00' W), and at the Ohio Agricultural Research and Development Center (OARDC) in Wooster (40° 25' N, 81° 75' W). In 1995, experiments were again seeded in late August at Wooster and at OARDC's Western Branch Research Center near South Charleston (39° 45' N, 83° 45' W). The soil at Columbus and S. Charleston was a Crosby silt loam (fine, mixed, mesic Aeric Ochraqualfs). The soil type at Wooster for both years was a Wooster-Riddles silt loam association (fine-loamy, mixed, mesic Typic Fragiudalfs and fine-loamy, mixed, mesic Typic Hapludalfs, respectively). Soil fertility levels for the four sites are given in Table 1. At the Wooster 1994 location, 290 kg ha⁻¹ K₂O was applied before seeding. At S. Charleston, 112 kg ha⁻¹ K₂O was broadcast uniformly in early April 1996.

All plots were band-seeded using an eight row drill with press wheels. 'WL 323' alfalfa, which is highly resistant to phytophthora root rot and resistant to aphanomyces, was seeded at a rate of 16.8 kg ha⁻¹. The seed was inoculated with *Rhizobium meliloti* before planting. Plot size was 3.05 x 6.10 m. The plots were divided lengthwise and one-half was used for plant sampling and the other was harvested for forage yield the year after seeding. A split-plot randomization of a randomized complete block design with four replications was used. Whole plot treatments were flooded and unflooded (natural precipitation). Split-plot treatments were no P and 62 kg ha⁻¹ of P₂O₅ banded 4 to 5 cm below the seed. Triple superphosphate fertilizer was used. Plots were irrigated as needed to ensure uniform and timely germination and emergence.

Common purslane (*Portulaca oleracea* L.) was controlled in the 1994 seeding at Columbus before the flooding treatment was imposed using 2,4-DB (4-(Dichlorophenoxy) butyric acid, dimethylamine salt) at 0.84 kg a.i. ha⁻¹. At Wooster in 1995, volunteer oat (*Avena sativa* L.) in the 1995 seeding were controlled before

flooding with sethoxydim (2-(1-(ethoxyamino)butyl-5-(2-(ethylthio)propyl)-3-hydroxy-2-cyclohexen-1-one) at 0.24 kg a.i. ha⁻¹. After flooding, 2,4-DB was applied at 1.68 kg a.i. ha⁻¹ for control of common lambsquarter (*Chenopodium album* L.). At S. Charleston, common chickweed (*Stellaria media* L.) was controlled using imazethapyr (ammonium salt of imazethapyr (±)-2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-5-ethyl-3-pyridinecarboxylic acid) at 0.07 kg a.i. ha⁻¹ in the spring following establishment.

The flooding treatment was imposed by placing trickle irrigation tubing between each row of alfalfa in the flooded plots. Water flow was regulated to keep the soil saturated and allow some ponding on the soil surface. A 3 m border between flooding treatments and a series of diversion ditches ensured that unflooded plots did not receive moisture from the flooded areas. Flooding was initiated in the 1994 seedlings when the seedlings had reached the 7th to 8th trifoliolate leaf stage (26 d. after seeding, 20 September 1994) at Columbus and the 4th to 5th trifoliolate stage (27 d. after seeding, 15 September 1994) at Wooster. The duration of flooding was based on the visible plant damage symptoms in the flooding treatment. Symptoms included yellowing of the plants, cupping of the leaves, and stunted growth. Plants were flooded for 15 d. at Wooster and 18 d. at Columbus in 1994. Flooding was initiated in the 1995 seedlings when seedlings were in the 2nd to 3rd trifoliolate leaf stage at both Wooster (21 d after seeding, 13 September 1995) and S. Charleston (27 d after seeding, 25 September 1995). Flooding was maintained for 11 d. at Wooster and 17 d at S. Charleston.

The following parameters were evaluated: 1) root and shoot dry wt., 2) plant density, 3) heaving, 4) forage yield the year following establishment, and 5) root nonstructural carbohydrates (NSC) concentration in the roots. In addition, a sample of plants representing all plots for a given location and year were sent to The Ohio State University Extension Plant and Pest Diagnostic Clinic in Columbus for phytophthora root rot testing using the ELISA test (Agri-Screen Phytophthora Detection Kit, Neogen Corp., Lansing, MI).

Plants were harvested for root and shoot dry wt. immediately before flooding, 2 to 3 d after flooding, and near fall dormancy (about 7 wk after the termination of flooding). Fall sampling dates for the 1994 Wooster seeding were 15 September, 5 October, and 18 November. Plots at Columbus in 1994 were sampled on 19 September, 13 October, and 28 November 1994. Sampling dates for the Wooster 1995 seeding were 13 September, 27 September, and 17 November. For the 1995 seeding at S. Charleston, samples were taken 25 September, 16 October, and 14 November. Whole plants with their roots encased in soil were carefully dug from 3 to 4 randomly chosen areas within each plot, placed on screens, and carefully washed. After washing, whole plant samples were placed on ice for transport to the lab. In the lab, the plants were separated at the junction of the crown and tap root. The shoots were dried in a forced air oven at 60°C for 3 to 4 d and the roots were freeze-dried for 3-5 d. Root and shoot dry wt. were expressed on a per plant basis.

Plant density was measured before flooding in the seeding year and the year after at the last harvest. Plants were dug and counted in 1.22 m of row at two randomly chosen areas within each plot, for a total of 2.44 m of row.

Winter heaving was assessed when it occurred by visually rating total subplot area heaved and severity of heaving. Severity of heaving was rated as slight if plants were heaved 1.5 cm or less above the soil surface and likely to recover, and severe if plants were heaved more than 1.5 cm above the soil surface and not likely to recover. Plots were harvested for forage yield the year after seeding when alfalfa reached the late bud to early bloom stage of growth. The harvest area was 1.07 x 6.10 m. At each harvest, forage was removed with a flail-type mower to a 7.0 cm stubble height and fresh weight was recorded for each plot. Samples of 600-800g were taken, weighed fresh, then dried at 60°C and reweighed to determine dry matter content. Forage dry matter yield was calculated as a product of the fresh weight for each plot and the percent dry matter. Four harvests were made.

Root samples for NSC determination were collected after flooding, in late November, and the following spring at 2 to 4 wk intervals up to the first harvest. Roots were dug, placed on ice, and brought back to the lab where they were washed and separated at the crown and 5.1 cm below it. The 5.1 cm upper root section was freeze-dried, ground through a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) with a 20 mesh screen and through a cyclone sample mill (Udy Analyzer Co., Boulder, CO) equipped with a 1 mm screen. Two 50 mg subsamples were analyzed for NSC. Free sugars were extracted using 80% (v/v) ethanol. Anthrone assay was used to quantify free sugars (Koehler, 1952). A modified enzymatic method from Smith (1981) was used to analyze for starch. Total NSC were calculated by addition of total free sugars and total starch, on a dry wt. basis.

Analysis of variance was used to test for statistical significance of environments (year-location combinations), treatment effects, and interactions. Flooding and P treatments were considered fixed variables, while years and locations were considered random variables.

RESULTS AND DISCUSSION

Data are analyzed and presented by environment (location-year) because treatment x environment interactions were significant ($P \leq 0.05$). Differences in soil type, soil fertility, and weather conditions may have contributed to the significant interactions observed in this study. Rainfall was approximately 50 mm below normal for the month following seeding (September) at all locations. For the 1994 seedings at Wooster and Columbus, rainfall in October was also more than 30 mm below normal. Although irrigation was used to ensure uniform and timely germination, plots were not irrigated on a regular schedule after emergence. Post-emergence irrigation was used only to facilitate digging of root samples. Death of the above ground tissue for the 1994 seedings occurred in mid-November at both locations. For the 1995 seedings, death of the above ground tissue occurred in early November at both Wooster and S. Charleston.

Effects on Seedling Growth. Root and shoot dry wt. averaged over P treatments was significantly reduced by flooding at all harvests in all environments, except for root dry wt. after flooding at S. Charleston in 1995 and shoot dry wt. at dormancy at Columbus in 1994 (Fig. 1 and 2). The observation of decreased dry wt. for flooded plants is in general agreement with the results obtained by other researchers (Barta, 1980; Cameron, 1973; Heinrichs, 1972; Rai et al., 1971; Thompson and Fick, 1981; Yu et al., 1969). At the start of flooding, plots banded with P were easily recognizable due to their increased size and vigor at all locations except Columbus in 1994. The lack of a P effect at Columbus in 1994 was most likely due to the extremely high level of available soil P (Table 1). Banded-P increased root and shoot dry wt. for most harvests, except at Columbus, which possessed an extremely high level of soil available P (Figs. 1 and 2; Table 1). Soils with less than 25 ppm P (Bray-1 extraction) are considered to be deficient for alfalfa production (Vitosh et al., 1995). Of the three locations responding positively to banded P, only S. Charleston had an initial soil test for available P below the critical level of 25 ppm (Table 1). This clearly demonstrates that the positive effects of banded P on early alfalfa seedling development are realized on soils which are not considered deficient in plant available P. However, on soils which are extremely high in plant available P, like those at the Columbus location, the positive effects of banded P may be subdued. The increase in root and shoot dry wt. due to banded-P observed in this study is in general agreement with the work of other investigators (Brown, 1959; Duell, 1964; Haynes and Thatcher 1951; Sheard and et al., 1971).

Approximately 5 to 7 d after the start of flooding, seedlings in the flooded treatment started to show visible signs of plant injury in the above ground tissue (yellowing of the shoots and cupping of the leaves). Flooded seedlings established with banded-P were larger at the termination of flooding at all locations, except Columbus (Figs. 1 and 2). In mid to late November, flooded seedlings established with banded-P possessed greater root dry wt. at Wooster and S. Charleston in 1995. Shoot dry wt. in mid to late November for flooded seedlings established with banded-P was significantly greater at S. Charleston only.

A flooding x P treatment interaction for root dry wt. after flooding and at dormancy ($P=0.10$) was found for the Wooster 1995 location only. Flooding x P treatment interactions were also observed for shoot dry wt. at S. Charleston in 1995 after flooding ($P=0.05$) and at dormancy ($P=0.08$), and at Wooster in 1995 at dormancy ($P=0.10$). The interactions were primarily due to small changes in magnitude of differences among treatments rather than changes in ranking of treatments.

Banding P directly below alfalfa seed resulted in larger seedlings at the start of flooding. Shoot and root growth continued during flooding, and at the end of the flooding, seedlings established with banded-P were still larger than the flooded, no P control. At fall dormancy, root dry wt. was still greater for flooded seedlings with banded-P at Wooster and S. Charleston in 1995.

Performance the Year After Seeding. Heaving was observed at all locations except Columbus. Severe heaving in late February 1996 at Wooster resulted in total stand death. Flooding increased heaving at Wooster in 1995 and S. Charleston in 1996 (Table 2). A P x flooding treatment interaction ($P=0.10$) for heaving was found at Wooster in 1995: banded-P reduced heaving in the flooded treatment, but had no effect in the unflooded treatment (Table 2). While banded-P significantly reduced heaving in the flooded plots at Wooster in 1995, its

ability to decrease heaving is at best variable and appears to have little effect under severe heaving conditions like those observed at Wooster in 1996. In contrast, Henderlong (1961) noted considerable heaving occurred in the unfertilized plots, while heaving was not noticeable in the fertilized plots. The effect of banded P on winter heaving may be influenced by environmental conditions following seeding, soil drainage, severity of heaving, type of seeding (no-tillage v/s conventional tillage), root development of the seedling, and snow cover.

Total yield for the year following establishment was reduced by flooding the previous fall at Wooster in 1995 and at S. Charleston in 1996 (Table 3). Banded-P increased total yield for the unflooded treatment at S. Charleston in 1996 and for the flooded treatment ($P=0.10$) at Wooster in 1995 (Table 3). The variable yield response to banded-P in the present study is in agreement with the findings of Carmer and Jackobs (1963), who observed that a yield advantage from banded-P was dependent on environmental conditions following seeding.

Stand density before flooding was similar for all treatments (Table 4). Final stand counts differed among P and flooding treatments at Wooster in 1995 only. In addition, a P x flooding treatment interaction for the final stand count was found at Wooster in 1995. The interaction was a result of opposite P-banding effects within the flooding treatments (Table 4). In contrast to the findings of Henderlong (1961), banded-P did not appear to result in significantly higher plant populations in these experiments. As with yield responses to banded-P, increases in stand density may be greatly dependent upon environmental conditions.

Total NSC accumulated in alfalfa during the fall provide energy for respiration during dormancy and regrowth the following spring (Jung and Smith, 1961). In addition, sugars play an important role in cold hardening. Sugar concentration increases during acclimation to cold stress in the fall and remains high until spring (Bula and Smith, 1954; Jung and Smith, 1961). Kust and Smith (1961) and Meyer and Nelson (1983) also noted a positive correlation between fall NSC concentration and yield the following year.

In the present study, NSC concentration declined from early December to late March (data not shown). Flooding reduced NSC concentration in December and March at all locations except Columbus. At the first harvest, NSC in the S. Charleston 1995 and Columbus 1994 seedings were significantly lower for the flooded plots. As noted earlier, flooding reduced total yield the year after establishment in the Wooster 1994 seeding. Total NSC concentration in December was positively correlated with total forage yield the following year for the Wooster 1994 seeding only. However, other factors such as stand density and heaving may have had a greater influence on subsequent dry matter yield than did fall NSC concentration. Although banded-P increased NSC concentration in the Wooster 1994 and S. Charleston 1995 seedings in the fall, its positive effect was lost by the first harvest the following year (data not shown).

All locations and years tested negative for phytophthora root rot for the flooded and unflooded plants. In the S. Charleston 1995 seeding, a number of plants possessed black girdling lesions on the main taproot. After a negative phytophthora root rot test, a *Fusarium* species was isolated from these lesions (L.H. Rhodes, 1995, personal communication). The small amount of disease observed in this seeding did not appear to be a major factor affecting stand productivity.

CONCLUSION

Flooding reduced root and shoot dry wt. regardless of P treatment. Banded-P increased root and shoot dry wt. of seedlings before flooding at all locations except Columbus, which had very high soil P levels. The greater seedling size with banded P was still present in the unflooded treatment in mid to late November, except for root dry wt. at Wooster in 1994. At the termination of flooding, the advantage of P-banding was still present. In mid to late November, the effect of banded-P in the flooded treatment was significant ($P=0.05$) for both root and shoot dry wt. at S. Charleston in 1995 and for root dry wt. only at Wooster in 1995. Flooding reduced stand density ($P=0.06$) and increased heaving ($P=0.001$) in the Wooster 1994 seeding. Although banded-P reduced heaving in the Wooster 1994 seeding, there was no benefit to banded-P under the severe heaving conditions observed at Wooster in 1996. Banded-P had small and inconsistent effects on stand density. Flooding usually reduced NSC concentrations going into winter. The difference in the fall NSC concentration correlated with increased yield the following year for the unflooded plots in the Wooster 1994 seeding only. However, other factors such as stand density and heaving may have had greater influence on subsequent yield at this location.

Although banded-P increased root and shoot dry wt. in the fall, its effect on heaving, stand density, and yield the year following establishment was minimal and variable. The size advantage obtained from

banded-P may increase winter survival and yield the year following establishment when environmental conditions do not favor seedling growth in the fall. The benefit of more rapid dry wt. accumulation from banded-P may be especially important when seedlings are made very late (Brown, 1959). Despite the variable response of subsequent productivity to banded-P in this study, banded-P is advantageous to seedling growth in the fall and should be employed as a relatively inexpensive means of ensuring seedlings have the best chance for successful establishment.

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Table 1. Soil fertility levels for the four experimental sites.

Year	Location	pH	P	K
			-----ppm-----	
			-	
1994	Columbus	6.9	198	210
1994	Wooster	6.6	39	48
	S.			
1995	Charleston	6.3	24	106
1995	Wooster	6.1	31	121

Table 2. Treatment effects on winter heaving the year after establishment.

Flooding treatment	Phosphorus treatment	Wooster 1995	S. Charleston 1996
		-----% Heaving-----	
Flooded	No P	80	27
	P	65	20
Unflooded	No P	22	2
	P	21	0
LSD (0.05)†		12	24
P-value P		0.06	NS
P-value flooding (F)		0.001	0.09
P-value P x F		0.10	NS

†LSD (0.05) to compare P treatment means within flooding treatment and location.

#NS=Not significant (P>0.15)

Table 3. Treatment effects on total dry matter yield the year after establishment.

Flooding treatment	Phosphorus treatment	Columbus	Wooster	S. Charleston
		1995	1995	1996
-----Mg ha ⁻¹ -----				
Flooded	No P	15.50	9.37	7.04
	P	16.05	11.02	7.18
Unflooded	No P	16.83	13.43	9.69
	P	16.42	13.64	11.21
LSD (0.05)†		1.38	1.75	1.35
P-value P		NS‡	0.12	0.08
P-value flooding (F)		NS	0.07	0.05
P-value P x F		NS	NS	0.13

LSD (0.05) to compare P treatments means within flooding treatment, location, and year

NS=Not significant (P>0.15)

Table 4. Treatment effects on stand density in the four experiments.

Flooding treatment	P treatment	Wooster		Columbus		Wooster		S. Charleston	
		Prefloodi ng 1994	Final harvest 1995	Prefloodi ng 1994	Final harvest 1995	Prefloodi ng 1995	Final harvest 1996	Prefloodi ng 1995	Final harvest 1996
-----Plants m ⁻² -----									
Flooded	No P	250	99	204	116	381	-	476	228
	P	269	110	214	146	362	-	490	259
Unflooded	No P	268	182	210	130	389	-	494	268
	P	255	161	216	116	348	-	495	344
LSD (0.05)		68	20	55	35	48	-	59	113
P-value P		NS	NS	NS	NS	0.08	-	NS	NS
P-value flooding (F)		NS	0.06	NS	NS	NS	-	NS	NS
P-value P x F		NS	0.03	NS	0.08	NS	-	NS	NS

LSD (0.05) to compare P treatment means within flooding treatment, location, and year

NS=Not significant (P>0.15).

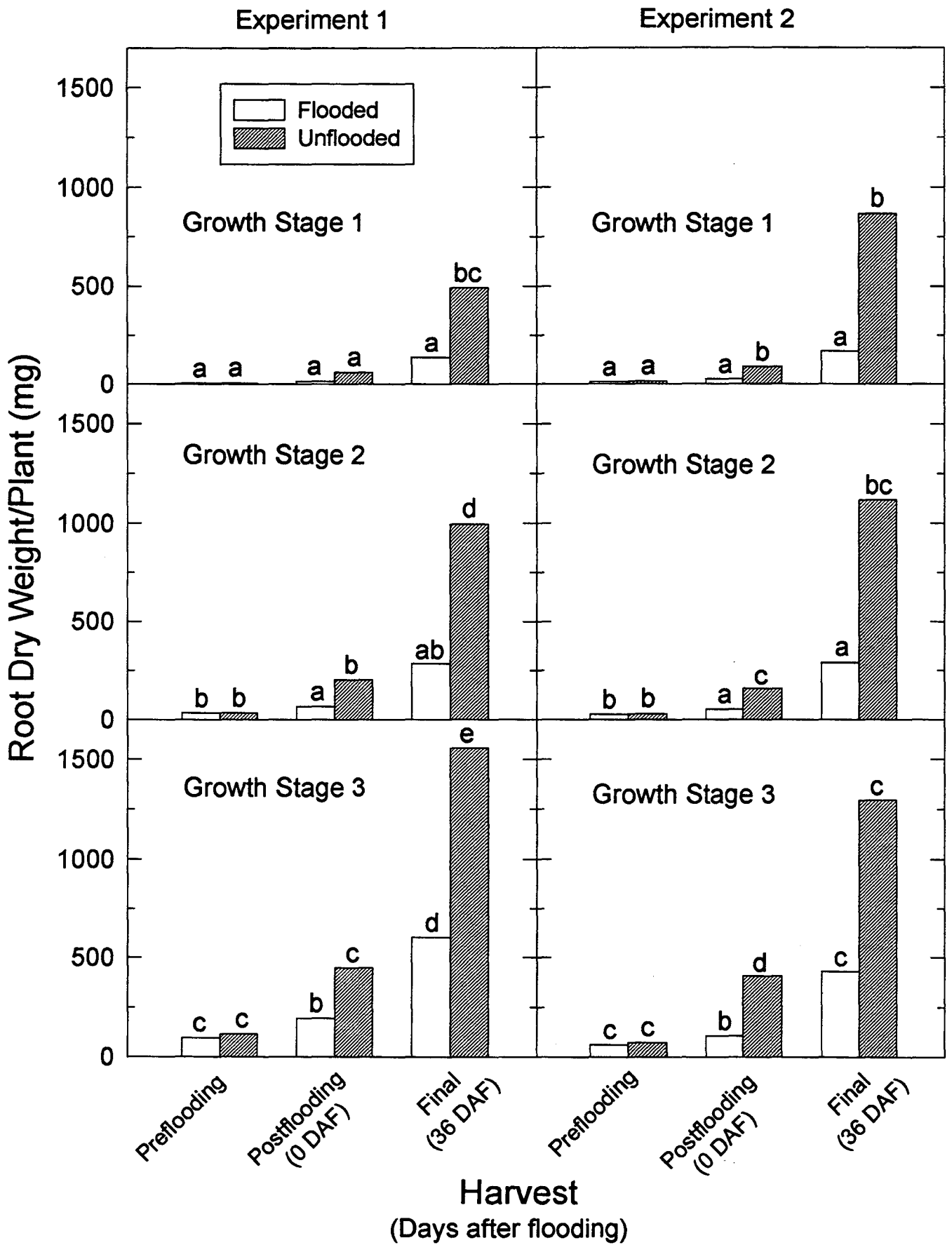


Fig. 1. Treatment effects on root dry wt. Growth stage treatments are defined in Table 1. Bars followed by the same letter within the same harvest and experiment are not significantly different according to LSD ($P=0.05$).

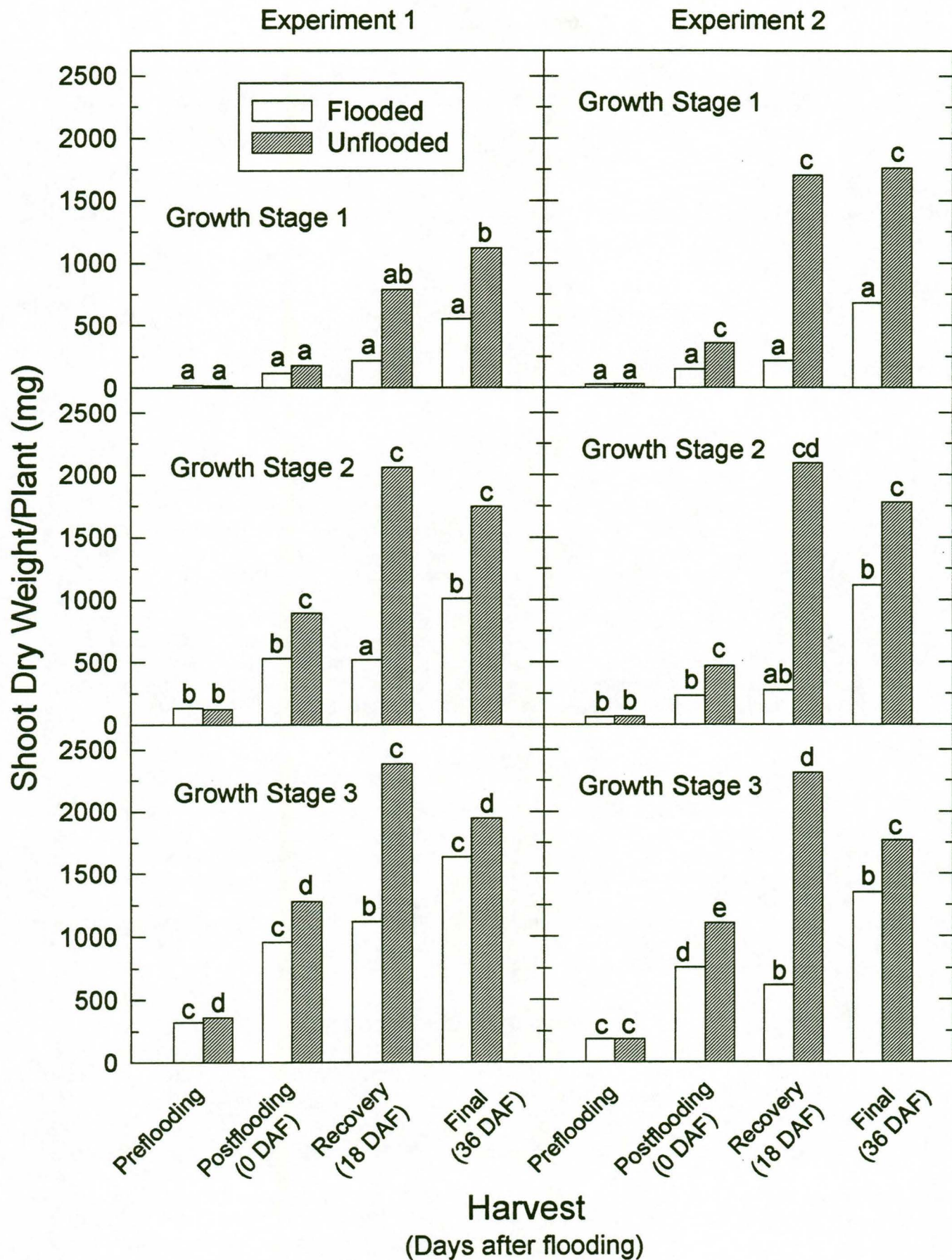


Fig. 2. Treatment effects on shoot dry wt. Growth Stage treatments are defined in Table 1. Bars followed by the same letter within the same harvest and experiment are not significantly different according to LSD ($P=0.05$).

Flooding Injuries in Soybean Are Caused by Elevated Carbon Dioxide Levels in the Root Zone

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ABSTRACT

Root flooding is damaging to plant growth. The injurious effects of root flooding have been widely attributed to the lack of oxygen in the root zone essential for root respiration. In addition to the lack of oxygen, the concentration of CO₂ in flooded soils may reach levels as high as 50% of the total gases. However, the effects of root zone CO₂ on flooded plants have not been fully investigated. In this paper, seedlings of two soybean genotypes 'Williams' and 'Williams 82' were grown in hydroponic solution bubbled with either air, nitrogen or CO₂. While the pH (5.5) and dissolved oxygen levels (<2mg/L) in the N₂ and CO₂ bubbling treatments were similar, seedlings of the CO₂ bubbling treatment became chlorotic and many died within 5 days. The seedlings that survived grew slowly and accumulated only small root and shoot biomass. Seedlings of the N₂ bubbling treatment, despite the low dissolved O₂ (<2mg/L) in the solution, grew almost as well as seedlings of the air bubbling treatment (dissolved O₂ = 8 mg/L). The results indicated that soybean is very tolerant to the lack of oxygen, but is sensitive to elevated CO₂ levels in the root zone.

INTRODUCTION

Flooding can cause a decrease in photosynthesis (Musgrave, 1994), in biomass accumulation (Oosterhuis et al., 1990), and in seed yield (VanToai et al., 1994). Damaged and dead roots in flooded plants (Setter and Belford, 1990; Huck, 1970) have been attributed to the lack of oxygen to support root respiration (Crawford, 1992; Kozłowski, 1984). Besides the lack of O₂, the concentration of CO₂ in flooded soils may reach a level as high as 50% of the total gases and could be toxic to plants (Ponnamperuma, 1972). The effects of root zone CO₂ on flooded plants have not been fully investigated. In 1962, Glinka and Reinhold reported that soil CO₂ affected the uptake of water in sunflower (*Helianthus annuus* L. Var. Jupiter) roots. In this paper, we present the evidence that high levels of root zone CO₂ were more injurious to soybean growth than the lack of O₂ alone.

MATERIALS AND METHODS

Plant materials. Seeds of two soybean near isogenic lines "Williams" and "Williams-82" were germinated in moist sand at 25 ± 2 °C constant temperature and darkness for one week. Healthy and uniform seedlings were transferred to hydroponic culture.

Hydroponic culture. The hydroponic culture was conducted in 35-L plastic containers filled with nutrient solution (Imsande and Ralston, 1981). The nutrient solution was made with 1mM MES containing 2.96 mM KCl, 0.099 mM K₂HPO₄, 2.1833 mM CaCl₂·2H₂O, 1.014 mM MgSO₄·7H₂O, 0.7132 mM MgCl₂·6H₂O, 0.320 mM NH₄NO₃, 40 mg/L iron sequestrene, 2.0212 mM MnCl₂·4H₂O, 0.8 mM CuSO₄·5H₂O, 4.527 mM H₃BO₃, 4.527 mM NaMoO₄·2H₂O, 0.619 mM ZnSO₄, and 0.695 mM CoSO₄·7H₂O mM. A total of 8 hydroponic containers was used in this study. Each

hydroponic container was covered with a thick styrofoam sheet having 12 holes at equal distances through which seedlings were placed and kept suspended in upright position by plugs made of soft polyurethane. Six seedlings of each genotype, or a total of 12 seedlings were grown in each container. Seedlings were allowed to acclimatize to hydroponic conditions for four days before the gas treatment was initiated. The conditions of the growth chamber were $25 \pm 2^{\circ}\text{C}$ constant temperature and 14 hr light at 515 PAR. Just before the gas treatment, the hydroponic solution was changed such that four of the containers had the complete solution described above (+ NH_4NO_3), while the other four containers had the same solution but without NH_4NO_3 (- NH_4NO_3). Dissolved oxygen in the medium was monitored continuously with an oxygen electrode (YIS model 5720A) connected to a dissolved oxygen meter (YSI model 58). The pH of the solution was determined with a pH meter (Beckman, model f45) at one, two, and four weeks after the initiation of the gas treatments.

Gas treatment. Three gas treatments were imposed by bubbling the hydroponic solution with either air, N_2 , or CO_2 . The stagnant treatment received no air or gas bubbling. Each gas treatment was applied to 2 containers. One contained the + NH_4NO_3 solution, the other contained the - NH_4NO_3 solution. The experiment continued for an additional four weeks after the gas treatment was initiated.

Plant responses. Plant responses to the gas treatments were determined by leaf greenness measured with a Minolta SPAD 502 Chlorophyll meter (Minolta Camera Co., Ltd., Japan) at 2 and 4 weeks after the onset of the gas treatment. Root, shoot and nodule biomass were determined at the end of the experiment.

Statistical analysis. The analysis of variance (ANOVA), means and LSD were determined using the SAS program (SAS Inst. Inc., Raleigh, NC).

RESULTS AND DISCUSSION

Dissolved oxygen and pH. The dissolved oxygen concentration was 8 mg/L in the air bubbling treatment and 4 mg/L in the stagnant treatment. The N_2 and CO_2 treatments had much less dissolved oxygen concentration (<2 mg/L).

At one week after flooding, the air treatment had the highest pH (5.77), while the N_2 and CO_2 treatments had similar but lower pH (5.51). It was unexpected that the stagnant treatment had the lowest pH (4.92) of all the treatments, especially since dissolved CO_2 can produce carbonic acid. The pH of the solution did not change during the rest of the experiment. Within each gas treatment, no difference in pH was detected due to NH_4NO_3 .

Effects of cultivars. Williams and Williams 82 are near isogenic lines. The difference is the presence of the *Rps1k* gene for Phytophthora resistance in Williams 82. Under root hypoxia (N_2 and stagnant treatments), Williams produced more root and shoot biomass than Williams 82. The results support our earlier data that Williams was more tolerant to flooding than Williams 82 in greenhouse screening tests (VanToai et al., unpublished data). Since Williams also provided more biomass than Williams 82 across all + NH_4NO_3 treatments (Table 1), it was not clear if the hypoxic tolerance of this genotype was due to specific gene expression, or its overall vigorous growth. Both genotypes were sensitive to the lack of nitrogen. The differences in shoot, root and nodule biomass between the two genotypes were negated when NH_4NO_3 was limiting. The results indicated an interaction between genotypes and NH_4NO_3 in the response of soybean to dissolved oxygen in the root zone.

Effects of NH_4NO_3 . The - NH_4NO_3 treatment was used to study the effects of gas treatments on nodulation and plant responses to hypoxia. During the first two weeks of the gas treatment, plants in the - NH_4NO_3 treatment were much more chlorotic than plants in the + NH_4NO_3 medium. Ammonium nitrate inhibited the formation of nodules in all gas bubbling treatments. The lack of NH_4NO_3 induced the plants to form nodules and fix nitrogen such that at 4 weeks after the treatment, leaves of the - NH_4NO_3 treatment were just as green as leaves of the + NH_4NO_3 treatment. Despite the recovery, plants of the - NH_4NO_3 treatment remained smaller than plants of the + NH_4NO_3 treatment at four weeks after the treatment (Table 1).

Effects of gas treatment on survival. While bubbling with nitrogen gas did not affect the survival of soybean seedlings, bubbling with CO_2 was highly detrimental, especially to the Williams genotype. Seventeen percent of Williams seedlings died within 5 days in the - NH_4NO_3 solution bubbled with CO_2 ; but none of the Williams-82 seedlings died in the same treatment. While Williams was more tolerant to hypoxia caused by N_2 bubbling and stagnant water (Table 1),

Williams-82 appeared more tolerant to dissolved CO₂ than Williams. Bubbling with CO₂ was even more injurious in the +NH₄NO₃ solution. In that treatment, 87% of Williams and 67% of Williams-82 seedlings died after five days. The toxicity of NH₄NO₃ in the CO₂ bubbling treatment could be due to the conversion of nitrate to nitrite at low redox potential.

Effects of gas treatment on leaf greenness. Hypoxia did not seem to affect the greenness of soybean leaves as determined by the SPAD meter. Leaves of the N₂ bubbling and stagnant treatments had the same leaf greenness as leaves of the air bubbling treatment (Table 2). However, leaves of the CO₂ bubbling treatment turned yellow within one day after the treatment and remained very chlorotic, except in the two plants that were able to produce stem roots above the solution.

Effects of gas treatment on biomass. Significant differences ($P < 0.05$) in root and shoot biomass were observed among the gas treatments in the +NH₄NO₃ medium. Plants of the air treatment had the highest shoot biomass, followed by plants in the stagnant and N₂ treatments, while plants in the CO₂ treatment had the lowest shoot biomass (Figure 1A). No significant differences for root biomass were detected in the air, N₂ and stagnant treatments, while the CO₂ treatment showed the lowest root biomass.

Without NH₄NO₃, the air and stagnant treatments had similarly high root and shoot biomass, followed by the N₂ treatment. Plants of the CO₂ treatment had the lowest shoot and root biomass (Figure 1B). Plants of the N₂ and stagnant treatments produced substantial amounts of nodules, but no nodules were found in plants of the CO₂ treatment.

Elevated CO₂ levels in the root zone significantly affected shoots, roots and nodules biomass. However, the effects were more severe in the solution with NH₄NO₃ than in the medium without NH₄NO₃. The results indicated that nutrient deficiency (-NH₄NO₃), lack of O₂ and excessive CO₂ under flooding are detrimental for soybean. Since soybean grew well in the N₂ bubbling treatment where the dissolved O₂ was very low, but was severely injured in the CO₂ bubbling treatment where the dissolved O₂ was at the same level, it was concluded that elevated root zone CO₂ was more detrimental to soybean than the lack of O₂ alone. Kramer and Jackson (1954) and Glinka and Reinhold (1962) indicated that high concentrations of CO₂ around plant roots inhibited water uptake by reducing the permeability of cell membranes. In addition, CO₂ has been known to antagonize the action of ethylene. Since ethylene is necessary for the formation of adventitious roots and aerenchyma in the acclimation responses of soybean to flooding, elevated CO₂ in the root zone is detrimental to the plants. The exact mechanism of CO₂ injury under flooding remains to be worked out.

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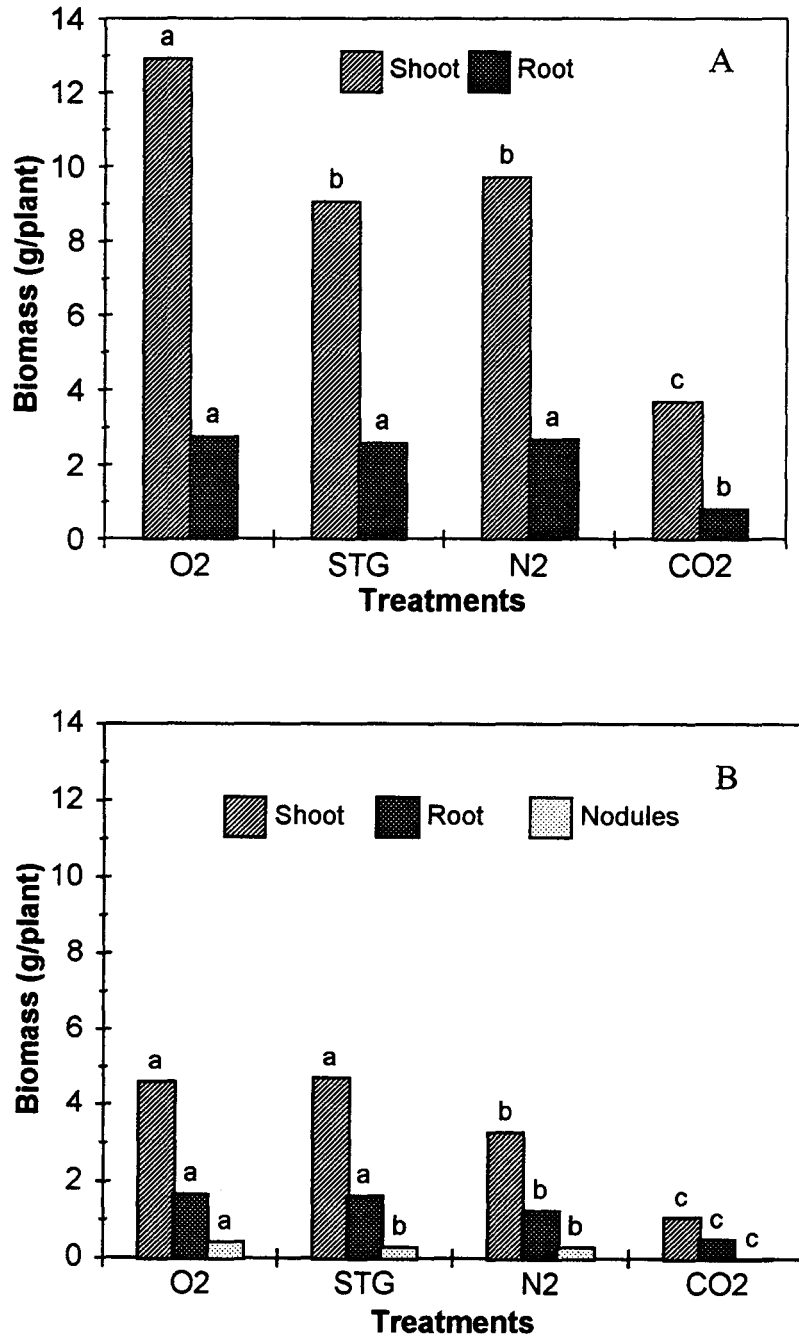
Table 1. Shoot, root and nodule biomass of two soybean genotypes grown hydroponically in medium with (+) and without (-) NH_4NO_3 .

Varieties	<u>+NH_4NO_3</u>		<u>-NH_4NO_3</u>		
	<u>Biomass (g/plant)</u>		<u>Biomass (g/plant)</u>		
	<u>Shoots</u>	<u>Roots</u>	<u>Shoots</u>	<u>Roots</u>	<u>Nodules</u>
Williams	11.04	2.74	3.80	1.42	0.27
Williams-82	8.71	2.27	3.21	1.15	0.23
LSD (0.05)	1.41	0.33	ns	ns	ns

Table 2. Effects of root gas treatment on leaf color (SPAD unit) of soybean grown hydroponically with and without NH_4NO_3 . Mean of 30-36 readings.

Gas Treatment	<u>Leaf Greenness (SPAD units)</u>			
	<u>Treatment Duration</u>			
	<u>Two weeks</u>		<u>Four weeks</u>	
	<u>+NH_4NO_3</u>	<u>-NH_4NO_3</u>	<u>+NH_4NO_3</u>	<u>-NH_4NO_3</u>
Air bubbling	31.62	8.80	33.80	35.65
N_2 bubbling	31.57	9.90	33.55	35.10
CO_2 bubbling	22.30	15.17	31.80	20.40
Stagnant	30.50	9.25	33.00	35.00
LSD (0.05)	3.72	3.42	3.29	2.84

Figure 1. Shoot, root and nodules biomass (g/plant) of hydroponically grown soybean in response to gas bubbling treatments (O_2 = air, STG= stagnant, N_2 = nitrogen gas, CO_2 = carbon dioxide gas). **A.** $+NH_4NO_3$ solution and **B.** $-NH_4NO_3$ solution.



Seeding Date Affects Sclerotinia Crown and Stem Rot Severity in Alfalfa Establishment

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ABSTRACT

Sclerotinia crown and stem rot is a serious threat to successful no-till summer establishment of alfalfa in the eastern and southeastern United States. This study was conducted to determine the effect of planting date on severity of Sclerotinia crown and stem rot (SCSR) and subsequent productivity of alfalfa. Alfalfa was seeded no-till in May, early August, mid August, and late August 1993 and 1994 in a sod uniformly infested with sclerotia of *S. trifoliorum*. Averaged over years, disease severity (percentage of plot area affected) was 4, 12, 23, and 41% for the spring, early August, mid August, and late August plantings, respectively. Forage yield the year after seeding reflected differences in disease severity ratings. The risk of severe SCSR damage in no-till summer seedings of alfalfa can be reduced dramatically if stands become established early enough so plants reach at least 10 wks of age by the time apothecia emerge in the fall.

INTRODUCTION

Sclerotinia crown and stem rot (SCSR), caused by *Sclerotinia trifoliorum* Erikss., is one of the most destructive diseases of alfalfa (*Medicago sativa* L.) and other perennial forage legumes in the eastern United States (1990). This disease, caused by the fungus *Sclerotinia trifoliorum*, is most damaging to alfalfa seeded in late summer, especially when reduced tillage and no-till seeding methods are used. Sclerotinia crown and stem rot is a serious threat to the continuation of this practice. This presents a production dilemma for producers, because no-tillage late summer seeding of alfalfa is an effective system for reducing soil erosion and potential pest problems and pesticide use during alfalfa establishment. Clearly, suitable control measures for this disease must be found to ensure that late summer establishment of alfalfa be maintained as a viable practice, especially when reduced tillage methods are used.

Infection by *S. trifoliorum* occurs in the fall, although symptoms may not be evident until the following spring. A general consensus exists among researchers that older alfalfa plants are less affected by *S. trifoliorum* (Rhodes and Gilbert, 1990). Welty and Busbice (1978) stated that, while plants of any age are susceptible, the incidence and severity of the disease is greatest in seedlings. Cappellini (1957, 1960) and Rowe and Welty (1984) indicated the disease is especially important in fall seedings the first winter after planting. Rhodes and Gilbert (1990) stated that late summer or early fall seedings of alfalfa are most vulnerable to SCSR because plants are still in the seedling stage at the time of ascospore release in the fall, whereas plants seeded in the spring are not exposed to ascospores of *S. trifoliorum* until fall, and by then will have passed the seedling stage when susceptibility is greatest. These statements were not substantiated by prior field research, but were based on field observations only. Brune (1988) and Reichard (1995) conducted studies in controlled environments demonstrating that plant age affects resistance to *Sclerotinia*. Brune (1988) found that alfalfa plants were severely affected by SCSR when 2 wks old, followed by a rapid decrease in disease severity as plant age increased to about 8 wks. Disease severity ratings remained consistently low in plants that were 8 to 10 wks of age or older. Reichard (1995) found a similar rapid decrease in disease severity as alfalfa seedling age increased from 2.5 to 5.5 wks.

The phenomenon of increasing resistance to *S. trifoliorum* with advancing plant age needs to be validated in field environments, because it may serve as the basis for a management tactic to reduce the risk of SCSR damage in alfalfa. The primary objective of this research was to determine the effect of planting date on severity of SCSR and subsequent productivity of alfalfa established with no-tillage

practices. In addition, we evaluated planting date effects with and without application of vinclozolin, which is highly effective in controlling SCSR in alfalfa (Rhodes and Myers, 1988).

MATERIALS AND METHODS

Field experiments were established in 1993 and 1994 at Columbus, OH in a grass-clover (*Trifolium* spp.) sod infested with sclerotia of *S. trifoliorum*. The grass-legume sod was killed 2 to 3 wks before each planting date. Alfalfa was no-till seeded at 16 kg/ha on four dates each year. Planting dates were 20 May, 30 July, 16 August, and 31 August in 1993, and 11 May, 1 August, 16 August, and 30 August in 1994. Hereafter, these will be referred to as spring, early August, mid August, and late August planting date treatments. Plots were irrigated immediately after seeding and then regularly as needed for at least 4 wks to ensure rapid and uniform establishment. Seedlings emerged within 1 wk in the spring and within 3 days in the August plantings. Two fungicide treatments, no fungicide and vinclozolin, were imposed in each planting date. Vinclozolin was applied at 1.12 kg a.i./ha on or near 15 September, 15 October, and 15 November in the year of seeding, and on or near 15 March in the year after seeding.

Treatments were arranged in a split-plot randomization of a randomized complete block design with four replicates. Planting dates were assigned to whole plots and fungicide treatments to subplots. Forage growth in the spring-planted plots was removed in July and the first week of September in the seeding year. Forage growth in the August plantings was not removed in the year of seeding.

Disease severity was assessed in May the year after seeding. Visual ratings of the percentage of the stand affected by SCSR (diseased and dead plants) in each plot were recorded independently by the authors. Forage yield was measured at four harvests the year after seeding. Analysis of variance was used to test statistical significance of years, planting dates, cultivars, fungicide treatment, and interaction effects for all data.

RESULTS AND DISCUSSION

Weather conditions were favorable for apothecial production during the fall months of 1993 and 1994. Apothecia were first observed in the experimental area on 13 October 1993 and 18 October 1994. Apothecial production continued until mid-December both years. Inoculum levels were sufficient to cause heavy damage by SCSR in both years. Four applications of vinclozolin effectively controlled the disease, as stand area visibly affected by SCSR was less than 1% in all fungicide-treated plots (data not shown). Rhodes et al. (1992) had previously found that a similar treatment regime with vinclozolin effectively protected alfalfa from damage by SCSR. The fungicide-treated plots, therefore, provided an essentially SCSR-free control within the two experiments.

Delayed planting dramatically increased disease severity in the absence of vinclozolin (Fig. 1). Planting date effects on disease severity ratings were consistent across years, i.e. the year x planting date interaction was not significant ($P = 0.14$). Mean disease severity ratings across the two years were 4, 12, 23, and 41% affected plot area for spring, early August, mid August, and late August seedings, respectively.

SCSR reduced forage yield in the absence of vinclozolin at the first harvest the year after seeding (Fig. 2A,B). The yield loss due to SCSR (yield difference between unprotected plots and corresponding vinclozolin treated plots) increased with each delay in planting. There was a year x planting date x fungicide treatment interaction ($P = 0.01$) for first-harvest yield, primarily because the alfalfa in the early August seeding in 1994 did not realize its full yield potential, regardless of fungicide treatment (Fig. 2A). Alfalfa plants in that seeding date were less vigorous because of weed competition during early establishment. In 1995, forage yield declined with delayed seeding in both fungicide treated and untreated plots; however, the decline was dramatically greater where vinclozolin had not been applied (Fig. 2B). Although there was a year x planting date x fungicide treatment interaction, the ranking of planting dates for yield loss due to SCSR was consistent across years. This is more apparent when yield in unprotected plots is expressed as a percentage of yield in vinclozolin treated plots. In 1994, first-harvest forage yield in unprotected plots was 95, 80, 73, and 62% of that in the corresponding vinclozolin-treated plots for spring, early August, mid August, and late August plantings, respectively (Fig. 2A). In 1995, first-harvest

yields in the unprotected plots were 92, 88, 64, and 48% of that in the corresponding vinclozolin-treated plots for spring, early August, mid August, and late August planting dates, respectively (Fig. 2B). Thus, there were changes in magnitude of differences among planting date treatments across years, but no changes in their ranking. These yield data confirm the differences in disease severity observed among planting dates (Fig. 1).

The greatest loss in yield due to SCSR occurred in the first harvest, when plants were directly affected by the pathogen (Fig. 2A,B). The unprotected plots in the early and mid August plantings recovered considerably during the summer, and the seasonal total yield reflected the extent of that recovery (Fig. 2C,D). In contrast, the late August plantings exhibited much lower seasonal yields (Fig. 2C,D). Apparently, the reduction in stand density in that treatment (data not shown) was great enough to limit the extent of yield recovery during the summer months.

The literature indicates that severity of SCSR in alfalfa varies with plant age or time of seeding (Capellini, 1957 and 1960; Rowe and Welty, 1984; Welty and Busbice, 1978). To our knowledge, this is the first data quantifying this phenomenon in the field. Based on the observational evidence in the literature, we expected the spring sown alfalfa to be less vulnerable to SCSR than late summer sown alfalfa. That was indeed the case, especially when spring and late August seedings were compared. Even more noteworthy were the incremental losses to SCSR with only 2 wk delays in planting during August. These data demonstrate that alfalfa plants older than 8 to 10 wks are more tolerant or resistant to infection than younger plants when exposed to ascospore inoculum of *S. trifoliorum* under natural field conditions. Plant age at the time of first apothecia appearance in the field was about 6 wks, 8 wks, 10 wks, and over 21 wks in the late August, mid August, early August, and spring plantings, respectively. Greatest damage of SCSR occurred in the late August plantings, corresponding closely with previous controlled environment studies in which disease severity increased exponentially in plants less than 8 wks of age (Brune, 1988).

When alfalfa is seeded no-till in late summer, the risk of SCSR damage is dramatically reduced in Ohio and surrounding regions if the seedlings emerge by early to mid-August and weather conditions are favorable for good growth during the fall months. Recommended late summer seeding dates are 1-15 August in northern Ohio, and 1-30 August in southern Ohio. This study demonstrates that seeding alfalfa toward the later part of these recommended time frames could result in a dramatic increase in disease losses to *S. trifoliorum*. Earlier seeding is encouraged where *S. trifoliorum* inoculum may be present, but the dilemma for producers remains in that timely establishment is dependent on adequate soil moisture supply. Delayed emergence due to lack of moisture will result in younger plants at the time of ascospore release, with the consequent increase in susceptibility to SCSR. Therefore, the benefit of achieving genetic resistance to *S. trifoliorum* cannot be minimized.

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Fig. 1. Planting date effects on severity of Sclerotinia crown and stem rot of alfalfa (area visibly affected) the year after no-till establishment in a sod uniformly infested with sclerotia of *Sclerotinia trifoliorum*. Values are for the no fungicide treatment. Vertical bars indicate 1 S.E.

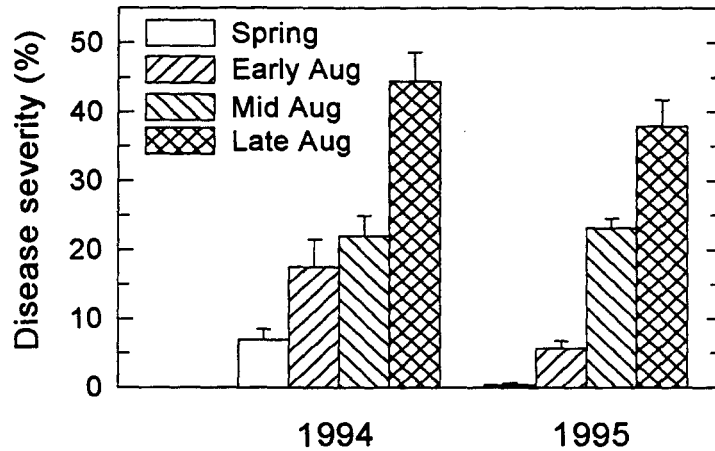
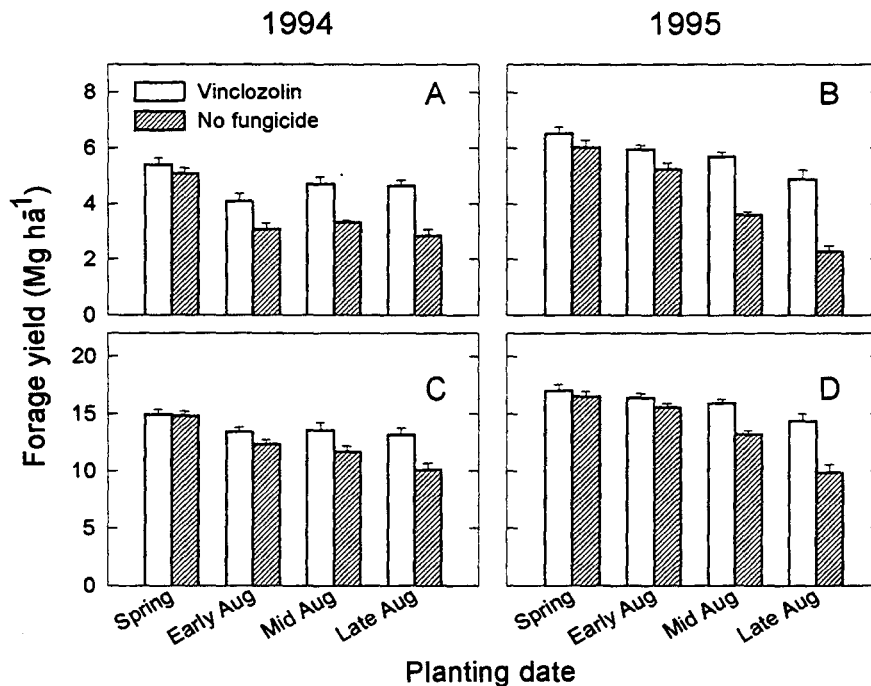


Fig. 2. Effects of planting date and fungicide treatment on forage yield of alfalfa the year after no-till establishment in a sod infested with sclerotia of *Sclerotinia trifoliorum*. (A,B) First-harvest yield, (C,D) total seasonal forage yield. Vertical bars indicate 1 S.E.



EMERGENCE OF SH2 SWEET CORN ON TWO SOIL TYPES AT VARYING DEPTHS

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ABSTRACT

As precision farming methods are gaining attention in large scale field crops, questions arise about the impact and potential for precision practices in other crops. In this study, the impact of soil type and planting depths on the emergence of three hybrids of shrunken 2 sweet corn with varying seed vigor were investigated. The seed was planted on September 18, 1996 in a field containing Crosby and Kokomo soil types in Columbus, OH. Three sh2 hybrids ('Starship', 'Skyline', and 'Confection') were planted at 1.3cm, 2.5cm, and 5.1cm depths with six replications on each of the two soils. Emergence counts as well as soil moisture and temperature were monitored. Seedling dry weights varied widely due to treatment combinations. The dry weight for a seedling harvested at the three to four leaf stage ranged from 10mg to 105mg. Potentially, this data could lead to variable planting depth prescriptions for sh2 corn. Future studies planned include mapping nutrient levels such as phosphorus and field characteristics including compaction over sh2 sweet corn fields. This information could then be used with emergence patterns to make better planting decisions.

INTRODUCTION

The purpose of precision farming is to give maximum economic yields while using sustainable practices. High precision includes timing of planting, placement in the soil, choosing suitable cultivars for field conditions, timing of inputs, and other factors (Wallace, 1994). In precision agriculture a field is not treated as a uniform entity but as many variable subunits that may require different treatments. Currently, precision farming is practiced mainly with corn and soybeans where many acres are being farmed. Precision farming may be beneficial to smaller fields where there is a high value crop being grown such as sh2 sweet corn.

Sh2 hybrids have been plagued by poor seed vigor and seedling disease, but otherwise are highly desirable (Wilson et al. 1992). Anything that could be done to establish a strong uniform stand would be very beneficial, especially when done with limited inputs of chemical treatments. Seed is particularly vulnerable between planting and germination (Carter and Chesson, 1996). It is important to plant in conditions that favor quick emergence (Price and Gaultney, 1993). Rapid germination and seedling growth are promoted by favorable soil temperatures (Willis et al. 1957) which vary directly with soil moisture and depth. Some seedling diseases are more likely to develop in cool, overly moist soil (Carter and Chesson, 1996). This study looks at the impact of planting depth and soil type on seedling emergence of three sh2 sweet corn hybrids of differing vigor.

MATERIALS AND METHODS

This was a fall study planted on September 18, 1996 in a field containing Crosby and Kokomo soil types in Columbus, OH. The study used nine treatments which consisted of the three hybrids planted at each of the following depths: 1.3cm, 2.5cm, and 5.1cm. These nine treatments were planted randomly in six replications in each soil type. Twenty seeds were planted in each row segment with hybrid remaining the same across a row and depth varying. Three meter gaps were left between row segments to assure separation of the treatments in sampling.

Seedling counts were taken every day until emergence had occurred in all nine treatments on both soil types. The counts were then taken once every three days until frost killed the entire stand on Nov. 5th. On October 15th when most of the seedlings were at the three to four leaf stage, five seedlings were harvested for each of the 108 plots. These seedlings were collected in labeled brown bags and dried in ovens at 57.2°C for 48 hours. The dry weights were then measured and recorded.

Soil moisture and temperature were measured on the day of planting at all 108 sites at the corresponding depth to the treatment at that site. Soil moisture at seed level was taken with approximately 1.3cm increments of

soil that were weighed for wet weight and dry weight after they had been drying for 24 hours in an oven at 60°C. Soil moisture was calculated using the equation: (wet weight - dry weight)/wet weight. Soil temperature was measured with a thermocouple at the tip of a copper stake that could be pushed into the ground at the appropriate depth.

Three indices were created for the field study. Maximum emergence was the total number of seedlings that emerged in a plot during the study. This was used instead of a final stand count because, this being a fall study, the stands eventually died off due to cold weather. The index for uniformity was the number of days from first emergence of the plot to the first day that the plot reached its maximum emergence. The seedling weight index is the average weight for the five seedlings harvested from the various treatment plots. Tests for significance were done using Systat for Windows (version 6.0). Analysis of variance was done for the three indices relating to the factors and the combinations of soil type, hybrid, and planting depth. For tests that were significant at the 95% level, pairwise comparisons were done with the Bonferroni adjustment.

RESULTS AND DISCUSSION

The hybrids tended to have higher emergence at 2.5cm depths on the Crosby soil (Figure 1) and best emergence at 5.1cm depths in the Kokomo (Figure 2). Shallowest planting depths were very poor for all hybrids on both soil types. 'Skyline' had the poorest performance on both soil types, averaging 30 to 45% emergence on Crosby and 40 to 50% emergence on Kokomo soil. 'Confection' tended to outperform 'Starship' on the Crosby soil with 83 to 100% emergence compared to 54 to 83% emergence for Starship. Both 'Confection' and 'Starship' had slightly lower emergence on the Kokomo soil but while the 2.5cm and 5.1cm planting depths for 'Starship' yielded about 70% germination; the 1.3cm depth had only 30% emergence on Kokomo.

Cultivar and seedling depth treatments on the Crosby soil showed a decline in seedling count well before all the seedlings died on November 5th. This may be due to a few factors. One is that after heavy rains the Crosby soil had crusting at the north end and some of the plots were completely under water for a few days. Another factor could be that a drop in temperatures to 0°C for a number of days in late October and the first few days in November may have had a more severe effect on the Crosby soil than the darker colored Kokomo soil. All the seedlings in the study eventually died by November 5th after a few days with temperatures reaching the lows around -5°C. Seedling emergence counts from October 9th were compared between treatments on Crosby soil (Figure 3) and Kokomo soil (Figure 4). The October 9th date was chosen because most treatments on both soil types had reached maximum emergence and had not yet declined due to cold temperatures (Figures 1 and 2).

Table 1 shows descriptive statistics for the three indices and moisture and temperature. Out of a total of 20 seedlings per plot, emergence rate ranged from 5%-100%. The mean peak emergence of all plots was approximately 65%. The uniformity index had a wide range with the relative peak emergence for a plot ranging from zero (due to a few very low emergence plots) to 35 days from first emergence. The average seedling weight per plot ranged from a minimum of 10mg to a maximum weight of 105mg.

Both moisture and temperature from table 1 refer to data taken at the time of planting. Moisture refers to the percent soil moisture at the seed planting depth, which averaged around 17%. Soil temperature was warm for the beginning of the study ranging from 17.5°C to 23.1°C with an average of approximately 19.6°C. Although not shown, soil moisture and temperature were only significantly affected by depth with the 1.3cm depths being warmer and dryer than the 2.5cm which were significantly warmer and dryer than the 5.1cm depths. Seedling emergence did not show a significant correlation with soil moisture or temperature (data not shown).

Soils, hybrids and depths were all significant factors in peak emergence (Table 2). Two interactions that were also significant at .05 level included soil x depth and hybrid x depth. Among the soils, significantly higher peak emergence was found on Crosby than on Kokomo. The hybrids ranked from highest to lowest emergence were 'Confection' > 'Starship' > 'Skyline'. The 2.5cm and 5.1cm planting depths emerged significantly better than the 1.3cm depth. For soil x depth, depths were not significantly different across soil types except in the 1.3cm where Crosby had higher emergence than Kokomo. At the 2.5cm and 5.1cm depths, 'Confection' and 'Starship' both ranked significantly better than 'Skyline' for the hybrid x depth factor but at the 1.3cm planting depth there was no significant difference in 'Starship' and 'Skyline', only 'Confection' had significantly higher peak emergence. Within the hybrids there was no significant difference in peak emergence at the varying depths except for 'Starship' which had significantly poorer performance at the 1.3cm depths than when planted at the 2.5cm or 5.1cm depths.

Hybrid had the only significant impact on uniformity as measured by this index (Table 3). 'Confection'

emerged in a significantly shorter time than the other hybrids (approximately five days less from first to maximum emergence). The reason that this index was used was that some of the plots had very low peak emergence (with only a few seedlings emerging in that plot) so that time to 75% emergence could not be measured. This may bias the plots somewhat because a plot with only one seedling emerging would have the best uniformity rating because it reached its peak emergence on the first day. This makes 'Confection' truly stand out as it had significantly better uniformity in emergence among the hybrids along with having the highest overall emergence rating.

Soils, hybrids and depths all had a significant effect on the average seedling weight in a plot (Table 4). With this index, the Kokomo soil produced significantly greater seedling weights than Crosby. 'Confection' and 'Starship' had higher mean weights than 'Skyline' at the 95% level, although there was no significant difference between them. The 2.5cm and 5.1cm depths again ranked as the better planting depths over the 1.3cm for the seedling weight index as they had in the maximum emergence index. The only significant interaction was soil x depth. The Kokomo soil had significantly higher mean seedling weights than the Crosby for the 2.5cm and 5.1cm depths with no difference between soil types at the shallow 1.3cm depth. Also, while Crosby showed no significant difference between depths in the seedling weight index, the 1.3cm depths on Kokomo had significantly lower mean seedling weights than the other depths.

It is interesting that Kokomo provided significantly higher mean seedling weights than the Crosby soil, when Crosby had higher peak emergence. As seen in Figure 1, the seedlings on the Crosby soil reached peak emergence early and then began to decline soon after. The decline was not seen as soon (and was less severe) for the Kokomo soil. The plots were harvested for seedling weight measurements on October 15th when seedlings on the Kokomo soil were still at high emergence levels. It is possible that extra cold stress had been placed on the Crosby seedlings accounting for the different seedling weights.

This study will be repeated in spring 1997 with some additions and changes. Soil temperatures will be much cooler in the beginning of the study and will warm as the season progresses. This will add more stress at the time of germination and emergence. Also, the decline in seedlings due to harsh weather towards the end of the study should not be seen. When planted at the shallowest depth, some of the seed was not completely covered by the soil. This may explain why the 1.3cm depths consistently ranked poorly in the maximum emergence and seedling weight indexes. To correct for this the planting depth will be shifted from 1.3, 2.5, and 5.1cm to 2, 4 and 6cm. The spring study will also include mapping the field for phosphorus and soil compaction levels which may help in understanding the emergence differences between soil types. Data loggers will be added so that soil temperatures can be taken throughout the study. In conclusion, significant effects were seen for seedling emergence outside of hybrid vigor differences. Soil type and planting depth along with various interactions all had significant impact on sh2 sweet corn emergence under field conditions. Precision seeding options and plant population targets in the future will rely on a better understanding of these seedling establishment factors.

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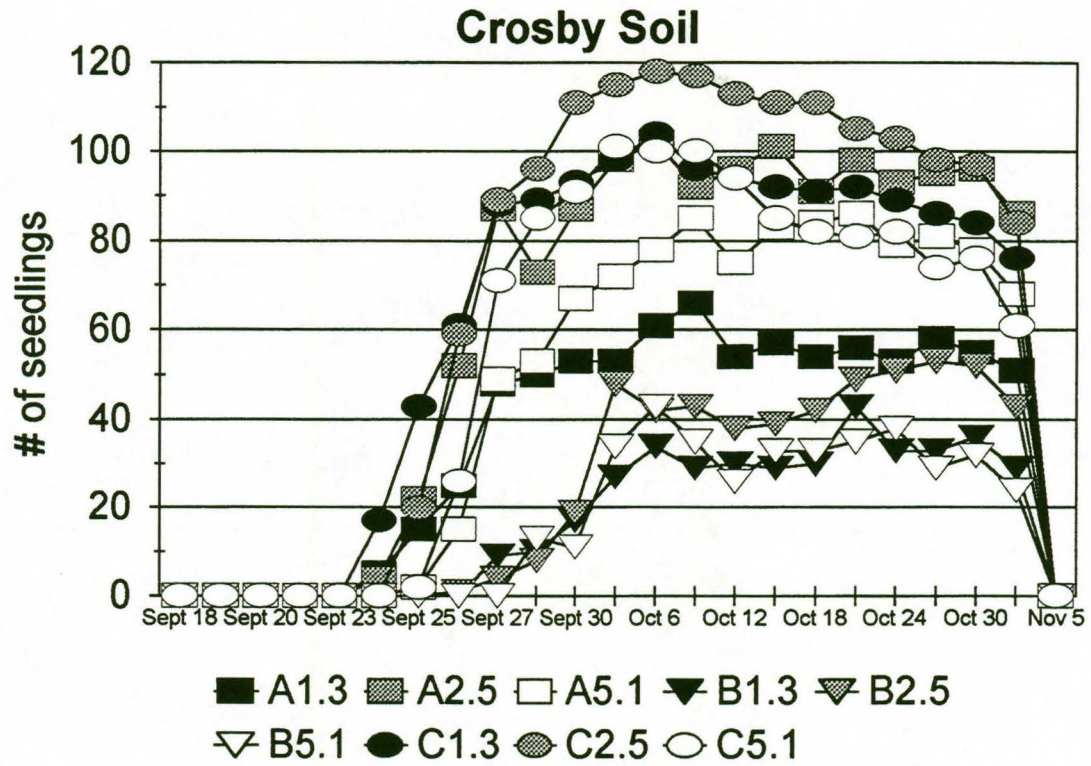


Figure 1. Summary of sh2 sweet corn emergence for all treatments throughout study by date on Crosby soil, Columbus, OH; 1996. A = 'Starship', B = 'Skyline', and C = 'Confection'. Numbers in legend refer to planting depth in centimeters.

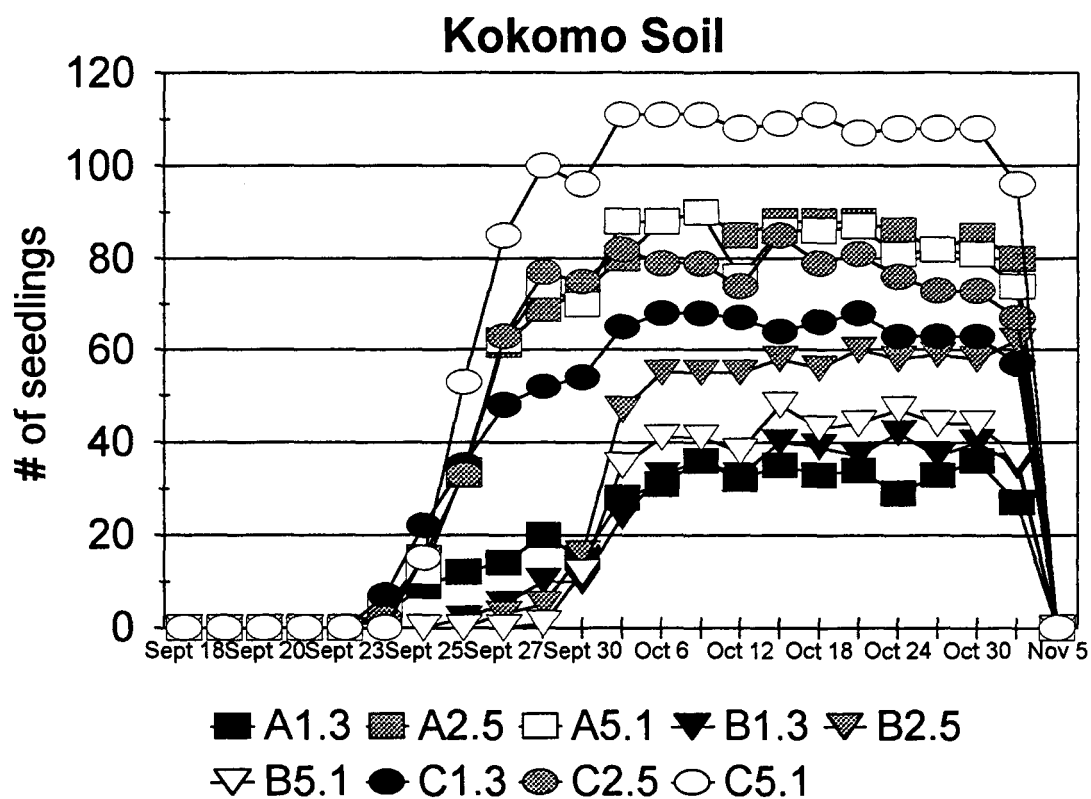


Figure 2. Summary of sh2 sweet corn emergence for all treatments throughout study by date on Kokomo soil, Columbus, OH; 1996. A = 'Starship', B = 'Skyline', and C = 'Confection'. Numbers in legend refer to planting depth in centimeters.

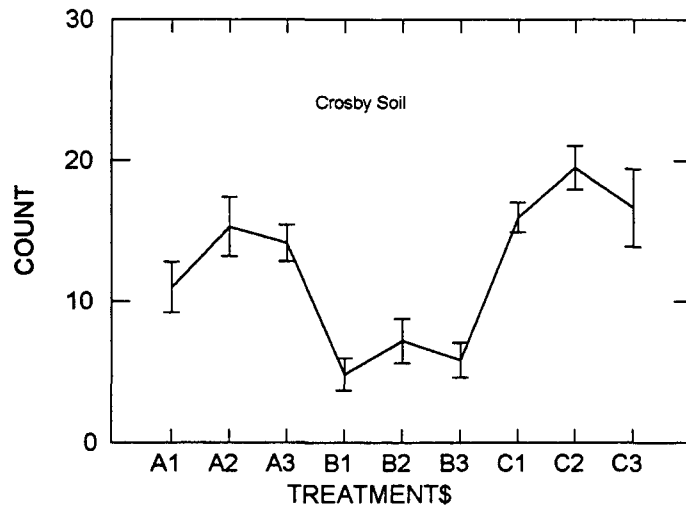


Figure 3. The nine treatments on Crosby soil with standard error bars at 95% level. A = 'Starship', B = 'Skyline', and C = 'Confection'. Numbers in legend refer to planting depth with 1 = 1.3cm, 2 = 2.5cm, and 3 = 5.1cm.

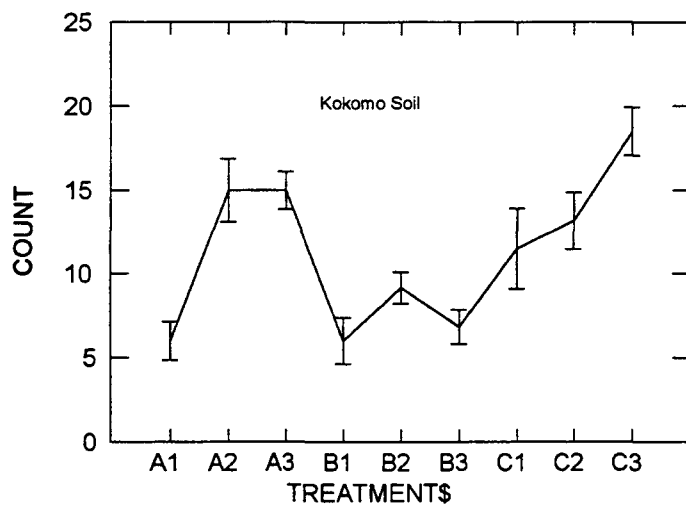


Figure 4. The nine treatments on Kokomo soil with standard error bars at 95% level. A = 'Starship', B = 'Skyline', and C = 'Confection'. Numbers in legend refer to planting depth with 1 = 1.3cm, 2 = 2.5cm, and 3 = 5.1cm.

Table 1. Descriptive statistics for three indexes and first day moisture and temperature readings.

	MAXIMUM EMERGENCE (%)	EMERGENCE UNIFORMITY	SEEDLING WT (mg)	SOIL MOISTURE (%)	SOIL TEMPERATURE (°C)
N of cases	108	108	108	108	108
Minimum	5.00	0.000	10.00	10.000	17.500
Maximum	100.00	35.000	105.0	23.729	23.000
Mean	65.41	14.944	38.00	16.838	19.565
Standard Dev	27.88	8.688	18.00	2.858	.987

Table 2. Analysis of variance for the maximum emergence index. Factors and interactions of those factors that were significant to emergence at $p = .05$ level (*).

<u>Factor</u>	<u>Interaction</u>
*SOIL	SOIL x HYBRID
*HYBRID	*SOIL x DEPTH
*DEPTH	*HYBRID x DEPTH
	SOIL x HYBRID x DEPTH

Table 3. Analysis of variance for the uniformity index. Factors and interactions of those factors that were significant to uniformity at $p = .05$ level (*).

<u>Factor</u>	<u>Interaction</u>
SOIL	SOIL x HYBRID
*HYBRID	SOIL x DEPTH
DEPTH	HYBRID x DEPTH
	SOIL x HYBRID x DEPTH

Table 4. Analysis of variance for the seedling weight index. Factors and interactions of those factors that were significant to seedling dry weight at $p = .05$ level (*).

<u>Factor</u>	<u>Interaction</u>
*SOIL	SOIL x HYBRID
*HYBRID	*SOIL x DEPTH
*DEPTH	HYBRID x DEPTH
	SOIL x HYBRID x DEPTH

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SOILLESS MEDIA: HOW IMPORTANT ARE PLUG MIX NUTRIENT ADDITIONS?

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ABSTRACT

Nutrient additions of lime (CaCO_3), phosphorus (as P_2O_5), and fritted-trace elements were added to a peat/vermiculite soilless medium, alone and in combination. Tomato seedlings were grown for 5 weeks in the amended media with high bicarbonate irrigation water. CaCO_3 additions tended to raise plug pH (>8.0), P_2O_5 increased plant height and general top growth, and trace elements increased leaf area and shoot dry weight compared to an unamended control during the course of culture. Amendment combinations heightened or lessened the impact of the individual additions. Findings suggest the grower can add or subtract these materials to achieve a desired plant habit without loss of tomato transplant quality.

Traditional thinking leads most vegetable transplant growers to lime their peat moss to counter the low pH, add phosphorus to encourage healthy roots, and add fritted-trace elements to supply necessary micronutrients. These practices are fairly standard throughout the industry and are based on sound research used to develop the Cornell Mix. But have these additions been thoroughly tested under conditions of high carbonate/bicarbonate irrigation water and stress management?

Carbonate and bicarbonate, plentiful in the irrigation water of southwest FL, tend to drive media pH up during the course of transplant production. A media pH > 8.0 is common in the finished (shipped) product. Such an alkaline pH can cause nutrient deficiencies, especially iron and phosphorus, during the later stages of the transplant crop. Grower efforts to circumvent these deficiencies often result in the installation of expensive acid-injection systems. However, even with acidified irrigation water, the "super buffering" nature of bicarbonate still tends to elevate pH. When aqueous bicarbonate increases the initial peat pH, is lime really necessary?

Recent evidence has shown that phosphorus (P), long held as "the builder of roots", actually increases seedling top growth (Lin et al., 1996; Vavrina, 1997) more effectively. Considering the highly leachable nature of P in organic soils (Lin et al., 1996), in a peat medium, is P really necessary or helpful?

No one disputes the fact that trace elements are required for good plant growth. Deficiency symptoms from a lack of trace elements are easily discerned. However, the amount of any particular trace element required for adequate growth is minuscule, especially in seedlings, so are they really necessary in the media?

In light of our understanding of P in soils and the quality of SW Florida irrigation water it was thought that the standard soilless media preplant-nutritional package needed to be reevaluated. This study was undertaken to test the effect of lime, phosphorus, and fritted-trace elements individually and in combination on tomato seedlings grown for 5 weeks in a peat/vermiculite soilless medium under high bicarbonate irrigation water.

METHODS AND MATERIALS

Verlite (Tampa, FL), a company that supplies many vegetable transplant production facilities, provided a 70% peat, 30% vermiculite mix (Vegetable Transplant Mix A) without nutrient additions. "Standard" amounts of CaCO_3 (67.24 g/ft^3), P_2O_5 (42.04 g/ft^3), and micronutrients [3% Cu, B, Mn, Mo, Fe, Mg] (0.84 g/ft^3) were added, alone and in combination, to the media until all combinations of the mix and the amendments were made (8 treatments).

Flats (242 cells @ 25 cm^3 each) were filled with the appropriate treatments, seeded with FTE 30 (Petoseed, Saticoy, CA), and grown for 5 weeks. All treatments received 50 ppm N from 20-20-20 (Miller Chem. Co., Feasterville, PA) twice weekly over the 5-week period and were irrigated on an as needed basis by overhead irrigation. All treatments were randomized and replicated (4) within the greenhouse. The experiment was conducted in summer, fall, and winter time slots. Prior to field setting, 10 plants from each treatment were assayed for plug pH, plant height, fresh and dry weight (stems, leaves, roots), stem diameter, leaf area, leaf number, root:shoot ratio, and leaf:stem ratio. Remaining plants were set in the field for growth analysis and yield determinations. Transplant data were analyzed by Anova with mean separation via Fisher's LSD (SAS, 1987). Not all of the transplant house growth parameters will be discussed here and only the fall data is presented. Field

and yield data will be published elsewhere.

RESULTS AND DISCUSSION

The results of the 8 treatment conditions are summarized in Table 1. This table reveals that liming the media significantly increased plug pH over 5 weeks compared to treatments without lime. It was also noted that CaCO_3 effectively increased plug pH 1.6 units above the pH increase caused by bicarbonate (200 to 400 ppm) in the irrigation (well) water alone (i.e., in the control). Without lime, plug pH stabilized around 6.5, an acceptable level to the plant-house grower.

Preplant phosphorus dramatically increased plant height and leaf area in tomato seedlings compared to no P in the media. Wherever P was included in the growing medium a taller plant and/or greater leaf area was achieved. Plant height control is a major concern of transplant growers (Vavrina and Summerhill, 1992), as there are no labelled products (i.e., growth retardants) for use with vegetables. Reducing the amount of preplant P in the media or even eliminating it altogether should provide an added degree of height control for growers.

Trace elements did not seem to impact plant morphology as strongly as CaCO_3 or P. Trace elements increased leaf area and shoot dry weight compared to the unamended control, and lessened the effect of P on growth parameters in general.

Surveying the data on root dry weight, one notes that liming the media results in root growth equal to that of P alone. Is it the high carbonate environment or the calcium by itself that contributes to the increase in root weight? Treatments containing P tended to have greater root dry weight accumulation than the control. However, CaCO_3 alone produced root weights higher than the unamended peat and most other treatments suggesting that Ca should be looked at for root enhancement. None of the treatments employed in this study seemed to affect stem diameter.

These results warrant the re-evaluation of media additions for small plug vegetable transplant production under conditions of high carbonate/bicarbonate irrigation water. The data here are only preliminary, and further trials under varying weather conditions are needed to delineate seasonal variation in these patterns. However, the findings from this study suggest the grower may have greater control of tomato growth and development through manipulation of transplant media nutrients.

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Table 1. The effect of nutritional additions to a standard peat/vermiculite vegetable mix on tomato growth and plug pH.

Treatment	Plug pH	Plant Height (cm)	Stem Diameter (mm)	Leaf Area (cm ²)	Root Dry Weight (g)
Peat	6.62 b	11.0 b-d	1.90	16.4 d	0.0186 c
Peat + CaCO ₃	8.28 a	10.1 d	1.93	17.1 b-d	0.0225 a
Peat + P ₂ O ₅	6.51 b	14.0 a	2.05	18.7 a	0.0209 ab
Peat + Trace Elements	6.48 b	11.0 b-d	1.95	18.1 a-c	0.0191 bc
Peat + CaCO ₃ + P ₂ O ₅	8.20 a	11.6 b-d	1.90	17.3 a-d	0.0200 bc
Peat + CaCO ₃ + Trace	8.28 a	10.5 cd	1.92	16.7 cd	0.0200 bc
Peat + P ₂ O ₅ + Trace	6.55 b	12.4 b	1.99	18.4 ab	0.0194 bc
Peat + CaCO ₃ + P ₂ O ₅ + Trace	8.17 a	12.0 bc	2.01	18.1 a-c	0.0229 a
LSD _{0.05}	0.20	1.6	NS	1.4	0.0021

DEVELOPMENT OF A STRAWBERRY PLUG TRANSPLANT SYSTEM

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Additional Index Words. *Fragaria x ananassa*, containerized transplants, tray plants, propagation, stand establishment, micropropagation

ABSTRACT

A strawberry (*Fragaria x ananassa* Duch.) transplant production system was developed that used micropropagated disease free mother plants in elevated horizontal culture. The mother plants were grown in suspended plastic gutters (10 cm width by 10 cm depth) with a soilless medium consisting of 4:1 (v/v) vermiculite and perlite. The mother plants were subfertilized through drip tubing to avoid leaf wetness. Runners produced by the mother plants hung over the gutter and continued to grow down toward the ground. This study compared proliferation rates of 'Sweet Charlie' and 'Oso Grande' micropropagated mother plants to non-micropropagated mother plants. Micropropagated plants produced more daughter plants than non-micropropagated plants. 'Oso Grande' produced more daughter plants than 'Sweet Charlie'. The benefits of the elevated system were: disease free plants, greater than 2500 daughter plant tips could be produced in a 1 m² area during a 16 week production period, all the runners could be removed at one time and separated for propagation, and the daughter plants had active root tips that established quickly.

INTRODUCTION

An estimated 1.8 billion strawberry plants are vegetatively propagated throughout the world each year (Boxus, 1989). Greater than 700 million of these plants are propagated in the United States. Commercial strawberry cultivars must be propagated vegetatively because seeds are not true to type. Strawberry transplants are produced conventionally by planting a field nursery in early summer, the nursery plants produce daughter plants on stolons in response to long day lengths and high temperatures. In late summer and early fall the daughter plants from the nursery field are dug, soil is removed from the roots, and then the plants may be cold stored for several months before planting, or planted immediately. These plants may become extremely stressed during this process. Additionally, inconsistency in strawberry transplant production and handling, coupled with post transplant conditions can contribute to delayed flowering and subsequent fruiting irregularity, and disease and spider mite epidemics due to pathogen infestation and plant stress. The percentage of marketable fruit may therefore be reduced. Mechanical digging and shaking (to remove soil from roots) often damages roots and breaks petioles, reducing the number of functional leaves for use by the transplant during establishment and creating possible sites for pathogen infection. Bare-root transplants often require large quantities of water at planting, especially in warm climates. This further exacerbates plant pest problems and can leach nutrients. One of the greatest challenges facing the current strawberry plant propagation system may be the elimination, by the year 2001, of the fumigant methyl bromide {Courter, 1993}. Methyl bromide has been used on the majority of commercial acreage for disease control and effective alternatives have not been developed. Additionally, there have been years when nurseries have had difficulty digging plants because of adverse weather conditions during the digging period. Weather conditions also affect the amount of chilling a plant attains. Chilling in strawberry is the exposure to a certain range of low temperatures, which depending on temperature level and duration, causes

changes in the reproductive/vegetative status of the plant. Chilling has been shown to increase the amount of carbohydrate storage in the root system (Maas, 1986). Inadequate chilling can result in reduced flower initiation, but excessive chilling can cause plant dormancy and decrease fruit production due to decreased plant growth (Durner et al., 1984). Therefore, varying weather conditions during the transplant production season can cause variability in transplant performance from year to year.

Through micropropagation, large quantities of uniform, vigorous, pathogen-free plants can be produced (Boxus et al., 1984). Micropropagated plants are more expensive than standard plants, but the increased runner production of micropropagated plants (Swartz et al., 1981) has justified the increased expense for some nursery growers. Micropropagated plants often produce smaller fruit and therefore have not been directly used for fruit production. In some cultivars, bare-root daughter plants of micropropagated mother plants had increased fruit production compared to conventional bare-root plants which were not micropropagated. The daughter plants from micropropagation, however, are produced in field nurseries and therefore can develop the same inconsistencies as conventional plants due to temperature fluctuations and exposure to pathogens.

The above problems of transplant variability could be avoided by the use of micropropagation derived plug transplants which can be mechanically transplanted into the field similar to other vegetable transplants. Plug (tray) transplants have been used successfully in Europe since the late 1980's (Hennion et al., 1996) and have been the subject of research in North America. In North Carolina, plugs have been reported to have several distinct advantages over bare-root transplants: plugs required only 10% of the water needed for bare-root establishment in spring production systems, a mechanical multiple-row plug transplanter could be used for planting, minimal root damage during transplanting which provided for quick root establishment, and plant survival was greater (Poling and Parker, 1990). 'Chandler' plug transplants grown in New Jersey had three times the fruit production of dormant crowns, but primary fruit size was lower with the plug transplant (Fiola and Lengyen, 1995). In Florida, plug transplants have been shown to have greater early and total season production than bare-root transplants (Bish et al., 1996). The purpose of the present research was to develop a system to produce vigorous, pathogen-free plants for plug production. The results from this work could be important in the development of a plug transplant system.

MATERIALS AND METHODS

'Sweet Charlie' and 'Oso Grande' micropropagated plantlets (donated from Nourse Farms, Inc.; South Deerfield, Massachusetts), first generation plantlets (daughter plants of micropropagated), and non-micropropagated plantlets (UF - GCREC, Dover, Florida) were planted in a horizontal gutter system which was developed at the University of Florida for runner plant propagation. The horizontal gutter system was constructed in a glass greenhouse (35/25 C day/night, 30 % shade cloth) at the University of Florida, Gainesville FL. Photoperiod was extended to 16 hours with high pressure sodium halide lamps.

The horizontal system consisted of white plastic gutters (10 cm width by 10 cm depth) that were elevated to 1.2 meters and filled with a soilless medium consisting of 4:1 (v/v) vermiculite:perlite mix (Verlite Company, Tampa, Florida; Airlite Processing Corporation, Vero Beach, Florida). Drip tubing, 30.5 cm emitter spacing with 62 ml / h / emitter at 55×10^3 Pa, was placed on the top of the rooting medium for fertigation in order to avoid leaf wetness. Plants were fertigated (Table 1) three times a day for ten minutes each fertigation. In 1996, the specific conductivity of the fertilizer solution was decreased (by decreasing the concentration of fertilizer) because of leaf tipburn in 1995. The tipburn was attributed to localized Ca deficiency which could have been caused by high osmotic potential of the nutrient solution (Doolan et al., 1983). White on black plastic mulch (0.03 mm thickness) was used to cover the rooting medium in order to deter algae growth. Runners produced by the plants could hang over the gutter and continue to grow down toward the ground.

A randomized complete-block experimental design was used with each treatment replicated five times. On 3 March 1995 and 1996, treatments were planted (10 plants/plot) on alternating sides of the drip tube with a 10 cm plant spacing in the gutter and 30 cm between center of gutters. Stolons were harvested at 8 and 16 weeks. Stolon, daughter plant, and crown number were recorded. Fifty daughter plants from each replicated treatment were then propagated under mist irrigation (12 seconds of mist every 6 min.) for 1 week in 18.8 cm³ container volume trays (Speedling Todd 100 flats). The rooting medium consisted of a 4:1 vermiculite:perlite mix. Percentage of daughter plant tip survival was recorded after two weeks in the flats. Data were grouped by year, and subjected to analyses

of variance (General Linear Models: Littell et al., 1991). Treatment means were separated by Duncan's Multiple Range Test, 95 % confidence level.

RESULTS AND DISCUSSION

'Sweet Charlie' and 'Oso Grande' plant growth for all treatments was very vigorous in the gutter system. Micropropagated (MP-0) plants produced a greater number of branch crowns than plants not micropropagated (MP-No) or first generation from micropropagation (MP-1) plants (Table 2). The increased number of branch crowns by MP-0 plants was most likely due to short photoperiod (12 h) and low temperature (25/15 day/night) conditions in the laboratory during micropropagation (Martinelli, 1992). This is supported by the observation of MP-1 plants that were grown only under the greenhouse conditions of 16 h extended photoperiod and 35/25 C day/night temperature. These plants produced no branch crowns.

Daughter plant number and number of stolons per plant was greater in MP-0 plants than both the MP-1 plants and the MP-No plants. MP-1 plants produced more daughter plants and stolons than MP-No plants. In most comparisons, 'Oso Grande' MP-0 plants produced more daughter plants and stolons than the MP-0 plants of the cultivar 'Sweet Charlie'.

Daughter plant survival after transplanting was very high. All treatments had survival rates of 96 % or above. The gutter production system used in this experiment had several advantages over traditional field propagation. The daughter plants produced were disease free, vigorous, had active root tips that established quickly, and the stolons could be harvested as long chains of daughter plants. The 10 cm x 30 cm spacing resulted in approximately 30 mother plants per square meter. Therefore the micropropagated 'Oso Grande' treatment in 1996 yielded over 2500 plants per square meter in a 16 week time period. The large amount of daughter plants produced and the very high survival rate of the daughter plants after transplanting suggested that this gutter production system developed at the University of Florida can be an excellent method for generation of vigorous, pathogen-free plants for plug production.

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Table 1. Nutrient concentrations of fertigation solution.

<u>1995</u>		<u>1996</u>	
<u>Element</u> ^z	<u>mg/liter</u>	<u>Element</u> ^z	<u>mg/liter</u>
N	120	N	30
P	40	P	10
K	120	K	30
Ca	120	Ca	30
Mg	40	Mg	10
S	64	S	16
B	0.8	B	0.2
Cu	0.2	Cu	0.05
Fe	4.8	Fe	1.2
Mn	0.4	Mn	0.1
Mo	0.04	Mo	0.01
Zn	0.4	Zn	0.1
EC = 1,720 :S		EC = 700 :S	
pH = 5.7		pH = 5.7	

^zNutrients derived from: calcium nitrate, potassium nitrate, potassium phosphate, magnesium sulfate, boric acid, di-sodium copper, sodium EDTA ferric, di-sodium manganese, sodium EDTA molybdate, sodium EDTA zinc.

Table 2. Strawberry daughter plant production and growth.

Harvest Date (weeks)	Cultivar	Generation from Micropropagation	No. crowns per plant	No. daughter plants per plant	No. stolons per plant	% survival of daughter plants
1995						
8	Oso Grande	0	2.4 a ^z	14.1 a	6.6 a	99.6 ab
	Oso Grande	1	1.0 b	9.4 b	4.1 b	99.2 ab
	Oso Grande	No	1.1 b	6.6 c	3.1 c	98.0 ab
	Sweet Charlie	0	2.5 a	9.4 b	4.2 a	100.0 a
	Sweet Charlie	1	1.0 b	6.8 c	3.4 c	99.6 ab
	Sweet Charlie	No	1.0 b	2.6 d	1.7 d	97.6 b
16	Oso Grande	0	3.3 a	35.9 a	8.7 a	98.8 ab
	Oso Grande	1	1.0 d	29.7 b	6.5 b	99.2 ab
	Oso Grande	No	2.1 b	26.8 c	5.0 d	97.2 b
	Sweet Charlie	0	3.6 a	30.4 b	6.7 b	99.6 a
	Sweet Charlie	1	1.0 d	27.2 c	5.8 c	100.0 a
	Sweet Charlie	No	1.6 c	25.7 c	6.0 c	98.8 ab
1996						
8	Oso Grande	0	2.3 b	15.3 a	8.4 a	100.0 a
	Oso Grande	1	1.0 c	12.8 b	5.0 b	99.6 ab
	Oso Grande	No	1.0 c	7.2 c	3.5 c	99.2 ab
	Sweet Charlie	0	2.7 a	15.7 a	4.7 b	99.6 ab
	Sweet Charlie	1	1.0 c	7.2 c	3.6 c	100.0 a
	Sweet Charlie	No	1.0 c	3.9 d	2.1 d	98.6 b
16	Oso Grande	0	3.2 b	68.7 a	14.8 a	99.6 NS
	Oso Grande	1	1.0 e	47.0 d	12.8 b	98.8
	Oso Grande	No	2.1 c	37.2 f	7.5 d	98.4
	Sweet Charlie	0	3.8 a	64.2 b	14.6 a	99.6
	Sweet Charlie	1	1.0 e	57.6 c	12.6 b	99.2
	Sweet Charlie	No	1.5 d	41.5 f	10.3 c	99.6

^z Treatment means within the same year, harvest date, and column with the same letters are not significantly different at the 5 % significance level according to Duncan's Multiple Range Test.

INTEGRATED TRANSPLANT PRODUCTION SYSTEM (ITPS)

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ABSTRACT

Many crops are grown through transplanting to improve stand establishment. These transplants may be produced in the field beds or greenhouses, with the trend towards the latter increasing progressively. Greenhouse transplant production may be accomplished through seed or tissue culture. At present complete separate protocols are used for both approaches. Further, the linked cell containers (i.e., trays or flats) in use at present have limited total mechanization of transplant production and transplanting. This paper describes a "cassette" culture approach which integrates seed based and tissue culture based transplant production to one operation and permits mechanization of all phases: media loading, seeding, culture, sorting, and transplanting. The cassette can be made of long-life materials or disposable materials and can be adapted to any size of operation without involving simple automation or complex automation with machine vision, artificial intelligence and robotics. The disposable cassettes would aid environment and resource conservation through solid waste recycling.

Most horticultural crops, forestry crops, and several agronomic crops are grown through transplanting to improve stand establishment. These transplants are produced in field beds or greenhouses depending on the species, marketing time, and value of the end product. Although more acreage are dedicated in the USA to field beds than greenhouses, the size of the latter is increasingly steadily because the greenhouse not only offers a protected environment but it also eliminates problems such as the need for prime land, soil compaction, erosion and salination, need for excessive irrigation and nutrients because of their loss to gravity and also because of the evaporative loss of water, groundwater contamination through the leaching of agrochemical, microenvironment variability, interruption of field operations by inclement weather, and need for fumigants and other agrochemical for pest management. The need for greenhouse transplant production will be even greater with gradual discontinuation of halogenated fumigants such as methyl bromide. For example, in North Carolina, more than 80% of tobacco transplants were being produced in field beds prior to 1985 whereas more than 80% of the transplants are being produced today in the greenhouse (Smith, 1996). Despite the increasing need for greenhouse technology, its used is limited to high value crops because of the high procurement, operating and maintenance costs. In order to make greenhouse technology affordable for common use for different crops, a systems approach needs to be taken to examine and optimize different components which constitute greenhouse technology. Such optimization should be based on an integrated approach which addresses all components of greenhouse technology: procurement, operation and maintenance. A mini greenhouse system (HMGS) developed in this laboratory has made the greenhouse technology 3 to 4 times more economical than the conventional greenhouse for the same area of floor space (Mohapatra, and Suggs, 1992, 1996; Mohapatra, 1997). This paper addresses the integration of seed based and tissue culture based transplant production and the significance of the container for this purpose.

MATERIALS AND METHODS

Transplants can be produced as seedlings from seeds or as plantlets through micropropagation, Each with various advantages and disadvantages (Fig. 1). The former involves seed technology while the latter involves cell, tissue or organ culture.

Seed-based seedling production

Seedlings are invariably mass produced in containers called flats which consist of linked cells. The flats are made of plastic or Styrofoam. Under each category, the flat is designated as a "plug-flat" when it consists of numerous small cells or as a "growth-flat" when it consists of larger cells. When the seedlings are grown in the plug-flat, the seedling plugs must be transplanted to the growth-flat, from where they are again transplanted to the

field. The linked cells save labor cost by permitting simultaneous handling of several hundred cells for media loading, seeding, growing, and transportation and storage. 1) Even in the growth flats currently in use, the plant spacing is too close to permit movement of mechanical manipulators. Thus, singulation must be accomplished manually. 2) Because singulation is not possible, mechanical sorting based on seedling quality is not possible, and this must also be accomplished manually. 3) Transplanting involves downward movement of the plant towards the soil while an upward movement of the plants is necessary from removing the plant from the cell. Operationally, it is difficult to synchronize two opposite movements with even the slowest tractor speed in the field, especially for close plant spacing. 4) As stated earlier, germination failure often leaves the flats with empty cells which create problems for mechanical transplanting.

Some of the above problems have been solved at least partially through different approaches. A two-piece growth flat permits separation of the base from the cells containing the plants which are then transferred to the soil through one down-ward motion (accomplished pneumatically or mechanically) toward the soil (Huang and Ai, 1992). Seedling transfer from donor flats to recipient flats through machine vision and robotics has been explored (Mohapatra et al., 1990; Tai et al., 1994). An automated approach based on the combination of "push" and "grab" mechanism has been attempted for tobacco transplants (Suggs et al., 1992). These approaches have either not been transferred commercial technology or when done the cost is prohibitive, thus limiting routine use. Some automated transplanting involve use of linked paper cells which are arranged like a flat for growing seedlings but are linearized and pulled apart for transplanting. However, special machinery is needed for each phase of the operation associated with these paper cells. Therefore, these containers have not found routine use for all crops.

Large quantities of containers are used in the food, beverage, pharmaceutical and cosmetics industries. These are, however, single containers. Even when sold in groups, single containers are linked through easily detachable straps. It appears that if singulated-containers can be used for transplant production the same degree of automation and quality control available for other industries can also be applied to transplant production and transplanting as such or with appropriate modification. Singulated container approach is not uncommon in the current greenhouse industry. But the smallest such container available is not less than 3" (7.5 cm) in diameter or height. Further, their conical shape is not conducive for easy automation. In fact, use of singulated containers for transplant production is not uncommon. But the dimension of these is not less than 3" x 4" (7cm x 9 cm). The greenhouse space needed to mass produce seedlings in these containers for commercial agriculture would be prohibitively costly. Further, the shape and the strength of these containers are not conducive for automatic transplanting. Therefore new approaches must be explored to produce seedlings in singulated containers. Such an attempt was based on a complicated two-container and multi-step system which has not found commercial application perhaps because the complexities and high cost (Brewer, 1994)

Transplant production through tissue culture

Plantlets are routinely produced in the laboratory through protoplast, cell, tissue and organ culture, collectively called tissue culture. Despite the unique advantage of tissue culture for transplant production, its commercial application is limited only to specialized operations. That is because i) there is a lack of clear understanding on the role of tissue culture in commercial transplant production, ii) the protocols used for tissue culture, including the containers, are so different from those used for seed based seedling production that the two approaches exist as two widely divergent approaches.

Integration of seed based and tissue culture based transplant production

With regard to automation, it does not make any difference whether the transplant comes from a seed or a vegetative part of the plant. On the other hand, if the two methods can be integrated to one approach, greenhouse use can be optimized by growing most plants from seeds and "filler" plants through tissue culture under a different environment. Because tissue culture gives a large number plants from a small quantity of starting tissue, in a small space, and under low light intensity, production of the "filler" plants can be easily accomplished in vitro without the need of greenhouse space. The "filler" plants can then be used to fill the empty cells resulting from germination failure or seedling death. The filling operation can be done automatically through robotics under development in this laboratory (Mohapatra et al., 1990) or other laboratories (Aitken-Christie et al., 1995; Tai et al., 1994). The filling operation would be much simpler if the transplant is grown in singulated containers instead of linked cells. As shown in Fig. 1, all tissue culture protocols do not allow singulated plant production. Therefore, different

protocols need to be combined to first produce large number of plants from small amounts of tissues and then produce singulated plants from these plants through nodal culture, where each node produces a single plant instead of clumps of plants. In view of the fact that each plantlet can yield at least four and as many as 10 nodes (depending on species and growth stage), the needed quantities of "filler" plants can be produced from a small population of plantlets. If the nodal culture can be accomplished in the same container as the seed based seedling production, it would be simple matter of replacing a container without a plant with that with a plant. Depending on the size of the operation, this greenhouse "filling" process can be accomplished manually or mechanically. These considerations were the basis of the "cassette" culture method described below and shown in Fig. 2

The cassette culture method

The cassette: The container is called a "cassette" to imply that not only the transplants are grown in singly in containers, but the handling of the container and removal of the transplant is a simple operation. The cassette can be made of materials to meet varied objectives and different levels of cash flow. For example, it can be made of sturdy materials with indefinite durability. These cassettes would be costly initially, but would be economical in the long run through repeated use. This would reduce the amount of plastic waste generated at present through the use of disposable plastic cells. However, use of long-life cassettes will need greater amount of initial cash than inexpensive disposable cassettes. The disposable cassettes can be made from solid waste such as paper and wood, thus aiding solid waste disposal. Because these containers would be biodegradable, they can be put in the soil (like the Jiffy pots) directly along with the transplant. The disposable/degradable cassettes would be ideal for low initial investment, but because the cost is recurrent yearly, it would eventually be more expensive than the long-life cassette.

As stated earlier, neither the concept of singulated transplant production nor the use of the term "cassette" is unique in ITPS described in this paper. However, unlike other systems (Brewer, 1994), the cassette used in the ITPS allows one continuous operation without requiring interim plant transfer from one container to another container.

The growth medium: While the growth medium for seed based transplant production is rather well defined, there is wide variability in the growth medium used for tissue culture based transplant production. Culture of isolated cells and protoplasts is usually accomplished through liquid culture (which is actually a form of hydroponics) whereas culture of tissue explants is accomplished through solidified agar medium enriched with various nutrients and growth regulators. In the proposed cassette culture, the cassette will be loaded with an inert media without any nutrient or hormone enrichment. For seed based transplant production, the media can come from peat and vermiculite mixtures. Because solidified agar will not meet this requirement, alternative media must be developed for tissue culture. For example, we have successfully used the same medium for transplant production from seeds and nodal explants.

The germplasm: This can come either from a seed or a leafy node. The "mother plants" used to obtain leafy nodes can come from callus, isolated protoplasts and single cells, tissue explants or even other leafy nodes. Thus traditional tissue culture protocols (including sterile culture) can be used to produce the mother plant whereas nonsterile protocols can be used for seed and nodal culture. If necessary, the cassettes containing the nodes may be cultured under sterile conditions to reduce plant mortality due to contamination.

RESULTS AND DISCUSSION

Commercial application of the ITPS: The schematics in Fig. 3 shows the steps involved to convert the ITPS concept to commercial technology. Briefly, the cassettes containing the seed or the leafy node are grouped to form a pallet, which is cultured in the in the float mode of the HMGS (hybrid mini greenhouse system) for seed based transplant production or cultured under sterile conditions for tissue culture based transplant production. The "Mother plants" needed for leafy nodes are produced through traditional tissue culture. The timing of the initiation of seed culture and node culture will depend on species and must be determined through preliminary trials. When the transplants are ready for use, the cassettes are sorted manually or mechanically to separate those with and

without plants or to separate plants of different sizes. Depending on the type of the cassette used, cassettes can then be used for automatic transplanting with a traditional "carrousel" type or "finger" type transplanter. The sorting process can also be used to staggered transplanting of large acreage, each area containing a uniform population of transplants.

Although adapting plant transfer from the cassette to the carrousel or finger type transplants would be a simple process of automation, such mechanisms do not exist at present. Because the ITPS has been accepted by the NCSU technology administration for patenting there is opportunity for commercial manufacturers to license the ITPS from the NCSU for their transplanters. Those who are interested should contact the author.

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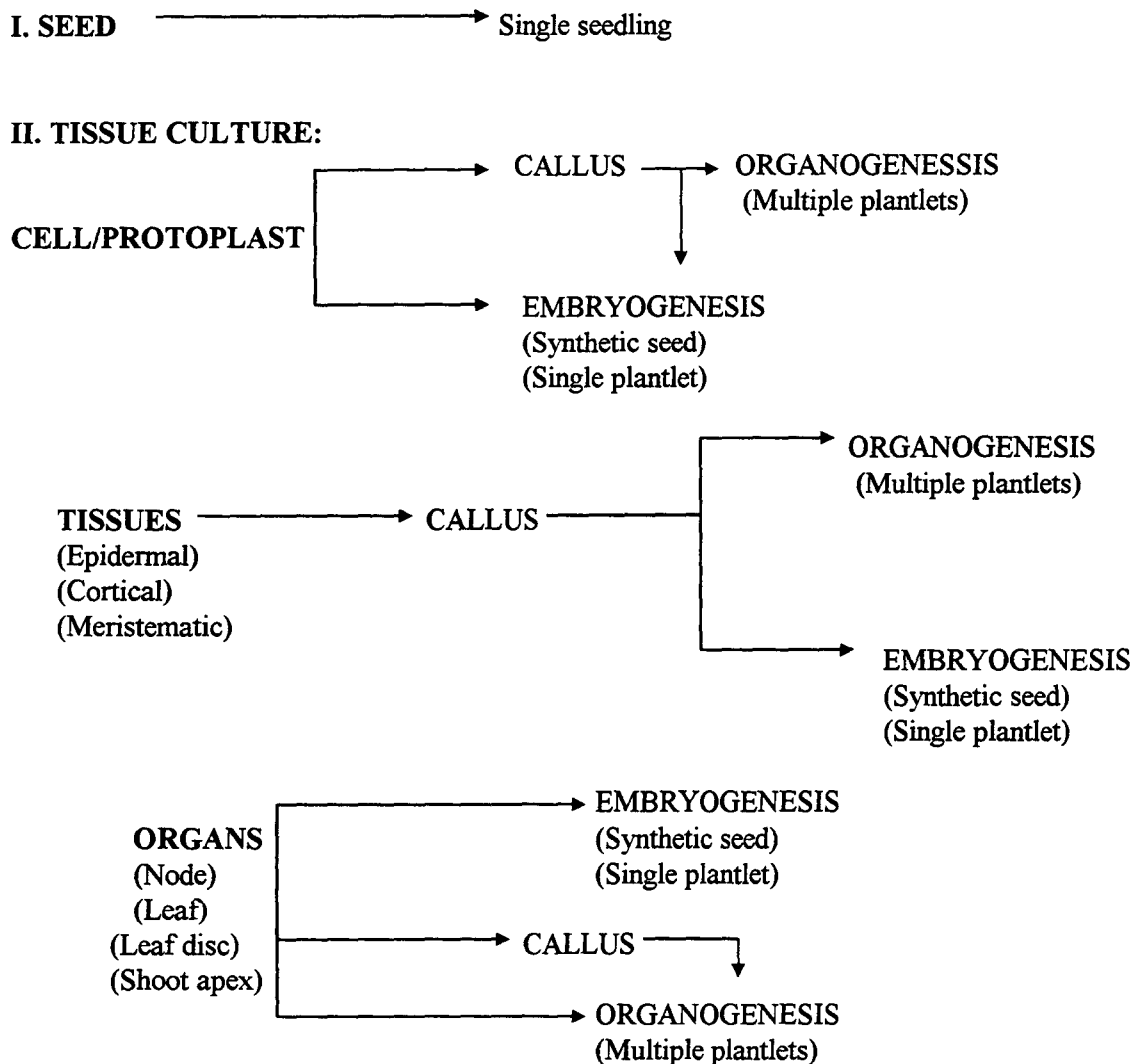


Fig. 1. Schematics of morphogenetic pathways for seed based and tissue culture based transplant production.

Note:

1. While multiple plantlets are desirable to produce mother plants for leafy nodes, single plants are desirable for mechanized handling and transplanting.
2. Some calluses can be made embryogenic
3. Although somatic embryos give single plants, multiple embryos are formed through embryogenesis and that needs additional steps for separation and encapsulation of somatic embryos to prepare synthetic seeds. Further, synthetic embryos pose the same problem as zygotic embryos (seeds) in the form of germination failure
4. Apical meristems and leafy nodes have the potential to give single plants, but only one apical meristem can be obtained from each plant where as several leafy nodes can be obtained from the same plant.

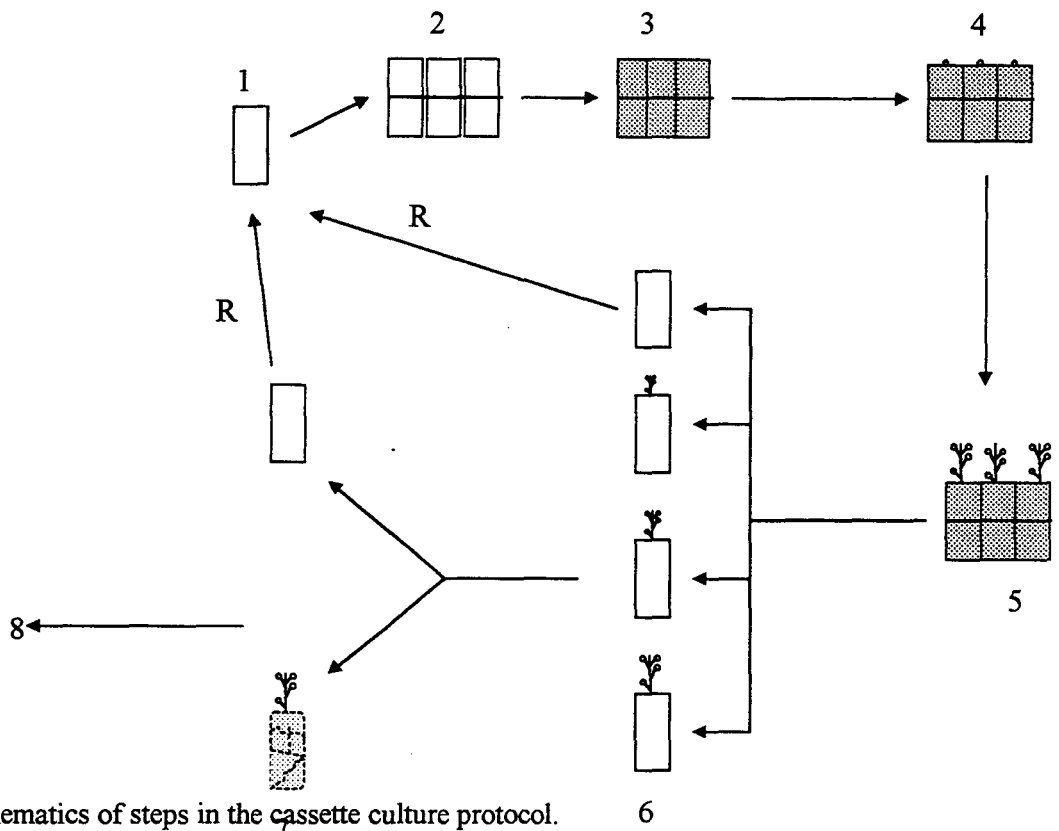


Fig. 2. Schematics of steps in the cassette culture protocol.

1. Single cassettes made from disposable or long-life materials.
2. Grouping of cassettes and media loading.
3. Loading cassettes with media
4. Planting of the cassette with seed or leafy node.
5. Culturing cassettes in the HMGS under sterile or nonsterile conditions.
6. Sorting cassettes on the basis of plant number and size.
7. Separation of cassette and transplant
8. Transplanting

R - Recycling of cassettes without plants and empty cassettes after plant removal.

Note: Each of the above operation can be performed manually or mechanically to fit the size of operation and objective.

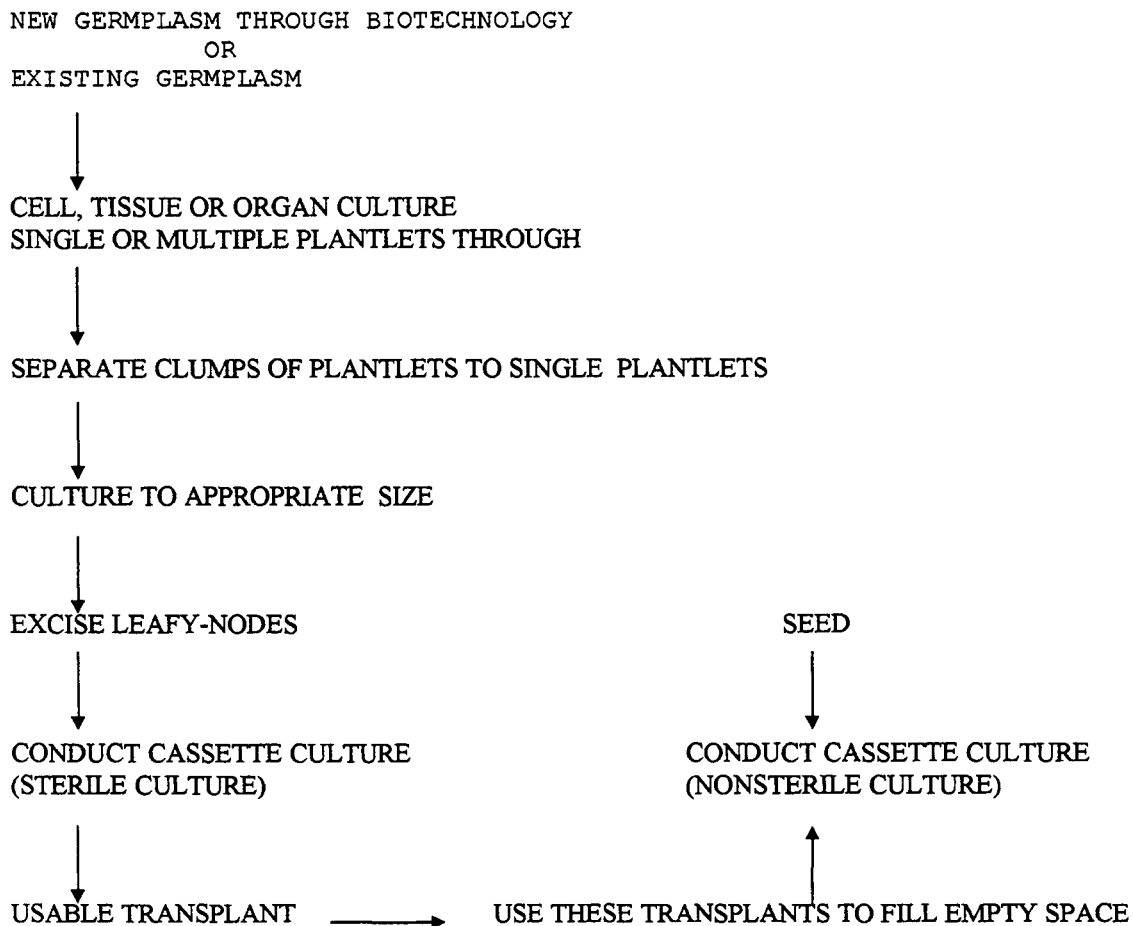


Fig. 3. Schematics of suggested steps in the Integrated Transplant Production System

Note:

1. For economy, convenience and operational efficiency, most of the transplants will be produced through seeds.
2. While seed based cassette culture can be done outdoors (in warm climate) and inside a conventional greenhouse, the HMGS will give best optimization.
3. Some of the organ (leafy node) cultures can be conducted under nonsterile conditions with peat-vermiculite growth medium.
4. See Fig. 2 for steps in cassette culture.

PLANT GROWTH REGULATORS, DATE AND NUMBER OF TREATMENTS IN TOMATO TRANSPLANT PRODUCTION

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ABSTRACT

The following studies were undertaken to evaluate some PGRs and the number of treatments for control of plant growth in greenhouse during transplant production, improvement of transplant quality and field establishment and subsequent crop yields.

Two greenhouse experiments were carried out in 1991. 'Hypeel' was sown on 18 April. In the first trial paclobutrazol (PCB, 12.5 ppm), PCB+chlormequat (CCC, 1000 ppm) or distillate water were used as foliar sprays at different ages: 25, 32 (optimal time to transplant) or 39 days after sowing (DAS). The first group treated seedlings were transplanted 7, 14 and 21 days after treatment (that is 32, 39 and 46 DAS); the second one 7 and 14 days after treatment, and the third group 7 days after treatment.

In a second experiment PCB (12.5 ppm), CCC (1000 ppm), PCB+CCC (12.5+1000 ppm), uniconazole (10 ppm), ethephon (100 ppm), sucrose and glucose (10%) were applied as foliar sprays 1, 2 or 3 fold (25, 25 + 32, 25 + 32 + 39 DAS respectively); an untreated control was included for comparison.

Experiment 1. Treatments reduced plant height, dry weight of shoot and root, leaf area and length of second internode of seedlings transplanted 46 DAS. The earliest treatment increased seedling compactness and particularly so in later transplanting date. PGRs did not influence marketable yield but with later transplanting date and earliest treatment it resulted lower than other treatment dates.

Experiment 2. Thirty-nine, 46 and 67 DAS the effects of PGRs did not increase increasing the number of treatments. The most effective PGRs in the height reduction were ETH and PCB+CCC. ETH had a long term effect on the marketable yield per hectare.

INTRODUCTION

Tomato for processing is an economically important crop in Apulia (southern Italy) where the land in production increased considerably during the 80s (from 20,000 ha in 1980 to 38,000 ha in 1989). At present tomato for processing is cultivated on 36-37,000 ha (34% of domestic hectareage and 44% of production).

Tomato is established through transplanting seedlings grown in multicell trays in greenhouse. To allow processors to work longer, planting takes place gradually from the end of March to mid June. So, tomato transplants are grown for a specific planting date, but sometimes they cannot be transplanted at the set date due to cool weather or rainfall delaying field preparation. A serious problem for transplant producers is to grow seedlings for later planting due to the high temperatures which can be attained in the greenhouse for some days in May (air temperature 25-27 °C). The seedlings then become elongated (spindly plants) which are unacceptable to farmers.

Plant growth regulators (PGRs) may be used as a useful tool to control growth of seedlings. The objectives of this study are: a) to determine the optimal date for preventive treatment of seedlings for late planting; b) to evaluate the effect of separate and repeated treatments on the seedling characteristics; c) to determine the residual effects of PGRs on the growth and productivity of tomato transplants under field conditions.

MATERIALS AND METHODS

Two experiments were carried out in 1991. 'Hypeel' seeds were sown on 18 April in multicell flats (180 cell/flat; cell volume = 17.4 cm³). After seeding, flats were placed in a pregermination chamber for 72 h (23 °C

and 90% R.U.), then they were transferred into polyethylene greenhouse. Twenty-five days after seeding (DAS), seedlings were split into 6 groups: three for the first experiment and three for the second.

In the first experiment, seedlings were treated with paclobutrazol (PCB, 12.5 ppm) or PCB + chlormequat (CCC, 1000 ppm) at different dates. Details are provided in table 1.

In the second experiment, PCB (12.5 ppm), CCC (1000 ppm), PCB + CCC (12.5 + 1000 ppm), uniconazole (UNI, 10 ppm), ethephon (ETH, 1000 ppm), sucrose (SUC, 10%) and glucose (GLU, 10%) were used. The first group was treated onefold on 13 May (32 DAS); the second one twofold on 13 and 20 May (32 and 39 DAS) and the last one threefold on 13, 20 and 27 May (25, 32 and 39 DAS). All seedlings were planted on 4 June. In both experiment an untreated control was included for comparison. Treated flats were sprayed with hand-held applicator at a rate of 150 ml per flat (0.78 l m⁻²). The check was treated with distillate water.

The following data were collected on three transplants per replication in the period from the first treatment to transplanting: height of seedling, height of first and second internode, stem diameter, shoot fresh and dry weight (SFW and SDW), leaf area, root fresh and dry weight (RFW and RDW), root length, root density, shoot:root dry weight ratio and weakness index (height:stem diameter ratio). Root were separated from the substrate following Cahoon and Morton method (Cahoon and Morton, 1961) and root length was determined by Tennant method (Tennant, 1975). Treatments were arranged in a randomized complete block design with one flat per treatment with three replicates.

Transplants were planted at the experimental farm "E. Pantanelli" at Policoro (MT). Plants were grown in double rows spaced 1.8 m (0.6 m between rows and 0.27 m within row, 4.1 plants.m⁻²) using a split-plot design with three replicates. Cultural operations and pesticide treatments applied to the crop were according to those commercially used in the area.

Twenty-one and 43 days after planting (DAP), three plants per plot were randomly selected and measured: height, stem diameter, SFW, SDW, number of leaves, leaf area; 43 DAP height, number of first fertile node and number of fruits were also recorded.

The plots were once-over harvested to simulate mechanical harvesting. Weights of marketable, green, blossom end rot or decayed fruits were recorded. Twenty fruits per plot were randomly selected and the extracted juice was analyzed for pH, total titratable acids and soluble solids.

RESULTS AND DISCUSSIONS

Experiment 1. Compared to untreated control, at each date of treatment PGRs reduced the height of seedlings (table 2). Forty-six DAS (day of planting) the earliest treatment (13 May) resulted more effective, which decreased seedlings height by 23%. The earliest treatment decreased also leaf area 32 and 46 DAS, and SDW and weakness index 46 DAS. The treatments applied on 20 May increased root length and root density 46 DAS.

At each date of planting, PGRs reduced the height of tomato transplants, but at the latest planting date PCB+CCC decreased the height by 23% (table 3). Growth suppression in the first and second planting dates was the result of the shorter second internode. PCB reduced root density at the first planting date (8 days after treatment) and PCB+CCC shoot:root ratio at the second one.

The effects of treatment dates were completely lacking 21 and 43 DAS on the seedlings planted on 21 May (8 d after treatment). Seedlings planted on 28 May (8 and 15 d after treatments) showed a residual effect on height, leaf area and SDW 21 DAP for the earliest treatment (table 4). At 43 DAP a slight reduction of plant height was observed when the treatment took place on 13 May. Transplants treated on May 20 and 27 and planted on 4 June exhibited a rapid field establishment; instead PGRs sprayed on 13 May continued to significantly regulate plant growth after field establishment. At planting, these transplants were 23% shorter, 15% lighter and with less leaf expansion (-17%) compared to control. After 21 days in the field establishment they were 11% shorter, 36% lighter and with 40% less leaf area than control. At 43 DAP the inhibiting effect was still significant for leaf area and SDW.

Twenty-one and 43 DAP no effect of PGRs was registered for June 4 transplants. For plantings on 20 and 28 May, PCB+CCC reduced the height 21 DAP. SDW, leaf area and number of leaves per plant were reduced 21 and 43 DAP when planting took place on May 28 (data not shown).

No significant effects of PGRs on productive and qualitative characteristics were observed. But when planting occurred 47 DAS the earliest of treatments reduced by 20% the total and marketable yield per hectare and per plant (data not shown).

Experiment 2. No influence of treatments was observed 32 DAS (7 d after first treatment). Thirty-nine, 46 and 67 DAS the effects of PGRs did not increase (table 5) with increasing the number of treatments. At date of planting (46 DAS), treated transplants showed a reduction in height, leaf area, SDW, root length and weakness index. The effect of treatment frequency was significant 89 DAS (43 DAP); transplants treated two and three times resulted shortened. Seedlings sprayed one and two fold had less leaf expansion, those sprayed one and three folds were lighter. The number of fruits per plant was higher in the control. Damato and Trotta (1991) reported a synergic effect of two treatments compared to one and three treatments on transplant features.

At planting time, PGRs and SUC inhibited height (table 6). The most effective in height reduction were ETH and PCB+CCC (-37%) and the least effective SUC and PCB (-22%). The height of second internode was 1 cm shorter with SUC, UNI, PCB+CCC and ETH compared to GLU and PCB tests. ETH decreased SDW compared to GLU, PCB and UNI tests. ETH, SUC, PCB+CCC and UNI treated transplants had less leaf area than untreated or GLU treated ones. Transplants sprayed with UNI showed a greater RDW; RDW was instead smaller with ETH and CCC treatments. ETH and PCB shortened root length compared to GLU. All PGRs and SUC treatments produced sturdy plants but ETH, PCB+CCC and UNI resulted to be more effective.

Some interesting interaction between treatment numbers x PGRs effects were noted. Transplants treated two fold with CCC, ETH and UNI were shorter and those treated with PCB taller (fig. 1). Leaf area and SDW were reduced in transplants treated two fold with ETH and UNI and increased with PCB. RDW decreased in transplants treated three fold with GLU; it decreased linearly with UNI treatment frequency and increased with two PCB treatments.

Within 43 after field establishment, PGRs controlled plant growth. Namely, ETH reduced SDW, leaf area, number of leaves and fruits per plant (table 6).

ETH had a long term effect on the marketable yield per ha (-38% compared to test) and per plant and green yield (+33%), table 7. The number of treatments did not modify yield characteristics.

CONCLUSIONS

The outcomes of this study indicate that earlier treatments are more effective in controlling transplant growth especially when the holding period is long. In such cases, there are long term effects on growth and marketable yield (-20%). PCB in combination with CCC inhibits the growth of transplants better than PCB alone.

In the second experiment PCB+CCC, UNI and ETH produced interesting effects in controlling transplant growth. GLU did not reduce the production of spindly transplants while ETH showed a strong effect in reducing height, SDW, RDW, leaf area and yield. One treatment is enough using PCB, and two treatments are needed for CCC, ETH and UNI.

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Table 1. Experiment 1. Date of treatment and planting.

Treatments		Planting			Dates of growth measures ^(x)
Date	Age of seedling	Date	Age of seedling	Days after treatment	
May 13	25	May 21	33	8	May 13 and 20
		May 28	40	15	May 13, 20 and 27
		June 4	47	22	May 13, 20, 27 and June 4
May 20	32	May 28	40	8	May 13, 20 and 27
		June 4	47	15	May 13, 20, 27 and June 4
May 27	39	June 4	47	8	May 13, 20, 27 and June 4

^(y) In each date of planting measure were made also 21 and 43 days after plant.

Table 2. Dates of PGR treatments and characteristics of transplants 32, 39 and 46 days after seeding.

Dates of treatments	Time (DAS) ^{(x)(y)}								
	32	39	46 ^(z)	32	39	46 ^(z)	32	39	46 ^(z)
	height (cm)			leaf area (cm ²)			shoot dry weight (mg)		
Control	9 A	12 A	13 A	33 a	38	41 A	228	264	323 A
May 13	7 B	8 B	10 C	30 b	35	33 B	200	262	274 B
May 20	-	9 B	11 B	-	35	40 A	-	264	345 A
May 27	-	-	12 B	-	-	41 A	-	-	342 A
	root length (cm)			root density (cm · cm ⁻³)			weakness index (-)		
Control	367	407	324 B	21 a	23	19 B	323	388 A	468 A
May 13	268	363	322 B	18 b	21	19 B	284	266 B	340 C
May 20	-	392	403 A	-	23	23 A	-	280 B	376 BC
May 27	-	-	388 AB	-	-	22 AB	-	-	403 B

^(x) DAS = days after seeding.

^(y) Mean separation within a column by Student-Newman-Keul's multiple range test at P = 0.05 (lowercase letters) and P = 0.01 (uppercase letters).

^(z) Planting date.

Table 3. PGRs and transplant characteristics in three different dates of transplanting.

Characteristics ^(v)		Dates of transplanting								
		May 21 ^(x)			May 28 ^(y)			June 4 ^(z)		
		test	PCB ^(w)	PCB+ CCC ^(w)	test	PCB ^(w)	PCB+ CCC ^(w)	test	PCB ^(w)	PCB+ CCC ^(w)
height	(cm)	9 a	8 b	7 b	12 a	9 B	8 B	13 A	11 B	10 C
2 nd internode	(cm)	6 a	5 b	5 b	7 A	5 B	5 B	6	6	6
root density	(cm · cm ⁻³)	21 a	17 b	19 ab	23	22	21	19	21	21
shoot root ratio	(-)	5.3	5.5	5.5	5.5 a	5.0 ab	5.1 b	4.9	4.8	4.6

^(v) Mean separation within a row using Student-Newman-Keul's multiple range test at P = 0.05 (lowercase letters) and P = 0.01 (uppercase letters).

^(w) PCB = Pachlobutrazol; CCC = Chlormequat.

^(x) Seedling treated on May 13 (25 days after sowing). ^(y) Seedling treated one fold on May 13 or May 20 (25 or 32 days after sowing). ^(z) Seedling treated one fold on May 13, May 20 or May 27 (25, 32 or 39 days after sowing).

Table 4. Dates of treatment, dates of transplanting and plant characteristics 21 and 43 days after planting.

Dates of treatments	Planting dates ^(x)			
	May 28		June 4	
	21	43	21	43
	height of plant (cm)			
May 13	18 B	43 b	23 b	43
May 20	20 AB	46 a	27 a	45
May 27	-	-	26 a	46
Control	22 A	46 a	26 a	45
	leaf area (cm ²)			
May 13	263 b	5154	489 b	5574 b
May 20	333 ab	6585	818 a	8217 a
May 27	-	-	716 ab	8759 a
Control	423 a	5154	656 ab	8513 a
	shoot dry weight (g)			
May 13	2.1 b	55	3.6 b	59 B
May 20	2.6 ab	73	5.7 a	79 AB
May 27	-	-	5.1 ab	84 A
Control	3.2 a	74	4.8 ab	75 AB

^(x) Mean separation within a column by Student-Newman-Keul's multiple range test at P = 0.05 (lowercase letters) and P = 0.01 (uppercase letters).

Table 5. Number of PGR treatments and some transplant characteristics 39, 46, 67 and 89 days after seeding.

Treatments (n)	Time (days after seeding) ^(x)						
	39	46 ^(y)	67 ^(z)	89 ^(w)	39	46 ^(y)	89 ^(w)
		height of plants (cm)			2 nd internode (cm)		fruits/plant (n)
0 (Control)	11 A	14 A	26	53 a	6	7 a	25 a
1	9 B	11 B	22	50 ab	6	6 b	16 b
2	9 B	10 B	21	47 b	6	6 b	17 b
3	-	11 B	21	46 b	-	6 b	18 b
		leaf area (cm ²)			root length (cm)		
0	38	42 a	593 A	8176 a	383	341 A	
1	35	38 b	395 B	5908 b	337	266 B	
2	32	35 b	413 B	6350 b	375	302 AB	
3	-	36 b	372 B	6463 ab	-	310 AB	
		shoot dry weight (g)			weakness index (-)		
0	0.26	0.33 a	5 A	79 a	385 A	447 A	
1	0.25	0.28 b	3 B	60 b	301 B	344 B	
2	0.23	0.30 b	3 B	66 ab	302 B	330 B	
3	-	0.28 b	3 B	56 b	-	349 B	

^(x) Mean separation within a row by Student-Newman-Keul's multiple range test at P = 0.05 (lowercase) and P = 0.01 (uppercase letters). ^(y) Planting date. ^(z) 21 days after planting. ^(w) 43 days after planting.

Table 6. PGRs and characteristics of transplants at planting, 21 and 43 days after.

Characteristics	Test	PGRs ^(x)							
		GLU	SUC	PCB	CCC	UNI	P+C	ETH	
at planting ^(y)									
height	(cm)	14.3 A	14.0 A	11.4 B	10.9 B	10.3 C	9.7 D	9.1 E	8.8 E
2 nd internode	(cm)	6.9 A	6.7 A	5.8 C	6.5 AB	6.0 B	5.9 C	5.6 C	5.6 C
SDW	(mg)	331 A	336 A	273 B	304 AB	293 B	303 AB	279 B	218 C
leaves	(cm ²)	42 A	42 A	35 C	40 AB	37 BC	38 B	36 BC	27 D
RDW	(mg)	64 AB	62 AB	57 B	61 AB	64 AB	66 A	63 AB	47 C
root length	(cm)	341 AB	353 A	300AB	277 B	316AB	328 AB	296 AB	180 C
shoot: root ratio	(-)	5.3 AB	5.4 A	4.8 AC	5.0 AC	4.6 BC	4.6 BC	4.5 C	4.7 AC
weakness index	(-)	447 A	450 A	372 B	354 B	348 B	310 C	300 C	253 D
21 days after planting ^(y)									
height	(cm)	25 AB	26 A	24 AB	22 BC	22 BC	22 BC	20 C	14 D
SDW	(g)	4.8 AB	4.9 A	3.7 BC	3.5 C	3.0 C	3.6 BC	2.7 C	1.1 D
leaves	(cm ²)	593 AB	624 A	449 BC	395 C	379 C	435 BC	340 C	131 D
leaves	(n.)	10 A	10 A	10 A	10 A	10 A	10 A	10 A	7 B
43 days after planting ^(y)									
height	(cm)	53 A	51 A	51 A	50 A	49 A	46 AB	46 AB	41 B
SDW	(g)	79 A	73 A	58 A	76 A	65 A	68 A	57 A	30 B
leaves	(cm ²)	8176 A	7831 A	5726 A	7418 A	6400 A	7222 A	6119 A	2964 B
leaves	(n.)	67 A	65 AB	53 B	61 AB	55 AB	59 AB	56 AB	31 C
fruits/plant	(n.)	25 A	17 A	16 A	20 A	21 A	21 A	17 A	5 B

^(x) GLU = Glucose; SUC = Sucrose; PCB = Paclobutrazol; CCC = Chlormequat; UNI = Uniconazole; P+C = Paclobutrazol + Chlormequat; ETH = Etephon.

^(y) Mean separation within a row by Student-Newman-Keul's multiple range test at P = 0.05 (lowercase letters) and P = 0.01 (uppercase letters).

Table 7. PGRs and yield characteristics.

Yield characteristics ^(y)		PGRs ^(x)							
		GLU	UNI	CCC	Test	PCB	SUC	P+C	ETH
marketable	(t ha ⁻¹)	60 A	57 AB	56 AC	55 AC	52 AC	50 BC	47 C	34 D
unmarketable	(t ha ⁻¹)	6.9 ab	5.6 ab	5.3 ab	6.0 ab	4.5 b	5.9 ab	4.9 b	8.4 a
marketable									
fruits/plant	(kg)	1.5 A	1.5 A	1.4 AB	1.4 AB	1.4 AC	1.3 AC	1.2 AC	0.9 D

^(x) GLU = Glucose; SUC = Sucrose; PCB = Paclobutrazol; CCC = Chlormequat; UNI = Uniconazole; P+C = Paclobutrazol + Chlormequat; ETH = Ethephon.

^{b)} Mean separation within a row by Student-Newman-Keul's multiple range test at $P = 0.05$ (lowercase letters) and $P = 0.01$ (uppercase letters).

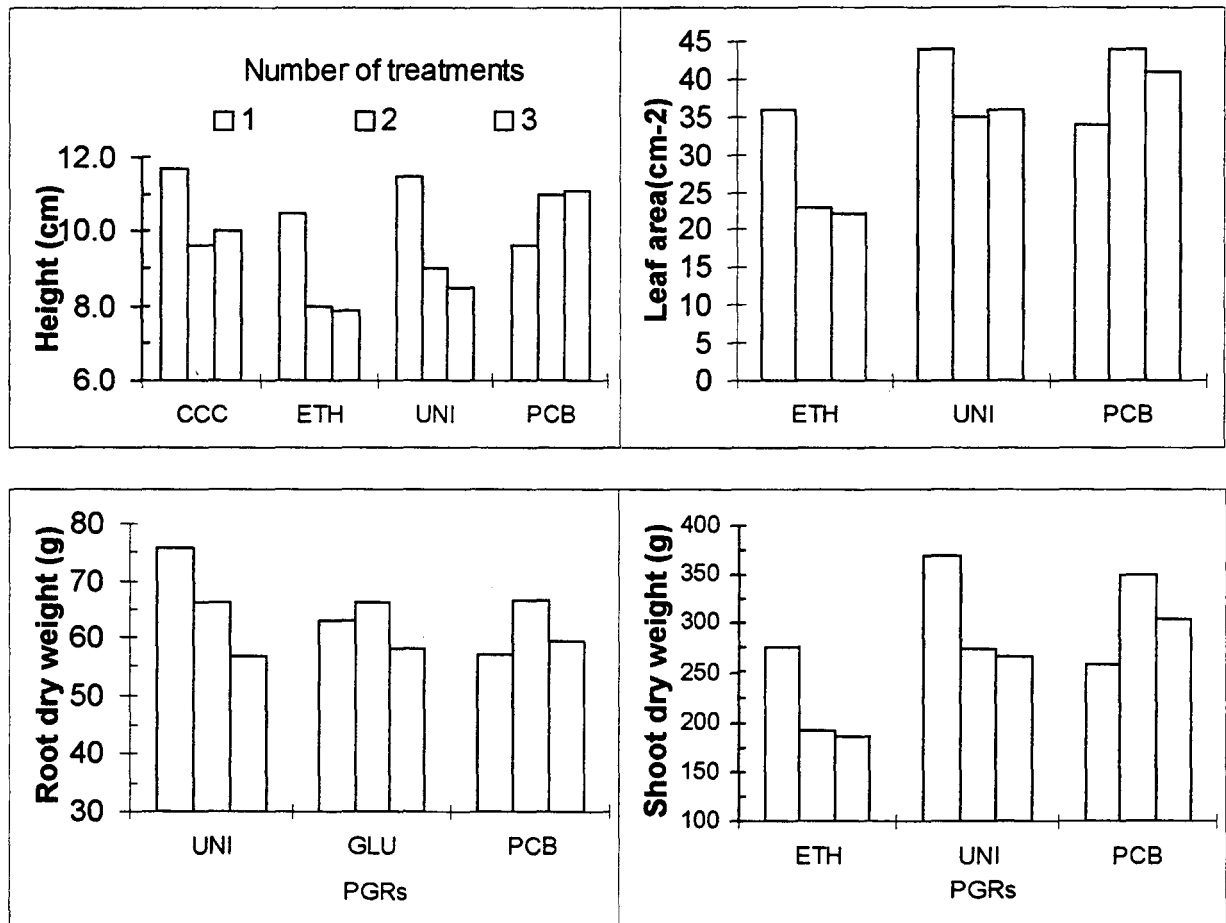


Figure 1. PGRs x number of treatment interaction effects. GLU = Glucose; PCB = Paclobutrazol; CCC = Chlormequat; UNI = Uniconazole; ETH = Etephon.

**STAND ESTABLISHMENT OF HIGH SUGAR SWEET CORN WITH
CLEAR PLASTIC MULCH AND TRANSPLANTS**

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ABSTRACT

Growers in the upper Midwest have used clear plastic mulch to improve early yield and advance the maturity of the high sugar sweet corn varieties. Results of this practice in Iowa have been mixed because of early spring temperature variability and the selected variety. In years of a clear plastic response, the results have been dramatic with days to harvest decreased by 7 and marketable yield increases of as high as 700 doz/acre. The 9 year/variety combination experiments resulted in a +20% marketable yield increase of +235 dozen/acre. For 1996, our objective was to improve performance consistency by examining transplant techniques with the early se, temptation. Treatments consisted of bare soil or clear plastic mulch, direct seeded or transplanted. The transplants were produced in either 50- cell trays, 1 7/8 in. X 2 5/16 in. Deep, or jiffy strips, 2 1/4 in. X 4 in. deep. The plastic harvest advantage was +3 days; but the transplant advantage, compared with direct seeded, was +9 days. The 50- cell tray hastened maturity by 3 days, compared with the deeper jiffy strip.

GROWING *sh*₂ SWEET CORN FROM TRANSPLANTS

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ABSTRACT

Sweet corn cultivars containing the shrunken-2 (*sh*₂) gene have superior kernel quality but often germinate poorly and display poor seedling vigor. Direct-seeded (DS) and transplanted *sh*₂ sweet corn were compared to determine whether transplanting could improve establishment. Shrunken-2 cv. Krispy King was direct-seeded (DS), or three-week-old plants, grown in 200-cell, polystyrene trays in either a "T-rail", float bed (FB), or ebb-and-flood (EF) system, were transplanted in July and May in 1995 and 1996, respectively. In 1995, final survival percentages were 83, 80, 82, and 83% for T-rail, FB, EF, and DS, respectively. All plants flowered prematurely when only 60 cm tall. In 1996, survival of all transplants was greater than 85% compared to 54% for DS. Transplants matured 10 to 13 days earlier than DS. However, over 90% of DS plants produced marketable ears compared to 63, 49, and 44 for EF, FB, and T-rail, respectively. Direct-seeded plants were also taller with better root development than transplants. Transplants produced smaller, lower-quality ears compared to DS which nullified the benefits of higher plant populations and earlier maturity. Of the transplant systems tested, EF offered the best control of water availability resulting in high-quality seedlings which produced the highest yield of marketable ears. In some areas, the increased value of early *sh*₂ sweet corn may be worth the extra cost of transplanting.

INTRODUCTION

Shrunken-2 (*sh*₂) sweet corn cultivars have higher sugar content, slower rates of sugar to starch conversion, and a more tender pericarp than sugary (*su*) or sugary enhanced (*se*) cultivars. However, many *sh*₂ cultivars germinate slowly and exhibit poor seedling vigor (Parera and Cantliffe, 1994). Poor germination is a particular problem with early spring plantings in cold soils. Seed priming, biopriming, prehydration, coating, and various fungicides treatments may improve the stand establishment of *sh*₂ sweet corn (Bennett et al., 1991; Parera and Cantliffe, 1994; Waters et al., 1990). Field and sweet corn has been transplanted experimentally in an attempt to improve stands and hasten maturity (Khehra et al., 1990; Miller, 1972; Waters et al., 1990; Wyatt and Mullins, 1989). However, transplanting sweet corn remains a questionable practice that often stunts plant development.

Some growers in the mid-Atlantic region transplant *sh*₂ sweet corn to improve stand establishment and hasten maturity. Transplanting corn is economically feasible because transplant technologies such as carousel-style planters and hydroponic transplant production in float beds enables large-scale transplant production with minimal labor (Miglianti, 1987). However, little information is available on the benefits of transplanting *sh*₂ corn or how to best produce sweet corn transplants. We compared direct-seeded (DS) and transplanted *sh*₂ sweet corn to determine whether transplanting can improve stands and hasten maturity. The suitability of T-rail, float-bed (FB), and ebb-and-flood (EF) systems for large-scale sweet corn transplant production were also evaluated.

MATERIALS AND METHODS

In 1995 and 1996, sweet corn (*sh*₂, *Zea mays* var. *saccharata*), cv. Krispy King (lot NW 3313, Rogers, Boise, Idaho) treated with thiram (tetramethylthiuram disulfide, Rhone-Poulenc Ag. Co, Research Triangle Park, N.C.), was seeded in 2-cm-deep, 200-cell (single-cell dimensions, 28 mm x 28 mm x 76 mm), polystyrene "Speeding" trays (Southern States Cooperative, Richmond, Va.). Trays for FB and EF systems were loosely filled with soilless mix (Tobacco mix, Carolina Soil Company, Kinston, N.C.). T-rail trays were filled with Sunshine mix I (Fissons, Vancouver, B.C.). Three trays each was placed into either T-rail, FB, or EF systems in a

greenhouse in Blacksburg, Va. with an average high and low temperature of 30.5° and 19.6°C, respectively. Each transplant bed was randomly arranged in the center of the greenhouse equidistant from the cooling pads. The FB and EF systems consisted of a galvanized, metal trough (3.3 m x 0.8 m x 0.3 m) lined with a double layer of 0.075-mm-thick, black plastic (Carlisle Plastics Inc., Minneapolis, Minn.). Tanks were filled with nutrient solution by dissolving 189 g of water soluble horticultural fertilizer (20-5-30, 20N-2.2P-25K, with Mg, B, Cu, Fe, Mn, Mo, and Zn, Peters Professional, The Scotts Co., Marysville, Ohio) in 570 L of tap water. The sources of elemental nitrogen were 10% ammoniacal, 40% nitrate, and 50% urea. The calculated nutrient concentrations were 66 mg L⁻¹ N, 7 mg L⁻¹ P, and 83 mg L⁻¹ K in both hydroponic systems. Actual concentrations of NO₃, K, and total dissolved salts were measured daily using hand-held meters (Spectrum Technologies, Plainfield, Ill.) (Hartz et al., 1994). Tap water or fertilizer were added periodically to adjust nutrient concentrations.

The EF bed was flooded for 2 h each 12 h with nutrient solution from a holding tank using submersible pumps (Rule Industries, Inc. Gloucester, Mass.) with a flow rate of 114-L-per-minute and automatic shutoff feature. Pumps were turned on or off by programmable, digital plug-timers (DT1, Intermatic Incorporated, Spring Grove, Ill.). To facilitate drying between flood cycles, trays in the EF beds were supported on polyvinyl-chloride pipe placed 23 cm above the bottom of each bed. Open areas on FB and EF beds were covered with black plastic film or sheets of polystyrene to minimize algal growth. Plants were acclimated for at least three days prior to transplanting by placing FB and EF trays outdoors. T-rail trays were supported on an open lattice bench top to air prune roots. The T-rail system was hand-watered daily and fertilized weekly with nutrient solution from FB.

Three-week-old seedlings were planted with a between-row spacing of 90 cm and an in-row spacing of 30 cm in a plowed field of Hayter loam (fine-loamy, mixed, mesic Ultic Hapludalf) near Blacksburg, Va. using a subsurface-tiller transplanter (B & B No-Till, Laurel Fork, Va.) (Morse et al., 1993). A liquid, starter-fertilizer solution was applied around the roots at 10N-4.3P-8.3K (kg ha⁻¹). Granular fertilizer was surface-banded 8 cm from both sides of each row at 112N-98P-93K (kg ha⁻¹) at planting in accordance with soil-test results. Direct-seeded plots were hand-seeded to a depth of 2.5 cm. Herbicides Dual (metolachlor) and Atrazine were applied at a rate of 2.2 and 1.6 kg ha⁻¹, respectively, and insecticides were applied as needed in accordance with published extension recommendations for sweet corn (Baldwin et al., 1994). The field was arranged in a randomized complete block design replicated three times. Four rows of 20 plants each were planted in each replication, and harvest data was taken from the inner two rows. Plants were harvested in the field or greenhouse and transported to the laboratory in self-sealing bags for leaf-area measurement (LiCor, model 3050A, Lincoln, Neb.). The mean time to harvest was determined graphically by plotting the cumulative number of ears harvested on a probit scale versus the time of each harvest in days. ANOVA and mean separation was performed using the computer program CoStat (CoHort Software, Minneapolis, Minn.).

RESULTS AND DISCUSSION

Establishment of *sh2* sweet corn using transplants was investigated in early July 1995 and early May 1996. The results of a previous study conducted in early June of 1994 showed that using T-rail or FB transplants reduced the mean time to harvest by one week compared to DS (Frantz and Welbaum, 1995). In 1994, all plants survived transplanting and the emergence of DS plots was 91%. The percentage of marketable ears was low for all treatments because of excessively high plant densities, but T-rail transplants produced the highest percent-marketable yield. There was no difference in yield between DS and FB plants (Frantz and Welbaum, 1995).

In 1995, seedlings from FB and EF systems grew more rapidly than plants from the T-rail system or DS plants until four weeks after planting (Fig. 1). However, FB and EF plants also experienced greater transplant shock than T-rail plants. The final survival percentages were 83, 80, 82, and 83% for T-rail, FB, EF, and DS, respectively. Leaf area of DS plants increased fastest between weeks four and seven while transplants were recovering from shock. Maximum leaf area was attained seven weeks after planting (Fig. 1) Direct-seeded plants had the greatest leaf area followed by T-rail, FB, and EF. All plants flowered prematurely when only about 60 cm tall. Since no premature flowering followed planting in May of 1996, we conclude that 'Krispy King' is sensitive to photoperiod and not suited for late planting in Virginia.

In 1996, FB seedlings had the greatest dry weight at transplanting and also higher survival in the field compared to other treatments (Table 1). Survival percentages were higher for all transplant treatments compared to DS plots, indicating that transplanting improved stand establishment. Transplants also matured earlier than DS plots with FB plants maturing the earliest of all treatments (Table 1). While all plants produced ears, DS plants

produced the highest percentage of marketable ears (Table 1). There was no difference in marketable ears between FB plots and T-rail, which had the lowest percentage. In general, ears on DS plants were longer, heavier, and had greater diameter than ears from transplants. Direct-seeded plants were also taller than transplants (Table 1). Transplanted corn was shorter in previous studies as well (Waters et al., 1990; Wyatt and Mullins, 1989). Ebb and flood plots produced the highest number of marketable ears because the higher plant survival rate more than compensated for the lower percentage of marketable ears (Table 1). However, when yield was calculated on the basis of marketable ear weight, DS and EF plots were the most productive and T-rail was the least (Table 1).

Corn does not transplant well because pruned roots do not branch and root replacement is generally poor compared to crops such as cabbage (*Brassica oleracea*) that transplant well (Loomis, 1925; Waters et al., 1990). At harvest, most plants in transplanted plots could be easily uprooted by hand while DS plants could not, indicating that transplanted corn had less extensive root systems. The inability of corn roots to regenerate after transplanting resulted in stunted plants that produced a high percentage of cull ears (Table 1). However, there were distinct differences among transplant treatments. The constant moisture allowed FB plants to produce massive root systems which resulted in greater seedling dry weight at transplanting in 1996 (Table 1). Nevertheless, many of these roots were lost when trays were removed from the FB or plants were pulled from the trays during transplanting. Plants from the T-rail system had smaller root balls than FB plants because roots were air pruned before they could grow through the bottom of each cell. Ebb and flood plants had intermediate root development between T-rail and FB because dry cycles limited root growth outside the trays (data not shown). The greater productivity of EF plants in 1996 may have been due to better root development and less root loss during transplanting (Table 1).

It is debatable whether transplanting *sh2* sweet corn is worth the extra expense and effort. Previous studies have shown no clear advantages to growing sweet corn from transplants (Waters et al., 1990; Wyatt and Mullins, 1989). The greatest benefits in the current study were obtained from early season transplanting which greatly increased plant populations compared to DS plots. The greatest reduction in mean time to harvest also occurred with earlier plantings. The increased value of early *sh2* sweet corn in some areas may be worth the extra cost of transplanting. However, transplants produced a higher percentage of culls and smaller, lower-quality ears compared to DS which may nullify the benefits of higher plant populations. Of the transplant systems tested, EF offered the best control of water availability which resulting in uniform root and shoot growth and high-quality seedlings.

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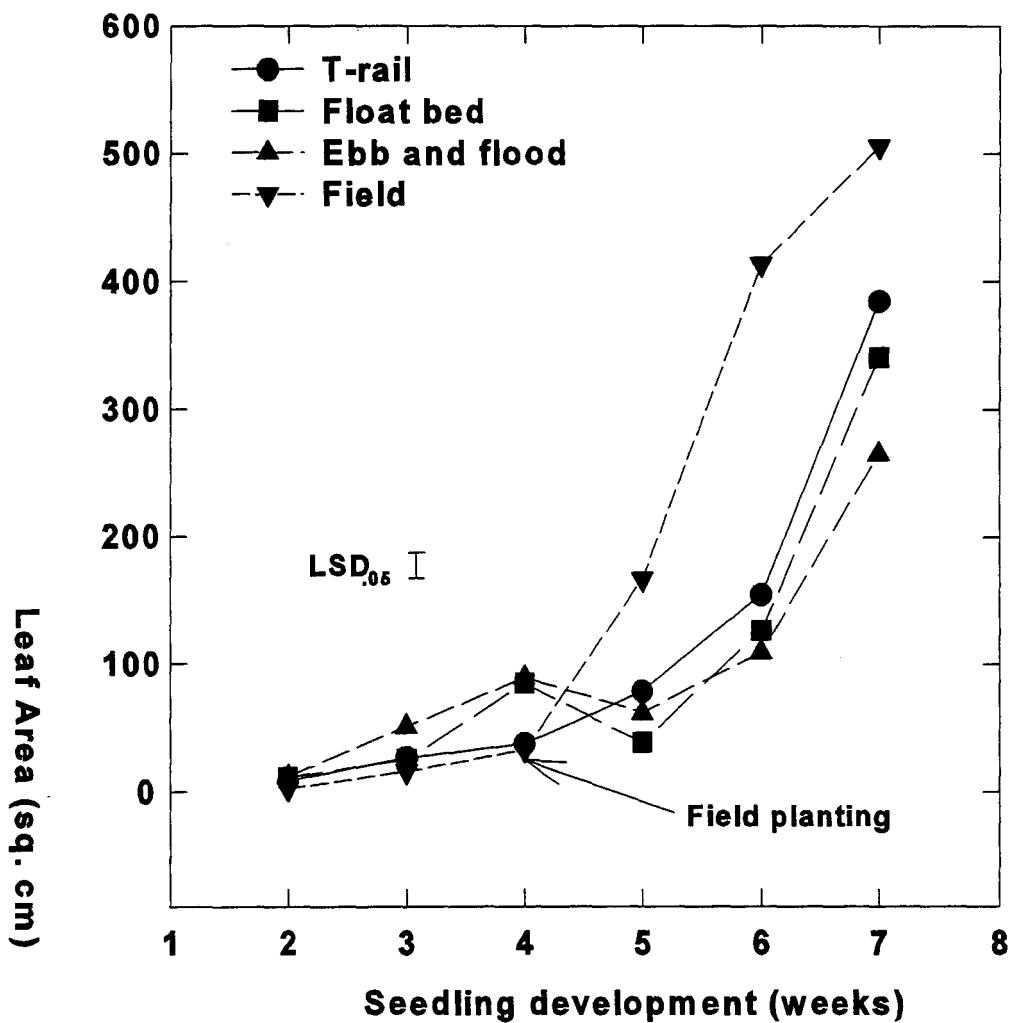


Fig. 1. Corn leaf area of transplants and field-seeded plants in 1995.

Table 1. 'Krispy King' *sh*₂ sweet corn mean transplant and harvest data from 1996.

Treatment	Seedling Dry Weight (g)	Surviving Plants ^y (%)	Mean Time to Harvest (days)	Mature Plant Height (cm)	Marketable Ears ^x (%)	Ear Length (cm)	Ear Weight (g)	Ear Diameter (cm)	Marketable Ears (ears/ha) ^w	Marketable Ear Fresh wt (kg ha ⁻¹) ^v
T-Rail	0.80b ^z	88.3a	72.0b	99c	43.8c	14.9b	127c	38.7c	13874c	1756c
Float Bed	1.10a	97.5b	68.8c	135b	48.7c	15.2b	138bc	40.0b	17031b	2352b
Ebb & Flow	0.86b	85.4a	71.2b	138b	63.0b	16.8a	152b	40.4b	19298a	2937a
Direct Seeded	-	54.2c	82.5a	181a	90.5a	17.0a	181a	44.2a	17594b	3192a

^zMeans followed by the same letter within columns are nonsignificant at LSD_{0.05}

^yDetermined four weeks after transplanting and seeding

^xUS No. 1 husked grade standards (USDA, 1992)

^wCalculated by multiplying percent survival times the number of plants in a hectare at a between-row spacing of 90 cm and an in-row spacing of 30 cm

^vMarketable ears/hectare times mean ear weight

MANAGING LIGHT TO CONTROL VEGETABLE TRANSPLANT GROWTH IN A GREENHOUSE (ABSTRACT)

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Controlling the height of vegetable transplants in a greenhouse is more difficult than other crops. Manipulating temperature is the technique that is most commonly used since the loss of chemical growth regulators. However in the greenhouse another environmental factor, light, contributes significantly to transplant quality. There are many aspects of light that can be controlled in a greenhouse but are often overlooked. Efficient use of available natural and supplemental light can be a means to maintain transplant quality. In addition, manipulation of the photoperiod can effectively reduce stem elongation. New technologies that allow selective removal of far-red light from the greenhouse environment of light plants receive show promise as another tool for growers to use to reduce stem elongation.

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Early Season Sweet Corn Emergence Using Open Furrows, Black Plastic Mulch, and Solid Matrix Priming

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ABSTRACT

Relative benefits from using open furrows, black/clear plastic mulch, and solid matrix priming on improving early season emergence and hastening maturity in a main season sweet corn variety (cv. Jubilee) while minimizing herbicide inputs was determined. The black/clear plastic mulch used had a clear plastic strip 10cm wide designed to lay directly over the furrow when installed. Germination was enhanced under the plastic mulch by 34% when compared to bare soil treatments. This translated into a 46% increase in yield over plants that were not grown under plastic. Germination was reduced by 13% when solid matrix primed seed was planted without plastic mulch. The use of solid matrix priming only resulted in a 4% increase in germination and 5% increase in yield when plants were grown under plastic mulch. Germination and yield from plants whose seed were solid matrix primed and grown under plastic mulch was not enhanced when using open furrows. However, when solid matrix priming was not used under plastic mulch, yield was increased by 3% when seed were planted in an open furrow, compared to a closed furrow. Days to maturity was also reduced by using plastic mulch and solid matrix priming.

INTRODUCTION

Early season stand establishment and harvest of sweet corn has always been a desirable goal for producers in the upper midwest U.S. Direct seeded crops are often subjected to very cold soil temperatures and often fall victim to imbibitional injury. Imbibitional injury may be due to one or more factors which are temperature, timing of temperature exposure and stage of germination, initial moisture content of seed, speed of imbibition, seed coat integrity, seed vigor, and cultivar or species (Herner, 1986). Seed priming has been an effective approach to controlling the temperature during, and rate of, initial imbibition in several vegetable crops. The priming technique, through the use of osmotic solutions, facilitates the ability to optimize conditions during initial imbibition, however it prevents radicle protrusion (Cantliffe, 1981). As a result of seed priming, germination rate, stand establishment, uniformity, and yield have improved in many vegetable crops (Khan et al., 1980). Previous studies investigating the potential benefit from priming sweet corn seed using polyethylene glycol (PEG) have had mixed results (Bodsworth and Bewley, 1981; Bennett and Waters, 1987). An alternative technique, solid matrix priming (SMP), employs an organic or inorganic solid matrix carrier to which a measured amount of water has been added (Harman and Taylor, 1988). Previous sweet corn emergence and stand establishment studies conducted using SMP also had mixed results (Cantliffe and Bieniek, 1988; Harman et al., 1989). Significant improvements in emergence and vigor were observed when seed were first surface sterilized using sodium hypochlorite before SMP (Parera and Cantliffe, 1992).

The use of plastic mulches have been used for several years. Specific benefits from using plastic mulches include earlier and increased yields, reduced evaporation, fewer weed problems, reduced fertilizer leaching and improved nutrient uptake, reduced soil compaction, elimination of root pruning, cleaner product, gas exchange (enhanced CO₂ microenvironment), reduced drowning, and ability to double/triple crop (Lamont, 1993; Caverio, Gil Ortega, and Zaragoza, 1996; Locascio et al., 1985; Grubinger et al., 1993; Wien et al., 1993). This intensive kind of crop management practice is often warranted by the price the early season produce attracts (Garton, 1995).

Our approach to improving early season stand establishment and harvest of sweet corn in the upper midwest includes the combination of SMP and black/clear plastic mulch system. In addition, open and closed seed furrows were also compared under plastic mulch culture to contrast the role of soil as a physical barrier to emergence.

MATERIALS AND METHODS

Sweet Corn (cv. Jubilee) was subjected to solid matrix priming (SMP) (Parera and Cantliffe, 1992). A black/clear plastic mulch combination was manually assembled by overlaying clear plastic mulch over strategically placed strips of black plastic mulch. Treatments in the study were comprised of solid matrix priming(+ vs. -), black/clear plastic mulch(+ vs. -), and furrow type(open vs. closed). Nitrogen fertilizer in the form of urea at a rate of 134 kg/ha was broadcast applied and incorporated with a field cultivator before planting and installation of the plastic mulch on a Webster clay loam soil (Typic Haplaquolls, fine loamy, mixed mesic) with a pH of 6.8 and P and K levels of 81 and 362 mg.kg⁻¹, respectively. Each treatment plot was comprised of 2 rows (1.5 meters wide), 6 meters long, and replicated randomly four times. After planting on April 24, plastic mulch was installed on selected treatment plots. The plastic(1.8 meters wide)covered both rows of the plot and had two clear plastic strips 15cm wide spaced 76cm apart so they would lay directly over both furrows. The remaining area of mulch had a layer of black plastic mulch underneath for the added benefit of weed control. Thermocouples were placed in the furrow to monitor temperature under the furrow, plastic mulch combinations and the bare soil control through the end of May. Once germination began to occur, seedlings under the plastic mulch were monitored closely and cuts were manually placed in the clear plastic mulch over the furrow. The experiment was routinely monitored for pest problems and treated as necessary. Germination, final plant stand, yield, and harvest date were recorded.

Data was analyzed using single degree of freedom contrasts for specific comparisons among treatment combinations.

RESULTS AND DISCUSSION

Seed furrow temperatures and temperature fluctuation were consistently higher in the open furrow under plastic mulch. An open furrow system without plastic mulch was not included in the study since it would not be a feasible production practice. Germination, final plant stand, and yield results are summarized in Table 1. The use of the plastic mulch system was significantly superior in all the parameters measured. Germination significantly declined (13%) in SMP seed when plastic mulch was not used, when compared to the control plot. In addition, SMP seed germination under plastic mulch was 48% better than SMP seed without plastic.

Final plant stand was also significantly effected from interactions between SMP seed and the use of plastic mulch. Final plant population in SMP seed suffered a 14% reduction when planted without plastic, compared to the control plot. In addition, SMP seed planted under plastic mulch resulted in a 47% increase in final plant population when compared to SMP seed without plastic mulch. It is logical to expect a reduction in marketable ears from this interaction since both germination and final plant population were effected.

Marketable ear production was 65% higher in SMP seed grown under plastic mulch versus SMP seed without the use of plastic mulch.

These results suggest that the use of SMP may not have any effect under a plastic mulch production system. However, when SMP seed were planted without plastic, a significant reduction in germination occurred, which ultimately resulted in yield loss. Solid matrix priming may enhance imbibitional injury in cases when **average soil temperatures** are around 10°C for after planting. Although the use of open furrows under plastic mulch increased seed furrow temperature, it did not have any effect on the end results. In general, sweet corn grown under plastic mulch was harvested 1 week earlier (August 7) than corn grown without plastic mulch.

The potential for the results from using these techniques to vary among sweet corn cultivars is significant. The variety chosen for this study, 'Jubilee', is more tolerant to cold soil conditions than many earlier maturing varieties. Additional studies are currently being planned for the '97 season with different varieties.

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Table 1. Single degree of freedom contrasts for the effects of solid matrix priming, use of plastic mulch and open furrows on early season performance of sweet corn.

Treatment	Germination (%)	Final Plant Population (10 ³ /ha)	Marketable Ears (10 ³ /ha)
- SMP	77	50.3	41.2
+ SMP	76	49.8	40.9
- Mulch	63	40.8	31.4
+ Mulch	84	54.7	45.8
Open Furrow ¹	85	55.4	47.4
Closed Furrow	72	47.4	38.4
C.V.(%)	7.4	7.5	11.2
+ SMP vs. - SMP	NS ²	NS	NS
+ Mulch vs. - Mulch	**	**	**
+ SMP & + Mulch vs. - SMP & + Mulch	NS	NS	NS
Closed Furrow & + Mulch vs. Open Furrow & + Mulch	NS	NS	NS
+ SMP & - Mulch vs. - SMP & - Mulch	*	NS	*
+ Mulch & + SMP vs. - Mulch & + SMP	**	**	**

¹ Mean was calculated only from open furrow plots under plastic.

² NS, *, ** = not significant, or significant at the p≤.05 or .01, respectively.

Soil Temperature (°C)

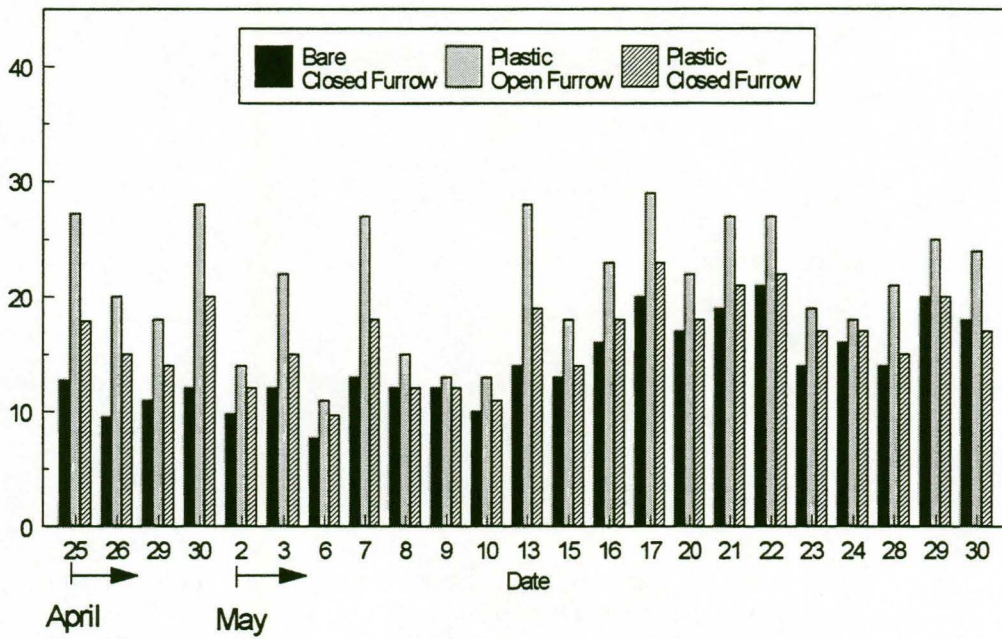


Fig. 1. Average seed furrow temperatures in different mulch and furrow combinations.

Soil Temperature Fluctuation (°C)

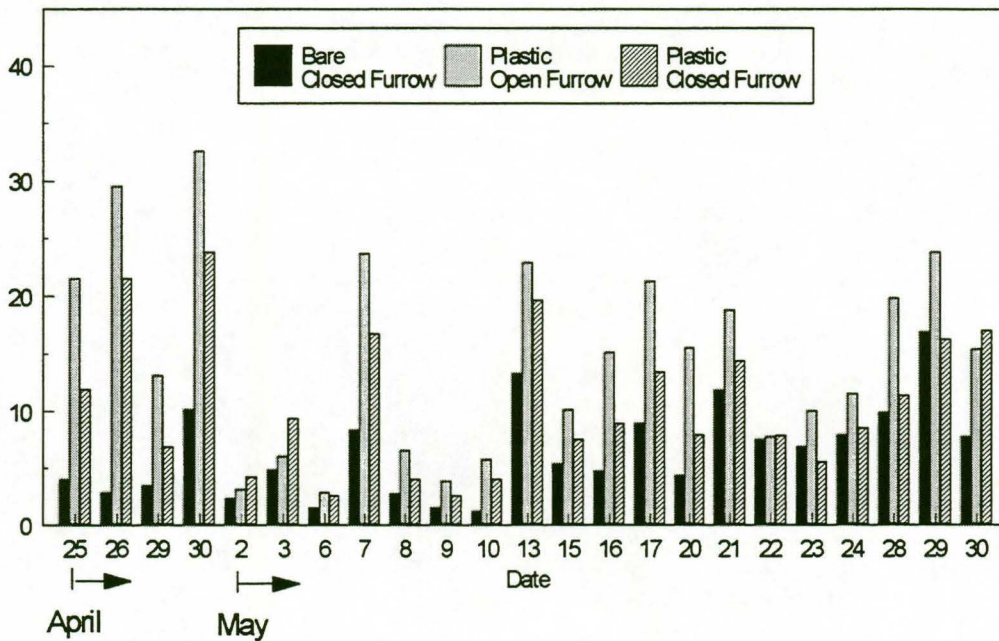


Fig. 2. Seed furrow temperature fluctuation under different mulch and furrow systems.

MICROORGANISM GROWTH DURING SEED PRIMING

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Additional index words. osmoconditioning, seed treatment, fungicide.

ABSTRACT

Seed priming has been reported to increase germination rate, total germination, and seedling uniformity in many species, especially under unfavorable conditions. However, the success of this technique depends on many factors, including the control of microorganisms during priming process. A cantaloupe (*Cucumis melo* L.) seed lot with 20 % of infected seeds was treated with fungicide Captan 50 WP (3 g / kg seeds) and primed for six days in darkness at 25 ° C in KNO₃ + KH₂PO₄ (1.5 + 1.5 %) in an aerated solution. After this period, seeds were rinsed in running tap water (2 min) and redried at laboratory conditions (25 ± 2 ° C ; 50 % RH) for four days. Part of the seeds were primed without fungicide treatment. The Blotter test was used to detect fungi, and the following fungi were identified : *Alternaria* sp., *Cladosporium* sp., *Epicocum* sp., and *Stemphylium* sp. Fungicide treatment eliminated microorganism on unprimed seeds. During priming, captan did not give good control of these microorganisms. Seed priming increased the microorganism incidence to 60 % on treated (with fungicide) seeds and 94 % on untreated seeds. The conditions established by seed priming contributed to seed fungal proliferation. Although the microorganisms detected in this lot are usually saprophytes, a high incidence of these fungi can severely affect the seed quality during storage as well as during seedling establishment. Studies are being carried out in our laboratory to achieve better microorganism control during cantaloupe seed priming.

INTRODUCTION

Seed priming has been shown to accelerate germination and improve uniformity of many species. This technique consists of imbibing seeds in an osmotic solution that allows pre-germinative metabolism to proceed, but prevents radicle protrusion through the seed coat (Heydecker et al, 1975). Among the factors affecting seed priming, microbial contamination has been cited (Copeland and McDonald, 1995). Biniek and Tylkowska (1987) verified a higher incidence of *Alternaria radicina* in carrot primed compared with unprimed seeds. Seeds free from microorganisms is an essential pre-requisite for good priming results (Cantliffe et al., 1987). However, as it is not always possible to obtain such seeds, the use of fungicides has become a common seed treatment. The practice of seed treatment by fungicides or addition their during seed soaking in priming technique have been done (Szafirowska et al., 1981; Taylor et al., 1985; Bujalski et al., 1989; Finch-Savage et al., 1991; Biniek, 1994). However, little is know about the microorganism growth during seed priming. The objectives this study were to: a) evaluate the microorganism growth on seeds and fungicide efficiency during melon seed priming, and b) analyze the effect these microorganisms on seed germination.

MATERIALS AND METHODS

Seed materia. A cantaloupe (*Cucumis melo* L.) seed lot cv. Mission (Asgrow Seed Co.) with 20 % of natural infested seeds was used in this experiment. Prior to seed soaking, half of the seed lot was treated with fungicide captan (Captan 50 WP, Southern Agricultural Insecticides, Inc., 3 g. kg⁻¹ of seed), and the remaining half was untreated.

Seed priming: seeds were primed for six days in darkness at 25°C in KNO₃ + KH₂PO₄ (1.5 + 1.5 %) in an aerated solution (10 ml of solution g⁻¹ of seed). The air was pre-hydrated by bubbling through water to minimize evaporation of soaking solution. The solution was changed each two days. After this period, seeds were rinsed in running tap water (2 min) and redried at laboratory conditions (25 ± 2°C ; 50 % RH) for four days.

Fungi growth and detection. The Blotter test was used, according to Neergaard (1977). Four replications of 20 seeds were placed in petri dishes containing three filter paper Watson n.1 wetted with deionized water. The petri dishes were incubated at 20° C, 12 h day-night fluorescent light cycle for seven days. Seed germination was inhibited by a freezing technique (24 h ; -20 ° C). The fungi were identified on the basis of colony and spore characteristics by using a stereo-microscope and microscopy, respectively.

Seed germination: four replications of 25 seeds were placed in petri dishes containing two germination papers wetted with 10 ml of deionized water each and incubated in a germination chamber, in the darkness, at 17 and 25 ° C. Radicle protusion was scored daily . After 7 and 14 days of germination at 25 and 17° C respectively, seedlings were evaluated according to Association of Official Seed Analysts (AOSA) rules (Anon., 1986).

RESULTS AND DISCUSSION

Seed infestation in the original lot was 20 %, and the following fungi were identified: *Alternaria* sp., *Cladosporium* sp., *Epicocum* sp., and *Stemphylium* sp. (figure 1). Captan eliminated the microorganisms on unprimed seeds. However, this fungicide did not give good control of these microorganisms during seed priming. The microorganism incidence, during seed priming, increased to 60 % on treated (with fungicide) and 94 % on untreated (absence of fungicide) seeds (figure 2). *Alternaria* sp. and *Stemphylium* sp., for instance, increased from 4 to 50% and from 2 to 52% respectively, during seed priming. The initial phase of imbibition has been regarded primarily as a physical hydration of seed tissues accompanied by loss of solutes. Leaching of seed exudates can stimulate microbial activity (Short and Lacy, 1976). Therefore, during seed soaking in the osmotic solution, seed leakage could contribute to the increase of fungal proliferation. In addition, during seed soaking and seed washing there was a possibility that the fungicide may have washed from the seeds, decreasing its efficiency.

Priming reduced the germination rate in both temperatures (table 1). However, the total germination (number of normal seedlings) was lower in primed seeds, possibly due to a high incidence of microorganisms. A high number of abnormal seedlings was observed in primed seeds, and this was markedly observed in untreated seeds at 17 ° C. A high seed infestation (94 %) associated with delayed germination and reduced seedling growth rate in a low temperature certainly contributed to the increase of abnormal seedlings. Deterioration of seed quality by action of *Alternaria* sp., *Cladosporium* sp., and *Stemphylium* sp. has been reported in other crops (Neergaard, 1977). The addition of fungicides to the soaking solution during seed priming has been used to control microorganism growth during seed priming (Szafirowska et al., 1981; Taylor et al., 1985; Bujalski et al., 1989; Finch-Savage et al., 1991; Biniek, 1994), as well as to control emergence damping-off in beet seedlings caused by *Pythium ultimum* and *Rhizoctonia solani* (Osburn and Schroth, 1989; Khan et al., 1992). However, precautions should be taken to avoid phytotoxic effects of fungicide added to the priming solutions (Finch-Savage, 1991). The conditions established by seed priming contributed to seed fungal contamination and these fungi, although they are usually saprophytes, severally affected seed quality.

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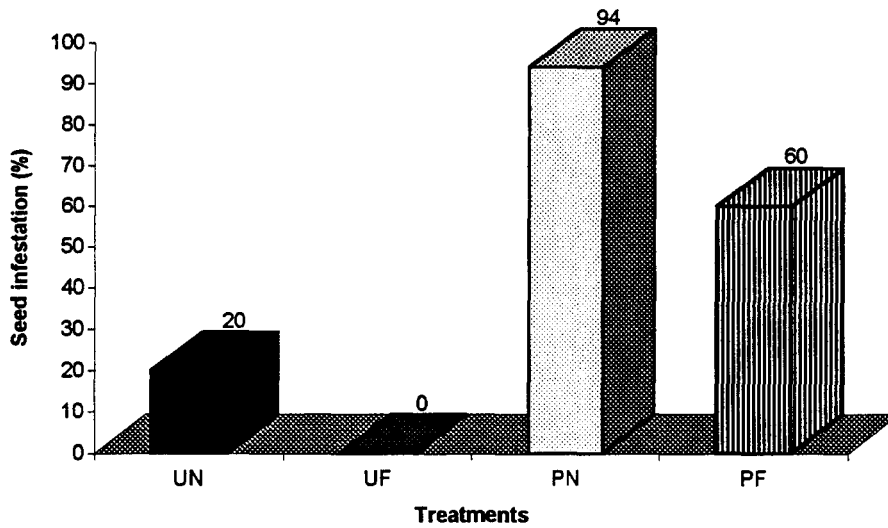


Fig. 1. Seed infestation on cantaloupe seeds. UN, unprimed, no fungicide; UF, unprimed, plus fungicide; PN, primed, no fungicide; PF, primed, plus fungicide.

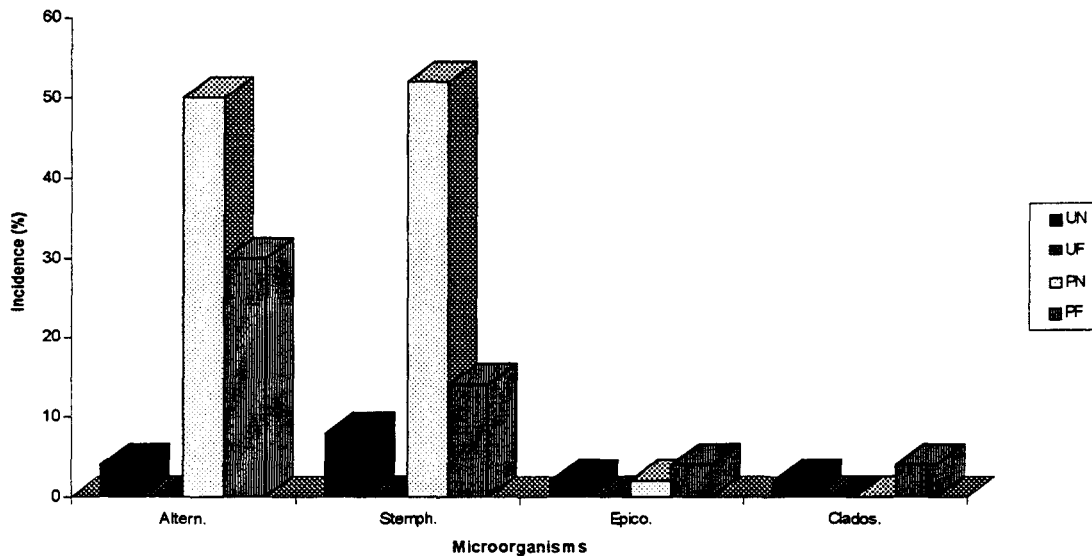


Fig. 2. Microorganism incidence (%) on cantaloupe seeds. Alter, *Alternaria* sp.; Clados., *Cladosporium* sp.; Epico., *Epicocum* sp.; Stemp., *Stemphyllum* sp. UN, unprimed, no fungicide; UF, unprimed, fungicide; PN, primed, no fungicide; PF, primed, fungicide.

Table 1. Germination (G), germination rate (days) and abnormal seedlings (Ab.) of cantaloupe seeds at 25 and 17 ° C.

Treatment	25° C			17 ° C		
	% G	days	% Ab.	% G	days	% Ab.
Unprimed, no fungicide	100.0 a*	2.0 a	0.0 b	100.0 a	4.7 a	0.0 b
Unprimed, plus fungicide	100.0 a	2.0 a	0.0 b	96.7 a	4.6 a	0.0 b
Primed, no fungicide	85.0 b	1.0 b	6.7 a	85.0 b	2.9 b	15.0 a
Primed, plus fungicide	91.7 ab	1.1 b	6.7 a	93.3 ab	2.8 b	3.3 b

* Values within a column followed by the same letter are not significantly different ($p=0.05$), according to Duncan's multiple range test.

Influence of Growth Stage on Flooding Injury in Alfalfa

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ABSTRACT

The severity of damage incurred by alfalfa (*Medicago sativa* L.) from waterlogging stress can be influenced by a number of factors including: temperature, duration of flooding, clipping management, and pathogens. This study was conducted to evaluate the effect of seedling growth stage on the severity of flooding injury in alfalfa grown under greenhouse conditions. Alfalfa seedlings were flooded for 14 d beginning at three progressively advanced vegetative growth stages: 1) early seedling development (1 to 2 trifoliolates), 2) early vegetative (3 to 6 trifoliolates), and 3) mid to late vegetative (5 to 11 branches from the main stem). Root and shoot dry wt. were recorded at the initiation of flooding, at 0 days after flooding (0 DAF), 18 DAF (shoots only), and 36 DAF (18 d of regrowth from 18 DAF harvest). The experiment was conducted twice. Flooding significantly ($P < 0.01$) reduced root and shoot dry wt. regardless of seedling age and adversely impacted regrowth potential of alfalfa. The regrowth was a significant ($P = 0.05$) stage x flooding treatment interaction for root dry wt. at 0 DAF and 36 DAF in experiment 1, but only at 0 DAF in experiment 2. Generally, flooding reduced root dry wt. least in the more vegetatively advanced seedlings. Averaged over experiments, flooding reduced root dry wt. at 36 DAF by 76, 72, and 64% for growth stage treatments 1, 2, and 3, respectively, compared with unflooded controls. A significant ($P = 0.05$) growth stage x flooding treatment interaction was observed for shoot dry wt. at 0 DAF, 18 DAF, and 36 DAF in experiment 1, but only at 36 DAF in experiment 2. Averaged over experiments, flooding reduced shoot dry wt. at 36 DAF by 56, 40, and 20% (compared with unflooded controls) for growth stages 1, 2, and 3, respectively. More vegetatively advanced seedlings were better able to recover shoot regrowth potential after waterlogging stress.

Abbreviations: DAF, days after flooding; P, phosphorus.

INTRODUCTION

Alfalfa is best adapted to well-drained fertile soils. Growth potential is restricted on many soils that do not meet these criteria (Lowe et al., 1972). While low fertility and pH can often be corrected through the use of fertilizer and lime, excess soil moisture is much more difficult to rectify (Alva et al., 1985). In addition, a large proportion of annual rainfall in the midwestern U.S. occurs in the spring and fall, precisely when new alfalfa stands are being established. This often results in temporarily waterlogged soils, especially in low lying fields or areas within a field. Excess soil moisture can profoundly affect the establishment and maintenance of alfalfa stands.

Common effects of excess soil moisture on alfalfa include a reduction in root and shoot dry wt., decreased stand density and persistence, and a general reduction in plant vigor (Letey et al., 1962; Thompson and Fick, 1981; Wahab and Chamblee, 1972). The severity of damage incurred by alfalfa plants subjected to waterlogging stress may be influenced by factors which include: temperature, duration of flooding, clipping management, pathogens, and seedling age.

Temperature can greatly influence the amount of time required for alfalfa plants to reach a given degree of damage under flooding stress (Cameron, 1973; Heinrichs, 1972; Thompson and Fick, 1981). Thompson and Fick (1981) found that higher temperatures hastened the start of waterlogging injury and increased the magnitude of yield losses. Similarly, Cameron (1973) found that at lower temperatures symptoms of flooding damage developed at a slower rate and stressed plants recovered faster when the flooding treatment was removed. The effect of temperature on flooding damage is thought to be a result of higher respiration rates of plants and soil microorganisms at warmer temperatures, thus causing a more rapid onset of anaerobic conditions in the root tissue (Cameron, 1973; Erwin et al., 1959). In addition, the solubility of oxygen in water is decreased as temperature increases (Ouellette, 1988).

The duration of waterlogging stress affects the damage incurred by alfalfa (Heinrichs, 1972; Thompson and

Fick, 1981). Thompson and Fick (1981) found that alfalfa shoot dry wt. decreased as duration of waterlogging increased in a controlled environment. A similar trend was demonstrated in the field by Bolton and McKenzie (1946).

Flooding immediately after clipping increases the damage incurred by alfalfa plants (Barta, 1988; Cameron, 1973; Erwin et al., 1959; Rai et al., 1971; Wahab and Chamblee, 1972). Rai et al. (1971) found that yield of field grown alfalfa was drastically reduced by flooding immediately after clipping. In contrast, flooding 14 or 28 d after clipping resulted in minimal yield losses. In a greenhouse study conducted by Cameron (1973), alfalfa flooded immediately after clipping was more adversely affected than alfalfa allowed to regrow for 5 d before flooding.

Most root rot diseases are favored by wet soils (Cook and Papendick, 1972). Excess soil water can influence the pathogen, soil microorganisms, and/or the host plant (Kuan and Erwin, 1980). Kuan and Erwin (1980) concluded that excess soil water predisposed alfalfa roots to infection by phytophthora root rot (*Phytophthora megasperma* f. sp. *medicagnis*). Barta and Schmitthenner (1986) found that 10-wk old plants with the shoots removed incurred more flooding injury and phytophthora root rot damage than 3-wk old plants with intact shoots. They concluded that the stress of shoot removal may increase plant susceptibility to injury from biological and/or physical stresses. Although diseases can reduce yields, in most cases they are only one component in the total stress load imposed on a plant. These stresses can interact with each other and other pests to cumulatively reduce production (Leath et al., 1988).

Seedling age may influence the degree of damage incurred from waterlogging stress. Fick et al. (1988) stated that some evidence exists that sensitivity of alfalfa to flooding stress increases up to 6-wk of age. This statement was not referenced and there are no replicated data to establish this point (G.W. Fick, 1995, personal communication). In contrast, Letey et al. (1962) concluded that low oxygen was most detrimental to cotton (*Gossypium hirsutum* L), green bean (*Phaseolus vulgaris*), and sunflower (*Helianthus annuus*) during early stages of vegetative growth. Letey et al. (1962) compared low oxygen injury at various stages of vegetative growth, but the stages were not clearly defined. To our knowledge, there are no published reports comparing severity of flooding injury in alfalfa at different stages of vegetative seedling development. The objective of this study was to evaluate the effect of seedling growth stage on the severity of flooding injury in alfalfa grown under greenhouse conditions.

MATERIALS AND METHODS

Plants were grown in a 2:1:1 (v/v) mixture of Crosby silt loam (fine, mixed, mesic Aeric Ochraqualfs), peat, and vermiculite. Fertilizer consisting of 90 g KCl and 118 g KH₂PO₄ dissolved in 12 L water was evenly applied to 240 L of soil as it was mixed. The soil was steam sterilized at 180°C for 5 hrs. Alfalfa cultivar WL 323, which is highly resistant to phytophthora root rot and resistant to aphanomyces, was seeded in 15 cm diameter pots at a rate of 30 seeds/pot. Seed was inoculated with *Rhizobium meliloti* before seeding. After seeding, the pots were placed in the greenhouse and watered thoroughly. Three sets of pots were seeded, one set for each destructive harvest. The greenhouse temperature was maintained at 20 to 25°C. Supplemental lighting was provided by 1000 watt high pressure sodium bulbs. The lamps were set at a 15-hr photoperiod and supplied 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light at pot height. After emergence was complete, pots were thinned to 15 seedlings per pot. The experiment was conducted twice. Seeding dates for experiment 1 were 24 January, 2 February, and 12 February 1996. Seeding dates for experiment 2 were 20 February, 27 February, and 5 March 1996.

A split-plot randomization of a randomized complete block design with four replicates was used. Whole plots were flooded and unflooded treatments. Subplots were three seedling growth stage treatments (Table 1). Flooding was accomplished by placing pots in 65 x 65 x 20 cm troughs filled with tap water. In an effort to displace oxygen in the water and hasten the onset of anaerobic conditions, nitrogen gas was bubbled through the water for 45 min at the initiation of flooding and twice daily for 10 min for the first 3 d after flooding began. The water level was maintained 1 cm above the soil surface for the duration of the flooding treatment. Unflooded plants were placed in troughs not filled with water. Pots were rotated daily within whole plots to reduce the impact of variations in the microclimate. Seedlings were flooded for 14 d in both experiments.

The following parameters were evaluated: 1) root and shoot dry wt., 2) aerenchyma formation, and 3) leaf chlorosis. The growth stage at the start of flooding was documented by counting trifoliolates or branches on the

main stem. After flooding, the mean stage count method of Kalu and Fick (1981) was used to stage plants in 2 replicates.

Plants were harvested for root and shoot dry wt. just prior to the initiation of flooding and at the termination of flooding (0 DAF). Shoots only were harvested at a 4-cm stubble height following an intermediate recovery period of 18 DAF. Directly following this harvest, pots were fertilized with a complete Hoagland solution (Hoagland and Arnon, 1938) at a rate of 400 mL pot⁻¹. Plants were allowed to regrow for 18 d when the final destructive harvest was taken (36 DAF). At each harvest, one pot for flooded and unflooded treatments for each of the 3 growth stages in each replicate was harvested. For the destructive harvests (initiation of flooding, 0 DAF, 36 DAF), pots were soaked in water and soil worked away in a manner that left the roots intact. The roots were rinsed in running water. The plant was separated at the junction of the crown and taproot. Shoots were dried in a forced air oven at 60°C for 3 to 4 d. Roots were freeze-dried for 3-5 d. After drying was complete, root and shoot dry wt. were recorded.

Root samples were examined for aerenchyma formation in both experiments. Samples were stabilized in a solution made up of 90% formaldehyde solution, 5% acetic acid, and 5% ethanol. A hand microtome was used to section roots for viewing through a light microscope. The main taproot was sectioned at 0.5 and 6 cm below the crown. A lateral root approximately 6 cm below the crown was sectioned at 2-3 cm from its junction with the taproot. Selected root tissue sections were photographed using an ISI 40 scanning electron microscope at 40 Kv (E.F. Fullmam, Inc., Schenectady, New York). No attempt was made to quantify aerenchyma, but only to document their presence or absence.

A Minolta SPAD Chlorophyll Meter (Minolta Corp., Ramsey, New Jersey) was used to estimate leaf chlorosis. Ten readings were taken on the leaves in the upper one-third of the plant canopy and averaged for each flooding-growth stage treatment in each of the 4 replications. SPAD readings were taken at the start of flooding, when chlorosis of seedlings became visible, and near the termination of flooding.

Root and shoot dry wt. data were expressed as mg plant⁻¹. Data were analyzed by analysis of variance to test for statistical significance of treatments and treatment interactions. Experiments were analyzed separately because experiment x treatment interactions were significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

Flooding was begun on 26 February for experiment 1 and 22 March 1996 for experiment 2 and continued for a 14 d period. Chlorosis of flooded seedlings was first observed 5 d after the start of flooding in both experiments. Thompson and Fick (1981) first observed yellowing of flooded plants 8 d after flooding began. The earlier observation of chlorosis in the present study may have resulted from efforts to deoxygenate the flood water, causing a more rapid onset of hypoxia in the root tissue, or it may have been due to differences in environmental conditions. Soil temperature during flooding for the flooded and unflooded treatments were 21 and 22°C, respectively. SPAD readings taken 13 d after the initiation of flooding showed an approximately two fold difference between flooded and unflooded seedlings within a given growth stage (Table 2). Severely chlorotic leaves began to senesce shortly after the termination of flooding. Flooded plants began to show visible signs of recovery (greening up) 10 to 12 d after the termination of flooding.

Flooded plants in growth stages 2 and 3 had significantly lower root dry wt. compared with the unflooded controls at 0 DAF and 36 DAF, while growth stage 1 showed lower root dry wt. only at 36 DAF (Fig. 1). Although flooding reduced root dry wt. in comparison to unflooded controls, root growth was not completely stopped by flooding as reported by Thompson and Fick (1981). Root dry wt. of flooded plants approximately doubled during the 14 d flooding period (Fig. 1). Root dry wt. before flooding averaged 8, 30, and 79 mg plant⁻¹ for growth stages 1, 2, and 3 respectively. After flooding, root dry wt. averaged 20, 59, and 151 mg plant⁻¹ for growth stages 1, 2, and 3 respectively. A growth stage x flooding treatment interaction for root dry wt. was found at 0 DAF and 36 DAF in experiment 1 and at 0 DAF in experiment 2 (Table 3). Generally there was a decreasing sensitivity of root dry wt. to flooding stress as seedling age increased. This is more apparent when the root dry wt. of the flooded treatments is compared to root dry wt. of the unflooded controls. For example, at 36 DAF flooding reduced root dry wt. by 75, 72, and 64% for growth stages 1, 2, and 3 respectively (averaged over experiments and compared with unflooded controls).

Although root mass of flooded plots was less than that in unflooded controls, the flooded roots appeared clean

and healthy with no signs of rotting. This is in contrast to the findings of other researchers in controlled environments. Thompson and Fick (1981) found that flooding caused death and rotting of the taproot. Cameron (1973) also noted severe flooding reaction as rotting of the central section of the main taproot. Erwin et al. (1959) found that alfalfa plants grown in unsterilized soil and flooded at 39°C were damaged by root rot and xylem necrosis, while plants grown in sterilized soil were not. They suggested that plants growing in unsterilized soil may incur more severe damage because some soil microorganisms may be capable of rapidly depleting soil oxygen, creating an anoxic environment more quickly. Erwin et al. (1959) also stated it is reasonable to suspect that organisms pathogenic to alfalfa may exist in unsterilized soil. The results from this study and of Erwin et al. (1959) indicate that rotting of root tissue and xylem necrosis observed by other researchers may not be entirely due to flooding damage, but rather a combined effect of flooding stress and soil microorganisms and/or pathogens remaining after incomplete soil sterilization.

Shoot dry wt. was reduced by flooding for harvests at 0 DAF, 18 DAF, and 36 DAF regardless of growth stage (Table 3; Fig. 2). A growth stage x flooding treatment interaction was found for the shoots harvested at 0 DAF, 18 DAF, and 36 DAF in experiment 1, but only at 36 DAF in experiment 2 (Table 3). Those interactions support the general trend of decreasing sensitivity of shoot dry wt. to flooding stress as seedling age increased. This is more apparent when shoot dry wt. of the flooded treatment is expressed as a percentage of shoot yield of the unflooded controls. For example, at 36 DAF shoot dry wt. expressed as a % of the unflooded control was 45, 60, and 80% for growth stages 1, 2, and 3, respectively (averaged over experiments). The 36 DAF harvest showed that regrowth potential of alfalfa seedlings subjected to flooding stress is affected by the morphological stage of development at the onset of the stress (Fig. 2). More developed seedlings were better able to recover shoot regrowth potential after a waterlogging stress.

Plant development was retarded by flooding (Table 4). At 36 DAF, the mean growth stages of flooded seedlings (averaged over experiments) expressed as a percentage of the unflooded control were 58, 68, and 88% for growth stages 1, 2, and 3, respectively. These observations support the general trend seen in shoot dry wt. expressed as a percentage of the unflooded controls, namely that more developed seedlings are better able to recover regrowth potential after flooding stress.

Aerenchyma formation under flooding stress has been documented for a variety of crop plants including alfalfa (McPherson, 1939; Yu et al., 1969; Zook et al., 1986). Zook et al. (1986) observed dissolution of parenchyma cells in alfalfa roots after 3 d of flooding. After 4 d of flooding, cavities (aerenchyma) were observed in the stele of the alfalfa plant. It is thought that these spaces are used as a pathway to transport oxygen from the shoots to the roots during periods of anoxia (Coutts and Armstrong, 1976). In the present study, aerenchyma formation was not observed, even after 14 d of flooding. Spongy white callus tissue formed at or just below the crown of flooded seedlings and appeared to be hypertrophied lenticels. Hypertrophied lenticels have been shown to function as primary entry points for oxygen diffusion to the hypoxically stressed root systems of several woody species (Hook et al., 1971). Zook et al. (1986) also observed hypertrophied lenticels in alfalfa after 4 d of flooding. Zook et al. (1986) noted that the morphological changes that make alfalfa more tolerant to anoxic conditions also increases the plant's susceptibility to phytophthora root rot. If this observation is real, the highly resistant alfalfa used in this study may not have undergone certain morphological changes leading to aerenchyma formation as described by Zook et al. (1986).

CONCLUSION

Flooding significantly ($P < 0.01$) reduced root and shoot dry wt. regardless of seedling age and adversely impacted regrowth potential of alfalfa. At the termination of the experiments, the impact of flooding was still evident by a reduction in both root and shoot dry wt. Final shoot dry wt. expressed as a percentage of the unflooded control illustrated that the morphological stage of seedling development at the onset of flooding affected the ability of seedlings to recover from a waterlogging stress. The results of the present study concur with the conclusions drawn by Letey et al. (1962), namely that low oxygen is most detrimental during the early stages of plant growth. On the other hand, results of the present study are not in agreement with the statement by Fick et al. (1988), that some evidence exists that the sensitivity to flooding stress increases until alfalfa seedlings are 6-wks of age. In these experiments and those reported by Letey et al. (1962), the detrimental effect of flooding injury on regrowth potential was less severe for more developed seedlings. The lack of xylem necrosis and rotting of root tissue observed in the present study raises the question of whether previous experiments were carried out under root pathogen-free conditions. Because the morphological stage of seedling development at the onset of flooding affects the ability of alfalfa seedlings to recover from a

waterlogging stress, it is recommended that in future experiments not only the seedling age in days after planting be documented, but also the precise stage of seedling development at the onset of flooding be reported. This may help to explain possible variations observed in future research on the effects of flooding injury.

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Table 1. Seedling growth stages and ages in days after planting at the onset of flooding for the two experiments.

Growth stage treatment	Experiment I		Experiment II	
	Growth stage	Days after planting	Growth stage	Days after planting
1	1-2 trifoliolates	14	2 trifoliolates	17
2	5-6 trifoliolates	24	3-4 trifoliolates	24
3	10-11 branches on main stem	33	5-6 branches on main stem	31

Table 2. SPAD readings for flooded and unflooded alfalfa seedlings at the 3 growth stages 13 d after the start of flooding. Growth stage treatments are defined in Table 1.

Growth stage treatment	Flooding treatment	-----SPAD reading-----	
		Experiment 1	Experiment 2
1	F	26a [†]	25a
	U	38b	48b
2	F	24a	21a
	U	55c	53bc
3	F	24a	21a
	U	61c	55c
LSD (0.05)		6	5

[†]Means followed by the same letter for the same experiment are not significantly different according to LSD (P=0.05).

Table 3. Analysis of variance of the effects of flooding and seedling growth stage on root and shoot dry wt. for the two experiments.

Source	df	-----Root dry wt.-----			-----Shoot dry wt.-----			
		Preflooding	0 DAF	36 DAF	Preflooding	0 DAF	18 DAF	36 DAF
-----Mean square-----								
<u>Experiment 1</u>								
Rep	3	39	510	92269	374	14864	114870	64470
Flooding (F)	1	281	125783**	2719795**	645	365454**	7591500**	1734228***
Rep x Flooding	3	55	1217	23142	176	7137	152945	10811
Growth Stage (GS)	2	21402***	168581***	1184752***	215857***	1906903***	3190720***	1839832***
GS x F	2	241	21498**	181489*	1537**	54779**	501197**	93857**
Error	12	63	1158	32442	124	4776	73495	10742
<u>Experiment 2</u>								
Rep	3	121	178	88753	360	8888	101631	79412
Flooding (F)	1	168	146291***	3824470**	20	424938**	16712248***	3104794***
Rep x Flooding	3	37	107	54073*	181	6382	64511	14312
Growth Stage (GS)	2	6507***	86896***	239411***	54451***	1074407***	510437***	235071**
GS x F	2	38	32393***	14761	30	10689	56545	225720**
Error	12	56	437	13825	318	3829	27977	32796

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

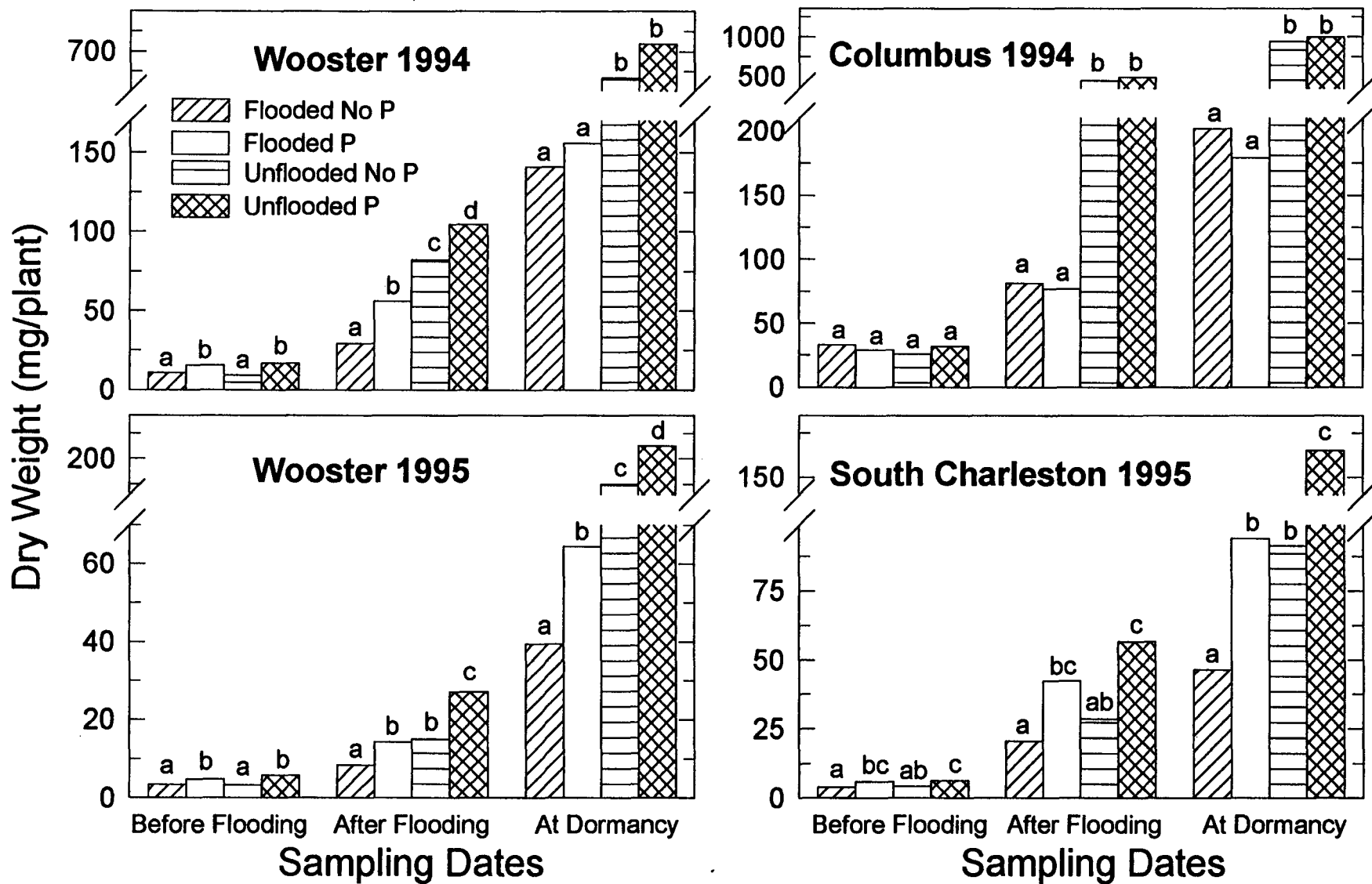


Fig. 1. Treatment effects on root dry weight in the establishment year. Bars followed by the same letter within the same sampling date, location and year are not significantly different according to LSD ($P=0.05$).

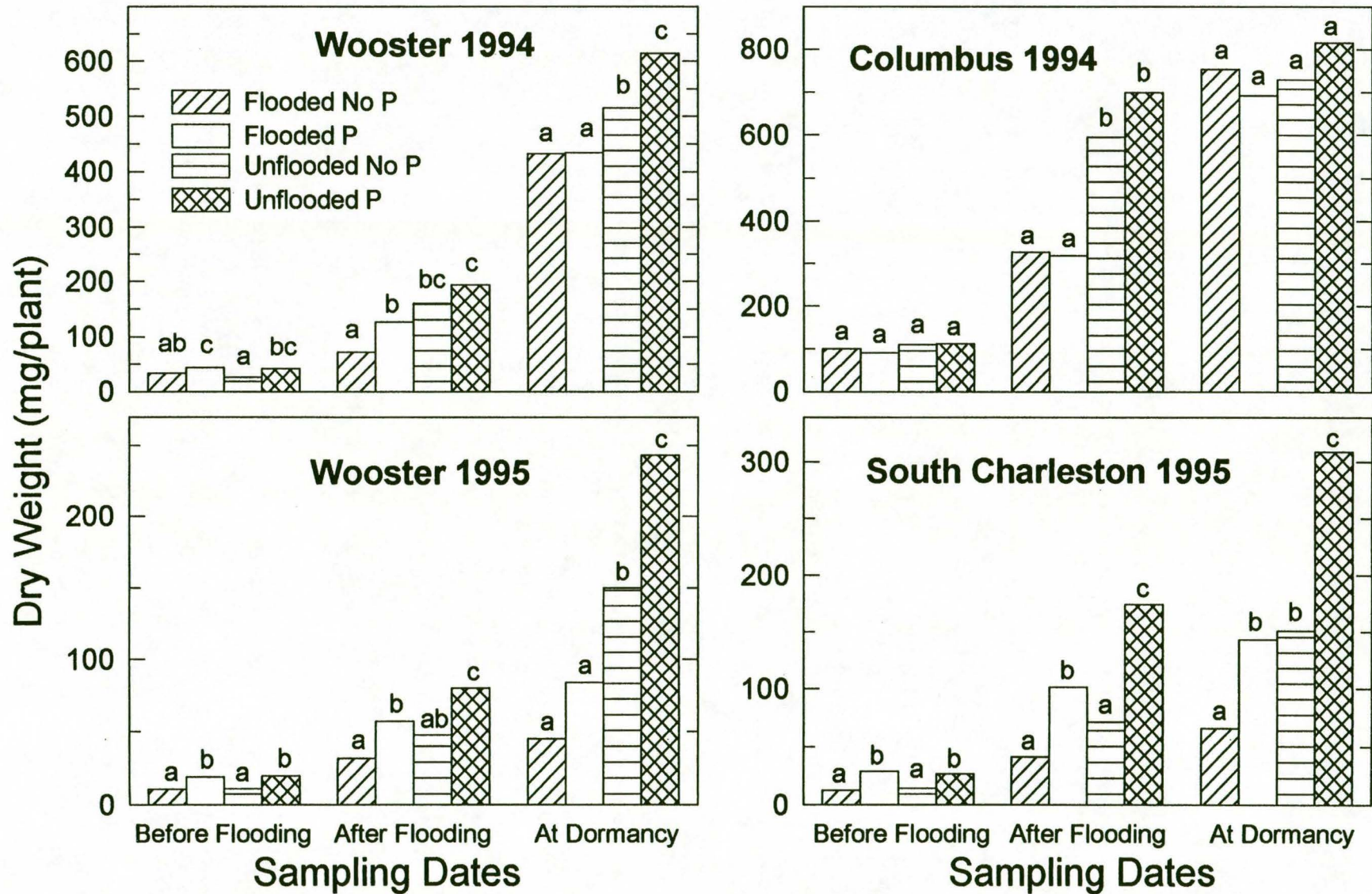


Fig. 2. Treatment effects on shoot dry weight in the establishment year. Bars followed by the same letter within the same sampling date, location and year are not significantly different according to LSD ($P=0.05$).

STAND ESTABLISHMENT PROBLEMS IN SOYBEANS: INVERTEBRATE PEST PROBLEMS

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INTRODUCTION

Problems with stand establishment in soybeans are usually associated with seed diseases, low seed quality, and difficulties with planting operations. Recommendations often suggest using a fungicide seed treatment and/or overplanting to compensate for stand losses caused by these factors.

Stand loss in soybeans caused by invertebrates is relatively unknown. Most growers are not aware that certain insects and other pests can cause significant reductions in plant stand. Losses in plant population caused by invertebrates is not nearly as well known or understood in soybean as compared with other crops in the Midwest. For example, the ability for cutworms and stalk borers to cause significant stand losses in corn has been extensively researched. The relationship between these two insects and the presence of weeds is well known. Growers are aware of various management practices that can alleviate the problems caused by these insects.

As soybean production become more diversified with the use of no-till practices and cover crops, it will be paramount that we become more aware of potential problems with stand loss in soybean that is caused by invertebrates. Since the early 1980s, we have studied the potential for two invertebrates, seedcorn maggot and slugs, to cause stand loss. Both can cause significant stand losses in soybeans, often without the knowledge of growers. Becoming more informed about the biology and management of these pests will aid growers in achieving good plant stands and maintaining high yields.

SEEDCORN MAGGOT

The seedcorn maggot (SCM), *Delia platura*, an Anthomyiidae fly belonging to the order Diptera, resembles a small housefly. SCM larvae, known as maggots, feed on germinating seeds causing serious injury to many crops resulting in significant stand loss. Their feeding causes two types of injury to soybean. The first is the death of the plant. When feeding on the cotyledons is severe, the germinating seed ceases further development and the plant dies. If the feeding has been to the plumule, a plant known as a Y-plant, or snakehead, is the result. This condition is where there are two main branches growing from the cotyledonary node. Although a plant results that will grow and produce seed, it does so with less vigor and a reduction in yield.

Originally, researchers thought that SCM problems would increase in field crops with the advent of no-till (Gregory and Musick 1976). Literature on arthropod pest problems in conservation tillage from the 1970s and 1980s list the SCM has a potential pest in no-till crops. However, research has determined that this is not the case. As the following discussion indicates, SCM has the potential to be a problem only when living, green organic matter is incorporated into the soil. These are the management practices where we see an enhancement in SCM population size.

Our involvement with SCM dates back to 1980 when we conducted a soil insecticide test at the Southern Branch of the OARDC near Ripley, OH. The field that we used was in alfalfa the previous year; that spring, the alfalfa was plowed in late April in preparation for our study. While the soybean plant stands obtained were considered normal in the treated plots, i.e., what was expected based on seed drop, the non-treated control plots suffered 50% or greater stand loss (unpublished data). Sampling was done to determine the cause for this severe loss. We found numerous SCM larvae, pupae, and empty pupal cases that indicated that the SCM was the culprit. Following this, we began a long-term project to examine the impact of various production practices on SCM. Numerous studies have been conducted in Ohio to determine the potential for SCM to cause injury to soybeans in different cropping systems. Herein is a brief synopsis of some of the studies and our findings.

Because of the rapid rise in no-till practices in Ohio, we thought it was important to confirm whether no-till did indeed increase SCM populations as the literature suggested. Numerous experiments explored this idea, including a multidisciplinary study that was conducted over a 12 year period at the North Appalachian Watershed in Coshocton, OH (Hammond 1997). The primary goal of this study was to determine if corn and soybeans could be grown in rotation using no-till practices on the hilly ground of east-central Ohio. During the course of these 12 years, various tillage practices were used, with no-till being employed each year. Results of SCM sampling showed that their population size never increased in the no-till plantings. Figures 1 and 2 indicate the relative size of the SCM populations between the various tillage management system used. Only when there was some degree of tillage, which included the incorporation of either crop residues or cover crops (the latter being used during 1990-1995), did we see increases in SCM numbers.

We also explored the impact on SCM when various cover crops were soil incorporated. Cover crops and crop residues were incorporated into the soil in the spring and SCM sampled (Hammond 1990). Table 1 shows data from 1986 indicating the effect of incorporating a living, green cover crop. Incorporating alfalfa and rye increased SCM size significantly more than did the incorporation of crop residues. As in all other studies, there was no increase in SCM populations in no-till. This pattern of SCM population enhancement was observed all three years of this study.

Table 1. Mean number of adult seedcorn maggots per trap in 1986.

Practice	Bare	Soybean Residue	Corn Residue	Alfalfa	Rye	Weeds
No-till	2.1 ef	1.9 f	0.5 f	0.9 f	0.8 f	0.6 f
Plowed	3.1 ef	14.3 bc	6.7 de	46.0 a	21.2 b	9.9 cd

Different letters indicate significant differences among the two management practices and cover crop/residues

The potential for SCM is highest when a grower incorporates a live, green living cover crop into the soil. As indicated, no-till does not call for any action against this pest. Current recommendations for management would be the application of a seed treatment containing an insecticide. Past research has shown that seed treatments are effective (data not presented).

Recently, we have explored alternative management tactics that relate to the development of SCM larvae. We studied the interaction of different tillage and planting times and their impact on the occurrence of SCM (Hammond 1995). Cover crops were incorporated into the soil in mid April and early May through plowing followed by disking. Following the second tillage operation, soybeans were planted in early May or 2 weeks later in mid May. Monitoring adult SCM emergence from these plots indicated that peak emergence followed the time of tillage (Figure 3). In both years of the study, two separate peaks were observed, one peak following the first tillage date and the second following the other tillage time. Planting date had no impact on time of SCM emergence, indicating no significant affect from planting on the insect population. This fact is further supported by sampling from areas where no soybeans were planted. Numbers of SCM collected in the non-planted areas were similar to those from where soybeans were planted (data not presented). Adult peak emergence was also the same as from planted areas and followed the times of tillage. When degree days were calculated from the time of tillage to peak emergence, we found that approximately 405 degree days had accumulated. This correlates well with other studies where emergence was usually between 400-410 degree days after tillage operations (Hammond and Jeffers 1983; Hammond 1990; Hammond and Cooper 1993).

Information on degree days becomes important when we relate it to the development of the insect. Sanborn et al. (1982) had reported that it takes approximately 230 and 400 degree days for SCM to reach the pupal and adult stage, respectively. Observations taken in the field support this; pupae are usually observed after about 250 degree days had accumulated following tillage. Because the presence and timing of SCM is dictated by tillage, we can use degree days to develop an alternative management practice. Following the accumulation of 250 degree days after tillage, we can assume that the SCM are predominantly entering the pupal stage. Because this is a non-feeding stage, growers can assume that they would be planting during a safe period, a time when the damage potential is low

(see Figure 4). Waiting to plant until this time offers growers an alternative management tactic against SCM when cover crops are incorporated.

SLUGS

With production of soybeans using no-till practices gaining greater acceptance, we have seen a dramatic rise in slug problems in the eastern corn belt, especially in east and east-central Ohio. Although slugs were associated with no-till corn, their ability to cause significant injury to soybeans was not realized until the 1980s (Hammond 1985). Since that time, we have intensively studied slugs in soybeans (along with corn).

Numerous slug species are found in field crops in Ohio, the most predominant being the gray garden slug, *Derocerus reticulatum*. Another slug of importance is the dusky slug, *Arion subfuscus*. We now believe that these two slugs can cause significant stand reductions in soybean, with injury occurring in two separate manners. Following the May and June egg hatch, small juvenile slugs have the ability to cause significant defoliation to germinating seedlings and small plants. We have observed numerous soybean fields where plants have reached the unifoliate or first trifoliate leaf stage and then were eaten to the ground leaving a very short stub. The result has been nearly 100% stand loss in small and large patches and occasionally major areas of the field.

The dusky slug has not been found to cause significant plant defoliation, but appears capable of causing severe stand losses by feeding on germinating seed and seedlings prior to the plant's emergence from beneath the crop residue. We often observe soybean fields with plant stands 60-70% of what would be expected under ideal conditions. These stand reductions are usually attributed to other causes. However, we recently obtained data that suggests otherwise. We conducted a study in 1995 in a field with a known dusky slug population. We established plots with an at-planting time application of a molluscicide. We obtained a significant increase in plant stands in treated plots compared with untreated areas that we could only attribute to the control of slugs. Of note, this study was not intended to examine stand loss. Although we attempted to explore this possibility in 1996, we experienced few slug problems with most fields having slug populations that were barely detectable. However, we noted that many of the fields that had a history of slug problems and reduced stands had some of the highest plant stands ever observed. Whether these increased plant stands are a result of little or no slug activity is unclear at this time, but warrants further investigation.

Slug control is difficult to achieve, with the only therapeutic tactics being the application of a molluscicide. Few materials are available to the grower, and all contain metaldehyde. The two most widely used baits in Ohio are Deadline Mini-Pellets and Prozap Snail and Slug Bait. We have conducted numerous studies with these materials and have found them to be very effective in controlling slugs when applied at the appropriate time (Hammond et al. 1996). Alternative cultural tactics do exist, but many defeat the purpose of using no-till. For example, the most direct method of control is the use of tillage. Few slug problems exist when little crop residue remains on the soil surface. The use of row cleaners have been recommended for corn as they enhance the growth of corn allowing it to outgrow slug injury. However, the use of row cleaners is limited because no-till soybeans are usually grown in narrow rows.

Studies on the relationship between slug injury and soybean yield indicate a growing need for control. We hope to develop predictive capabilities that will allow us to inform growers when large populations of slugs are anticipated. Figure 5 shows data from a field in Wayne County in northern Ohio suggesting that large populations of slugs in the fall (determined by sampling) result in large populations the following spring, while low numbers indicate a relative low population. It will take many more years of research before we have a complete picture of slug biology and life history. Until that time, growers should be aware of the potential for stand losses due to slugs and that scouting and correct identification are important parts of slug control.

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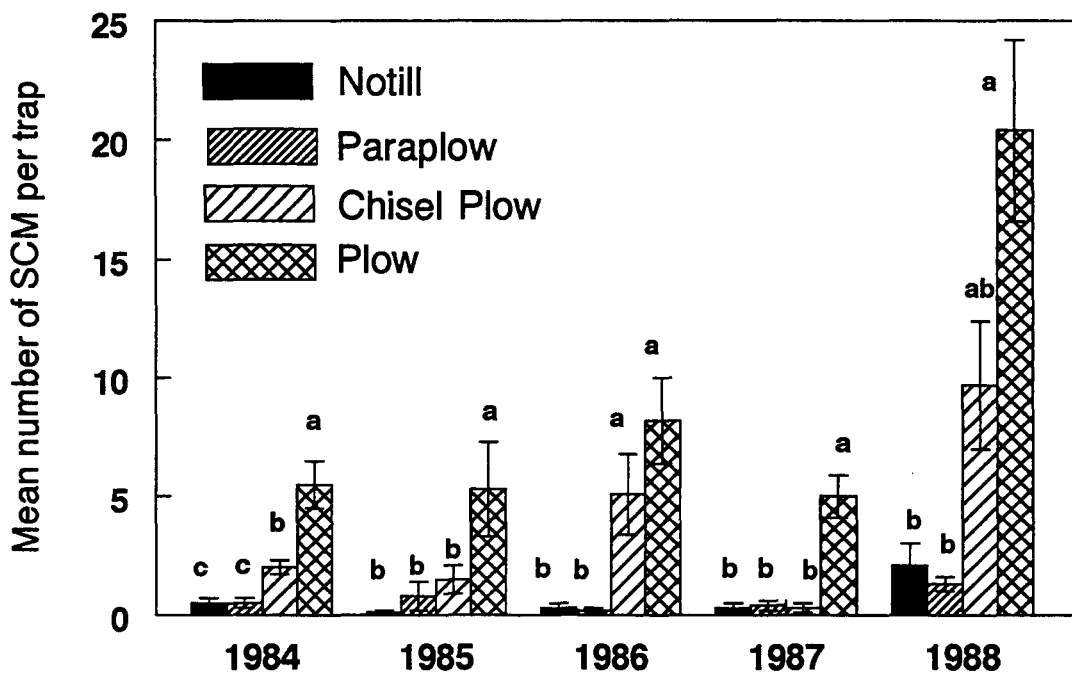


Figure 1. SCM per trap in Coshocton Study, 1984-1988

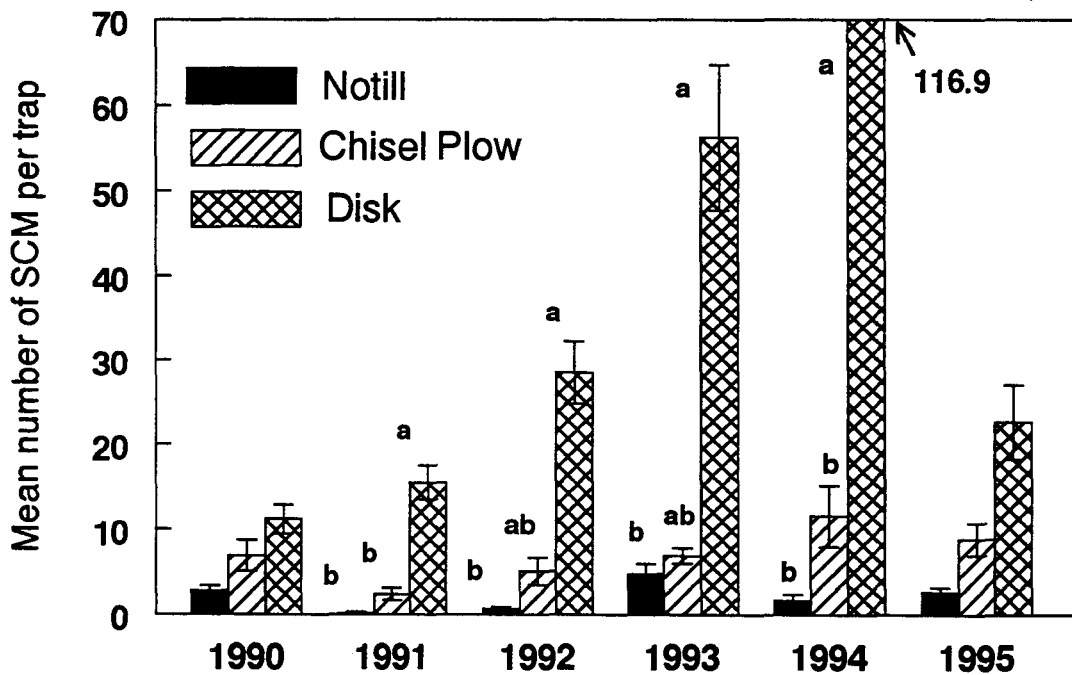


Figure 2. SCM per trap in Coshocton Study, 1984-1995

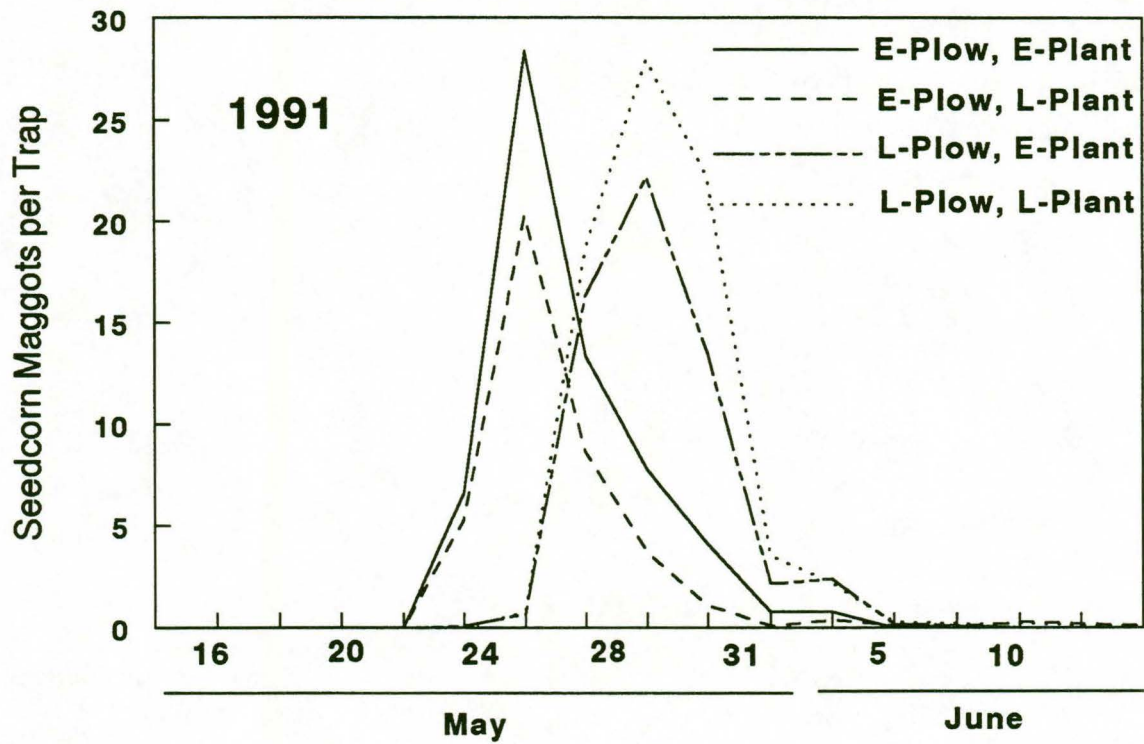


Figure 3. SCM emergence per trap from tillage study; E= early, L = late

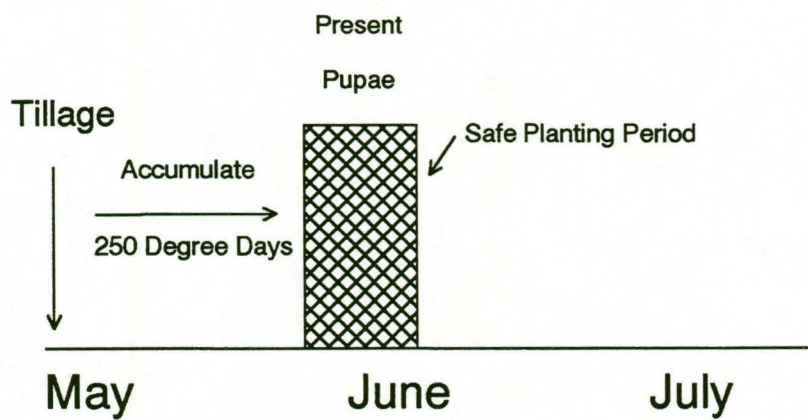


Figure 4. Planting scheme based on SCM degree day development

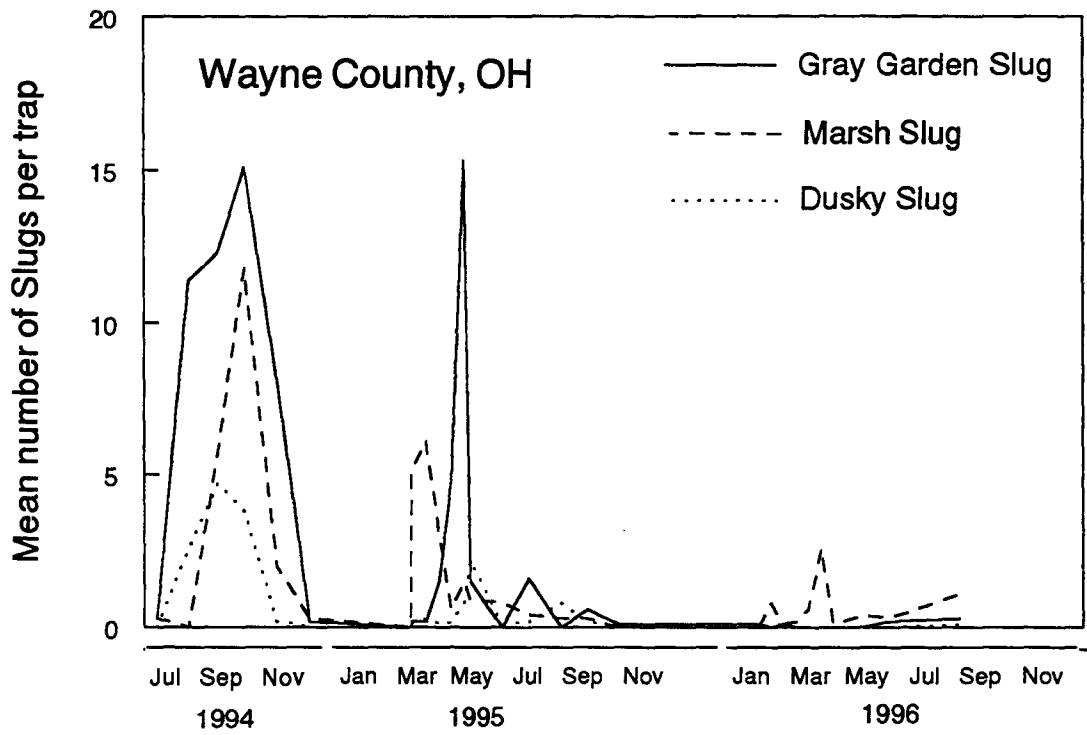


Figure 5. Slugs per trap from 1994 to 1996 in a field in Wayne County, OH

Developmental changes associated with acquisition of desiccation tolerance in maize

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Abstract

In this report, desiccation tolerance is described in terms of germinability which for agronomic purposes is considered the production of a normal seedling and its inherent vigor. Acquisition of tolerance to desiccation during development was investigated in two maize inbreds which differ in sensitivity to desiccation, A632, tolerant, and B73, sensitive. Inbreds were grown in 1995 and 1996. Ears were hand pollinated, harvested at 7 to 8 intervals from 7 to 64 days after pollination and dried at room temperature, in experimental thin-layer dryers at 35°C or 45°C, or held in high relative humidity chambers. Seed samples were removed at 0, 24, or 48 h, or after seeds reached 100 to 130 g H₂O kg⁻¹ fw for protein studies, assays and tissue mc determination. Seed quality and respiration studies were carried out on seeds dried to 100 to 130 g H₂O kg⁻¹ fw. The effects of genotype, harvest maturity, and drying environment on the acquisition of desiccation tolerance were characterized by measuring changes in SDS-PAGE protein profiles, protein synthetic capacity by radioisotope incorporation, respiratory competence of excised axes, axis and whole seed moisture content, and seed quality estimates including standard warm and cold germination tests, normal seedling dry weights, shoot to root dry weight ratios and electrical conductivity.

Introduction

Germinability in orthodox seed is acquired early in development partially in response to changes associated with acquisition of tolerance to desiccation. Studies characterizing changes in molecular and protein profiles, carbohydrate and membrane lipid composition, plant hormone levels and sensitivities and water relations that occur during specific developmental stages have been important to understanding the mechanisms of desiccation tolerance and subsequent germinability. However, the agronomic importance of normal seedling production extends that need for understanding to the relationship of these changes associated with desiccation tolerance as they impact seed quality. In addition, in maize seed production, ears are commonly harvested early in maturation and desiccation is completed under relatively high temperatures imposing important implications for seed quality. In this study, germinability, in terms of normal seedling production, is evaluated in two maize inbreds differing in sensitivity to high temperature drying that are harvested at several developmental stages and subjected to different drying regimes. Subsequent seed quality estimates are related to physiological changes that occur during development and in response to natural and artificial maturation drying.

Because drying temperature has a fundamental impact on rate of moisture loss, moisture contents of intact seed and axes samples were measured during drying to determine relative rates of moisture loss experienced by the tissues and to identify moisture contents at which subsequent analyses were carried out. Tests for seed quality included standard warm and soil-free cold germination tests and electrical conductivity. Metabolic potential of excised axes was measured by oxygen uptake during imbibition. Drying-induced qualitative and quantitative changes in synthetic potential were assessed using radiolabeled amino acid incorporation into embryo proteins at drying intervals followed by SDS-PAGE of water-soluble, non-heat-denatured proteins.

Materials and methods

Plant materials

Two inbreds, A632 and B73, desiccation tolerant and sensitive, respectively, were grown at Iowa State University, Ames, Iowa, in 1995 and 1996. Controlled pollinations were carried out at approximately 50% pollen shed. A632 achieved 50% pollen shed five to seven days before B73 in both years. Pollination of each inbred was completed within two days.

Harvests were made at 7, 10, 15, 20, 30, 40, and 50 days after pollination (DAP); additional harvests were made prior to frost. Ears were hand-husked and three to five ears randomly assigned to each drying treatment.

Drying treatments

In 1995, ears were placed in experimental thin-layer dryers (Navratil and Burris, 1982) at 35° C and ~35% relative humidity (RH) or 45° C and ~25% RH. In 1996, two additional drying treatments were included: ears held upright on the benchtop at ambient conditions of 25° C, and approximately 35% RH (Room); or held at >90% RH above water:glycerol (4:1) saturated Kimpak in a sealed plastic crisper (HRH). With the exception of ears held at HRH, ears were dried to 100 to 130 g H₂O kg⁻¹ fresh weight (fw).

Moisture content determination and seed quality

Moisture loss in intact seed (three replicates of ten seeds) and excised axes (three replicates of five axes) was determined (oven method: 105° C for 24 h) on samples removed at 0, 24, and 48 h of drying and at frequent intervals thereafter down to 100 to 130 g H₂O kg⁻¹ fw.

Standard rolled towel warm (7 d at 25° C) and soil-free (Loeffler *et al.*, 1985) cold germination (7 d at 10° C followed by 7 d at 23° C) tests were conducted on seed dried to 100 to 130 g H₂O kg⁻¹ fw from all harvest and treatment seedlots for both years. Seedlings were evaluated, normal shoot and root tissue separated, dried (105° C for 24 h) and weighed to obtain seedling dry weights and the ratio of shoot to root tissue dry weights. All tests were replicated three times.

Conductivity of individual seed leachate (3.75 mL distilled H₂O per cell) was measured on three replicates of 20 (1995) or 25 (1996) seeds per seedlot using the Genesis-2000 Seed Analyzer (Wavefront, Inc., Ann Arbor, WI). Care was taken that prior to soaking, seeds transferred from the cold room (12° C, 50% RH) were allowed sufficient time to come to room temperature without experiencing moisture loss. Readings were taken after 1, 4, 8, 12 and 24 h soak time. Mean and median values of readings are reported for 1996 seedlots; means alone are reported for 1995 seedlot data. Means and median values presented are corrected for seed weights. In order to compare individual conductivity values with subsequent germination performance following conductivity measurements, seeds were removed individually to wetted towels and warm germination scores evaluated.

Respiration

Oxygen uptake by axes was measured after 4, 8, 12, and 24 h imbibition using a Clark-type polarographic oxygen electrode (Rank Brothers, Cambridge, England). Seven to 10 axes from 20 DAP seedlots and 5 axes from all others were excised, weighed and imbibed on wetted blotter paper in glass petri dishes. Axes were transferred to the electrode chamber for measurements and returned to the dishes to continue imbibition between measurements.

Protein synthetic capacity during drying

In 1995, protein synthetic capacity was studied in 20, 30, and 40 DAP seeds removed after 0, 24, and 48 h drying by uptake and incorporation of a 5:1 mixture of L-[³⁵S]-methionine and L-[³⁵S]-cysteine (> 1000 Ci mmol⁻¹, Pro-MixTM, Amersham, Arlington Heights, IL) into aseptically excised embryos. Three replicates of five 20 DAP embryos or three embryos from 30 or 40 DAP seeds were incubated in 25 µL H₂O containing 20 µCi activity at 23° C two or four hours. The embryos were then rinsed well, carefully blotted dry, placed in microcentrifuge tubes and stored at -70° C until use.

Water-soluble proteins were extracted from embryos in 500 µL Tris buffer (50 mM Tris, 5 mM MgCl₂, pH 7.5) on ice with mortar and pestle. Homogenate was centrifuged at 20 000 x g for 20 min, the supernatant removed and non-heat-denatured proteins obtained by heating at 100° C for 15 min, followed by centrifugation at 20 000 x g for 20 min. Protein in the supernatant was concentrated by TCA precipitation, resuspended in buffer and the concentration determined by the method of Bradford (1976).

SDS-PAGE was carried out by the method of Laemmli (1970) on 12% gels loaded with equal amounts (20-25 µg) of embryo protein. Gels were stained with Coomassie Blue, impregnated with PPO in DMSO, then transferred onto filter paper and dried at 60° C under vacuum for approximately two hours. The gels were exposed to Kodak X-OMAT AR film at -70° C for four to twelve weeks, developed and the image analyzed using OS-ScanTM Lite image analysis software by Oberlin Scientific Corporation (Oberlin, OH).

For comparison of synthesis with accumulation, SDS-PAGE was similarly carried out on water soluble, non-heat-denatured proteins extracted from axes not incubated with ³⁵S-labeled amino acids. Stained gels were analyzed as above.

Results and discussion

Freshly harvested ears of both inbreds at 7 and 10 DAP displayed uniform appearing seed whereas after 48 h drying at 35°C, 5 to 7 days at room temperature, or longer for ears held at HRH, a small number of randomly scattered seed remained 'blistered' in appearance while the remainder appeared wrinkled and dried. Interestingly, 30% of the 'blistered' 7 DAP seed that had dried slowly at room temperature produced viable seedlings upon germination. In contrast, 7 and 10 DAP seed that appeared dried and wrinkled after drying were not viable regardless of drying treatment. Variability in seed response to drying at this early stage may reflect differences in inherent pollen or seed vigor, or, more unlikely, contaminant pollen. Nonetheless, this variability is not observed in seeds of later stage ears.

Rates of moisture loss in intact seed were generally steady within drying treatments among developmental stages in both A632 and B73. Differential rates of drying were observed between seed and axis tissue. Axes generally maintained a higher mc relative to intact seed, particularly earlier in development in the case of A632 (Table 1) and in both inbreds at all DAP in axes dried at 45°C relative to 35°C.

Viability, in terms of warm germination percent, increased significantly from 7 to 40 DAP across inbred and drying treatment (Table 2), whereas vigor continued to improve with developmental stage in terms of increasing cold test germination percent and seedling dry weight and decreasing shoot to root ratio. Tolerance to high temperature drying was acquired earlier by A632 as shown at 30 DAP by significantly higher warm germination percentages relative to B73 (81.3% compared to 63.7% in 1995 and 62.7% compared to 8.7% in 1996, respectively). Sensitivity to high temperature drying in B73 was accentuated by cold germination conditions.

The rate of leakage of cellular contents measurable by conductivity decreased with maturation and with lower drying temperature (slower drying rates) across both inbreds (Figure 1. A-F). However, mean conductivity readings were generally greater for B73 compared to A632 at each developmental stage and for each drying treatment though those differences decreased with maturation.

Oxygen uptake was responsive to developmental stage and drying treatment. In axes at all stages dried at 25 °C or 35 °C oxygen consumption increased in an essentially linear manner during the first 8 h of imbibition (Figure 2. A-F). For axes dried at 45 °C the rate of oxygen consumption slowed after 4 h and after 12 h did not increase except in the case of B73 at 57 DAP, and in 20 DAP A632 axes oxygen consumption declined dramatically after 8 h imbibition.

The high rates of oxygen consumption observed in 30 DAP B73 axes dried at 25 °C or 35 °C may be due to more readily available metabolic substrates at that developmental stage in B73 relative to A632. Further, the rate of drying at 35 °C seemed to enhance uptake in 30 DAP axes while impairing uptake in younger axes.

Synthesis of non-heat denatured proteins was clearly induced by both natural and artificial drying. Non-heat denatured proteins accumulated between 20 and 40 DAP as shown in Figure 3 and from 0 to 48 hours drying across all treatments, but significantly more in response to 35 and 45°C drying. Notable is induction of bands at approximately 66, 40 to 44, 37 and 25 to 27 kDa. Radiolabel incorporation into protein showed a consistent pattern (Figure 4) that could account for some of the accumulation of Coomassie-stained protein seen in Figure 3. The abundance of methionine and cysteine in embryo protein will of course limit the amount of label incorporated and therefore signal detected on film. The induction and accumulation of specific proteins in response to high temperature drying shown in 20 DAP embryo axes may be a heat shock response rather than associated with desiccation tolerance and seed quality. Alternatively, desiccation tolerance may involve a minimum level of accumulation that natural maturation permits or is associated with the increased time in slow drying in the case of 20 DAP seed. The relationship of these proteins to other desiccation response proteins and to seed quality needs further investigation.

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Table 1. Relative moisture content loss of seed tissues during drying (1995)

Inbred	Developmental stage																	
	20 DAP							30 DAP					40 DAP					
	Trt Temp	Tissue	hours of drying				rate of moisture loss	hours of drying				rate of moisture loss	hours of drying				rate of moisture loss	
		0	24	48	dry	$\text{H}_2\text{O kg}^{-1}\text{fw h}^{-1}$	0	24	48	dry	$\text{g H}_2\text{O kg}^{-1}\text{fw h}^{-1}$	0	24	48	dry	$\text{g H}_2\text{O kg}^{-1}\text{fw h}^{-1}$		
A632	TL 35°C	seed	654	608	505	97	0.310	432	363	279	89	0.319	360	265	208	87	0.317	
		axis	692	674	654	134		669	537	538	138		512	509	455	110		
	TL 45°C	seed	654	567	331	99	0.675	454	288	131	103	0.675	365	192	109	85	0.535	
		axis	692	676	575	150		669	564	200	131		512	406	123	94		
	Room	seed	654	618	566		0.183	433	400	336		0.202	370	312	267		0.214	
		axis	692	662	665			669	576	510			512	493	501			
	HRH	seed	654	641	623		0.065	435	424	408		0.056	372	353	341		0.062	
		axis	692	670	669			669	547	576			512	520	532			
	B73	TL 35°C	seed	647	599	492	104	0.323	502	432	320	75	0.379	423	357	294	92	0.269
			axis	633	649	649	87		558	556	590	93		522	550	521	76	
		TL 45°C	seed	645	541	321	99	0.675	504	382	346	96	0.508	430	283	201	89	0.475
			axis	633	677	537	94		558	534	583	76		522	554	269	73	
Room		seed	648	608	568		0.169	506	476	418		0.183	418	403	356		0.127	
		axis	633	666	637			558	582	559			522	549	522			
HRH		seed	639	643	603		0.075	500	484	476		0.05	422	416	390		0.069	
		axis	633	683	637			558	552	506			522	551	523			

Table 2. Influence of developmental stage and drying treatment on seed quality in A632 and B73 (1996)

Inbred	DAP	HMC g H ₂ O kg ⁻¹ fw	Trt/Temp (°C)	Warm Germination			Cold Germination		
				Germ (%)	SDW (mg)	S:R	Germ (%)	SDW (mg)	S:R
A632	15	820	Rm 25	13.3	8	8.95	21.3	9	8.69
			TL 35	0	0	0.00	0	0	0.00
			TL 45	0	0	0.00	0	0	0.00
	20	720	Rm 25	68	16	3.97	73.3	22	5.15
			TL 35	65.3	15	4.60	83.3	17	5.13
			TL 45	0	0	0.00	0	0	0.00
	30	535	Rm 25	98.7	30	1.83	98	45	2.22
			TL 35	96.7	26	1.64	82	31	1.99
			TL 45	62.7	15	2.21	67.3	22	2.62
	40	414	Rm 25	99.3	35	1.4	94.7	58	1.78
			TL 35	98	33	1.37	89.3	50	1.54
			TL 45	96.7	26	1.57	79.3	31	1.56
	50	320	Rm 25	97.3	35	1.2	100	61	1.72
			TL 35	97.3	33	1.23	98.7	58	1.70
			TL 45	97.3	33	1.27	78	46	1.67
	64	253	Rm 25	100	34	1.27	97.3	57	1.53
			TL 35	97.3	35	1.22	94.7	57	1.56
			TL 45	99.3	36	1.31	98	57	1.73
B73	15	800	Rm 25	14.7	11	7.80	20	11	9.84
			TL 35	0	0	0.00	5.3	5	13.83
			TL 45	0	0	0.00	0	0	0.00
	20	707	Rm 25	86.7	15	6.04	96	20	5.25
			TL 35	58	14	0.00	66.7	17	7.08
			TL 45	0	0	3.37	0	0	0.00
	30	552	Rm 25	97.3	29	2.34	98.7	43	2.18
			TL 35	94	28	2.05	99.3	39	2.35
			TL 45	8.7	17	3.14	10	10	2.53
	40	460	Rm 25	99.3	39	1.59	99.3	55	1.74
			TL 35	98	40	1.51	100	60	1.60
			TL 45	84	22	2.12	81.3	34	2.41
	50	432	Rm 25	99.3	45	1.57	100	67	1.58
			TL 35	99.3	41	1.39	99.3	63	1.49
			TL 45	96.7	32	1.67	84	53	1.94
	57	365	Rm 25	99.3	39	1.43	98.7	61	1.62
			TL 35	100	41	1.48	98	64	1.61
			TL 45	100	41	1.59	74.7	50	1.85

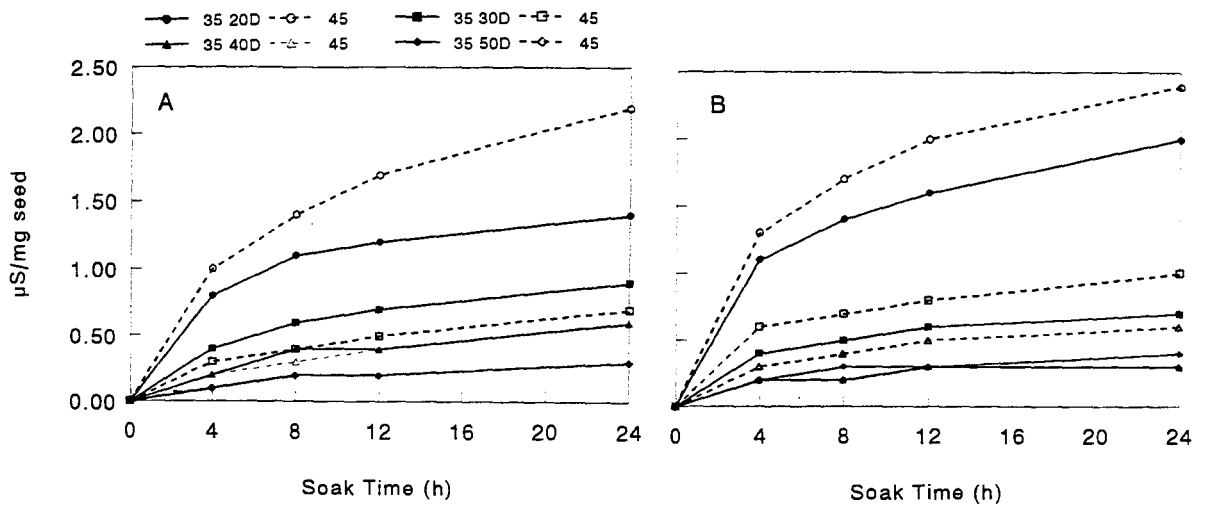


Figure 1. Electrical conductivity of leachate in $\mu\text{S mg}^{-1}$ seed of A632 (A) and B73 (B) harvested at different developmental stages and dried at 35°C or 45°C.

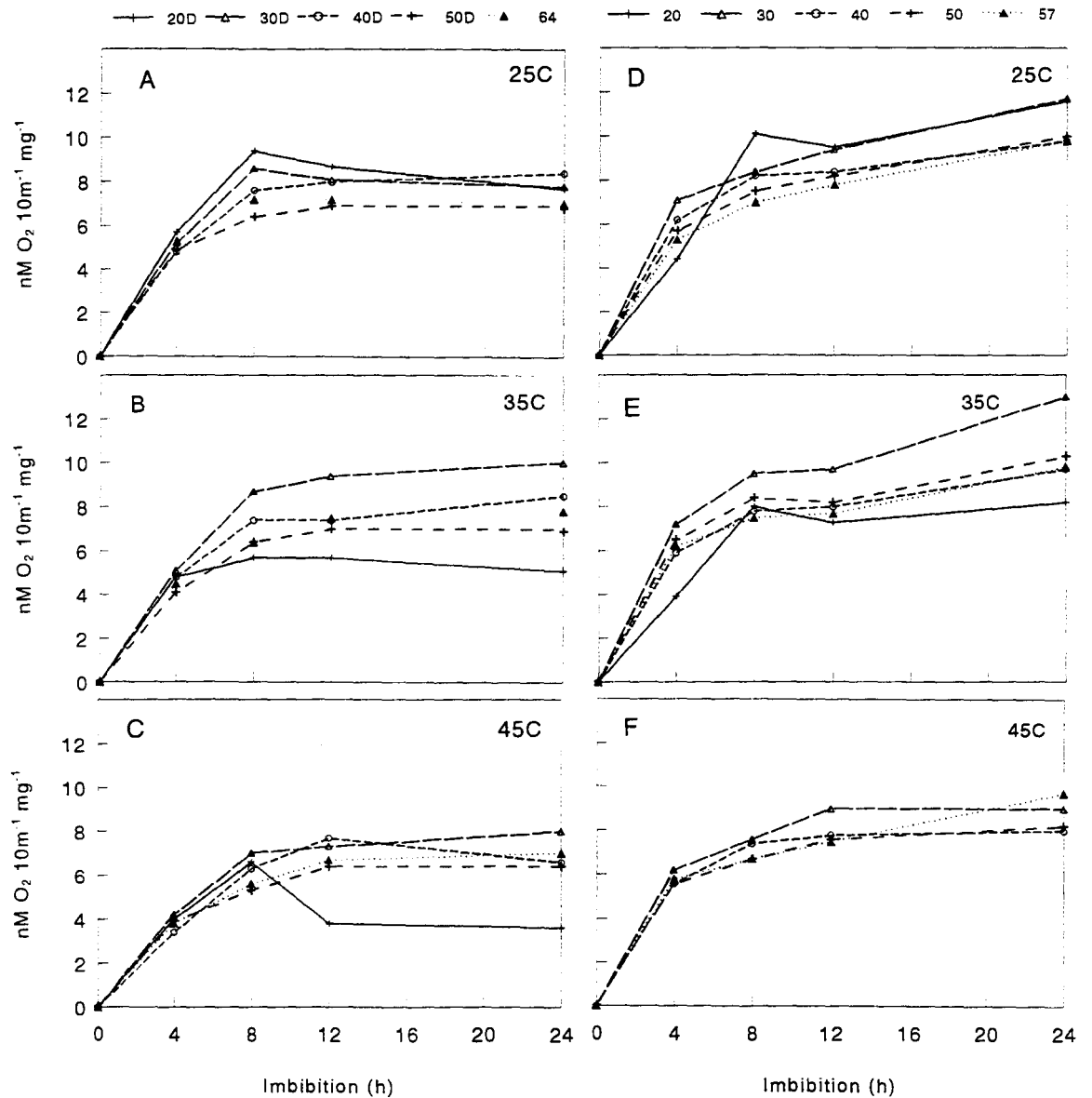


Figure 2. Oxygen uptake by imbibing axes harvested at different developmental stages and dried at three different temperatures. A632: A-C; B73: D-F.

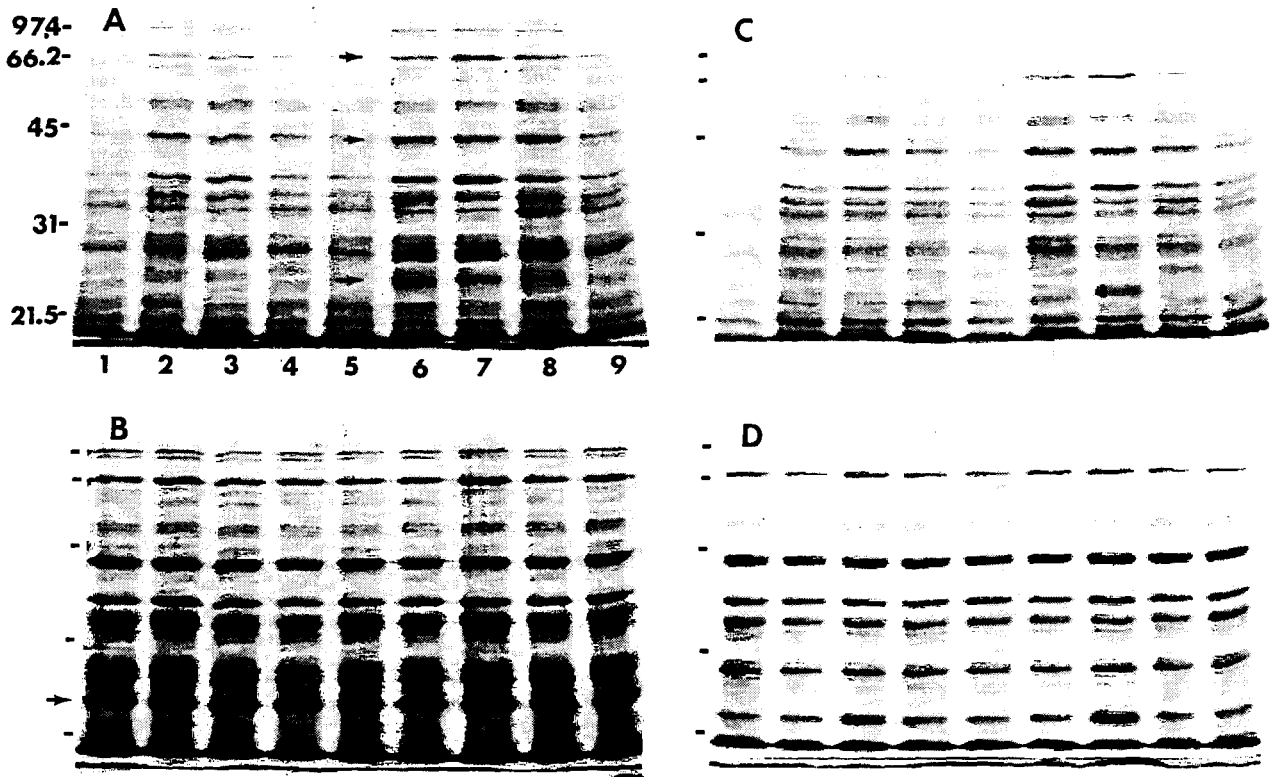


Figure 3. Coomassie-stained SDS-PAGE gels of water soluble non-heat denatured proteins extracted at 0, 24, and 48 h intervals from axes harvested at 20 or 40 DAP and dried at four different rates. Gel A: A632 20DAP; B: A632 40DAP; C: B73 20DAP; D: B73 40DAP; Lanes of all gels shown are loaded in same order of treatment-hour combination: Lanes 1) 0 h; 2) TL35C 24 h; 3) TL45C 24 h; 4) Rm25 24 h; 5) HRH 24 h; 6) TL35 48 h; 7) TL45 48 h; 8) Rm25 48 h; 9) HRH 48 h. Molecular weight standards are noted.

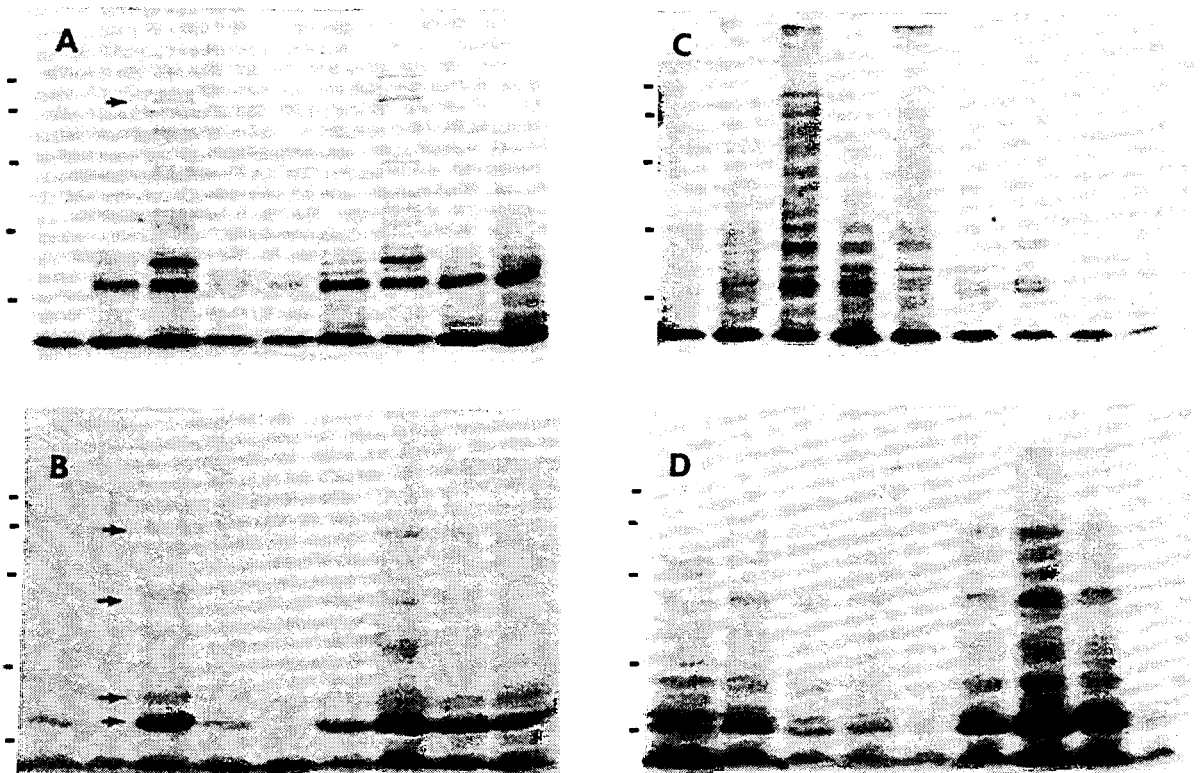


Figure 4. Autoradiographs of SDS-PAGE gels of radiolabelled water soluble non-heat denatured proteins extracted at 0, 24, and 48 h of drying from embryos of A632 (A,B) and B73 (C,D) harvested and dried as described in Figure 3. Lanes were loaded in the order given in Figure 3.

MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES ASSOCIATED WITH DESICCATION IN MAIZE EMBRYOS.

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ABSTRACT

The relationship of genotype, harvest maturity, drying temperature and preconditioning processes have been described. This study proposes to further clarify the effect of drying environment, system geometry and harvest maturity in a susceptible genotype; and to describe the expression in subsequent seed quality and vigor. Hybrid maize of the seed parent B73 by H99xH95 as pollen parent was harvested periodically, husked and dried in thin layer dryers at 35 or 45°C, mounted vertically in the laboratory (23°C) or hand shelled and dried in a fluidized bed (25 or 35°C) to 12% mc. Drying times varied from approximately 4 hr in the fluidized bed to 65-72 hr at 35 or 45°C to approximately 300 hr at room temperature. The standard germination was generally very high and cold test values were similar regardless of the temperature of drying. Seedling dry weights from seedlings grown at 25°C were responsive to drying temperature while dry weights from seedlings produced in the cold test showed little difference except for those from the rapid drying treatment; in general the root development was depressed more than shoot. Membrane integrity expressed as conductivity of steep water was similar for the thin layer and room dried material in contrast to the rapid dried material which exhibited a linear increase in conductivity with time. Electron microscopy showed marked differences in alignment of lipid bodies in the rapid drying treatments as compared to all others. This may effect the rate of moisture loss from the seed during drying and more importantly fail to regulate water uptake during imbibition and result in substantial leakage.

INTRODUCTION

In contrast to many other seed crops, hybrid maize is harvested at high moisture and must be dried artificially. The general concepts of reserve deposition, acquisition of desiccation tolerance, transition from developmental to germinative modes and membrane stabilization are well accepted. Modern hybrid maize seed production attempts to manage the later stages of maturation and to control the desiccation processes by artificial drying. The impact of genotype, harvest maturity, drying temperature and some preconditioning process have been described(Navratil and Burris, 1984, Herter and Burris, 1989 and Chen and Burris, 1990). Until recent studies, the geometry of the drying process had not been examined as a treatment variable. The industry typically dries the high moisture seed on the ear which limits the rate of water diffusion away from the seed surfaces adjacent to the embryo during the early part of the process. Although drying rate has been addressed in a limited way, this study proposed to further clarify the impact that dramatic modifications in drying environment have on

morphological changes in both tolerant and susceptible genotypes; investigate the impact of drying rate and temperature on subsequent seed quality and vigor and to suggest a histological explanation for these responses.

MATERIALS AND METHODS

Hybrid maize seed was produced at Iowa State University, Ames, Iowa, using the inbred line B73 as the seed parent, and single cross H99XH95 as the pollen parent. Random ear samples were harvested periodically with husk intact, to obtain ears with kernel moisture contents ranging from 50 to 30% (w/w). Except where noted, ear samples were brought into the laboratory, husked, and immediately placed in thin layer dryers (35 or 45C), mounted vertically on a nail bed (23C), kernel samples were carefully hand shelled and dried in thinlayer or fluidized bed driers (25, 35 or 45C) to 12% moisture. Subsequently, shelled dried seed was placed in paper bags and stored in conditioned storage at 10C and 50% RH.

Seed quality was measured by standard germination and a soil free cold test (Loeffler et al., 1985) carried out on lots of 50 kernels per sample. Shoots and roots were removed from the seedlings, weighed and dried (oven method) to obtain seedling dry weights. Conductivity of leachate was measured on 50 kernels per sample and measured with the Genesis 2000 (Wavefront Inc. Ann Arbor, MI) at various time intervals. All tests were replicated three times.

Transmission electron microscopy (TEM) was done on embryonic axes fixed in 3% glutaraldehyde/3% paraformaldehyde in 0.1 M sodium cacodylate at pH 7.2 for 18 h at 4C. After rinsing, the samples were stained in 4% aqueous uranyl acetate, dehydrated, then embedded in Spurr's embedding medium. Sections were examined and photographed with a JEOL 1200EX STEM.

RESULTS

Drying Rates

Drying rate is controlled by the environmental temperature, humidity, and the geometry of the drying process and is modified by the initial moisture and the genotype. The results presented in Figure 1 demonstrate the range of rates achieved by the various treatment combinations. The extremely rapid rates provided by the fluidized bed system clearly demonstrate the effect of increased surface area exposure. But even under the high humidity conditions of the HRH the removal of the kernels from the ear increases the rate of moisture loss. When humidity conditions are moderate (Rm or TL 25), the drying rates are similar. This suggests that the rate of moisture removal from kernels on the intact ear is not limited by diffusive resistance associated with air velocity. A dramatic increase in rate is associated with increasing temperature when the seed remains on the ear as shown by the rates of TL25, TL35 and TL45, respectively. Temperature has a much less pronounced impact when the kernels are

removed from the ear. Still by far the most rapid rate is exhibited by the detached kernels in the fluidized bed environment.

Seed Quality

The drying treatments represented radical contrasts in temperature and rate and traditional measures of seed quality showed equally dramatic differences (Table 1). The slow drying conditions of the Rm 25C dried material showed a slight advantage to seed dried on the ear at harvest moistures above 50%. At harvest moisture levels above 40% there was a consistent improvement in germination and vigor associated with drying at 35C as compared to 25 or 45C on the ear. The detached kernels show less sensitivity, indicating that the increase in drying rate associated with 35 versus 25C can be substituted for by the removal of kernels from the ear. The detrimental impact of the rapid drying imposed by the fluidized bed system is evident throughout all harvest moistures. The seedling dry weights from the warm germination tests exhibit a consistent advantage to drying on the ear. Whether this is a response to translocated metabolites or changes in hormonal balance requires additional investigation.

The cold test germination values show a significant advantage to ear drying at harvest moistures of greater than 50% which persists even as moisture decreases at the higher drying temperature of 45C. At drying temperatures of 35C or less there is no improvement in cold test performance at moisture contents of 45% or less. This plateau in seed quality is reached prior to physiological maturity which is reached at about 35-37% harvest moisture. The fluidized bed system was the only treatment that resulted in a decrease in cold test performance when the seed was harvested at 40% moisture. The seedling dry weights produced in the cold test reached a maximum at or below 44% harvest moisture. At higher moisture levels the differences between ear and kernel drying treatments were specific to the drying temperature although in general ear drying was superior to the excised kernels.

Membrane Damage

The characteristics of membrane leakage as measured by conductivity of steep water was measured and found to be consistent with those reported earlier by this laboratory. In a paper presented in 1995, we proposed that the migration of lipid bodies to the plasma lemma occurred naturally during maturation or under reasonable rates of drying. The dramatic increases solute leakage associated with the rapidly dried material suggested that the lipid body alignment adjacent to the cell wall contributed to control of membrane leakage. Such an increase in leakage is shown by the conductivity values given in Figure 2.

Histology

Early work in this laboratory reported the impact of high temperature drying on mitochondrial competence but was unable to address the effect of drying rate alone (Madden and Burris, 1995). Because the mc was relatively low, the mitochondria did not appear to be

damaged by drying rate. Mitochondria are abundant in the rapidly dried seed (Figure 3) and their outer membranes appear to be intact. The most striking histological difference evident in the rapidly dried seed is the lack of lipid body migration to the cell wall. Lipid body migration was complete in seed harvested at either 45 or 30% moisture as long as it was dried in any system other than the fluidized bed. If the harvest moisture content was 30% or less the lipid body migration was complete and the rapid drying treatment had no effect. However, at the elevated moisture level the lack of lipid body migration was clearly evident and resulted in substantially increased leakage from the imbibing seed. The mitochondria did not exhibit any damage by any drying treatment as long as the temperature did not exceed 40C (micrographs not shown). The contribution of the lipid bodies to moisture regulation during drying and imbibition remains under investigation in this laboratory

SUMMARY

Work in this laboratory has focused on understanding maturation and the drying of maize seed. Maize acquires desiccation tolerance very soon after fertilization and attempts to describe the days after pollination (DAP) required are only legitimate in the context of the conditions the seed is exposed to after being removed from the parent plant. In addition to the classical desiccation response, we have reported the gradual acquisition of tolerance to high temperature drying with natural maturation or in response to artificial drying conditions which include temperatures of less than 40°C and humidities of less than 75%. Until recently it was not possible to separate the effect of high temperature drying from drying rate. High temperature would appear to impact the competency of the conserved mitochondria and to a lesser extent the impact on membrane integrity. Rapid drying rates would appear to prevent the migration of the lipid bodies to the cell wall which we speculate precludes the regulation of cellular drying rates which the aligned lipid bodies may provide. Further, the contribution that the aligned lipid bodies may provide to the regulation of water uptake during imbibition is eliminated. Rapid drying, if it occurs at a safe temperature, does not damage the mitochondria and the rapid rate of water uptake of moisture associated with the rapidly dried seed may actually accelerate the activation of the mitochondria. It should be noted that the rates of drying used in the rapid treatment are not possible if the seed remains on the cob during the drying process. The contribution of seed/ear geometry to the regulation of drying rate needs to be considered when discussing the impact of harvest moisture, drying temperature and rate on subsequent seed quality.

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Table 1. Influence of harvest maturity and drying treatment on seed quality in B73 hybrid (1996)

Har	hmc g H ₂ O kg ⁻¹ fw	Drying treatment °C	Sample	Warm Germination			Cold Germination		
				Germ	SDW	S:R	Germ	SDW	S:R
				(%)	(mg)		(%)	(mg)	
1	520	Rm 25	kernel	92	36	1.85	92	38	2.08
			ear	98	37	2.35	99	45	1.83
		TL 25	kernel	88	29	2.11	87	32	2.23
			ear	84	36	2.64	95	28	1.78
		TL 35	kernel	71	26	2.57	73	27	2.64
			ear	93	33	2.52	98	38	2.01
		TL 45	kernel	17	18	3.02	12	11	2.58
			ear	83	21	3.5	87	21	3.20
		FB 25	kernel	0	0	0	0	0	0
		FB 35	kernel						
2	490	Rm 25	kernel	95	39	1.55	98	45	1.72
			ear	100	41	1.93	100	44	1.82
		TL 25	kernel	95	37	2.07	93	42	1.82
			ear	100	28	1.37	99	30	1.79
		TL 35	kernel	97	33	2.15	97	40	1.94
			ear	99	44	1.65	99	50	1.46
		TL 45	kernel	61	20	3.37	37	20	2.24
			ear	89	18	1.44	97	30	2.60
		FB 25	kernel	3	14	3.00	0	0	0
		FB 35	kernel	15	18	2.80	0	0	0
3	440	Rm 25	kernel	100	47	1.42	100	59	1.42
			ear	99	45	1.53	100	55	1.41
		TL 25	kernel	99	40	1.36	99	50	1.48
			ear	83	44	1.54	100	54	1.50
		TL 35	kernel	100	23	1.09	97	45	1.68
			ear	99	46	1.54	100	59	1.4
		TL 45	kernel	94	26	2.1	88	36	2.05
			ear	83	33	2.04	95	40	1.93
		FB 25	kernel	3	15	2.7	1	26	1.60
		FB 35	kernel	20	21	2.88	1	30	2.33
4	400	Rm 25	kernel	98	54	1.46	100	60	1.53
			ear	100	52	1.47	100	59	1.43
		TL 25	kernel	95	46	1.65	100	62	1.46
			ear	100	74	1.63	100	57	1.44
		TL 35	kernel	91	46	1.76	100	56	1.62
			ear	96	52	1.56	100	61	1.39
		TL 45	kernel	100	48	1.74	97	50	1.79
			ear	99	42	2.11	99	56	1.68
		FB 25	kernel	57	15	2.76	55	25	2.44
		FB 35	kernel	72	17	2.74	66	24	2.80

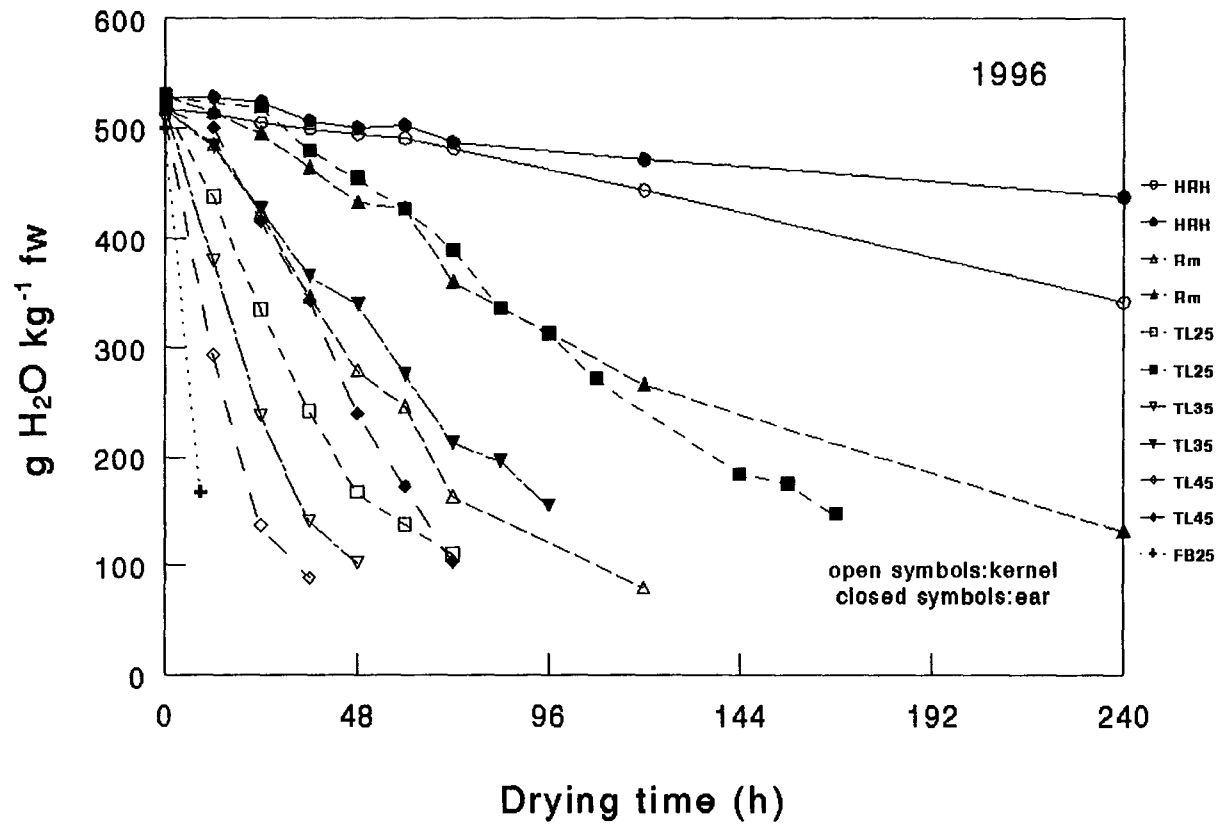


Figure 1. Drying rates as effected by temperature and geometry

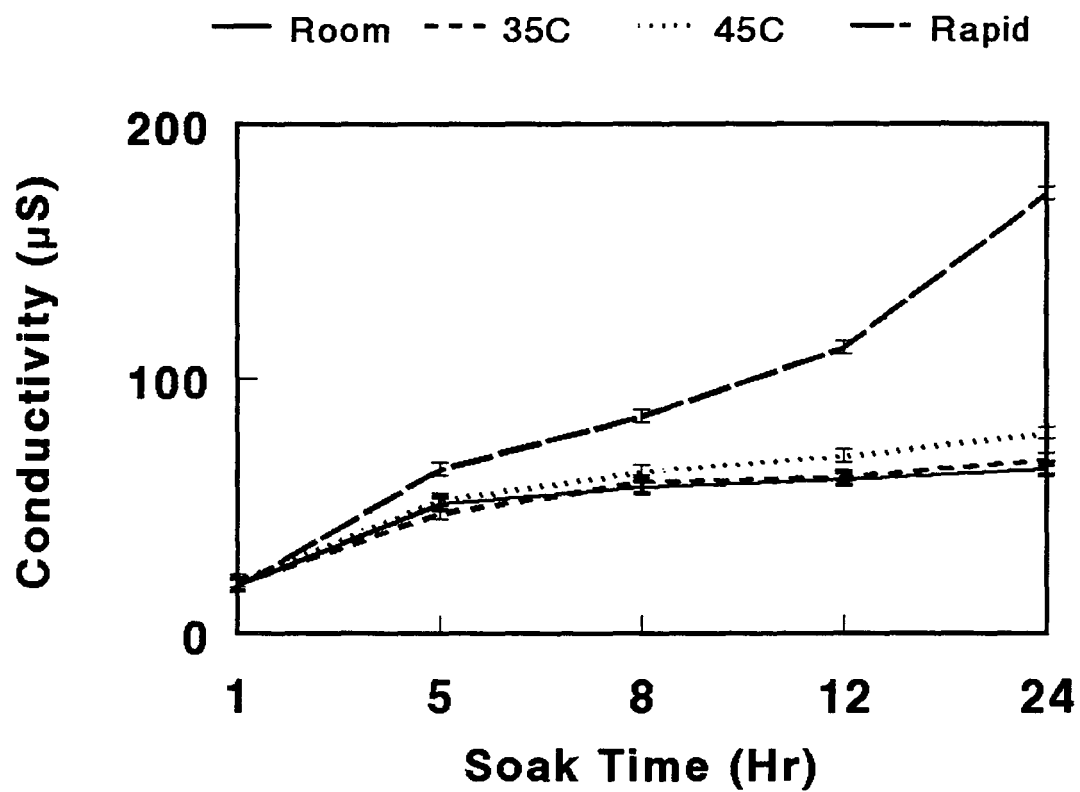


Figure 2. Effect of drying treatment on membrane leakage at different soaking times.

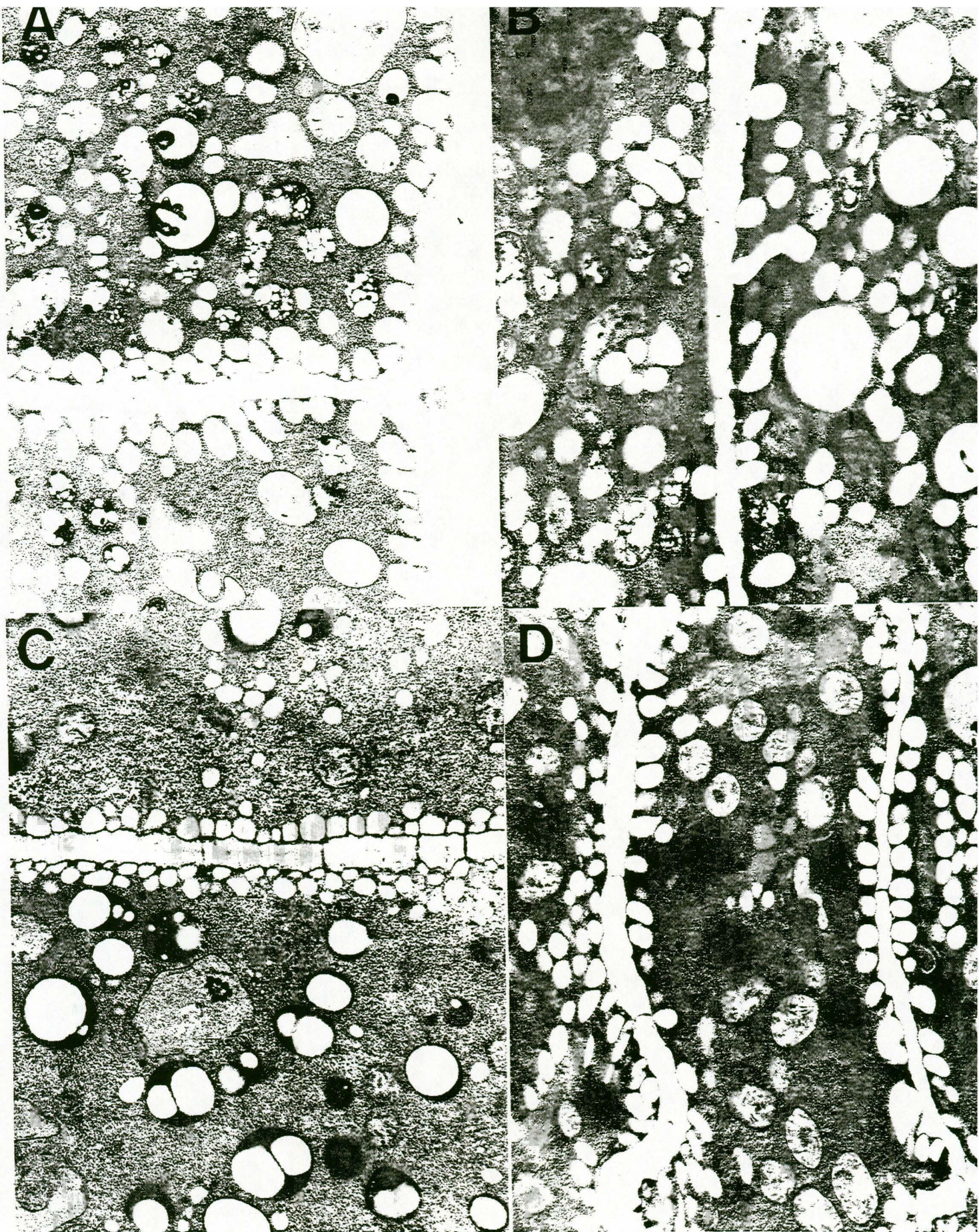


Figure 3. The impact of harvest moisture and drying rate on cytoplasmic differences in maize embryo axis tissue following drying at : A = HM 45% dried at RM, B = HM 45% dried at 45C in a fluidized bed, C = HM 30% dried at RM, D = HM 30% dried at 45C in a fluidized bed.

**LEEK PERFORMANCE EVALUATION BASED ON GREENHOUSE PLUG SIZE AND FIELD
WITHIN ROW SPACING ON
OVERALL MARKETABLE YIELDS**

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ABSTRACT

Two cultivars, five plug trays and three within row spacings were evaluated at the Muck Crops Branch near Willard, Ohio. Tray size ranged from 288 (10cc vol.) To 512 (4.0cc vol.). Plant height was unaffected by tray size. Root and top mass and was significantly reduced with the 512 tray both in fresh and dry weights. However, final yields were unaffected by plug size at transplanting. Within row field spacings consisted of 10, 5 and 2.5 cm. Shank width and trimmed plant fresh weight decreased with increased plant populations. Plant height and shank length increased with an increase in plant populations. Since trimmed weight and shank size directly affects the marketable quality, an increase in within row plant populations is not recommended.

GROWTH AND YIELD OF CONTAINERIZED ONION TRANSPLANTS AS AFFECTED BY CELL SIZE, AGE, AND CLIPPING

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ABSTRACT. Onion (*Allium cepa* L. cv. Texas Grano 1015Y) transplants were evaluated for seedling survival, root growth, shoot growth, and yield response to container cell size and age. Seedlings were grown in flats O80A (338 cells; 7.1 cm³) and 595M (595 cells; 4.0 cm³) for 6, 8, 10, and 12 weeks (W). A 12W-old bareroot transplant was also included. Survival was reduced for 6W compared to \geq 8W containerized or 12W bareroot transplants. At planting, root count increased linearly with age. Cell size did not affect root count, plant height or leaf number, but shoot dry weight was greater for seedlings grown in O80A compared to 595M flats. Total, jumbo and large size yields were higher for bareroot, \geq 10W in O80A and \geq 8W in 595M flats than younger transplants. Total yields were unaffected by cell size but seedlings on 595M flats had a 16% decrease of jumbo size. A separate study determined the effects of leaf clipping on growth and yield of four onion cultivars grown on 595M flats. Clipping did not affect bulb diameter but increased survival in the less vigorous onion cvs. Red Granex and Henry Special. Clipping also increased yield in Henry Special, but not in Texas Grano 1015Y, Early White Supreme or Red Granex.

INTRODUCTION

In 1996, Texas planted 5,260 ha of spring onions (*Allium cepa* L.), contributing 35 % of the total 14,900 ha in U.S. (J. Peña, personal communication). Other important onion production states are Georgia, California and Arizona. The Winter Garden/Laredo and Lower Rio Grande Valley are the two major areas with 1,360 ha and 3,900 ha, respectively. Total production in Texas amounted to 168 M kg with a gross income of about 50 M dollars in Texas. Presently onions are established by direct seeding or bareroot transplants at a minimum plant density of 166,000 plants/ha. Seedling growth is retarded under extreme conditions such as excess or deficit of water or temperature extremes. Transplants grown in small cell sizes could be an alternative and more manageable system of plant establishment with a potential increase in pre- and post-planting stress tolerance, with a lower cost of irrigation but at higher cost of establishment than direct seeded. Potential added benefits for transplant onions are a superior bulb size uniformity for large high-value sizes and earlier maturity. Transplanted onions will also require less inputs of irrigation water and pesticides, therefore reducing costs and potential pesticide load in the environment. For the nursery, the use of small cell size offers the advantage of less space in the greenhouse, less time and consequently less cost compared with the traditionally higher cell volumes. To our knowledge, no information is available on effects of cell size on growth and yield of onions. Herison et al. (1993) reported that 10- and 12-week-old containerized transplants growing at 150 and 225 mg N/liter produced high yields of fresh bulbs \geq 76 mm in diameter.

Bareroot onion transplants are severely clipped in production fields before shipping to production areas to aid in producing compact plants and for easiness in transplant manipulation (Lipe and Thomas, 1980; Sabota and Downes, 1981). This practice has generally been done in open-field southern U.S. tomato nurseries before shipping to Northeastern regions (Taha et al., 1980). To date information about the effects of clipping on containerized onion transplant growth is lacking.

The objective of this study was to evaluate onion seedling survival, root growth, shoot growth, and yield in response to cell size and age of containerized onion seedlings. A second objective was to determine how leaf clipping in the greenhouse affect growth and yield of onion cultivars.

MATERIALS AND METHODS

Transplant age. Onion seedlings cv. 'Texas Grano 1015Y' were grown at Speedling Inc., Alamo, Texas on Speedling trays 080A (338 cells, 7.1 cm³) and 595M (595 cells, 4.0 cm³). Seeding was done on a biweekly basis starting on 4 Sep. 1993 to obtain 12, 10, 8, and 6 week (W) old transplants. Field grown 12W bareroot transplants produced near Uvalde, Texas, was also included. Standard irrigation and nutritional procedures for that location were used. Plants were pulled from trays, packed in boxes, and shipped to the Texas A&M University Agricultural Research and Extension Center (TAMU-AREC), Uvalde, where transplants were machine transplanted (Lannen RT-2 Model, Lannen Plant Systems, Finland) between 3 and 8 Dec. 1993. A buried drip irrigation system with drip tape positioned at 30 cm depth was used. Plants were grown on single raised beds with two rows/bed at 12 cm spacing between plants. The experiment was conducted using a randomized complete block design with four replications. Each replication consisted of four beds, two records and two buffers. Plants were sampled 8 Nov. and 8 Dec. 1993, and 24 Feb. 1994, for root, bulb and shoot measurements. Plant survival was evaluated after frost on 23 and 30 Dec. 1993 by counting seedlings on 12 meters per plot/replication. Onions were harvested 26 May 1994. They were pulled, clipped and placed in burlap sacks for a week for field curing, and then were graded by diameter into size classes: medium (< 76mm), large (76-102 mm), or jumbo (>102 mm) and total yield determined.

Leaf clipping. Onion seeds of cvs. 'Texas Grano 1015Y', 'Early White Supreme', 'Henry Special' and 'Red Granex' were sown on 19 Oct. 1993 on 595M trays (595 cells; 4.0 cm³) at Speedling Inc., Alamo, Texas. Standard growing procedures for that nursery were used. Plant samples were taken prior to clipping on 1 Nov. 1993 for plant height, root number, leaf number, bulb diameter, root and shoot dry weights measurements. Half of the remaining trays were allocated to four replications per cultivar and were mechanically clipped on 14 Nov. and 4 Dec. 1993 to a plant height of 12 cm. A second and third sampling for unclipped and clipped seedlings on all cultivars were performed on 30 Nov. and 16 Dec. 1993. Then seedlings were transplanted at TAMU-AREC, Uvalde on 20 Dec. 1993. Onions were harvested on 28 June 1994. Experimental design, planting procedures, and field practices were as described before.

RESULTS AND DISCUSSION

Transplant Age. Plant survival was reduced for 6W onion transplants compared to older transplants (Fig. 1). There were no differences between containerized and bareroot 12W transplants. The reduction of plant survival for 6W seedlings may have been accentuated by 3 days elapsed time and stress conditions between delivery from the greenhouse and field transplanting. At transplanting, root numbers had increased constantly with age for both 7.1 cm³ and 4 cm³ cell size (Table 1). Root number was not affected by cell size. Root dry weight tended to increase rapidly after seedlings reached 10 weeks, especially in seedlings grown in 7.1 cm³. Similar results were found by Herison et al. (1993), who reported a linear increase in root and shoot fresh weight to increase seedling age from 8 to 12 weeks. Bulb diameter response was similar. Cell size and age influences on the of bulb development phenology and bulb carbohydrate accumulation are unknown. However, early bulbing may increase if seedlings are grown for more than 12 weeks. Leaf number and leaf dry weight increased constantly with increasing age for plants grown in 7.1 cm³ but not for seedlings grown in 4.0 cm³ cell size. The significant contrast comparison of bareroot vs. containerized 12W transplants for all growth parameters measured at transplanting indicated that seedlings grown in both cell sizes had larger root systems, smaller bulb diameter and less shoot dry weight than bareroot transplants (Table 1).

After 10 weeks in the field, root number, root dry weight, bulb diameter and shoot dry weight increased with increasing age from 6W to 12W only in seedlings grown in 7.1 cm³ cell size (Table 1). Transplant age did not affect root and shoot components when grown in 4 cm³ cell size. Most growth parameters for containerized seedlings were similar to bareroot transplants, except that containerized transplants had heavier roots and lower shoot:root ratio. Therefore, the initial growth differences associated with age at transplanting were minimized after 10 weeks in the field. Leaf number was independent of transplant type, transplant age or cell size. Flower induction and bolting responses are known to be not only temperature and photoperiod dependent (Steer, 1980) but also plant size dependant (Yamaguchi, 1983). Small plants, still in the juvenile stage, with < 5 leaves have less probability to bolt in the field when exposed to low winter temperatures. During this experiment, temperatures of -5.5C and -3.3C occurred on 20 and 30 Dec. 1993, respectively, and bolting was < 0.05%.

Total yield increased with increased transplant age for both cell sizes, with the highest yields for 12W containerized and bareroot transplants (Table 2). Sabota and Downes (1981) reported a 58% bulb increase for large bareroot transplants (≥ 0.63 cm neck diameter) compared to small transplant sizes (≤ 0.48 cm). Pooled across age, there were no differences in total yield between 7.1 cm³ and 4 cm³ cell size, but 4 cm³ cell transplants had a 16% decrease of Jumbo onions compared with 7.1 cm³ cell transplants. Bareroot and containerized transplants had similar yields, but the latter had an increase of medium size bulbs (Table 2).

Leaf clipping. There was a significant treatment \times cultivar interaction for plant survival (Fig. 2). 'Henry Special' and 'Red Granex', the two less vigorous cultivars in this study, had lower plant survival compared

to 'Texas Grano 1015Y' and 'Early White Supreme'. Survival was related to cultivar differences in root characteristics. Root numbers were higher, but dry weights lower, for onion cultivars 'Texas Grano 1015Y' and 'Early White Supreme' than 'Henry Special' and 'Red Granex' (Fig. 3A, 3B). Across cultivars, clipping slightly reduced root dry weight but not root number or bulb diameter (not shown).

The partitioning of the significant treatment × cultivar interaction for total yields indicated that clipping reduced total yield of 'Early White Supreme' but increased in 'Henry Special' (Fig. 4). The latter response was related to the increase in plant survival associated with clipping. Lipe and Thomas (1980) found that when more than one half of the top of bareroot transplants was removed, yields were significantly reduced. Clipping terminal bud, upper leaves, and the first flower cluster 12 days before transplanting increased pepper yield (Jaworski and Webb, 1971), but pepper yield response to clipping was cultivar dependent (McCraw and Greig, 1986). Multiple clippings was also reported to improve quality of tomato transplants (Taha et al., 1980).

The use of 10- and 12-week-old containerized onion transplants grown on 4 cm³ cell size may be a viable alternative for onion growers. However, gross income from fields established by containerized onion transplants must offset the initial transplant cost investment compared with direct seeding. This can be accomplished by increased production and/or by entering the market earlier. Clipping leaves to 12 cm on containerized onion transplants in the greenhouse did not reduce field plant survival and provided some hardening against low freezing temperatures in the least vigorous cultivar 'Henry Special', thereby increasing yields. The mechanism underlying this response remains unknown.

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Table 1. Root and shoot characteristics of 'Texas Grano 1015Y' onion transplants in response to cell size and transplant age, Uvalde, TX.

Cell size (cm ³) ²	Age (weeks)	Root		Bulb	Shoot		Shoot
		No.	Dry wt. (mg)	diam. (mm)	Leaf (no)	Dry wt. (g)	root ratio
At transplanting							
7.1	6	19.6	5.5	3.7	3.9	0.023	4.2
	8	25.0	9.3	4.1	4.3	0.034	3.7
	10	32.0	11.6	4.3	4.8	0.040	3.5
	12	36.6	21.1	5.7	5.9	0.074	3.5
<i>Significance</i>		L**	Q**	Q*	L**	Q*	NS
4.0	6	18.3	5.0	3.2	3.6	0.022	4.5
	8	28.2	8.2	4.2	4.4	0.029	3.6
	10	30.8	9.1	3.8	4.4	0.030	3.4
	12	41.9	15.6	4.7	5.5	0.050	3.3
<i>Significance</i>		L*	Q*	L**	NS	NS	NS
Bareroot (BR)	12	23.6	7.0	8.3	5.8	0.087	19.4
<i>Contrasts</i>							
7.1 vs 4.0		NS	**	**	NS	**	NS
BR vs (7.1 + 4.0)		**	**	**	NS	**	**
Ten weeks after transplanting							
7.1	6	39.0	126	8.0	4.0	1,150	9.3
	10	49.0	203	9.1	4.6	1,420	7.1
	12	47.0	215	9.6	4.5	1,490	6.9
<i>Significance</i>		L**	L**	L**	NS	L**	NS
4.0	6	41.0	142	8.7	4.3	1,150	8.1
	8	41.0	147	8.6	4.5	1,190	8.1
	8	43.0	159	8.8	4.4	1,270	8.6
	10	46.0	155	8.9	4.5	1,180	7.7
	12	47.0	150	8.2	4.8	1,190	8.2
<i>Significance</i>		NS	NS	NS	NS	NS	NS
Bareroot (BR)	12	41.0	113	8.5	4.1	1,150	11.5
<i>Contrasts</i>							
7.1 vs 4.0		NS	NS	NS	NS	NS	NS
BR vs (7.1 + 4.0)		NS	**	NS	NS	NS	**

²Cell sizes were 7.1 and 4.0 cm³ for O80A and 595M flats containing 338 and 595 cells, respectively.

NS,*,** Nonsignificant or significant F-test at P=0.05 or 0.01, respectively. Significant age effects were linear (L) or quadratic (Q).

Table 2. Yield and quality of 'Texas Grano 1015Y' onion transplants in response to cell size and transplant age, Uvalde, TX.

Cell Size (cm ³) ²	Age	Total	Jumbo	Large	Medium
		----- (MT/ha) -----			
7.1	6	40.9	31.8	7.3	1.9
	8	56.4	41.2	13.1	2.1
	10	59.7	46.8	10.4	2.5
	12	68.4	50.8	15.2	2.4
<i>Significance</i>		L**	L**	L*	NS
4.0	6	30.4	24.9	4.3	1.2
	8	60.7	41.2	15.5	4.0
	10	52.0	33.9	14.5	3.6
	12	61.9	42.6	14.6	4.8
<i>Significance</i>		L*Q*	L**	L*	NS
Bareroot (BR)	12	69.8	45.7	18.0	6.1
<i>Contrasts</i>					
7.1 vs 4.0		NS	**	NS	*
BR vs (7.1 +4.0)		NS	NS	NS	**

²Cell sizes were 7.1 and 4.0 cm³ for O80A and 595M flats containing 338 and 595 cells, respectively. Plants were harvested on 26 May 1994.

^{NS,*,**} Nonsignificant or significant F-test at P=0.05 or 0.01, respectively. Significant age effects were linear (L) or quadratic (Q).

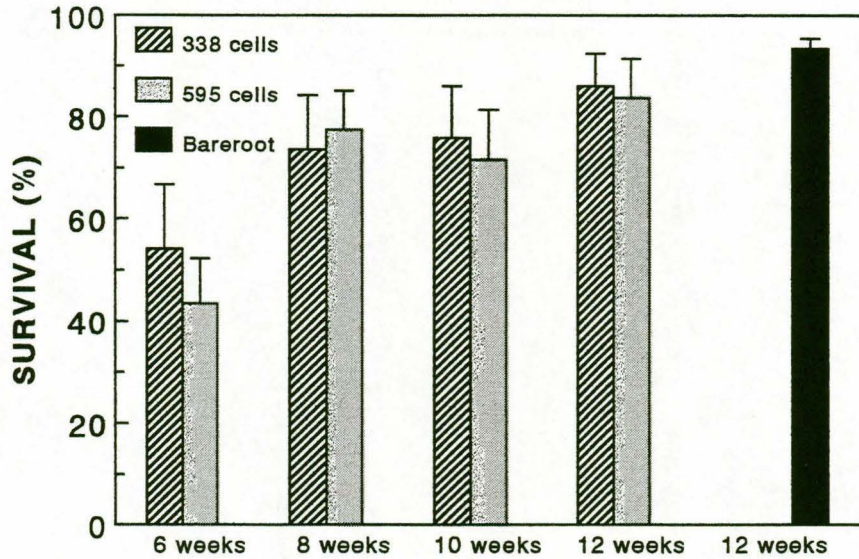


Fig. 1. Field survival of onion transplants grown in O80A (338 cells; 7.1 cm³) and 595M (595 cells; 4.0 cm³) transplant flats in response to transplant age at Uvalde, TX. Error bars represent a mean \pm SE.

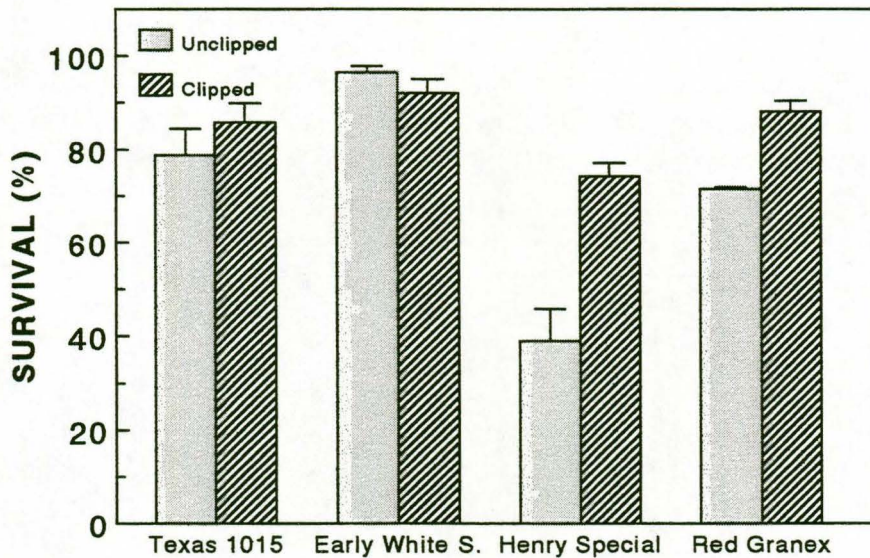


Fig. 2. Field survival of onion transplants grown in 595M transplant flats (595 cells; 4.0 cm³) as influenced by cultivars. Transplants were set in the field on 20 Dec.1993 and were exposed to freezing temperatures (-3 to -5C) one week after transplanting at Uvalde, TX.

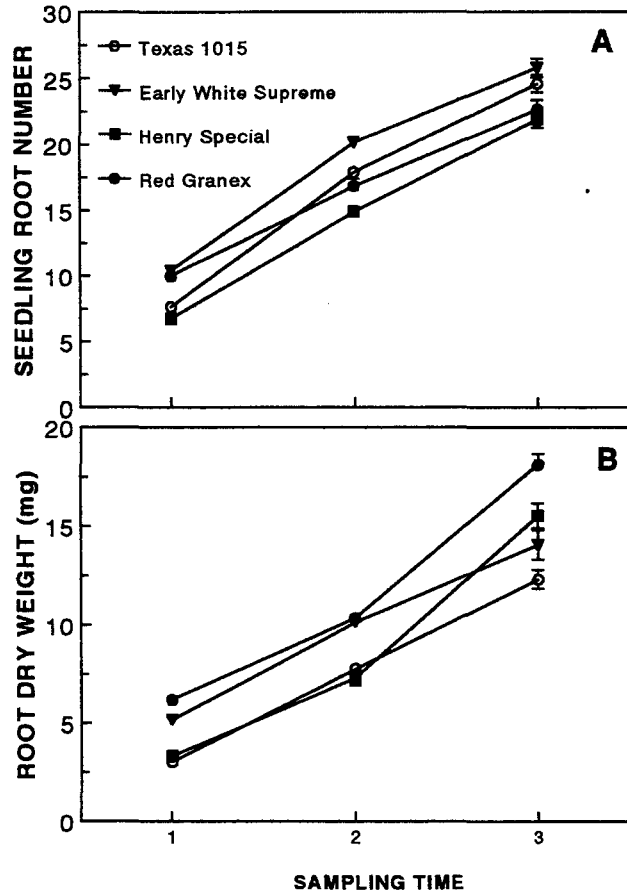


Fig. 3. (A) Seedling root number and **(B)** seedling root weight of onion cultivars. Seedlings were seeded in 595M (595 cells; 4.0 cm³) transplant flats on 19 Oct. 1993. Sampling times correspond to 11 and 28 Nov. and 16 Dec. 1993.

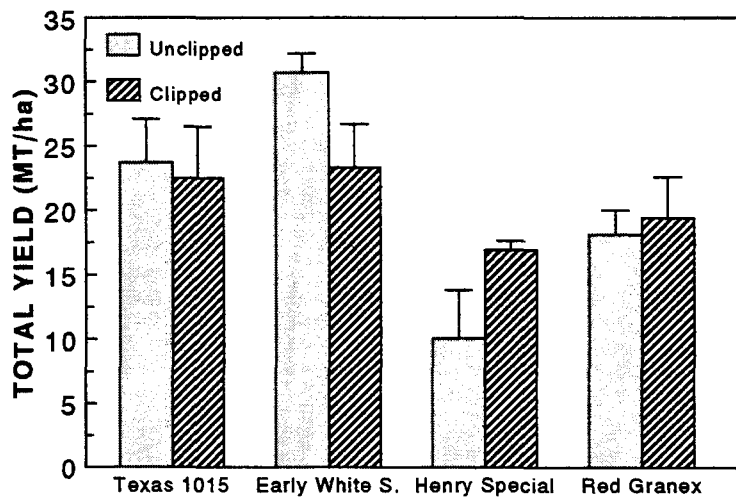


Fig. 4. Marketable yield of onion transplants as influenced by cultivar and clipping. Seedlings on 595M (595 cells; 4.0 cm³) were transplanted on 20 Dec. 1993 and onions were harvested on 28 June 1994.

EFFECT OF GREENHOUSE CONDITIONING ON GROWTH AND LANDSCAPE PERFORMANCE OF PERENNIAL AND ANNUAL BEDDING PLANTS

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ABSTRACT

Columbine (*Aquilegia X hybrida* 'McKana Giants'), New Guinea impatiens (*Impatiens X hybrida* 'Agadoo'), marigold (*Tagetes erecta* 'Little Devil Mix') and ageratum (*Ageratum Houstonianum* 'Blue Puffs') plants were conditioned with mechanical stress (brushing), drought, high (500 ppm) or low (50 ppm) N fertilizers, B-Nine (5000 ppm daminozide), or Bonzi (45 or 180 ppm paclobutrazol) during greenhouse production. Untreated controls were maintained well-watered and fertilized with 250 ppm N three times per week. In two of the four species, marigold and New Guinea impatiens, low N during greenhouse production delayed the growth of the plants in the landscape. High N improved the landscape performance of only one species, New Guinea impatiens. Marigolds subjected to drought in the greenhouse were shorter than controls at 4 weeks after planting in the landscape. Brushing reduced the height of all species except ageratum in the greenhouse and had no persistent effect on plant growth in the landscape. Persistent growth reductions in response to B-Nine were observed in ageratum and to Bonzi in New Guinea impatiens.

INTRODUCTION

Management of plant growth during greenhouse production generally involves a combination of cultural and chemical regulation of plant processes to condition plants to the handling and stresses involved in shipping, marketing, planting, and establishment of the plants in the landscape. The objective of the tests reported here was to evaluate the effect of individual cultural and chemical methods of growth regulation of herbaceous perennial and annual bedding plants on plant growth in the greenhouse and subsequent performance in the landscape.

MATERIALS AND METHODS

Shade plants used in the study were columbine and New Guinea impatiens and sun plants were marigold and ageratum. Plugs for each species were obtained from a commercial grower in late March and early April 1994 and planted into 10-cm plastic pots (soil volume 580 cm³) filled with Metro-Mix 300 (Grace Horticultural Products).

Conditioning treatments were initiated about 2 weeks after planting to allow plant establishment and resumption of growth. Treatments included: untreated plants which were maintained well-watered with overhead irrigation and received 250 ppm N three times per week using Peters 20-10-20; a fertilization variable with low-N fertilized plants receiving 50 ppm N, and high-N fertilized plants receiving 500 ppm N; an irrigation variable where untreated plants were maintained well watered with ebb-and-flow irrigation or allowed to experience drought stress for as much as 2 h wilt each day; or chemical growth regulation by a single application of 5000 ppm B-Nine (daminozide), a single application of 45 ppm Bonzi (paclobutrazol, 180 ppm applied to columbine); and a nonchemical treatment of brushing 40 strokes twice daily. All plants not assigned to 50 or 500 ppm N treatments were fertilized with 250 ppm N three times per week. Each plant species was set up in a randomized complete block design with three plants per experimental unit and five replications. Plant height and width of all three plants per treatment (n = 15) were measured at 2 and 4 weeks after treatment.

At 4 weeks after initiation of conditioning treatments, plants were planted in full-sun or 50% shaded landscape beds (Cecil sandy loam, Typic Halpludult) at the Griffin Campus. Plant height and width were measured on two plants per

treatment/replication (n = 10) at 2, 4, 8, and 12 weeks after planting (WAP) to determine long-term effects on plant performance. Only plant height for 4 and 8 WAP are reported.

All data were subjected to analysis of variance using SAS's general linear models procedure (SAS Institute, Cary, NC) and mean separation was by protected least significant difference test (LSD), $P < 0.05$.

RESULTS

Columbine. At 2 and 4 weeks after initiation of conditioning treatments (WAT), plant height of columbine treated with drought, brushing, or B-Nine was reduced relative to controls (Table 1). At 4 WAT, plants treated with low (50 ppm) or high (500 ppm) N also were shorter than the untreated controls which had received 250 ppm N over the treatment period. However, there were no significant differences in plant height at 4 or 8 WAP in the landscape.

New Guinea impatiens. At 2 WAT, plant height of New Guinea impatiens was reduced only by treatment with 30 ppm Bonzi, while plants grown undisturbed on the ebb/flow bench were significantly taller than the controls (Table 1). At 4 WAT, plants provided with 50 ppm N were shorter than controls fertilized with 250 ppm N, but there was no difference between controls and plants grown with 500 ppm N. Plants subjected to drought stress, brushing, or treated with the plant growth regulators were all significantly shorter than controls at 4 WAT. At 4 WAP in the landscape, plants subjected to low N during greenhouse production were still shorter than controls, but plants grown with 500 ppm N in the greenhouse were significantly taller than controls. A significant height reduction of plants treated with 45 ppm Bonzi also persisted through 8 WAP in the landscape.

Marigold. At 2 and 4 WAT, marigold plants that were brushed or treated with B-Nine or Bonzi were significantly shorter than controls (Table 2). At 4 WAT, plants treated with drought or low N were also shorter than controls. The effects of low N or drought persisted through 4 WAP in the landscape, but there were no significant differences in plant height at 8 WAP.

Ageratum. Height of ageratum plants grown with ebb/flow irrigation was greater than that of controls at 2 WAT (Table 2). Ageratum also was very responsive to fertilization level; plants grown with 50 ppm N were much shorter than controls and those treated with 500 ppm were taller than controls. Effects of N level during greenhouse production did not persist in the landscape. Ageratum plants treated with B-Nine or Bonzi were significantly shorter than controls at 2 and 4 WAT, but only the effect of B-Nine persisted at 4 WAP in the landscape. There were no significant differences in plant height at 8 WAP.

DISCUSSION

The goal in conditioning bedding plants is to reduce plant height in the greenhouse while improving the plant's ability to establish in the landscape. The data presented here do not provide sufficient detail to assess plant establishment, but do offer information on treatments that cause undue delays in plant growth in the landscape. In two of the four species, low N (50 ppm) during greenhouse production delayed the growth of the plants in the landscape. On the other hand, high N improved the landscape performance of only one species, New Guinea impatiens which are considered to be "heavy" feeders.

Marigolds were sensitive to drought conditioning, resulting in an 18% height reduction, relative to controls, at 4 WAP in the landscape. Brushing was effective in reducing plant height (relative to controls) of columbine (35%), New Guinea impatiens (20%), and marigold (21%) at 4 WAT, but significant height reductions did not persist at 4 WAP in the landscape in any of these crops. On the other hand, ageratum was not responsive to brushing, probably due to the very dense growth habit of this crop. Minor leaf damage from the brushing treatment was observed on ageratum and New Guinea impatiens, while flower damage was significant and unsightly on New Guinea impatiens during greenhouse production. The moderate height reduction and lack of persistence have been noted benefits of brushing of vegetable transplants (Latimer, 1991a).

A single application of 5000 ppm B-Nine provided moderate reductions in plant height of ageratum (16%), marigold (20%), and columbine (24%) at 4 WAT in the greenhouse, but caused persistent reductions in ageratum plant height at 4 WAP in the landscape. Columbine was not responsive to 180 ppm Bonzi, but 45 ppm Bonzi caused excessive persistence of New Guinea impatiens height reductions in the landscape. Landscape persistence of plant growth

retardant effects has been seen with Bonzi, but B-Nine has not generally caused any persistent growth effects (Latimer, 1991b).

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Table 1. Effect of conditioning treatments on growth of 'McKana Giants' columbine and 'Agadoo' New Guinea impatiens at 2 and 4 weeks after treatment (WAT) in the greenhouse and at 4 and 8 weeks after planting (WAP) in the landscape.

Species Treatment	Plant height (cm)			
	2 WAT	4 WAT	4 WAP	8 WAP
Columbine				
Control	12.4 a	24.4 a	29.3	37.9
50 N	10.3 abc	17.5 cd	25.2	35.1
500 N	11.4 ab	20.7 bc	28.5	36.9
Ebb/flow	12.2 a	25.6 a	31.6	35.9
Drought	9.9 bcd	20.6 bc	30.1	37.8
Brushed	8.2 cd	15.9 d	27.1	31.3
B-Nine	7.7 d	18.6 cd	31.1	34.6
Bonzi	10.7 ab	22.8 ab	32.0	37.6
F-test	***	***	NS	NS
New Guinea impatiens				
Control	8.2 bc	13.1 ab	18.6 bc	25.2 a
50 N	8.1 bc	11.5 cd	17.1 d	23.6 a
500 N	8.5 ab	13.8 a	20.5 a	23.6 a
Ebb/flow	8.9 a	13.3 ab	18.8 bc	24.6 a
Drought	7.7 c	12.3 bc	19.2 b	24.0 a
Brushed	7.7 c	10.5 d	18.1 bcd	24.6 a
B-Nine	8.1 bc	12.6 b	18.9 bc	23.8 a
Bonzi	6.6 d	7.4 e	9.7 e	12.4 b
F-test	***	***	***	***

NS, *** Not significant or significant at $P < 0.001$. Mean separation by protected LSD, $P < 0.05$.

Table 2. Effect of conditioning treatments on growth of ‘Little Devil Mix’ marigolds and ‘Blue Puffs’ ageratum at 2 and 4 weeks after treatment (WAT) in the greenhouse and at 4 and 8 weeks after planting (WAP) in the landscape.

Species Treatment	<u>Plant height (cm)</u>			
	2 WAT	4 WAT	4 WAP	8 WAP
Marigold				
Control	4.8 a	10.8 ab	20.2 ab	25.5
50 N	4.5 abc	7.7 d	17.4 c	25.5
500 N	4.5 abc	10.3 bc	21.0 a	25.2
Ebb/flow	4.7 ab	12.2 a	20.7 a	23.2
Drought	4.2 abcd	8.4 d	16.6 c	22.4
Brushed	4.1 bcd	8.5 d	18.0 bc	22.8
B-Nine	4.0 cd	8.6 cd	19.0 abc	23.5
Bonzi	3.7 d	9.0 cd	17.8 bc	23.1
F-test	*	***	*	NS
Ageratum				
Control	6.7 bc	13.1 b	14.7 abc	15.8
50 N	6.1 c	10.5 d	13.4 cd	17.6
500 N	6.1 c	14.5 a	15.5 a	17.2
Ebb/flow	7.7 a	14.8 a	15.2 ab	19.6
Drought	6.9 b	14.0 a	15.5 a	17.4
Brushed	6.4 bc	12.3 bc	14.0 abcd	16.8
B-Nine	4.8 e	11.0 cd	12.6 d	16.4
Bonzi	5.2 de	12.0 cd	13.7 bcd	15.5
F-test	***	***	*	NS

NS, *, **, *** Not significant or significant at $P < 0.05$, 0.01 , or 0.001 , respectively. Mean separation by protected LSD, $P < 0.05$.

TRANSPLANT PRODUCTION AND PERFORMANCE: TRANSPLANT AGE

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The effect of transplant age on crop yield is an issue often broached by growers of horticultural and agronomic crops in an effort to maximize production potential. This question appears to be of greatest interest to olericulturists and agronomists where the commodity produced is consumed. Despite general interest in this area, the literature is surprisingly sparse. For example, Bedding Plants IV (Holcomb, Ed., 1994), a highly regarded manual on the culture of bedding plants as a greenhouse crop, makes only passing reference to transplant age, usually from a postharvest shelf-life standpoint.

Agronomic interest in transplant age is most prominent in rice (*Oryza sativa* L.), a bare-root transplanted crop of major economic importance. However, transplant age studies have been conducted on tobacco [*Nicotiana* sp.] (Greenfield and Paterson, 1994; Tancogne, 1991), cotton [*Gossypium* sp.] (Abou-Zeid et al., 1995; Sherief et al., 1995), rape [*Brassica napus* subsp. *oleifera* var *napus*] (Gupta, 1994), and forest species (Battaglia and Reid, 1993; Chaney and Byrnes, 1993).

Olericulture supports the largest volume of literature on transplant age. Publications such as Knott's Handbook for Vegetable Growers (Lorenz and Maynard, Eds., 1988) and various extension bulletins (e.g., Vavrina, 1995) suggest ages for field planting of numerous vegetable transplants (Table 1.) These recommendations are generally based on years of horticultural observation and some research, but the scientific investigation of vegetable transplant age is far from complete.

McKee (1981) provided a brief review of studies conducted on transplant age. He referenced work by Loomis (1925) and Dullforce (1954), noting that "almost all vegetables" can be transplanted as early seedlings with little effect on growth, but with increasing age, this situation changes. He stated: "The later the transplant check [i.e., cessation in growth] occurs in the ontogenetic development of the plant, the more serious the effect in terms of its normal development. Following transplanting, older plants have only a limited time for the readjustment of their vegetative development before the initiation of reproductive growth or the maturation of the vegetative phase." (p. 270). How is age viewed by the producer and receiver (e.g., farmer) of vegetable transplants? As a general rule, receivers prefer young, actively growing transplants. While the traditional time frame for vegetable transplant production (4 - 6 weeks) is generally adhered to by the producer, a delay in the receivers' planting schedule results in aging transplants. Additionally, the producer may tend to slow plant growth in an effort to stay within height constraints often dictated by the receiver. Finally, in the event of a catastrophe (freeze, flood, etc.) receivers demand transplants to reset, regardless of age. When transplants are thought to be "too old", concerns are raised about their subsequent growth and yield potential.

This paper will review the literature on transplant age in vegetable crops and the effect of transplant age on performance in the field. Because it is implausible to consider all the information that exists in research station bulletins, proceedings papers, and media releases, the focus of this review will be on data found in journal articles and abstracts (English summaries). It is also recognized that there are many production practices that may affect transplant establishment and subsequent yields (e.g., media, watering, pruning), but it is not the intent of this article to address these practices. A summary of transplant age research on 15 crops is presented.

SOLANACEAE

Tomato - *Lycopersicon esculentum* Mill. A review of the literature on tomato transplant age was undertaken by Vavrina and Orzolek in 1993 and the reader is referred to their article for specific information presented from the studies examined. A revised summary table from that work (Table 2) shows that little could be distilled from the 70 years of research. Lack of standardized methods, multiple cultivars, processing vs. fresh-market types, bare-

root vs. containerized, various container cell sizes, superimposed studies, postharvest storage, and other aspects all led to varying results.

Conclusions. Vavrina and Orzolek (1993) concluded that transplant age apparently had little bearing on tomato production. Some points stressed by the authors include:

1. Young transplants (3 - 4 weeks) reduce production costs, but may not pull from the container without injury, and will need to be carried longer in the field to reach optimum yield (Hoffman, 1929; Leskovar et al., 1991; Orzolek et al., 1991).
2. Older transplants (7 - 9 weeks) tend to produce earlier yields (Hoffman, 1929; Liptay, 1987; Nicklow and Minges, 1962; Vavrina, 1991) but may result in more problems during the greenhouse phase (i.e., insects and diseases).
3. Commercial transplants (4 - 7 weeks) are appropriate for immediate field setting, however, if significant mortality occurs after transplanting, the use of "older" plants as resets should not reduce yield, size, or earliness.

Pepper - *Capsicum annum L.* Nicklow (1963) found that pepper transplants without flower buds or with unopened flower buds produced more large fruit (early and total) than transplants with open blooms or small fruit. This research was conducted in New York state.

McCraw and Greig (1986) used 8 and 11 week-old transplants of four cultivars in a pepper transplant age study in Kansas in 1975 - 76. Pooling the data from the four cultivars, they found no differences due to transplant age in early yield (number, weight) the first year, but a greater number of heavier fruit with 8-week-old transplants the following year. Three of the four cultivars tested showed that the 11-week-old plants produced more total fruit per plant than the younger transplants (12 vs. 10 fruit).

Weston (1988) conducted a study in Kentucky using containerized pepper transplants of 4, 6, 7, and 9 weeks. She found 70% more early U.S. Fancy and U.S. No.1 fruit with 9-week-old transplants. However, total U.S. Fancy and U.S. No.1 fruit yield and total overall yield was unaffected by transplant age.

Vavrina and Armbruster (1991) conducted a one-year trial with transplant ages of 4, 6, and 11 weeks in Florida. They found no effect of transplant age on yield (number, weight) in three of four harvests, but a significant yield increase at fourth harvest with 4-week-old plants. This single harvest increase was significant enough to result in a greater overall yield for the 4-week-old transplants. The yield effect here was due to a greater number of fruit not greater individual fruit weight. McCraw and Greig (1986), as noted above, had a similar finding with 11-week-old transplants.

Conclusions. Three of the studies cited here imply that pepper transplants of 8 - 11 weeks may have a yield advantage for early size and number of fruit (McCraw and Greig, 1986; Nicklow, 1963; Weston, 1988). Yet Vavrina and Armbruster (1991) provide evidence that younger transplants may eventually exceed yields produced by older plants. These researchers used different numbers of harvests (McCraw and Greig - 3; Vavrina and Armbruster - 4; Weston - 10), making comparisons among the studies difficult. Perhaps a standardization of the number of harvests for early and total yield is necessary to critically determine the impact of transplant age on pepper production. Considering the slower growth habit of pepper compared to tomato, older transplants (i.e., > 4 - 6 weeks) may still be advised.

Eggplant - *Solanum melongena.* Lou et al. (1993) indicated that "younger" eggplant grew more vigorously after transplanting and yielded higher than "older" seedlings in China. (Their abstract did not define young and old.) Hotta et al. (1993) determined that 40-day-old eggplant seedlings were the most successful in summer trials conducted in Japan. Harmon et al. (1991) investigated eggplant yield with transplants in Kentucky. Testing 4-, 5-,

6-, and 7-week-old transplants, they suggested transplants 5-weeks-old or younger produced only minimal yields, and that the earliest yields were obtained with 7-week-old plants in large cells (>30.7 cm³).

Conclusions. The information available from these studies yields no definitive information on which to base decisions on eggplant transplant age. However, the data support a 5- to 7-week-old transplant which conforms to the tomato data.

Husk Tomato - *Physalis ixocarpa*. Pena-Lomeli et al. (1991) used husk tomato transplants of 2, 3, 4, 5 and 6 weeks of three cultivars in Mexico. They found the 3-week-old transplants had the highest yields across cultivars. Direct-seeded husk tomatoes out-yielded the 5- and 6-week-old transplants.

CUCURBITACEAE

Watermelon - *Citrullus lanatus* (Thumb.) Matsum & Nakai. Vavrina et al. (1990) and Vavrina et al. (1993), reporting on four years of data, showed that watermelon transplant age had no effect on either early or total yield in Florida. They used transplants of 3, 5, 7, 9, 11, and 13 weeks of two cultivars and carried out the study at multiple locations. These data somewhat contradict the traditional Extension recommendation of a 4-week-old watermelon transplant without flowers. The authors did not recommend the use of older transplants but suggested that in the event of a crop loss, age of the resets need not be a consideration.

Squash - *Cucurbita pepo* L. NeSmith (1993) utilized 1-, 2-, 3-, 4-, and 5-week-old summer squash (yellow crookneck and zucchini) transplants in greenhouse and field studies in Georgia. He found no difference in early or cumulative yield due to transplant age in yellow squash, but slightly higher cumulative yields with 4-week-old zucchini transplants. He recommended 3 weeks as a target pull date, which supports the Knott's Handbook for Vegetable Growers (1988) suggested date. NeSmith stated that a 3-week-old plant could be held for 10 days before planting, if necessary, without subsequent yield loss.

Muskmelon - *Cucumis melo* L. NeSmith (1994) also designed a muskmelon transplant-age trial using plants 2-, 4-, 6-, and 8-weeks old. He found age had little effect on either early or total yield.

Conclusions. As these data suggest, transplant age does not adversely influence yield in cucurbits. The commercial industry uses 3- to 4-week-old transplants for general cucurbit production. However, these data support the idea that cucurbits can be held, in the event of poor planting conditions, beyond this time frame without fear of yield loss.

BRASSICACEAE

Broccoli - *Brassica oleracea* var. *Italica*. Olson and Locascio (1990) ran four experiments in Florida with broccoli transplants of 3-, 4-, 5-, and 6-weeks of age. In two of the four trials, age had no effect on yield. In the remaining two trials, the 6-week-old plants produced the highest yields in one trial and the lowest yields in the other trial.

Jones et al. (1991) studied broccoli transplant age (specified only as 3 - 7 weeks), in spring and fall crops for two years in Kentucky. This work, presented at the 1991 ASHS annual meeting (abstract), found "older" plants (>5 weeks) produced higher early fall yields one year, but age had no effect on spring, early, or total yield.

In a study that stretched the limits of transplant age, Lamont (1992) compared broccoli transplants of 31 and 29 weeks to plants of 2 and 6 weeks of age, respectively, in trials in North Carolina. He found minor statistical differences in head weight and diameter in a spring trial only, but considered the differences too small to be of concern to the consumer.

Damato et al. (1994) used broccoli transplants of 5, 6, and 7 weeks in a study with three cultivars for fall production in Italy. These authors found a linear decrease in individual head weight with increasing age, but treatment effect on head weight was not significant when tested via mean separation. Time-to-first harvest

significantly increased with increasing age, and the incidence of hollow heart lessened with increasing age in this study.

Conclusions. These studies sufficiently cloud the issue of transplant age in broccoli. Jones et al. (1991) suggest older is better, Damato et al. (1994) indicate younger is better, Lamont (1992) implies age makes no difference, and Olson and Locascio's (1990) results confirm each of the other researchers work. Neither Olson and Locascio, Lamont, nor Jones et al. addressed time-to-harvest, an important factor to growers. However, it appears more work is necessary before recommendations for broccoli transplant age can be made.

Cabbage - *Brassica oleracea* var. *capitata*. Jones et al. (1991) found that transplant age did not influence cabbage early or total yield in the spring of their two-year study in Kentucky. No mention was made of fall yield effects in the abstracts though two seasons were indicated.

Cauliflower - *Brassica oleracea* var. *botrytis*. Salter and Fradgley (1969a) found if they delayed using bare-root-raised cauliflower transplants by 14 days, marketable yields tended to decline, but time-to-maturity was unaffected (Salter and Fradgley, 1969b).

Wurr et al. (1986) conducted two experiments with modular-raised cauliflower in England: 5- and 6-week transplants vs. 7- and 8-week transplants, and 5- and 7-week transplants vs. 6- and 8- week transplants. They determined transplant age had a small effect on time-to-50%-maturity (old plants matured 3 days later than young plants in one year only), but transplant age had no effect on yield. They stated "changes in production practices within the normal range are unlikely to have any significant practical effect on ... the timing of crop maturity" (Wurr et al., 1988, p.427).

Jones et al. (1991) found that containerized cauliflower transplants of 5 weeks or older produced higher early and total yields in one of two fall trials and higher early yields in the spring.

Lewandowska (1992), in a three-year trial in Poland involving three cultivars and transplants of 3-, 4-, 5-, 6-, and 7-weeks of age, demonstrated higher yields of better quality with younger transplants (age unspecified). Lewandowska noted that as transplant age increased, so did the time to early and mid harvest.

Conclusions. The data supplied by these cauliflower studies indicate more may be at work here than transplant age. Salter and Fradgley (1969a) used bare-root plants while Wurr et al. (1986) used containerized plants. The data from Jones et al. (1991) and Lewandowska (1992) were in abstract form. Therefore, more information is necessary concerning cauliflower transplant age.

Chinese Cabbage - *Brassica campestris* ssp. *pekinensis*. Kratky et al. (1982) in Hawaii found little difference in time-to-maturity, individual head weight, and total yield from Chinese cabbage transplants of 3-, 4-, 5-, and 6-weeks of age.

Brassica napus subsp. *oleifera* var *napus*. Gupta (1994) showed that rape seedling age influenced seed yield in India. Of 30-, 40-, 50-, and 60-day-old transplants, the highest yields were obtained from the 60-day-old plants. This conclusion was based on three years of trials with fall-planted rape.

ALLIACEAE

Onion - *Allium cepa*. Vachhani and Patel (1988, 1989) used red onion transplants of 4 - 10 weeks (weekly intervals) in a one-year trial in India. They found yield increased with increasing age to 7 weeks at which point it began to decrease gradually through 10 weeks.

Lujan-Favela (1992), in a three-year study looking at planting date and transplant-age in Mexico with Early White Grano, showed highest yields were obtained with 7-week-old transplants set in mid-September. Specific transplant ages were not given in the abstract (range 50 - 140 days), but Lujan-Favela correlated yield with transplant size (larger was better) and subsequent vegetative growth.

Wojtaszek et al. (1993) grew 30-, 40- and 50-day-old onion transplants in peat blocks (5 seed per block) for spring onions in Poland. They found no effect of age on marketable yield.

Herison et al. (1993) used three cultivars of containerized Spanish-type onions of age 8, 10, and 12 weeks in Michigan. These researchers found that although bulbs essentially matured at the same time, the 10- and 12-week-old transplants yielded larger bulbs. This response was positively correlated with larger plants at setting.

Conclusions. Yield response in onions may be more the result of plant size than age as both Herison et al. (1993) and Lujan-Favela (1992) suggested. Herison et al. (1993) noted higher N rates during transplant production resulted in larger seedlings (regardless of age) and greater yields. Most onion transplants are field-grown due to the population density required per unit area. Wojtaszek et al. (1993) and Herison et al. (1993) conducted studies with containerized plants but Vachhani and Patel (1988) and Lujan-Favela (1992) gave no indication whether their studies involved containerized or bare-root transplants.

Leek - *Allium porrum*. Kunicki (1993) in a three-year study in Poland with leek transplants of 11-, 13-, and 15-weeks of age, found no effect of age on crop height or quality. The abstract indicated a yield range of 17.1 - 33.6 t/ha, but did not specify a treatment effect.

COMPOSITAE

Lettuce - *Lactuca sativa*. Boa et al. (1979) suggested a minor effect of transplant age on butterhead lettuce, finding older transplants tended to produce lighter heads.

Wurr et al. (1987) conducted seven experiments over three years in England on the influence of plant raising and transplant age on crisp (Iceberg) lettuce. Finding inconsistent results, they concluded that the plant age (13, 19, 25, 32 days) could effect mature head weight. However, they suggested the use of "younger" transplants (< 25 days) to minimize variation in head weight across seasons (i.e., product consistency.)

SUMMARY

The studies reviewed above represent the majority of the research on transplant age found in the literature today. Yet with all this work, we still fall short of our goal ... the "ideal" transplant age. When the results of the studies presented here are distilled down to a single number for an individual crop, we arrive at recommendations similar to those found in Knott's Handbook for Vegetable Growers in its 1962 edition.

The conflicting results in the literature on transplant age may be due to the different environmental and cultural conditions to which plants were exposed, while being raised and in the field. Certainly, even under the most controlled experimental conditions, older plants are exposed to greater levels of hardening and water stress. Root pruning, which may be excessive in bare-root production, and is encouraged by the Speedling system, will differ in magnitude among plants of varying ages. Such factors result in hormonal changes that influence plant growth, and as McKee (1981) suggested, there is a point beyond which normal growth will not occur.

Yet the studies reviewed here reveal that the transplant age "window" for certain crops may be wider than we had previously thought. These findings suggest that it may be our modern cultivars, production systems, technical know-how, or a combination of factors that enable us to produce high yields regardless of transplant age. Producers will continue to provide their clientele with quality transplants of a particular age as dictated by their production practices. Receivers, when in need of multiple resets will not fear "older" plants, knowing yields will not suffer.

Research on vegetable transplant age provides a valuable service, yielding new information while building on the existing literature foundation. Several observations noted while reviewing these studies should help in developing a stronger data base in the future:

Abstract. The abstract should provide full, clear, and concise information, especially for work compiled in an abridged form (e.g., proceedings paper), obscure source, or where a translated summary is requested. A translated abstract may be our only access to information contained in a publication from another culture.

Standardization. Realizing the impact of aspects other than transplant age on transplant growth (cell size, fertilization, water quality, etc.), production practices should be more standardized across studies. Qualified commercial operations might be enlisted for the actual growing of test seedlings. Additionally, replications of and comparisons with established work should occur.

Age Range. Efforts should be made to broaden experimental time frames. Researchers are often timid in their approach, limiting studies to 3, 4, 5, and 6 weeks. If Lamonts' (1992) results were not significant for transplants varying in age from 2 weeks to 6 months, then what can we hope to learn from short time-frame studies?

Yield Aspects. All studies should incorporate at least time-to-maturity, early yield, and total yield, and include fruit size and/or grade characteristics. Some standardization in number of harvests (for multiple-harvest crops) and determination of earliness is also suggested.

Statistical Significance vs. Commercial Reality. Researchers should give consideration to distinguishing between significant results and their application to commercial production. Small significant differences may not be of great commercial importance.

Helpful Hints. In addition to transplant age effects on yield, secondary horticultural findings of value often result from the research. Examples of useful information gained from the studies reviewed here include: younger tomato transplants must be carried longer in the field to reach optimum yield (Hoffman, 1929; Orzolek et al., 1991), onion transplants of various ages essentially mature at the same time (Herison et al., 1993), pepper transplants without flower buds or with unopened flower buds produce more large fruit (early and total) than transplants with open blooms or small fruit (Nicklow, 1963), and watermelon fruit set is synchronous regardless of transplant age (Vavrina et al., 1993).

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Table 1. Times required for growing plants for field transplanting.*

Vegetable	Time (weeks)
Broccoli	5-7
Brussel Sprouts	5-7
Cabbage	5-7
Cauliflower	5-7
Celery	10-12
Corn, sweet	3-4
Cucumber	3-4
Eggplant	6-8
Lettuce	5-7
Muskmelon	3-4
Onion	10-12
Pepper	6-8
Summer Squash	3-4
Tomato	5-7
Watermelon	3-4

*Table adapted from "Knotts Handbook for Vegetable Growers", Third Edition. O.A. Lorenz and D.N. Maynard (Eds.), 1988, New York: Wiley.

Table 2. Yields associated with tomato transplant age studies as determined by multiple researchers.*

Researcher	Cultivar	Transplant Age Range	Highest Yield			Significant Difference
			Age	Early (lb/plant)	Total (lb/plant)	
Hoffman (1929)	---	5, 6, 9, 10	5	---	10.6	No statistics
Casseres (1947)	Earliana	7, 11, 15	7	0.89	11.9	7>11, 15 early and total
Sayre (1948) ^y	John Baer	6, 8, 10	6	3.86	---	Average over three locations, 8>10 early
			8	---	18.04	NS - total
			10	3.49	---	Only one location showed difference, 7>12>14 early
Chipman (1961) ^y	Scotia	7-12	10	---	9.87	NS - total
			7	2.6	---	7>all; 9>10, 11, 12; all >12 early
			8	---	8.8	8>all; 7>9, 10, 11, 12; 11, 9>10, 12 total
Nicklow & Minges (1962)	Fireball	6, 8	7, 8	15.4	---	7, 8>9, 10, 11, 12; 9>10, 11, 12; 10>11, 12
			7	---	28.5	7>all; 8>10, 12 total
			6	---	11.2	No statistics
Cooper & Morelock (1983) ^x	Traveler '76	5, 7, 9, 11	4	---	2.2	4> 8 total
			9	0.24	---	9, 7>3, 5 early
			5	---	12.9	3, 5>7, 9 total
Liptay (1987)	H-2653	4-7	7	1.35	---	7, 9>5, 11 early
			5	---	8.0	5>11 total
Weston & Zandstra (1989)	Pik-Red	3-6	7	4.20	---	NS - early
			6	---	7.9	NS - total
Leskovar et al. (1991)	Sunny	3-6	4	2.23	---	4, 5 >6, 3; L* 5>4, 6>3 early
			6	---	17.1	6>4, 3; L*6>5>4>3 total
			5	10.20	---	Q*= 5>4>6>3 total early yield
Vavrina (1991)	Sunny	3, 5, 7, 9, 11	4	---	25.5	Q*= 4>5>3>6 overall yield
			4	4.11	---	NS -early
			4	---	15.0	NS - total
Orzolek et al. (1991)	Sunny	3, 4, 7, 9, 11	9	8.33	---	NS - early ; Q*= decreasing xl l fruit with increasing age
			9	---	11.9	NS - total
Orzolek et al. (1991)	Colonial	5, 7, 9, 11, 13	4	---	6.9	NS - total yield
			13	---	10.1	NS - total yield

*Table adapted from *Tomato transplant age: A review by C.S. Vavrina and M. Orzolek, 1993, HortTech. 3(3):313-316.*

^zNS, L*, Q* = Nonsignificant, linear, and quadratic, respectively.

^yPlant populations not indicated; therefore, yields given in tons/acre.

^xPlant populations determined through personal communication.

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