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Studies on the premature bolting of the chicory cultivar 'Daliva'

Jomo MacDermott

To the Graduate Council:

I am submitting herewith a dissertation written by Jomo MacDermott entitled "Studies on the premature bolting of the chicory cultivar 'Daliva'." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

David L. Coffey, Major Professor

We have read this dissertation and recommend its acceptance:

Charles A. Mullins, John H. Reynolds, John Foss, Robert Auge, Arnold Saxton

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Charles A. Mullin

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

STUDIES ON THE PREMATURE BOLTING OF THE CHICORY CULTIVAR 'DALIVA'

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee

Knoxville

Jomo MacDermott

May 1997

DEDICATION

Dedicated to the Lord Buddha in each of us.

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The author would like to thank those who helped make this dissertation possible. Among the most immediate at the University of Tennessee are the members of his committee: Drs. David L. Coffey, Charles A. Mullins, John H. Reynolds, John Foss, Robert Auge' and Arnold Saxton. Each has contributed to my success and my dissertation. I am also very grateful to the Department of Plant and Soil Science for the financial support.

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ABSTRACT

The flower initiation and bolting responses of the extra-early F-1 hybrid chicory cultivar 'Daliva' (*Cichorium intybus* L. var witloof) were investigated under controlled daylength and field conditions at Knoxville, TN (35°, 53'N and 83°, 57'W). Histological sectioning and biochemical screenings from apical shoot tips for soluble sugars (glucose, fructose, and sucrose) and total free amino acids prior to and after floral transition, in addition to various plant size measurements, were conducted.

Under controlled daylength (10 hours light) for 143 days, pot grown plants did not exceed 22 leaves and no plants initiated a floral transition. After 143 days similar plants were subjected to long photoperiods (>14 hours) for up to 15 days. Over the 15 days of long photoperiods six harvests were made but no floral initiation was found. Total amino acids from all shoot tips ranged from 15 to 30 mg / g dry weight (DW). Soluble sugars leached from apical shoot tips followed no apparent trends. Glucose and fructose each ranged from 25 to 120 umol / g DW while sucrose ranged from 30 to 130 umol / g DW. Immediately after the imposition of the long day photoperiods the total free amino acid level in the shoot tips appeared to rise.

In a field experiment, some field grown 'Daliva' chicory sown on 29 May had signs of bolting within 90 days. Vegetative meristems appeared flattened,

even sunken beneath the overarching leaf primordia while induced meristems were domed and hemispherical in shape; this is consistent with other reports from the Cichorieae.

In vegetative shoot tips, total free amino acids from shoot tips ranged between 23 and 40 mg / g DW with the maximum value at the middle harvest. Glucose and fructose each were less than 100 umol / g DW and remained steady through three harvests. Sucrose from the same tissue dropped linearly from 450 to near 50 umol / g DW over a 27 day harvest period.

Shoot tips identified as transitional had levels of free amino acids which rose steadily from 15 to 25 to 35 mg / g DW over three harvests within 13 days. From the same shoot tips all sugars also rose steadily; glucose from 90 to 700, fructose from 60 to 260, and sucrose from 10 to 400 umol per g DW. Transitional plants exceeded 33 leaves and had root diameters greater than 20 mm.

A separate field experiment investigating 'Daliva' plant growth and bolting response was begun on 19 June when seeds were planted into an Etowah clay loam. Five weeks later half the plants were mulched with 10 cm of straw mulch. Harvests began 14 days after planting and continued for 14 weeks. Bolting plants were observed after 8 weeks.

Measurements of bolting plants showed that although the mulched plants grew faster and larger they did not bolt with any more frequency than plants in the bare soil. However, a comparison of various plant parameters (leaf number, area, DW, root diameter, root DW and crown diameter) between bolting

and non-bolting plants showed that the leaf number differed ($P<0.01$). Bolting plants had, on an average, 34 leaves while non-bolting plants had 28 leaves.

In summary, these experiments investigated the bolting responses of 'Daliva' chicory and found that the juvenile stage of this cultivar is passed at approximately 30 leaves and thereafter the plant may proceed directly into flowering. Once induced to flower the apical shoot meristem appeared distinctly domed, in contrast to the vegetative meristem which was flattened at the top.

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PART I

INITIAL CONSIDERATIONS ON WITLOOF CHICORY IN TENNESSEE

INTRODUCTION

Increased vegetable consumption is encouraged by the USDA and fresh salad items are widely eaten in North America. Witloof chicory (aka Belgian or French endive) is a labor intensive, high value potential alternative vegetable crop for East Tennessee. In central Europe the crop is grown on thousands of hectares and is widely eaten as a fresh salad green or as a cooked vegetable, including a gourmet soup. Chicory heads (chicons) are about one-third the size of lettuce heads and provide comparable food value. Chicons have a shelf life of several weeks if kept humid and cold, but not wet. Some cultivars have reddish leaves which may offer marketing niches for producers.

Witloof chicory production requires 3 major steps: 1) field growing and digging of suitable roots, 2) storage of roots in cold conditions, either in refrigerated rooms or left in the ground during winter and, 3) forcing of the apical vegetative bud in dark, temperature controlled rooms where roots are kept at 15-18 ^{o}C and tops at 12-16 ^{o} C. Chicons are ready to harvest from the roots after three weeks in the forcing houses.

Chicory growing is much like sugar beet culture; both crops do best under cool conditions. Tennessee may be far enough north to grow suitable roots (unlike Florida) and yet far enough south to avoid high heating costs of forcing structures (unlike New York). Currently nearly all witloof is imported from Europe, which accounts for some of the high retail cost.

In 1992, six chicory cultivars were planted to investigate root yield potential at two Tennessee locations, the Plateau Experiment Station at Crossville and the Knoxville Plant Science Farm. The six cultivars were chosen from seedmen's selections and were based on 'earliness in forcing', suitability for hydroponic forcing, and leaf color. A planting design included two planting dates, 1 June and 1 July, two planting densities, 80,000 and 160,000 plants per hectare, and three replications at each location. The data taken were principally root yield, including root diameter. Through the summer some plants unexpectedly bolted and developed flowers, so percentage bolting in mid-September was also recorded. Bolting plants cannot be forced and thus represent a lost effort for the grower. A sample of roots of each cultivar was stored and forced for chicons.

Table 1 gives the cultivars and bolting percentages for plants sown 1 June, at Knoxville. The table indicates that the 'earliness for forcing' is a factor in number of plants bolting. The extra-early cultivar 'Daliva' had more than 40% of the plants bolting but the late cultivar 'Rinof' had almost no plants bolting. In every case the low density planting had more bolting plants than the high density, suggesting that less competition increased the opportunities for bolting. At Crossville, none of those planted 1 June bolted and those planted 1 July all failed to reach minimum marketable size, three cm in root diameter. Figure 1 shows the association of root diameter and percentage bolting in 'Daliva' plants from Knoxville. As the root diameter increases the percentage bolting increases in a linear fashion. The plants which had the greatest root diameter, those

TABLE 1. Chicory cultivars and bolting by plant density.

		PERCENTAGE BOLTING	
		PLANT DENSITY	
Cultivar	'Earliness for forcing'[z]	HIGH	LOW
DALIVA	EXTRA EARLY	44	49
FLASH	EARLY	23	36
ZOOM	EARLY, HYDROPONIC	16	37
ROBIN	MID, RED LEAFED	17	34
FARO	LATE, HYDROPONIC	4	28
RINOF	LATE, HYDROPONIC	4	0

Plants grown at Knoxville, 1992 from 1 June planting. Values are the mean of 3 replications.
Z, as described by seedmen's catalogues.

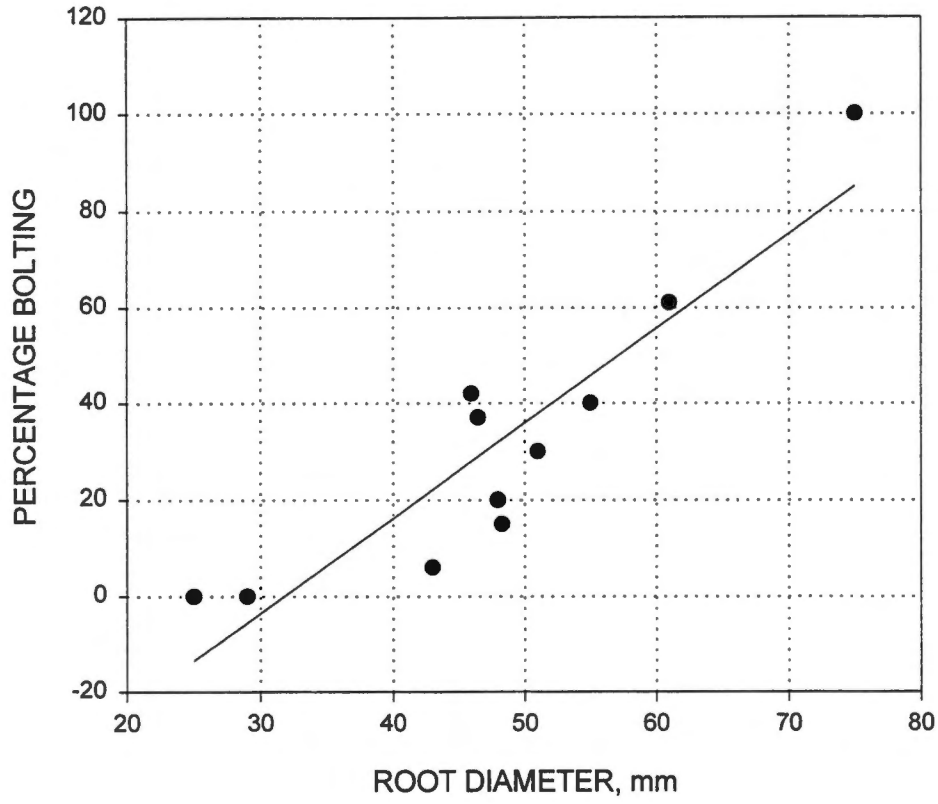

FIGURE 1. Association between root diameter and percentage bolting.

Means of root diameters and bolting of 'Daliva' plants from two densities planted on two dates, 1 June and 1 July, 1992, at Knoxville. Roots were measured in December and bolting plants in September.

planted 1 June, also had the highest bolting percentage. Only plants which reached a minimum root diameter size, about 33 mm, showed a tendency to bolt.

Chicory is considered by many authorities as a plant which requires a cold period followed by long days for flowering, the typical response of most biennials. The behavior of 'Daliva' in the field suggested that other factors were influencing the flowering response besides vernalization (cold treatment for flowering) since these plants never experienced a cold period yet many did flower. Since bolting was associated with increasing root diameter this suggested that the 'Daliva' plants outgrew the juvenile growth stage within the seedling year.

These preliminary studies suggested that size or age of the plant is a factor in bolting. Therefore additional field and laboratory studies were necessary to further substantiate if the environmental conditions of Tennessee, particularly the Knoxville area, were suitable for potential production of chicory roots for chicon production and to characterize growth and phenology of this unfamiliar vegetable plant under Tennessee conditions.

Recent literature on the nature of the endogenous floral messenger suggests that soluble sugars and free amino acids at the apical meristem may play a role in the transition to flowering. These biochemicals may have an influence on floral transition at the apical meristem as plants exceed a minimum size and grow into an adult phase.

RESEARCH OBJECTIVES

Based on these considerations, the research objectives were:

1) to determine whether 'Daliva' chicory could be induced to flower under controlled daylength conditions without a cold treatment,

2) to determine if soluble sugars and free amino acids could be extracted from apical shoot tips and correlated with transition to flowering, and

3) to determine if plant growth rate or plant size has an influence on bolting.

LITERATURE REVIEW

BOTANY AND HISTORY

Chicory (*Cichorium intybus* L.) is a member of the Asteraceae family, subfamily Liguliflorae, tribe Cichorieae. The subfamily members all have ligulate flowers and sticky, milky sap. Taxonomically, the tribe identifies members of this family with ligulate perfect flowers as the only ones present on the receptacle. Each elongate corolla is typically five toothed; in chicory the corolla is sky blue and lasts but a day. Others in this tribe are sowthistle (*Sonchus*), hawkweed (*Hieracium*), dandelion (*Taraxacum*) and lettuce (*Lactuca*), probably the most economically important member of the entire family of nearly 1000 genera (Smith, 1977; Waycott, 1993).

The genus Cichorium has been divided into nine species: the two of economic value are *C. endiva* L. and *C. intybus* L. (Bailey and Bailey, 1976). The former are the annual and biennial endives of cultivation, with their deeply serrate, lobed and often curly leaves, known since ancient times around the Mediterranean as a salad green and potherb. *C. intybus* L. is a perennial plant with a deep tap root, growing as a basal rosette of large dandelion-like leaves the first year. Following the winter the plant sends up a tall, up to one meter, erect flower stalk with small clasping, lanceolate leaves hugging the stalk. *C. intybus* is also native to the Mediterranean but has a history of root uses as tonics, blood purifiers, and when roasted, as a coffee additive and hot drink

ingredient. Steiner (1983) outlines some of the historical uses of chicory root and mentions that the ancients Aristophanes and Dioscorides both speak of chicory in their writings with uses as wild vegetables and medicinals. The Roman writers Horace, Vergil, Pliny, and Galen mentioned chicory and during that era both the tops and the roots were used as food and prescribed for liver ailments and inflamed eyes (Mitich,1993).

ECONOMIC USES

Currently, chicory is cultivated extensively in Europe for its roots, which are the source of inulin, a fructose polymer not digestible by humans. Cooking will break such polymer linkages and boiled roots taste something like carrot or parsnip. Inulin can be easily broken down by fungal enzymes into high fructose syrups suitable for soft drinks (Chubey and Dorell, 1977). Inulin is also finding uses as a natural food ingredient with value as a low-calorie dietary fiber and bulking agent used as a fat and sugar replacement in cold dairy products especially (Franck,1992). One chicory cultivar, 'Grasslands Puna' has been selected and developed in New Zealand as a forage crop with high nutritive value. This forage cultivar is being investigated with sheep in West Virginia (Tietz, 1992) and steers in Oklahoma (Volesky, 1996). Chicory has been investigated as a biofuel energy crop in Canada (Chubey and Dorell, 1978), New Zealand (Douglas and Poll, 1969) and Florida (O'Hair, 1982)

One of the most important economic uses of chicory is the forcing of the etiolated, vegetative apical bud, known as a chicon, from the mature root. These tender little heads of pale yellow leaves can be eaten raw, cooked as an entree

or as soup, or as excellent 'dippers'. Imports into the USA from Belgium, France, New Zealand and Chile totaled over 3,000 tons in 1983 (Hill, 1987). Current Knoxville prices for Belgian endive or witloof are about $3.49 per pound, or about three times the price of lettuce. Its food value is about the same as lettuce (Yamaguchi, 1983). Hill (Hill, 1987, 1986) investigated many aspects of chicory culture in Connecticut, including growing and forcing 26 cultivars. He found substantial variation in yield of roots and chicons but was especially struck by the organoleptic acceptance by tasters of fresh, locally grown chicons over the imported ones which must have been days, perhaps weeks older.

CHICORY CULTURE

The culture of chicory is much like sugar beets, according to Steiner (1983). The soil should be well-drained; any texture except heavy clay or very rocky will do but no soil should be overly fertilized with nitrogen, as this is known to result in excessive leaf growth at the expense of root development. Plant densities of 110,000 per hectare, in rows 45 cm apart, or in double row beds are desirable. Phosphorous, potassium and magnesium are recommended (Anon, 1995). Weeds can be controlled with herbicides (Mersie and Eliot, 1993) or by cultivation. Seedling emergence and stand establishment are critical factors and crusty soils or dry periods during the first month should be avoided. Mature plants, those whose roots are ready for harvest, can be had in 120 days, or sooner if early maturing hybrids are used (Nunhems, 1994). Harvest of roots requires leaf removal above the crown, lifting the roots, about the size of parsnips, up to 30 cm long, sorting out culls (small and forked roots) and storing

in cold rooms for a time. The duration of cold storage depends on the time of harvest (those dug in December may need no further cold treatment) and the maturity status of the variety grown. Varieties are classified as 'early, medium or late' according to the time to maturity and the time required in cold storage. Late maturing hybrids take longer in the field to reach maturity and require more time in cold storage prior to forcing. However, late varieties can be kept in cold storage for many months, up to seven, before forcing (Nunhems, 1994).

Traditional techniques of forcing were discovered by accident in Belgium around the mid-nineteenth century when a Belgian farmer left chicory roots buried in his dark cellar over the winter and found as the spring arrived the mild-flavored blanched heads appeared. At that time few winter vegetables, especially greens, were available for the table. The techniques improved over time and now hydroponic forcing is common in Belgium, France and the Netherlands (Hill, 1987), but does require special cultivars which retain their head shape and density without the mechanical pressure imposed by a soil or sand covering (Tan and Corey, 1990). Biochemical changes in the root while in cold storage and during forcing have a decided impact on the quality of the resulting chicons (Bhatia et al., 1974; Rutherford and Phillips, 1975).

PRODUCTION CONCERNS

Difficulties associated with production of chicory roots for forcing include stand establishment as the seeds (achenes) are small, about the size of a carrot seed (1 x 2 mm) and are sensitive to depth of planting and soil moisture conditions. Once up and growing, field densities are important in order to obtain

proper sized roots suitable for the forcing beds. Small (under three cm diameter) and forked roots are not good, nor are roots too large as these may develop multiple, smaller heads. Each bud on the chicory crown, about 25 total, is capable of developing a shoot of some size, but the trade requirements for chicon size and head density mean that growers must obtain the central or apical bud from each root. The harvest of roots is much like that of beets or potatoes and mechanical equipment is often used. The characteristics of biennial tap root growth include high carbohydrate storage during the declining days of summer and autumn, so the harvest of roots can be delayed beyond the first frost (Cyr et al., 1990). Such timing of harvest, especially in Tennessee, implies that if the crop requires 120 days and can be established and grown during the summer it may serve as a double crop following the harvest of a winter grain, for example. After digging, roots can be shortened to a uniform length, usually 20 cm, are often dipped in 10% bleach to control surface fungi, and stored in humid, near freezing rooms to stimulate the subsequent forcing response.

FORCING OF CHICONS

Forcing of a chicon from the vegetative apical bud is carried out under controlled temperatures for both roots and tops. The roots, whether in soil or water, are kept at about 15-18° C. If production is hydroponic the water is nutrient fortified (DeProft et al., 1986). Since the witloof likes 'warm feet and a cold head' the air temperature is kept 5° C cooler than the water. The conditions are kept constant and light is fully excluded. The chicons are ready for harvest in three weeks and can be cut or broken from the root. Cleanliness and uniformity

of the chicons is very important for sales appeal and often the individual heads are wrapped in waxed paper for boxing and shipping. The chicons can be held for weeks if kept dry and cold. They are subject to some internal browning over time and surface rots may develop if the tender leaves are wetted.

BOLTING IN THE FIELD

Field production experiments, in addition to greenhouse trials with various cultivars, have shown that cool temperatures, below 15° C, promote rapid flower stalk extension, known as bolting, during the seedling year (Kalloo, 1993). Other factors, besides cool temperatures, are involved with the bolting response. For example, when chicory was planted into the warm soils of Connecticut (Hill, 1987,1988) or Tennessee (MacDermott et al., 1993) in mid-June, a certain percentage of plants bolted. Bolting plants are worthless for forcing and so represent a lost opportunity for the grower. Generally, Hill and MacDermott found that those hybrids listed as early and extra-early bolted most readily and that the earlier they were sown the greater the percentage of bolting plants. MacDermott et al. (1993) found nearly 50% of the extra-early F-1 cultivar 'Daliva' bolting in mid-September from a 1 June planting.

Bolting is a normal response of biennial and herbaceous perennial plants which overwinter close to and in the ground. Chicory is a perennial grown as a biennial; all cultivars will bolt and flower the second season in response to the long days of late spring and early summer. During the seedling year chicory grows as a rosette of low but large leaves hugging the ground and encircling its deeply compressed stem, known as a crown. Stems are composed of nodes,

those places where leaves and axillary buds are attached to the vascular system, and internodes. When the cells of the internodes expand the stem grows tall. Internode cells are highly responsive to gibberellic acid (GA), a plant growth regulator (PGR), and exogenous applications of GA on rosette, long day plants often result in dramatic stem extensions (Cleland and Zeevaart, 1970; Metzger, 1995) and in many cases floral induction (Zeevaart, 1984). The flowering stalk of chicory grows tall the second year and exposes the flowers to the aerial insects, especially bees, needed to accomplish pollination (Kalloo, 1993). In chicory, the pollen cannot germinate on the stigma of the flower from which it comes, a form of genetic self-incompatibility (Paulet, 1985). Bolting and flowering are separate but closely associated physiological phenomena.

FLOWERING RESPONSE

Many authors write that *C. intybus* is a plant which requires vernalization followed by long days in order to flower (Hartman, 1956; Martin-Tanguy et al., 1984; Wellensiek, 1964). However, first year plants are known to bolt and flower from every production region thus disputing the claim that vernalization (a cold treatment) is necessary in order to flower. Kalloo (Kalloo, 1993) agrees that all cultivars need long days for flowering but states that a cold period is needed only for bolting. This contradiction was resolved by Joseph and Paulet (cited in Paulet, 1985) who divided the chicory cultivars into 3 groups: 1) the 'late' varieties, those which have an absolute cold requirement for flowering, 2) the 'early' varieties, those which can flower without a cold treatment and, 3) the 'reversible' varieties, those able to switch according to the most recent treatment

(an obscure group). Hill (1987) believes that plant breeders, through selection of plants for shorter storage times, have also chosen plants which fall into the 'early' category and so these are able to bolt and flower the first year if other conditions are favorable. Many hybrids, called F-1s, are on the market and vary, among other factors, in time of maturity. Ryder (1984) believes excessive heterozygosity exists in the inbred lines needed to generate F-1 hybrids because of self-sterility in the species; therefore such hybrids show more phenotypic variation than found in F-1 hybrids of other crops.

JUVENILITY

Perennial plants do not flower unless the energy demands of producing and supporting the floral organs and developing seeds and fruits can be maintained, at least for some of the progeny. This support can come from a minimum photosynthetic area or from stored reserves. Until such time as the plant is able to flower it remains in a juvenile state, unable to flower, regardless of the environmental conditions. The duration of the juvenile state can range from one day in some annuals (Cumming, 1959) to many years in forest trees (Hackett, 1985). The juvenile stage in most annual crops is days or weeks (Swiader et al., 1992). In Brassicas, for example, because many plants in this genus are induced to flower by cold in the adult state, a juvenile phase of weeks or months permits greenhouse grown transplants to be set out into the field early in the season when the chance of cold nights is greatest (Friend, 1985). Once beyond the juvenile stage if those same plants experienced a cold night all might flower. The morphological conditions which signify the end of juvenility range

from a minimum number of leaves, 16 leaves for cauliflower and brussels sprouts, to a minimum stem size, five cm in cabbage. In many cases, a minimum number of leaves or nodes along the stem is the criterion listed as determining the end of juvenility (Bernier, 1983). Sadik (1967) found that early cauliflower varieties had shorter juvenile periods than did late varieties. In these cases, longer juvenile phases help prevent losses to early flowering. In cauliflower, the duration of juvenility is determined primarily by growth rate, the speed at which the new leaves are produced, and genotype (Atherton et al., 1987)

In fruit trees, however, early flowering is greatly desired and shorter juvenile periods are sought. In pome fruits, the formation of flower initials in the buds can only occur after a certain number of vegetative nodes have been laid down, about 16-20 nodes (Gur, 1985). If the vegetative leaves are produced faster, then flower buds can form sooner. Besides the inability to flower, the juvenile stage is best known for its rapid growth rate, probably due to lack of competing meristems (Leopold and Kriedemann, 1975). Regardless of the causes, eventually the plant passes out of the juvenile stage into the adult vegetative phase and often simultaneously directly into the reproductive phase. If the plant is in an inductive environment when it leaves the juvenile phase then it may proceed directly into the reproductive phase, making the transition inseparable. Inductive environmental conditions which have the most effect on adult vegetative plants are photoperiod and temperature. Robinson and Wareing (1969) believed that the meristem itself must undergo a minimum

number of mitotic cycles before the juvenile stage can be complete.

PHOTOPERIOD

Photoperiod, that combination of light and dark in any 24 hours, varies throughout the year in accordance with the season and many plants are highly responsive to its slow daily changes. As a tool towards investigating the floral responses of plants, it has produced a staggering amount of information over the last 75 years. Because flowers must come before almost all seeds and fruits, mankind's basic foodstuffs, the manipulation of flowering has had a substantial economic thrust. The flowering process has been the topic of recent books (Atherton, 1987; Bernier et al., 1981; Halevy, 1985; Hillman, 1963; Jordan, 1993;) and review articles (Bernier, 1986, 1988; Bodson, 1984; King, 1983; McDaniel et al., 1992; Sachs and Hackett, 1983; Sussex and Kerk, 1990; Zeevaart, 1976, 1984). Garner and Allard (1920) recognized three classes of photoperiodic responses: short-day plants (SDP), long-day plants (LDP) and indeterminate plants. Some examples of SDP are ragweed, soybeans, Poinsettia and cocklebur; all flower in the late summer and autumn as the hours of daylength wane. Some LDP are spinach, plantain, and chicory; these flower as the daylength increases with oncoming summer. Nearly 20 years after Garner and Allard published their paper on photoperiod responses, Hamner and Bonner (1938) reported that it was the length of the night that was the determining factor in plant response, not the length of day. Since then, research has shown that pigments, known as phytochromes, in the mature leaves are receptors of light and are able to perceive time through a chemical process

(Vince-Prue, 1983). Since chicory is known to be a LDP, some interactions between the length of day (and night) and the plant responses must be operating.

MERISTEMATIC TRANSITION

During the vegetative phases of plant growth the apical meristem remains in an iterative mode, producing new leaves and axillary buds, over and over again. The apical meristem (a Greek word meaning 'to divide') is a region of approximately 1000 cells (Medford,1992) overlain by dividing epidermal cells. It is at this meristem that the entire life of the plant shoot is made. The meristem cells not only make new organs, e.g. leaf primordia, but also continue to create new identical members of their own kind. The passage from juvenile to adult vegetative, a condition of the meristem when floral induction is possible, implies that once the adult stage is reached either 1) a shift in the receptiveness of the meristem cells to be transformed has been accomplished, or 2) a modification of the leaves to accept the environmental flowering cues has happened, or 3) some change in the internal balance of PGRs and/or nutrients is in force since something is known to happen at the meristem, namely floral induction, that could not when the meristem was juvenile.

At the shoot apex of *Rudbeckia hirta* (Asteraceae) a morphological change of the meristem can be seen within a few days of inductive flowering treatments (Harkess and Lyons, 1994). Normally, the vegetative meristem of *Rudbeckia* is a flattened, slightly concave growing point deeply covered by new unfolding leaves. Floral induction changes the shape of the entire region to one

of a bulging hemisphere, convex and pushing up through and beyond the leaf primordia. Figure 2 is a drawing showing examples of apical regions and highlighting the differences in size between the meristem itself and the shoot tip. Not all plants show an identical response, as some are convex even when vegetative but many in the Asteraceae are of the former kind.

The 1000 cells of the meristem occupy a minute volume and the meristem is extractable only with the greatest difficulties. Yet it is these cells only which are the (probable) receptors of any floral signal emanating from the leaves. It is known that the leaves make and transmit a floral signal to the meristem (Bernier, 1988) but the nature of that signal remains unknown. The elusive nature of the signal is partly due to the size of the receptor and to the fact that once received the signal may stop coming.

FLORAL SIGNALS

Early studies (see Leopold and Kriedemann, 1975) on meristem transition to the reproductive state eventually revealed the nature of photoperiodism operating by means of leaf phytochromes, the transmission of a signal to the meristem through the vascular system, and some of the post-induction biochemical and cellular responses of the apical shoot tip. Generally two possible genetic responses to the floral signal have been considered: 1) the floral signal activates specific dormant genes (so called 'flowering genes') which are responsible for the initiation and continuation of the reproductive activities (Wellensiek, 1977), or 2) something internal is preventing the floral genes from being active or dominant over the vegetative genes.

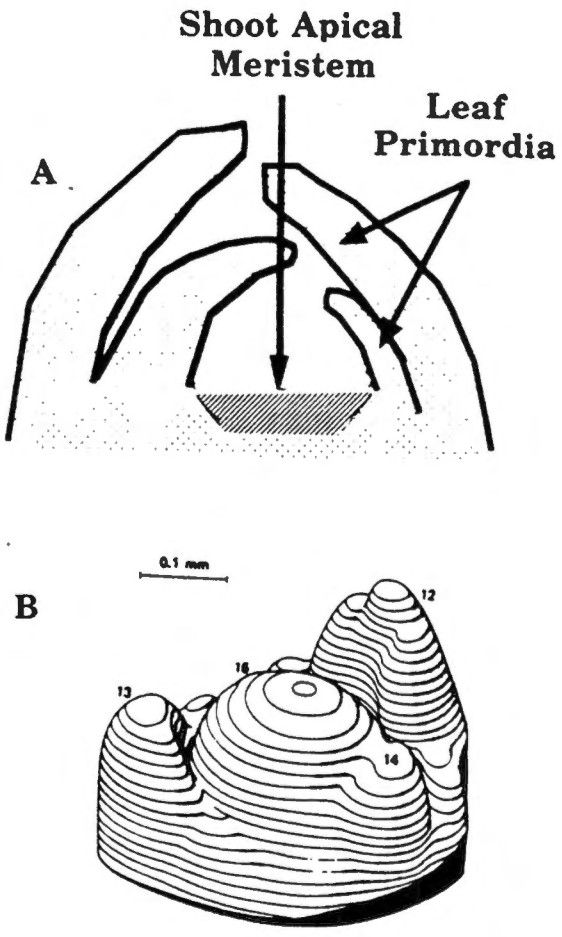

FIGURE 2. Longitudinal and three dimensional drawings of shoot apical regions.

Figure 2A depicts a longitudinal section of an entire shoot apex; the shoot apical meristem is represented by the denser, shaded area. Such a depiction is consistent with the vegetative meristem of *Cichorium intybus* L. and other genera in the Tribe Cichorieae of the Family Asteraceae.
Figure 2B is a three-dimensional drawing of a typical vegetative shoot of the Family Fabaceae. The size, as noted by the 0.1 mm bar, is consistent with reports of other shoot tips. The small numerals identify expanding leaf primordia with the lowest number the oldest leaf. This vegetative meristem region is domed and the primordia rather erect, contrary to the condition found in chicory, e.g., Figure 2A. This drawing, Figure 2B, may bear a resemblance to the induced floral state of chicory.
Sources: Figure 2A modified from Medford, J. I. 1992. Vegetative apical meristems. The Plant Cell 4:1029-1039.
Figure 2B from Dale, J. E. and F. L. Milthorpe. 1983. The growth and functioning of leaves. Cambridge Univ. Press, Cambridge.

Sachs and Hackett (1983) make the case that the latter situation is the one which opens more possibilities of inter-relationships between various plant organs and can help explain the lack of progress experienced in isolating any specific floral 'hormone'. In place of any specific induction chemical, Sachs and Hackett (1969,1983) postulated their 'nutrient diversion' theory which states that an internal modification of the source:sink relationships alters the levels of soluble sugars reaching the shoot tip, thus making induction possible. This scheme recognizes the competition between various meristems, both shoot and root in the plants and the sum of the photosynthetic area supplying the sugars. This line of thought mirrors the arguments brought forward by Trewavas (1986) who maintained that since 'in biology we deal with the most complex situations known' any model dependence on anything less than a 'metabolic network behavior' is incompatible with the vagaries of nature. Bernier (1988) agrees in principle with Sachs and Hackett but argues for a 'multifactorial control' which includes carbohydrates, PGRs, PGR antagonists, and other unidentified chemicals working in unison throughout the plant.

Although the idea of a 'florigen' (flower-maker) hormone, as postulated by Chailakian (cited in Hillman, 1969) has proven attractive, partly because it arose at a time of high research interest in other hormones, such as auxin, and because it offered a simple solution to a complex biological situation, to date there has been no evidence that any one chemical, either endogenous or externally applied, can induce all plants to flower. Although GA can, at times, induce flowers on some plants, nothing resembling a universal flowering

hormone has been found to date (Poethig, 1990). Auxins, abscisic acid (ABA), ethylene, and cytokinins have all been shown to be, mostly, inhibitory to flowering (Cleland and Ben-Tal, 1983).

Two recent candidates, phenolamides and polyamines, have appeared as floral signals, both reduced nitrogen compounds. Martin-Tanguy et al. (1984) reported that an accumulation of phenolamides is linked to the induction of flowering of *in-vitro* chicory (*C. intybus* L.) buds. Kaur-Sawhney et al. (1988) later reported a correlation between high spermidine (a polyamine) levels and floral bud initiation in tissue cultured tobacco. Following those reports, Harkess et al. (1992) measured polyamines (free putrescine and spermidine) in mature leaves of induced and vegetative plants of *Rudbeckia hirta* L. They found a significant increase in polyamines during floral initiation and concluded that these nitrogen compounds may form part of the floral stimulus, especially since polyamines are known to be phytochrome mediated (Dai and Wang, 1987).

Carbohydrates, especially soluble sugars, have been linked with flowering and fruit development since Kraus and Kraybill (1918). Sachs (1987) has outlined the considerations that soluble sugars are definitely involved in the floral transition and maintenance of the floral state: 1) plants need more light to flower than to continue vegetative growth, 2) flowering plants often revert to vegetative when placed in low light conditions, 3) simple sugars, glucose, fructose or sucrose can (sometimes) substitute for photoperiodic requirements, 4) more soluble sugars are needed *in vitro* to promote reproduction than is needed to

continue vegetative growth and, 5) transition meristems have been found to have increased concentrations of soluble sugars. The overall premise of Sachs and Hackett (1969,1983) is that once sugars at the apical meristem are increased floral induction is promoted. Any factors which tend to limit the supply of carbohydrates to the meristem tend to slow the transition to the reproductive state and any agents or factors which restrict or remove competing sinks tend to promote floral initiation (Bernier, 1988).

Although many authors concede that soluble sugars may be involved either during or after floral transition recent items of evidence suggest that sugars alone cannot be responsible for floral induction. Jones (1990) showed that only small, insignificant carbohydrate changes were found in the meristem of red clover during the onset of flowering. He did not rule out entirely the involvement of carbohydrates in a more complex signal but believes the evidence tends to weigh against floral carbohydrates being the sole controlling factors in flowering, as suggested by Sachs and Hackett (1983).

In ryegrass (*Lolium temulentum* L.), King and Evans (1991) reported that an increase in sucrose, the major sugar found in the apical tissue, was not required for floral induction, although induction was highly responsive to more sucrose. Working in the same laboratory, McDaniel et al. (1991) reported that despite the fact that sucrose is taken up rapidly by excised induced shoot apices of *L. temulentum* L., the requirement for a LD floral stimulus is absolute.

Pulling these two avenues of possibilities, namely high sugars and reduced nitrogen at the apex, into a cohesive theory of floral control was initiated

by Raper and his group. Raper et al. (1988) reported on the responsiveness of tobacco induction by an imbalance in the relative availabilities of sugars and reduced nitrogen at the shoot apices. They found the periods of floral transition were not correlated with size, soluble sugars or reduced nitrogen but rather floral initiation was associated with those periods when levels of either one or the other of these nutrients was reduced at the apex. They concluded that the demands of competing sinks regulate the availability of these nutrients for translocation to the apex, thus influencing floral transformation. Working in Raper's laboratory, Rideout et al. (1992) correlated microscopic evidence of tobacco meristem transition with inequalities in soluble sugars and/or free amino acids at the apices. When rates of assimilate transported to the apex remained parallel, the changes in the pools of nutrients at the apex stayed identical, and vegetative development was maintained. When the partitioning of the two nutrients diverged due to external stress or internal ordering, the resulting imbalance in the pools of nutrients at the meristem promoted floral transition. In essence, when a constant proportion of nutrients is maintained at the meristem, vegetative growth continues. When the relative proportion of nutrients is either increased or decreased, the meristem is unable to sustain vegetative growth and floral development is stimulated as a default state. This proposal has been termed the 'modified nutrient diversion' theory by Raper to account for the nitrogen components which have been added to the 'nutrient diversion' scheme of Sachs and Hackett.

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PART II

LONG DAY PHOTOPERIODS, TRANSITION TO THE FLORAL STATE AND BIOCHEMICALS IN THE SHOOT APICES OF 'DALIVA' CHICORY

INTRODUCTION

Perennial plants flower only after they establish a photosynthetic or storage capacity which is able to support the energy demands of flowers and developing fruits, a decided adaptive advantage. During this establishment, known as the juvenile stage, the plant is unable to respond to the environmental cues which induce floral transition. Some perennials can achieve sufficient size in a few months whereas others (e.g. forest trees) require many years. Witloff chicory (*Cichorium intybus* L., Asteraceae) is a perennial plant grown for the first year root and subsequent forcing of the apical vegetative bud for edible heads. All chicory cultivars require long days for flowering (Kalloo, 1993; Paulet, 1985). Some chicory cultivars are capable of flowering the first year, these are known as 'early' cultivars and some genotypes are obligate in their demands for vernalization, these are known as 'late' cultivars (Paulet, 1985). The duration and measurement of the juvenile state in 'early' chicory cultivars is a concern for growers since plants which depart the juvenile stage can readily bolt and thus lose their value. The 'extra early' chicory cultivar 'Daliva', although having many valuable attributes (Nunhems, 1994) is known to bolt early, especially when planted before approximately 15 June, in either Connecticut (Hill, 1987, 1988) or Tennessee (MacDermott, 1993).

The achievement of a mature developmental stage in long-day plants can be shown by rapid floral initiation when the plants are placed in floral inducing

conditions, i.e. long-days. One correlative measure of maturity in long-day plants is the number of leaves expanded prior to maturity as this reveals previous environmental conditions such as temperature, light and water availability. For example, in the annual Asteraceae *Rudbeckia hirta,* Orvos and Lyons (1989) found that, based on the least number of inductive cycles needed to induce flowering, 12 leaves was the minimum number needed to describe the mature stage. Once the mature stage is reached, the proper floral inducing treatment will permit the generation and transmission of a signal enabling the meristematic cells to shift from the iterative production of vegetative organs, leaves and axillary buds, to the production of radically different, and often terminal, floral organs. This shift at the *Rudbeckia* meristem was microscopically visible within four days after the inductive cycles began. Many genera in the Cichorieae, including lettuce (Constantin and Mullenax, 1966), hawkweed (Yeung and Peterson, 1972) and dandelion (Rauh and Rappert, 1954) have a flattened, slightly sunken vegetative apical meristem but once induced to flower the meristem assumes a convex, hemispherical shape.

In photosensitive plants the floral signal, as received by the meristematic cells when the plant is placed in an inductive long-day environment, has been the object of substantial research. What is known thus far is that the mature leaves perceive the duration of the daylight and night hours through the phytochrome pigment system and transmit a diffusible substance from the leaves to the meristem via the vascular connections, usually thought to be traveling with the assimilates in the phloem (Bernier, 1986; Vince-Prue, 1983).

The nature of the diffusible substance has remained controversial for a long time and, as yet, is not defined. Among the possible candidates for the role of chemical signal are the soluble sugars and the soluble, reduced forms of nitrogen since both originate in the leaves, both are highly mobile in the phloem stream, and both have been implicated in the initiation and development of flowers.

Kraus and Kraybill (1918) established a positive relationship between the flowering response and the carbohydrate and nitrogen nutrition of the leaves, but their work did not address whether any changes at the meristem were preceded by causal variations in sugars or nitrogen levels was not addressed. In their work, Kraus and Kraybill noted that the ratio of carbohydrates to nitrogen increased as the photoinduction period progressed, suggesting more carbohydrates reaching the apical bud. Over the years several observations have reinforced and modified the theory that the supply of carbohydrates greater than that needed to maintain vegetative growth is required to initiate floral evocation, not merely to promote floral growth as Kraus and Kraybill found. Sachs (1987) outlined five known considerations: 1) greater radiant energy is needed for reproductive than continued vegetative growth, 2) when placed in low irradiance levels flowering plants often revert to the vegetative, 3) simple sugars, sucrose, glucose or fructose can (sometimes) substitute for photoperiodic requirements, 4) *in vitro* cultures promoting reproductive effort require more sugars than those promoting vegetative development, and 5) transition meristems have been found to have increased concentrations of soluble sugars

and associated enzymes.

The nutrient diversion theory of floral initiation of Sachs and Hackett (1969, 1983) proposed that carbohydrates are the primary controlling agents in flowering and that floral initiation is promoted if carbohydrates at the apical meristem are increased, but inhibited if they are decreased. This theory recognizes that the mature leaves, as sources of assimilates, and the sinks, regions of high metabolic activity, principally the shoot and root meristems and developing leaves, are in continued dynamic equilibrium. Any internal or external factors which keep the supply of carbohydrates to the apical meristem limited, whether by competing sinks or restricted sources, tend to slow the meristematic transition to the reproductive state. Agents or factors which restrict or remove competing sinks tend to promote floral initiation (Bernier, 1988).

Other authors have assigned carbohydrates a lesser role. Cleland and Ben-Tal (1983) argued since rapid development ensues once evocation has occurred, it is logical to expect that an increased supply of assimilates to the apical meristem is a normal change. These authors maintained that hormonal flowering control at the apex results in a stronger sink signal or greater source strength, thus accounting for any assimilate increases after evocation. Likewise, Bernier (1988) was reluctant to abandon plant growth regulators, (PGRs) or relegate them to an inferior role, in any discussion of floral control. He proposed that several factors, including PGRs, PGR antagonists and various endogenous assimilates provide a more sophisticated and inclusive theory of floral evocation.

Recent items of evidence that carbohydrates are not solely responsible for

evocation are presented by Jones (1990) who showed that changes in carbohydrates at the apex were not associated with the onset of flowering in a red clover (*Trifolium pratense* L.) mutant, although he does not rule out possible carbohydrate involvement in a more complex signal. Additionally, King and Evans (1991) found that floral evocation in ryegrass (*Lolium temulentum* L.) did not require an increase in sucrose at the apex, although inflorescence development was highly responsive to it. Both these lines of work suggest that although carbohydrates may be part of the evocation signal, they alone cannot be totally responsible.

Raper et al. (1988) proposed that floral transition in tobacco (*Nicotiana tabacum* L.) is stimulated by an imbalance in the relative concentration of carbohydrates and reduced nitrogen in the apical meristem, not an absolute amount of either. By ranking stem apices before, during and after floral inductive treatments and measuring soluble carbohydrates and reduced nitrogen in those apices and the leaves which serve them, they determined that floral initiation was associated with growth intervals when either soluble carbohydrates or reduced nitrogen concentrations fluctuated outside of previous bounds, thus upsetting the relative balance of the nutrients. The principal conclusion reached was that vegetative development is maintained as long as the supplies of soluble carbohydrates and reduced nitrogen remain in a constant proportion at the apical meristem. However, once the relative proportion of either shifts up or down, the meristem can no longer support vegetative growth and floral development is

stimulated as a default state. A second paper from the same laboratory (Rideout et al., 1992) implied very similar conclusions. By growing plants under regimes of temperature and nitrogen stress and observing shifts in soluble sugars and free amino acids, Rideout et al. showed that the relative availability of soluble sugars or free amino nitrogen at the apex was effective in promoting floral transition; whereas, concentration stability of those compounds permitted maintenance of the vegetative condition. Depending on the environmental stresses encountered by the plant, various changes in production or transport of assimilates do occur, thus shifting the partitioning and magnitude of the labile pools of carbon and nitrogen at the apex. Raper et al. (1988) and Rideout et al. (1992) believe such a relative imbalance of assimilates, when correlated with observed floral transition, lends support to the nutrient diversion hypothesis of Sachs and Hackett (1969, 1983), although Raper et al. and Rideout et al. used the term 'modified nutrient diversion' to draw attention to the role of reduced nitrogen in the scheme.

The objectives of this experiment were:

1) to determine whether 'Daliva' plants, when grown under non-inductive conditions to a minimum size, at least 15 leaves, can be induced to flower by exposure to long photoperiods,

2) to determine by histological sectioning of the apical shoot tips whether the change to long photoperiods resulted in floral transition,

3) to harvest shoot tips, extract and measure soluble sugars and total free amino acids from the apical shoot tissue.

MATERIALS AND METHODS

PLANT CULTURE

Seeds of the F-1 hybrid, extra early chicory cultivar 'Daliva' (Nunhems Seed Co., Lewisville, ID) were sown on two dates, 1 April and 2 May, 1994, into 8 liter (2 gal) pots filled with a 50-50 mix of Fafard ProMix potting media and aged pine bark amended with 130 g pulverized limestone and 60 g of 14-12-14 plus micronutrients Osmocote beads per 72 liters (18 gals). Seeds of the first sowing germinated within seven days in the greenhouse and were kept indoors for five weeks at which time all pots were removed to an outdoor cold frame fitted with a tight fitting, yet removable, opaque 'Mum' cloth (Hummert's, St.Louis, MO). The second planting germinated outside and after four weeks was placed under the opaque tent. Outdoors, the opaque covering was pulled over, but not touching, the plants from 1800 hours to 0800 hours, for a 14 hour dark period daily. The pots were thinned to approximately six plants each and all pots received a 60 g portion of Osmocote beads after thinning and were otherwise treated identically, except for a whitefly infestation late in the season on the second block which required insecticidal treatments. The photoperiod regime of 14 hours dark per day continued throughout the summer. The temperature beneath the cloth was monitored and at no time exceeded 35° C.

HARVEST PROCEDURES

The first harvest for the 1 April planting was made on 21 August (143

Days After Planting, DAP) when 13 pots were randomly chosen and the 35 largest plants were selected, rinsed, and placed in ice water. Thirty of these plants were individually washed and leaves greater than five mm in length were removed, counted, chopped, bagged, oven dried and weighed. The roots were measured for maximum diameter, severed about three mm below the crown junction, chopped, bagged, oven dried and weighed.

At the apex, any remaining leaves from one to five mm were removed under magnification (#10 Optivisor, 3.5X, Lenora KS). The apical shoot tip, an approximate cube one mm on a side was excised and dropped into a thermos of liquid nitrogen. All apices were kept together in the thermos, thus pooled. They were transferred to a ultra cold (-80° C) freezer, and later lyophilized. The freeze dried tissues were heated to 60° C for 120 minutes to inactivate enzymes. Leaching of soluble sugars and free amino acids followed the protocol of Thomas (1990). To each 25 mg of freeze dried tissue, three ml of HPLC grade methanol was added and kept at 0° C for 24 hours. An additional two ml of HPLC grade water was then added and also kept at 0° for 24 hrs. The liquor was drawn off and kept at -18° C.

For total free amino acids measurement, 10 ul of the liquor was placed in a micro-reaction vial and allowed to react with 20 ul fluoraldehyde (Pierce Chemical Co., Rockford, IL) for exactly 60 seconds at which time the reaction was terminated with an additional 50 ul of HPLC water. A 25 ul loop on an ISCO 2550 HPLC (ISCO Co., Lincoln, NE) pump was filled with the now derivatized amino acid mixture and the contents of the loop were pumped through a Waters

Model 24-C fluorescence detector (Cambridge, MA) fitted with a 334 nm excitation filter and a 440 nm emission filter. The results obtained were the quantity of total free amino acids as leached from the pooled liquor of 30 apical shoot tips, as compared to standards (Sigma Chemical Co., amino acids standard #18) drawn under similar analytical conditions. Three separate observations of each sample were taken and the means are presented as mg per g dry weight (DW). Additional information on the chemistry of derivatization, detection and quantification of amino acids is given in the appendix.

Soluble sugar detection and measurement from the liquor was established through the use of an ion chromatograph equipped with a Pulsed Amperometric Detector (PAD) and a Carbopac PA-1 anion chromatography column required to separate glucose, fructose and sucrose from other sugars. Twenty ul of the liquor was added to 480 ul of HPLC grade water and this 0.5 ml of sample was analyzed in a Dionex Model 230 ion chromatograph. The 0.5 ml sample was internally divided in half and two observations from the same sample were taken. The means of the two observations are presented as umol sugar per g DW. All data and displays were carried out with Dionex software. Sigma Chemical Co. analytical sugars were used to establish standards for each and all sugars at various concentrations and used as comparisons to the sugars in the pooled liquor. Additional information on the principles of sugar separation and PAD detection are given in the appendix.

In this experiment a total of six harvests per planting date were made. Each harvest was of 13 randomly selected pots, and from those pots the 35

plants with the largest roots were chosen. Thirty of the 35 went for apical tip harvest and five of the 35, randomly chosen, went for histological examination. The first two harvests, Table 1, represented control plants, i.e. baseline groups devoid of any short night treatments. The third harvest occurred one day after the plants were taken into the greenhouse and placed one meter below a double string of 12, 60 Watt incandescent light bulbs fitted with light-directing shades. On the first, and each succeeding night in the greenhouse, the indoor plants received an additional six hours of light from the bulbs above them, from 2000 hrs to 0200 hrs, thus creating a photoperiod greater than 13 hours. Plants harvested from the third, fourth, fifth and sixth harvests were treated exactly as those from harvests one and two, described above. On the first Day After Treatment (DAT) harvest three was collected. The total collection schedule is given in Table 1.

HISTOLOGICAL SECTIONING

At each harvest five plants were randomly chosen from the selected 35 for histological observation of the meristem itself. A section of crown tissue containing the meristem and surrounding tissue (about three mm on a side) was dropped into HistoChoice fixative (Amresco Co., Solon OH), cleared in a series to 100% of isopropyl alcohol, embedded in PariPlast paraffin, sectioned on a Reichert-Jung retracting microtome set at 10 um and stained through the wax with alcian blue (IBA Chemicals, Stain #456, Cleveland OH) in 5% benzoate buffer (Graham, 1994). The wax was removed with Micro Clear clearing agent (Micro Environmental Industries, Fairfax VA) and a cover slip was affixed with

Table 1. Harvest collection schedule of plants grown under controlled photoperiods.

	PLANTING DATE					
	1 APRIL			2 MAY		
	DATE	DAP[Z]	DAT[Y]	DATE	DAP	DAT
HARVEST						
1	21 AUG	143	-8	20 SEPT	143	-6
2	28 AUG	150	-1	22 SEPT	145	-4
3	30 AUG	152	+1	27 SEPT	150	+1
4	1 SEPT	154	+3	29 SEPT	152	+3
5	5 SEPT	159	+7	4 OCTO	157	+8
6	12 SEPT	166	+14	11 OCTO	164	+15

[Z] DAP= Days After Planting
[Y] DAT= Days After Treatment (the imposition of long-day photoperiods)

Eukitt fixative. The sections were observed through a Zeiss microscope (model 232, Buffalo, NY) and photographs were taken with a Nikon camera and Kodak film. Shoot apices from all harvests were prepared in a similar manner.

RESULTS AND DISCUSSION

SIZE OF PLANTS COMPARED.

Plants harvested from the early planting date consistently exceeded 20 leaves per plant over the six harvests and exceeded ($P<0.01$) the leaf number from the second planting date at every harvest (Table 2). Plants from the second date averaged between 12.6 and 15.2 leaves per plant, depending on harvest. In addition to leaf number, differences were found between planting dates, at every harvest, in the other plant variables measured. Leaf dry weights from the early planting date ranged from 18.6 g to 21.3 g and differed ($P<0.01$) from dry weights of the second date which ranged between 12.8 g and 16.4 g (Table 2). Between planting dates, root diameter and root dry weights showed slightly closer values than those of the leaves, but differences between root diameter and root dry weight existed ($P<0.01$) at every harvest date (Table 3).

These results indicate that plants sown on 1 April were larger than those sown a month later, despite closeness of age at various harvest dates (Table 1). These differences suggest substantial variation in growing conditions through the summer and it is theorized that such differences were the result of: 1) crowding of plants in the structure which caused excessive shading of the younger plants, 2) the whitefly infestation on the second planting date, and 3) diminishing daylength and shade from nearby buildings late in the season.

Table 2. Harvest means and standard errors of leaf number and leaf dry weights by planting date.

	LEAF NUMBER		LEAF DRY WEIGHT, g.	
	PLANT DATE		PLANT DATE	
	1	2	1	2
HARVEST NUMBER				
1	20.6±0.6	12.7±0.4*	20.5±0.7	14.3±0.3*
2	20.8±0.8	15.2±0.4*	21.3±0.6	16.2±0.4*
3	21.7±0.9	13.8±0.9*	19.3±0.8	16.4±0.6*
4	21.2±1.0	13.3±0.6*	19.2±0.8	14.1±0.3*
5	20.3±0.6	12.6±0.5*	18.6±0.5	13.1±0.3*
6	21.4±0.8	13.3±0.5*	19.3±0.6	12.8±0.3*

Means (n=30) between columns at each harvest number followed by * differ ($P<0.01$).

Table 3. Harvest means and standard errors for root diameter and root dry weights by planting date.

	ROOT DIAMETER, mm		ROOT DRY WEIGHT, g.	
	PLANT DATE		PLANT DATE	
	1	2	1	2
HARVEST NUMBER				
1	18.7±0.6	17.0±0.4*	25.7±1.4	21.7±0.6*
2	20.5±0.7	18.6±0.4*	29.4±1.5	24.8±0.8*
3	21.7±0.7	18.0±0.5*	30.8±1.4	24.2±1.3*
4	22.6±0.6	17.6±0.4*	34.5±2.0	22.1±0.7*
5	21.2±0.5	18.6±0.4*	30.8±1.6	24.0±1.9*
6	22.3±0.6	18.8±0.4*	31.7±1.4	25.4±1.1*

Means (n=30) between columns at each harvest number followed by * differ ($P<0.01$).

HISTOLOGICAL EVIDENCE OF FLORAL TRANSITION.

Although plants from the first planting date had more than 20 leaves prior to the initiation of the long photoperiod treatments, no histological evidence was found that any plants experienced a transition to the floral state. All examined apical shoot tips, 30 in total, collected over the course of a five week harvest period, were vegetative and identical in morphology to that shown in Figure 1. This same histological condition, vegetative only, was also found in all shoot tips harvested from the second planting date. Since plants from the second planting date were significantly smaller in all respects, floral induction in these plants was not expected since plant size was used a correlative measure of maturity or 'ripeness-to-flower' and plants from the second date were too small.

In conclusion, the results indicate that plants grown under these conditions either remained juvenile and did not reach 'maturity' and were thus unable to respond to the photoperiod treatments or did not receive a proper initiating stimulus from the photoperiod regime.

TOTAL FREE AMINO ACIDS FOUND IN SHOOT TIPS

The total free amino acids leached from the shoot tips of the early planting ranged between 15 and 30 mg per g (DW) (Figure 2). Figure 2 shows a peak immediately following the imposition of long day treatments, harvest three (+1 day), with a subsequent decline and eventual stabilization over the next three harvests. The lines connecting the points merely display a possible trend over the time of the harvests, a total of 22 days. Too few data points, six only, are available to warrant fitting a curve to the connected points.

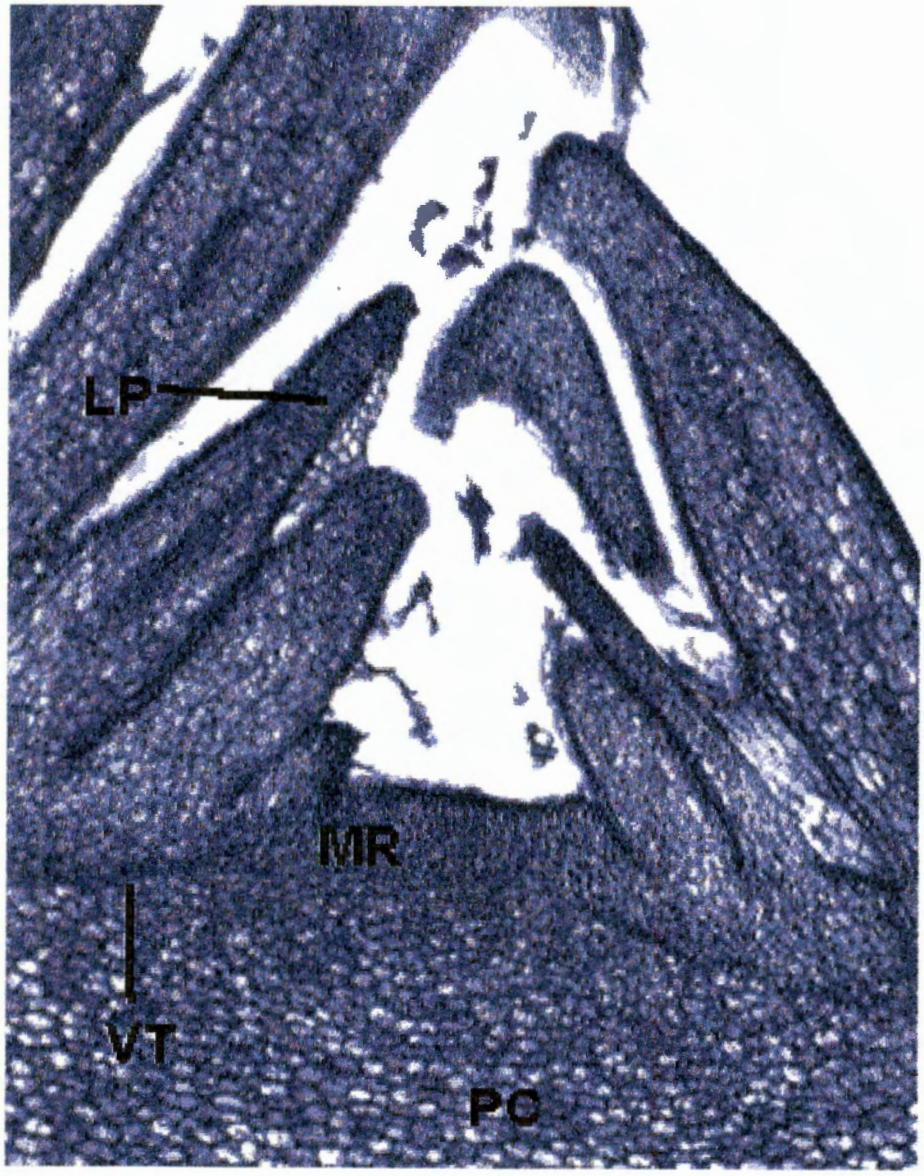

FIGURE 1. Microphotograph of 'Daliva' chicory vegetative apical region.

Longitudinal section, at 40X, of 'Daliva' chicory apical region showing newly expanded leaf primordia (LP), tightly packed meristem cells in the flattened meristem region (MR), and larger, vacuolated pith cells (PC). Also visible are the vascular traces (VT) leading horizontally out from the meristem region.

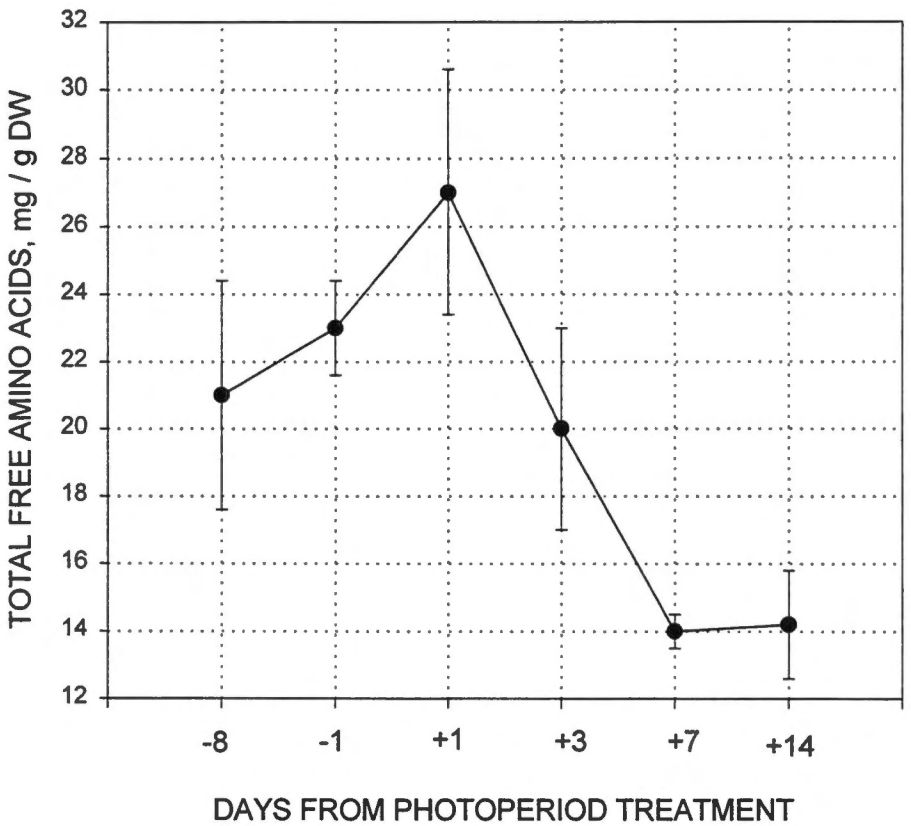

FIGURE 2. Total free amino acids in chicory shoot apices harvested from planting date 1 April.

Total free amino acids in apices of 'Daliva' chicory plants, over 6 harvests, before and after long day photoperiod treatments. Data points represent the mean, with error bar, of three observations from one pooled sample of leachate of 30 apical shoot tips.

Figure 3 displays the amino acids leached from apices of the second planting, with totals also ranging from 15 and 30 mg per g DW. These data points show a steep decline between the first and second harvests with a reversal in direction immediately after the imposition of the long day photoperiod treatment. The upwards trend continues over the next three harvests and finally another decline is seen. The connecting lines of Figure 3 display a different picture from Figure 2, and no curve has been fitted here for the same reasons. The error bars in both figures indicate wide variation between the three observations of the same sample and reflect the sensitivity of the method and difficulties in fluorescence detection experienced by this author.

Others have pointed out the dynamic nature of the nitrogen supplies at the apical meristem which represents a balance of an import-export plus synthesis-degradation complex and which may vary considerably during the day (Rivjen and Evans, 1967). Although the harvest protocol required all plants be harvested 'at the same time', the actual harvest of the 13 pots, selection of the preferred plants and getting them into the ice bath did take some time, often more than an hour from beginning to end. Once in the ice bath one might expect certain enzymatic reactions in the meristem and leaves to slow more than others (as the cold penetrates) thereby influencing the nitrogen balance, but this research did not address those questions. These considerations may have accounted for some of the variation in amino acid levels between harvests and planting dates. The quantities of total free amino acids measured, 15-30 mg per g DW, are in close agreement with those in the meristematic leaf bases of the

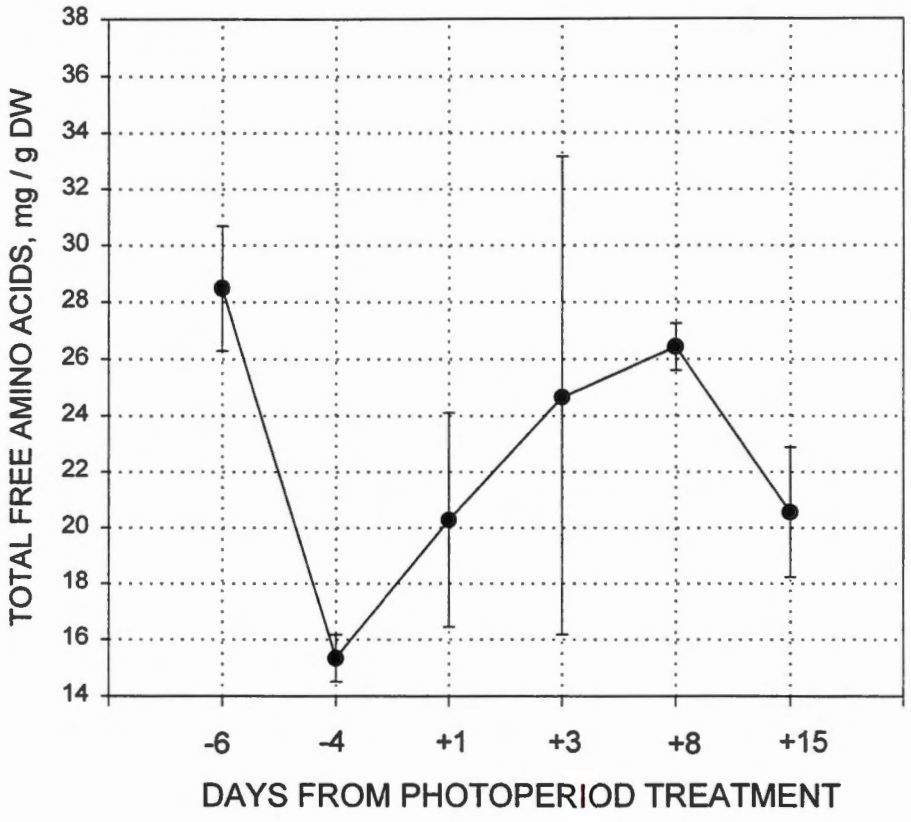

FIGURE 3. Total free amino acids in chicory shoot apices harvested from planting date 2 May.

Total free amino acids in apices of 'Daliva' chicory plants, over 6 harvests, before and after long day photoperiod treatments. Data points represent the mean, with error bar, of three observations from one pooled sample of leachate from 30 apical shoot tips.

grass *Lolium perenne* L., as detected by the ninhydrin method and reported by Thomas (1990, 1991).

SOLUBLE SUGARS IN SHOOT TIPS

Figure 4 traces the course of the three measured sugars, glucose, fructose and sucrose as leached from the shoot tips at each harvest from plants sown on 1 April. Individual sugars ranged between 25 and 85 umol sugar per g DW, with total values between approximately 200-400 umol sugars per g DW. In Figure 4, glucose and fructose, both monosaccharides, can be seen to follow (roughly) similar declines till the fourth harvest (+3 days) then both rebound. Sucrose concentration marks a steady upwards course over the initial four harvests, then drops to its lowest values at harvests five and six.

The graph shows no evidence that the photoperiod treatments, starting at harvest 3 (+1 day), had any effects on any of the measured sugars. However, one can see a possible inverse correlation between the disaccharide sucrose, (composed of glucose and fructose) and the two monosaccharides. As the sucrose level falls the levels of glucose and fructose tend to rise, which may be indicative of the breakdown of the disaccharide into its simple components.

The sugars from planting date two (Figure 5) display an even closer tracking of the monosaccharides as the levels of glucose and fructose follow a very similar path through the six harvest dates. The most conspicuous peak is the sharp decline between harvests four and five (+3 and +7 days) with an equally dramatic rebound at the sixth harvest. In Figure 5, the sucrose values vary but slightly from the others until the fifth and sixth harvests when the

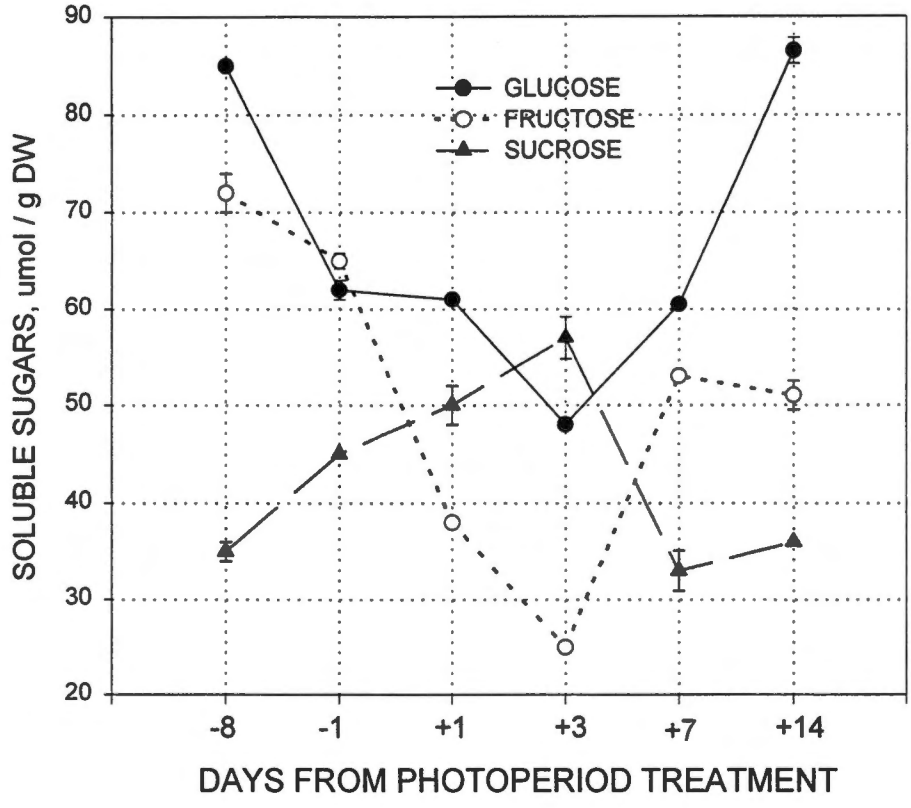

FIGURE 4. Soluble sugars leached from chicory shoot apices harvested from planting date 1 April.

Soluble sugars, glucose, fructose and sucrose, leached from shoot apices of 'Daliva' chicory plants, over six harvests, before and after long day photoperiod treatments. Data points represent the mean, with error bar, of two injections of one observation from one pooled sample of leachate of 30 apical shoot tips.

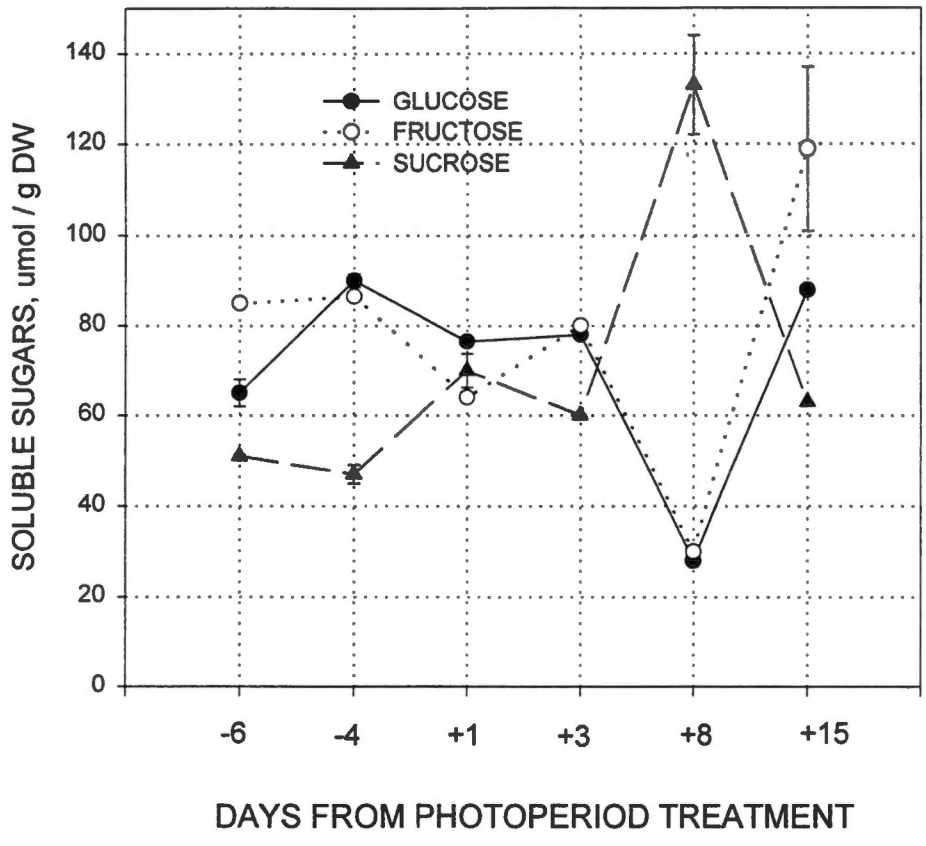

FIGURE 5. Soluble sugars leached from chicory shoot apices harvested from planting date 2 May.

Soluble sugars, glucose, fructose and sucrose, leached from shoot apices of 'Daliva' chicory plants, over six harvests, before and after long day photoperiod treatments. Data points represent the mean, with error bar, of two injections of one observation from one pooled sample of leachate of 30 apical shoot tips.

sucrose rises steeply and then falls, a nearly perfect inverse picture of the pattern displayed by glucose and fructose.

As stated above in the section on amino acids, too few data points are available to warrant fitting curves for either planting date and the plant size variation between planting dates may have caused biochemical differences unrelated to the photoperiodic treatment, thus rendering comparative results unreliable.

As seen in Figures 4 and 5 the error bars for soluble sugars are, in most instances, very small, especially when compared to total free amino acids. This is due to the nature of the internal sampling techniques from one vial, automated equipment and troubleshooting expertise of Mr. Terry Walker, Research Associate, Department of Food Science and Technology.

CONCLUSIONS

The primary objective of this research was based on the premise that pot grown 'Daliva' chicory plants could be induced to flower by long day photoperiod treatments once the plants had reached a certain size, namely 15 leaves. However, no plants flowered even though many had more than 20 leaves. The most likely reason for this was that plants with even 20 leaves remained juvenile. Had the plants grown larger, to perhaps 30 leaves, the results may have been different. The root diameter of the largest pot grown plants, 23 mm, was below the minimum root diameter, 33 mm, associated with bolting plants in the previous field study; further suggesting the plants were too small.

The second objective of examining apices by histological sections to determine if floral induction was related to long photoperiods proved to be a valuable technique in observing early events in floral transition. However, no histological evidence was found that any of the sample of 30 large plants (20+ leaves), over three weeks, did shift to the floral state. The long photoperiods themselves were insufficient to induce floral transition and it is theorized that the plants were still in the juvenile stage and unable to respond to the photoperiod treatments.

The third objective, measuring biochemicals from the leached shoot tips and correlating the total free amino acids and soluble sugars with the photoperiod treatments, was inconclusive. Although the size of the plants

differed between planting dates, one may look for comparative trends in the data presented. In the case of the amino acids, the large errors from many of the sample observations implies that the laboratory work was exacting. However, since the total amino acid values detected were in agreement with those of other researchers, this technique of measuring total free amino acids from leached plant material may be useful and lead to further inquiry. The one point in common between planting dates is the elevation of amino acid levels one day after the initial long day light treatments began. This elevation may suggest that the meristem is receiving a 'pulse' of amino acids from the leaves due to the long photoperiod.

The soluble sugars levels are perhaps more reliable due to established procedures but still represent a minimal statistical picture. Fluctuations in the levels of sucrose and its component monosaccharides were observed but if any such movements were due to the photoperiod treatments they were not readily identified.

Medford (1992) discusses the difficulties in excising shoot tips and the near universal failure to recognize the minute size of the meristem itself, only approximately 1000 cells which is at or below the limits of even highly skilled botanical microsurgeons. The consequences of using probably 10 times (even perhaps 100 times) too much tissue, as certainly was used, means that most of the leachate did not represent the meristem itself but the surrounding tiny leaves and the structural and storage pith cells. The overall objectives of answering questions about both floral transition in general and chicory in particular, were

quite broad and led to some unsupported assumptions, particularly the number of leaves indicating the end of the juvenile phase and the number of shoot tips needed for biochemical extraction. These, in turn, led to poor plant performance, lack of replication and no floral transition. Additionally, the lack of knowledge in the practical operation of the fluorescence detector led to high laboratory variation. The positive aspects of successfully applying a simple technique for leaching and measuring soluble sugars in low concentrations, and total free amino acids from plant tissues may lead to additional use of these protocols.

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PART III

VEGETATIVE AND FLORAL SHOOT APICES: MORPHOLOGICAL AND BIOCHEMICAL DIFFERENCES

INTRODUCTION

Many plants have a juvenile phase during which time they are unable to respond to environmental conditions which would otherwise promote flowering. The duration of the juvenile phase is quite variable but in most woody plants it lasts at least a few years and in some forest trees may last decades. Herbaceous plants tend to have shorter juvenile phases, some lasting months, some days and a few have none at all as flower primordia are found in the seed. During the juvenile phase the apical meristem is engaged in the repetitive production of vegetative parts: new leaves and axillary buds at the base of the leaves and supporting structural tissues which form the stem of the plant. The apical meristem is itself a compact, highly compressed group of actively dividing cells, approximately 1000 cells (Medford,1992), located at the tip of the stem. The transition to the adult stage (known as a phase change) centers around the life of the dividing meristem cells which are rendered receptive to certain endogenous physiological signals which are able to reprogram the cells toward floral induction. It has been shown that all the cells in the meristem are actively engaged in vegetative mitosis; no cells wait in reserve for the reception of a floral signal (Jegla and Sussex, 1989), as once thought. The attainment of the adult state is often accompanied by simultaneous reproductive development, a situation which makes the separation of the two events impossible. In many plants, the adult state is correlated with the size of the plant, regardless of time

to reach that size (McDaniel, 1980; Schwabe and Al-Doori, 1973; Werner, 1980).

Rosette plants are herbaceous and grow close to the ground and eventually, perhaps after years, send up a tall flower stalk. During the rosette stage the stem remains compressed and the leaves are crowded around this compressed stem, known as the crown. On the crown the oldest leaves are farthest from the meristem and the youngest leaf primordia grow somewhat over the top of the meristem itself. Biennial plants often grow as rosettes and form a substantial overwintering storage root which supports the rapid growth of the flower stalk and floral organs in the spring. Werner (1980) has shown that in teasel, a rosette plant, flowering will only occur when the rosette has reached a certain minimum size, which is highly correlated with whole plant dry weight.

The chicory cultivars known as witloof (*Cichorium intybus* L., Asteraceae) are cultivated for their forced leafy salad heads and are low growing perennial rosette plants treated in agriculture as biennials. Many chicory genotypes exist which display varied responses to the environment but all chicory cultivars require long days for flowering (Kalloo, 1993). However, not all cultivars require overwintering treatments or vernalization, which is a common trait among biennials as it offers a physiological mechanism to prevent flowering in the late summer when the photoperiod is identical to that found in the late spring. Paulet (1985) lists 'early' and 'late' chicory cultivars which are catalogued by their need for a cold treatment prior to the initiation of flowers. Early cultivars can flower the first season in the field, whereas the late ones require a cold treatment of a few weeks minimum before they will respond to the long day length.

Although the attainment of the adult state implies a 'ripeness-to-flower', it in itself does not ensure that flowering will occur, as the correct environmental conditions must also be satisfied. In those plants which are promoted to flowering by the correct photoperiod, it is generally accepted that the leaves are the organs which measure the duration of darkness through the phytochrome pigment system found in the leaves (McDaniel et al., 1992). Once the cells in the meristem reach the responsive state and the leaves perceive the correct photoperiod, a message or signal, probably chemical (although none yet has been identified), is sent from the leaves eventually to reach the meristem where the response occurs, and at no other site is this signal thought to be meaningful (Sussex and Kerk, 1990).

As the signal arrives at the site of action, the cells at the meristem are genetically reprogrammed to initiate permanent responses leading to the development of the determinate floral axis. Once induced to shift to the production of floral organs, the cells begin a rapid increase in mitosis, with significant needs for amino acids and carbohydrates both to support energy demands and as raw materials for new organelles and especially membranes (McDaniel et al., 1992). These demands, especially for soluble carbohydrates, have been found to increase at the apical bud as early as 12 hours after an induction period (Bodson, 1977). Additionally, a doubling of the rate of activity of glucose-6-phosphate dehydrogenase, an enzyme associated with simple sugars and the production of NADPH for fatty acid membrane biosynthesis has been found to occur 15 hours after an induction period (Auderset et al., 1980).

Although both Bodson (1977) and Auderset et al. (1980) reported on results found only after induction, they both indicated that soluble sugars may play a required role in floral transition as part of the 'floral signal'. Increases in sucrose particularly have been claimed to have a 'message-like role' as an early event in the floral transition of *Sinapsis alba* L. (Lejeune et al., 1993), and cauliflower (Sadik and Ozbun, 1968).

Although metabolic activities of shoot apical meristems increase rapidly soon after photoperiodic induction (Bernier et al., 1981) and include increases in nucleotides into RNA, few reports on reduced nitrogen at the apical shoot tips prior to and during floral transition are in the literature. Rivjen and Evans (1967) found no increase in soluble ninhydrin-positive fractions in the four days after inductive treatments in *Lolium temulentum* L. A recent review article makes no mention of the possible role of free amino acids as physiological floral signals (Bernier et al., 1993).

In addition to the metabolic shifts within the cells, the shape of the entire meristematic region often assumes a new appearance as the morphology of the shoot tip is transformed. This is known to be true in many Asteraceae which when vegetative, display only a sunken, buried or flattened apical region, as found in *Rudbeckia* (Harkess et al., 1992), dandelion (Rauh and Rappert, 1954) and lettuce (Colinson and Mullanax, 1966). Once these plants are induced to flower however, the shoot tip assumes a domed, hemispherical shape (Harkess and Lyons, 1994; Yeung and Peterson, 1972). The doming of the apical shoot

tip has been identified within four days of inductive treatments in the Asteraceae *Rudbeckia hirta* (Orvos and Lyons, 1989). This domed condition is accompanied by an expansion of the pith cells which lie below the meristem and act to elevate the flower stalk. This rapid growth of the flower stalk, known as bolting, is a separate physiological response from flowering, although they are usually associated in time and often seen together. Occasionally, vegetative plants may bolt without flowers.

Substantial effort has been invested in the search for the chemical factors which induce the floral state but none has yet been identified. Among the plant hormones, better known as plant growth regulators, (PGR), the one most closely identified with flowering, and certainly bolting, is gibberellic acid (GA). This PGR often induces dramatic results in flower stalk extension (Sachs et al., 1959) but its role at the meristem is not clear and exogenous applications of GA often delay flowering. Bernier (1988) stressed the need for a comprehensive outlook which includes not only the PGRs but also PGR antagonists and internal plant nutrition.

The isolation of (possibly) transient chemical signals presumably flowing in the nutrient stream from the source (the leaves) to the sink (the apical meristem) has proven a complex task. Sachs (1987) believed that the nutrient stream itself, carbohydrates especially, would be the key to understanding the signal from leaves to meristem. Sachs and Hackett (1983) have developed the 'nutrient diversion' scheme which utilizes the facts known about the relationship between flowering and soluble carbohydrates. Higher levels of sugars are

needed and found at the apical shoot tip upon induction (King and Evans, 1991), but whether these sugars are the cause of induction has not been proven. Raper et al. (1988) and others (Rideout et al., 1992) have expanded the nutrient diversion theory to include reduced nitrogen, especially free amino acids as these are closely correlated with the increases in mitotic division found in the meristem upon induction. Accordingly, Raper et al. offer evidence that an imbalance between the levels of soluble carbohydrates and reduced nitrogen reaching the apical cells causes the shift from vegetative to floral. Both components of this scheme, the soluble sugars, glucose, fructose and sucrose and the free amino acids, are highly mobile in the nutrient stream and certainly reach the apical shoot tip with minimum delay. How these chemicals work to induce genetic reprogramming at the cellular level is not known, but Sachs and Hackett (1983) theorized that the apical meristem is relatively starved for either nutrients or reducing power and cannot proceed through the accelerated growth phases required for reproductive development.

The objectives of this research were:

1. to identify, by histological means, the morphological differences between vegetative and florally induced apical shoot tips, and
2. to identify differences in soluble sugars and total free amino acids between vegetative and florally induced apical shoot tips.

MATERIALS AND METHODS

PLANT CULTURE

Seeds of the 'extra-early' chicory cultivar 'Daliva' (*Cichorium intybus* L.) (Nunhems Seed Co., Lewisville, ID) were sown on 29 May 1995 into two rows, 45 cm apart, at the Knoxville Plant Science Farm. The soil was an Etowah clay loam (fine-loamy siliceous thermic Typic Paleudult), had received no fertilizer applications since the prior year, and was rotovated twice three days before planting. Germination was rapid and uniform in the moist and warm soil and after five weeks of growth a 10 cm straw mulch was applied to stabilize soil moisture levels and reduce heat absorption.

PLANT HARVESTS

Beginning 60 days after planting (DAP), randomly dug plants were stripped of leaves longer than five mm and scored for vegetative, transitional or floral, based on whether any sign of stem extension was evident. If no stem extension was seen the plant was judged vegetative and if a conspicuous floral stalk was seen it was judged floral. Somewhere between these extremes, not readily evident to the unaided eye, were the transitional meristems. If a slight hint of stem extension was noticed, the plant was judged transitional. All plants were evaluated through a pair of #10 Optivisor glasses (3.5X). Because the ultimate authority on the condition of a transitional meristem was destructive (microscopic sectioning), visually enhanced evaluations became the standard

and allowed the collection of apical tissue which at least on the exterior either had undergone the floral transition or was vegetative. This difference was (nearly) certain and permitted a comparison between the two states from a morphological and biochemical viewpoint. Once the decision was made as to the state of the plant, that apical shoot tip was excised out and dropped into a bottle of liquid nitrogen labeled either vegetative or transitional.

For each shoot tip collected, the number of leaves exceeding five mm in length and the root diameter at the fullest part, just below the crown junction, were recorded.

Each day's collection of apices was pooled in the thermos bottle, drained and stored at -80° C and later lyophilized. Once dried, all were heated to 60° C for 120 minutes to deactivate enzymes. The tissues were then weighed and leached of soluble sugars and free amino acids according to the protocol of Thomas (1990) which prescribed three ml of methanol per 25 mg of dry weight (DW) at 0° C for 24 hours followed by an additional two ml water for another 24 hours at 0° C. The leachate was stored at -18° C until analyzed.

Soluble sugar analysis of glucose, fructose and sucrose consisted of dilution of the leachate and injection into a Dionex ion chromatograph fitted with a Carbopac PA-1 column consisting of high performance anion exchange resins, and a pulsed amperometric detector (PAD). Sugars were identified and quantified according to retention time and peak areas of authentic standard sugars. Further details of this technique are discussed in the appendix. Total free amino acids in the leachate were derivatized by the addition of OPA and

pumped through a Waters brand fluorescence detector. Total amounts of amino acids were quantified by comparisons to peak areas of authentic amino acid standards. Further details on the use and detection of OPA amino acids are discussed in the appendix.

A total of four harvests were made over a 27 day period in August. The harvest days after planting (DAP) and number of apices of each kind gathered are given in Table 1.

TABLE 1. Shoot apices harvested by condition.

VEGETATIVE		TRANSITIONAL	
DAP	APICES	DAP	APICES
60	3	74	13
79	5	79	5
87	10	87	2

For each kind of shoot tip, vegetative or transitional, a graph was prepared with values plotted for each variable (glucose, fructose, sucrose and free amino acids) over the three harvest periods to examine possible trends between sugars and amino acids.

Plots of leaf number and root diameter over the harvest period were also made. Possible associations of plant size and biochemical status may be suggested by evaluating the graphs.

SHOOT APICES COLLECTED FOR SECTIONING

During the harvest period, but not necessarily on the same day as biochemical harvests, shoot tips from each kind, vegetative and transitional, were selected for histological preparation according to the protocol of Graham (1994). A section of crown tissue containing the meristem and nearby surrounding tissue (about three mm on a side) was dropped into HistoChoice fixative (Amresco Co., Solon OH), cleared in a series to 100% of isopropyl alcohol, embedded in PariPlast paraffin, sectioned on a Reichert-Jung retracting microtome set at ten um and stained through the wax with alcian blue (IBA Chemicals, Stain #456, Cleveland OH) in 5% benzoate buffer. The wax was removed with Micro Clear clearing agent (Micro Environmental Industries, Fairfax VA) and a cover slip was affixed with Eukitt fixative. The sections were observed through a Zeiss microscope (model 232, Buffalo, NY) and photographs were taken with a Nikon camera and Kodak film. Shoot apices from all harvests were prepared in a similar manner.

RESULTS

HISTOLOGICAL EXAMINATIONS

Midway between the harvest dates, on August 15, the length of daylight was 13.3 hours. No artificial inducements to flower were made and all plants were treated alike. A microphotograph of the apical region from a vegetative plant is shown in Figure 1. The central, darkly staining portion, the apical meristem, is flattened, even sunken beneath the overarching leaf primordia. The vascular traces, or procambium, extend nearly horizontally from the central zone; the pith rib meristem, a region of large, vacuolated and slowly dividing cells lies below the central zone. Differences in staining density or color can be attributed to cell type or age. Some cells contain thicker or more lignified walls, and some have active cytoplasmic contents attractive to the stain; both features produce variation in the resultant appearance.

A representative microphotograph of a floral apical region is shown in Figure 2. The meristem area here is seen as a domed, rounded and darkly staining area. Along the edge of the meristem itself are seen newly formed floral buds, also round but smaller than the central region. At the central meristem no distinction can be made between cell types leading to floral organs, a feature which would be seen later during floral initiation. The doming of the meristem region is driven, at least partially, by the cell division and expansion in the pith area beneath the meristem itself.

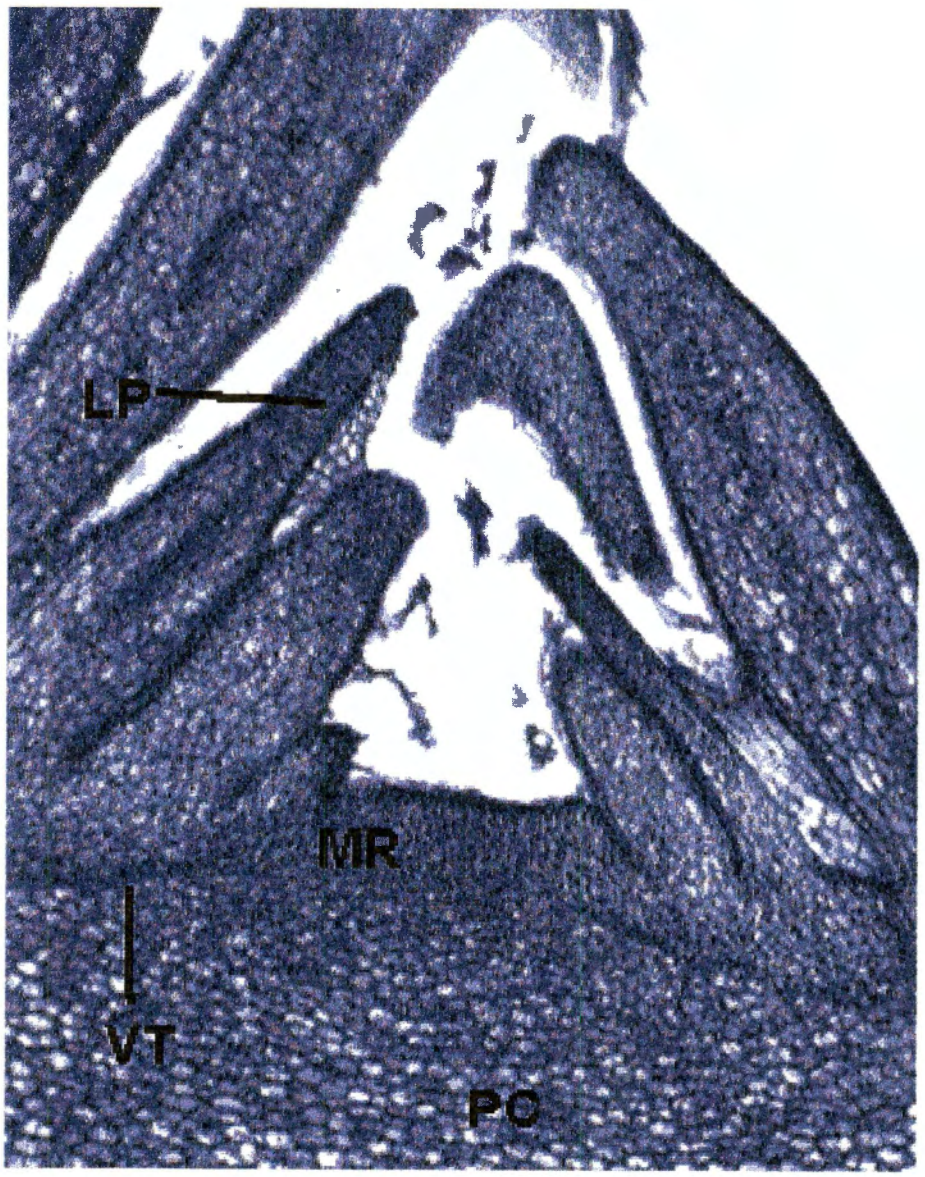

FIGURE 1. Microphotograph of vegetative chicory meristem region.

Longitudinal section, at 40X, of 'Daliva' chicory apical region showing newly expanded leaf primordia (LP), tightly packed meristem cells in the flattened meristem region (MR), and larger, vacuolated pith cells (PC). Also visible are the vascular traces (VT) leading horizontally out from the meristem region.

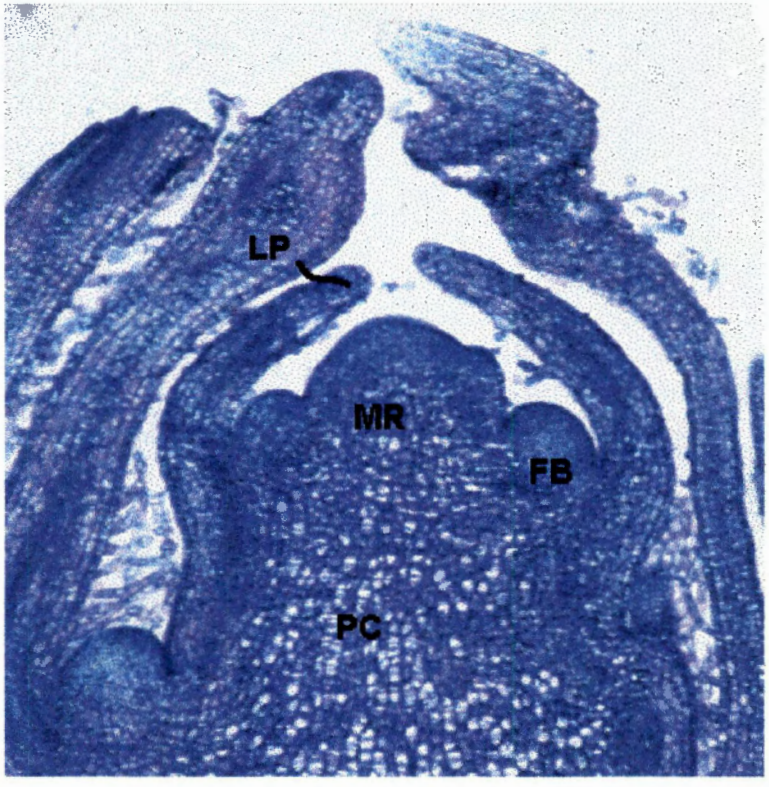

FIGURE 2. Microphotograph of 'Daliva' chicory floral apical region.

Longitudinal section, at 100X, of 'Daliva' chicory apical region showing newly expanded leaf primordia (LP), tightly packed meristem cells in the hemispherical meristem region (MR), and larger, vacuolated pith cells (PC). Also visible are the rounded floral buds (FB) along the edges of the meristem region.

These results are conclusive evidence that within 90 days of field growth some 'Daliva' chicory plants are committed to flowering and that the meristem region itself becomes a hemispherical dome-shaped upswelling visibly distinct from the previously flattened condition. All shoot tips selected and sectioned as florally transitional showed histological evidence of this condition and no shoot tips selected and sectioned as vegetative had a domed meristem. These results support the claim that the plants selected as transitional for biochemical evaluation had some basis in fact of being in that condition. Chicory here is shown to behave like many other Asteraceae during the shift from vegetative to floral morphology, that is from a flattened to domed meristem.

BIOCHEMICALS IN VEGETATIVE SHOOT TIPS

The values of the leached total free amino acids in vegetative shoot tips ranged from 23 to 40 mg per g DW over 27 days (Figure 3, right axis). The trend shows a start at the lowest value followed by a dramatic rise 14 days later to the highest value, then a decline to 30 mg per g DW.

Measured glucose values over time started at 94 umol per g DW, dropped to 42 umol per g DW, then rose to 66 umol per g DW (Figure 3, left axis). Fructose values followed a similar trend and started at 106 umol per g DW, but declined to 4 and 6 umol per g DW at the final two harvests (Figure 3).

Sucrose values started at the highest value, 456 umol per g DW and dropped linearly to near 20 umol per g DW, then rose slightly to 66 umol per g DW over the 27 day harvest period (Figure 3).

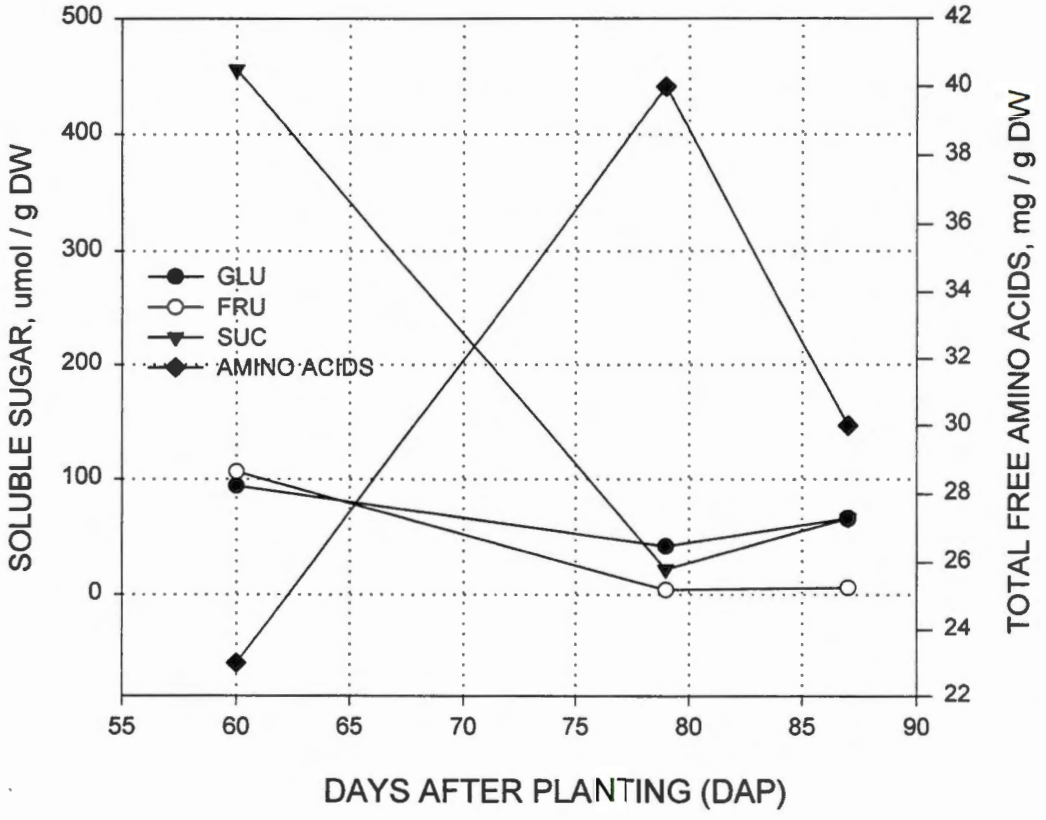

FIGURE 3. Soluble sugars and total free amino acids in vegetative shoot tips at three harvest dates.

Soluble sugars, glucose, fructose and sucrose, and total free amino acids leached from vegetative shoot tips. Values are the mean of two (sugars) or three (amino acids) observations from one pooled sample of leachate from varying number of shoot tips. Note values for ordinate axes differ.

BIOCHEMICALS IN TRANSITIONAL SHOOT TIPS

The values for free amino acids in transitional tissues rose continually across the time period of 13 days (Figure 4, right axis). On the first harvest, 15 mg per g DW were recorded, then 25 mg per g DW, and finally, on the third harvest, 35 mg per g DW.

All soluble sugars followed an upwards trend over the harvest period (Figure 4, left axis). Glucose rose linearly from 88 umol per g DW, to 444 umol per g DW, and finally, to 688 umol per g DW. Fructose rose from 61 umol per g DW to 133 umol per g DW, and finally to 261 umol per g DW. Measured sucrose values started at 11 umol per g DW, rose to 98 umol per g DW and on the final harvest, to 400 umol per G DW.

LEAF NUMBER

Vegetative plants increased in leaf number at each harvest date (Figure 5). The number of leaves was 23 at DAP 60, this number rose to 30 at DAP 79, then to 34 leaves at DAP 87. Leaf increase was 0.4 leaves per day, over the 27 days.

Transitional plants also had more leaves at each harvest (Figure 5). At the first harvest, plants had 32 leaves; the number rose to 33 at harvest two and finally to 35 leaves at DAP 87. Leaf increase was 0.23 per day, over 13 days.

In a comparison of the number of leaves on vegetative and transitional plants on the two harvest dates in common (DAP 79 and DAP 87) only at DAP 79 did the number of leaves differ ($P<0.05$).

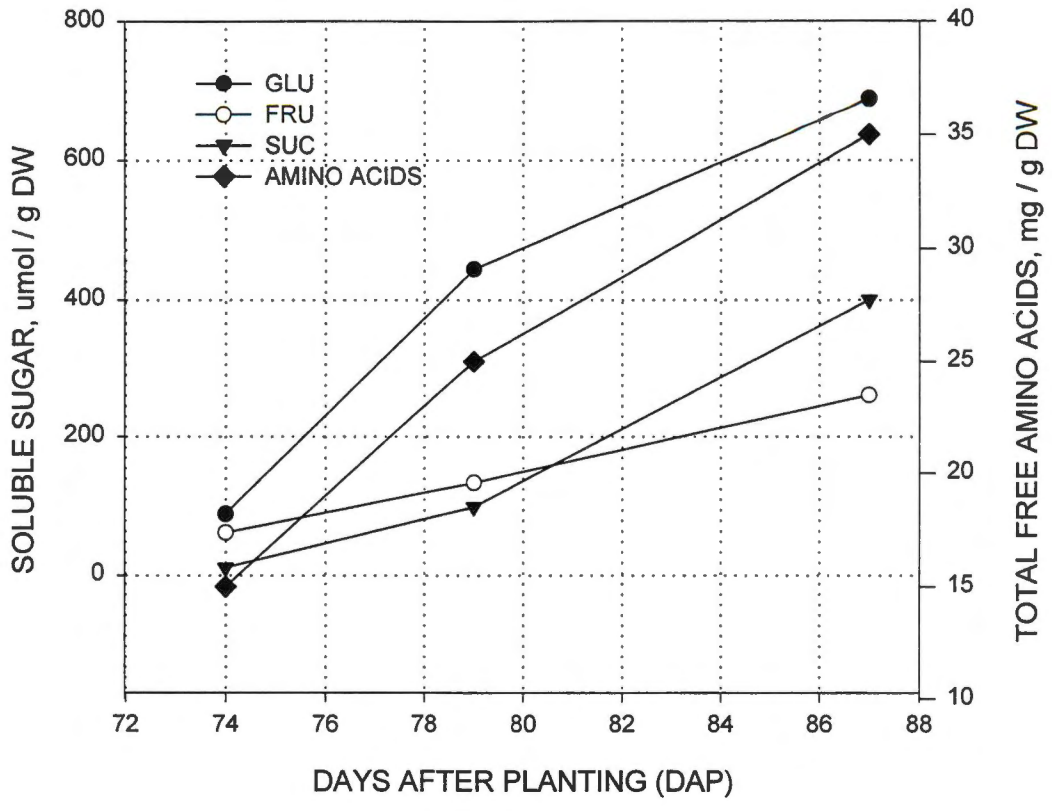

FIGURE 4. Soluble sugars and total free amino acids in transitional shoot tips at three harvest dates.

Soluble sugars, glucose, fructose and sucrose, and total free amino acids leached from transitional shoot tips. Values are the mean of two (sugars) or three (amino acids) observations from one pooled sample of leachate from varying number of shoot tips. Note values for ordinate axes differ.

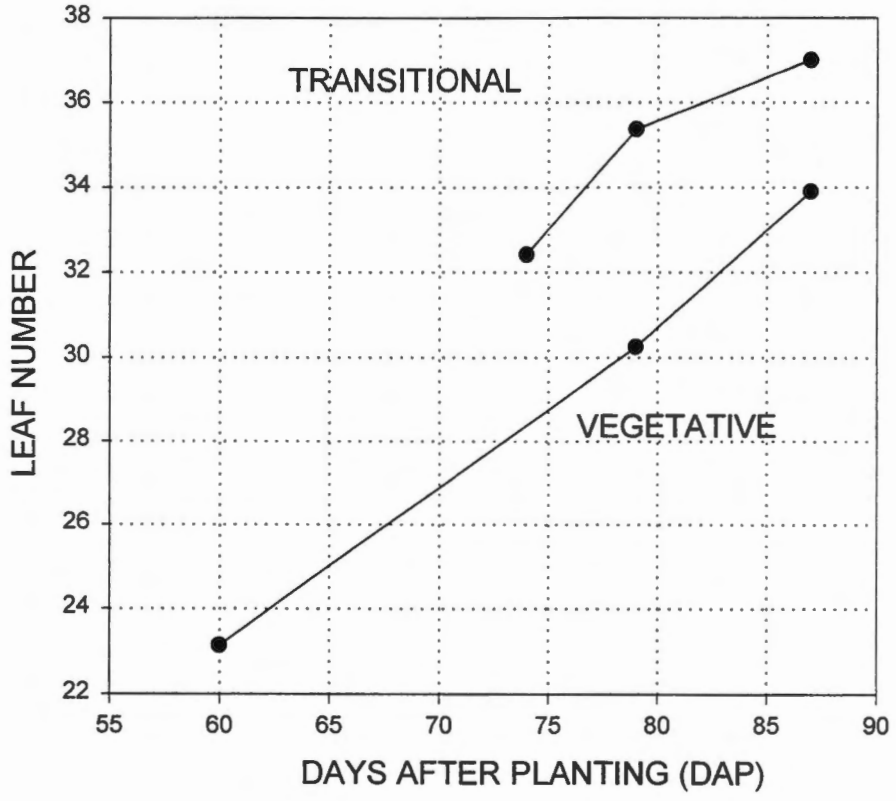

FIGURE 5. Association between leaf number and days after planting.

Number of leaves, greater than five mm in length, on transitional and vegetative plants harvested over 27 days in August. Between the two dates in common, number of leaves differ ($P<0.05$) at DAP 79. Number of plants varied by condition and date.

ROOT DIAMETER

Figure 6 shows that the root diameter on vegetative plants followed a strong upward trend which started at 12 mm, then rose to 18 mm, and ended at 20 mm on the final harvest. The root diameter increase was 0.3 mm per day over 27 days. The root diameters of transitional plants also followed an upwards trend over the three harvest dates, but these plants started at 20 mm, rose slightly over five days and eight days later had grown to 22 mm (Figure 6). The root diameter increase was 0.15 mm per day over 13 days. In a comparison of the root diameter on vegetative and transitional plants on the two harvest dates in common (DAP 79 and DAP 87) only at DAP 79 did the root diameters differ ($P<0.05$).

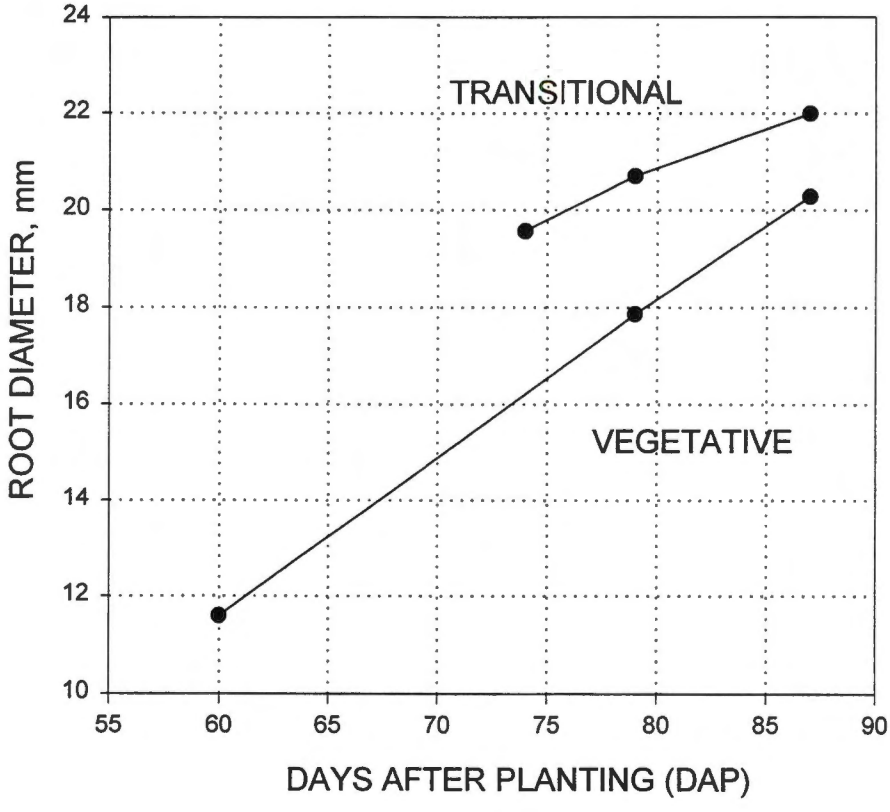

FIGURE 6. Association between root diameter and days after planting.

Diameter of roots on transitional and vegetative plants harvested over 27 days in August. Between the two dates in common, root diameters differ ($P<0.05$) at DAP 79. Number of plants varied by condition and date.

DISCUSSION

These values of total free amino acids from plant tissues, approximately 15 to 40 mg per g DW, are well within the range given by other authors. For example, Thomas (1990) found in meristematic *Lolium* leaf bases approximately 15 mg per g DW, but leaf blade tissue was higher, around 25 mg per g DW. From chicory roots, Cyr et al. (1990) leached amino acids through the year and found between 5 and 30 mg per g DW, with the highest levels in the winter. From xylem sap of woody plants Anderson et al. (1995) measured only 1.5 mg amino acids per ml of sap and Alm et al. (1990) found only 0.2 mg amino acids per ml of floral nectar. The apical meristem region represents one of the strongest of all sinks, or regions of high metabolic activity with great demands for energy and raw materials, therefore, it may naturally have substantial quantities of free amino acids.

The differences in total free amino acids between the vegetative and transitional tissues are small, but the range of the transitional shoot tips is from 15 to 35 mg per g DW and the range for the vegetative is from 24 to 42 mg per g DW, suggesting that the transitional plants may have had fewer total amino acids. For the transitional shoot tips, the graphs show an increase in all measured variables, soluble sugars, free amino acids, leaf number and root size, over the harvest period. Although the data has been presented over time, 13 days, it is not known whether the size increases of the plants over that time had

any bearing on the levels of amino acids or sugars. Plants were not selected for size but rather for the appearance of floral transition only. It is possible that the size of the plant and the upward trends of the sugars and amino acids were correlated; perhaps larger plants have higher levels of extractable biochemicals.

Also unexplained is why the biochemicals from the vegetative plants did not also rise with their consistent size increase over the harvest period. The longest time between harvests, 19 days, saw large size increases in leaf number and root diameter but a steep decline in sucrose and minor declines in glucose and fructose. During that same time period the amino acids rose sharply to their maximum value.

The vegetative plants showed a different amino acid trend over their harvest period. In this case, the sharp peak at the middle harvest appears associated with the equally sharp drop in sucrose. Whether these two biochemicals are associated in any way, or whether the trend lines can be explained solely by the responses of the plant, can not be stated from these results.

Laboratory variation between observations, both for soluble sugars and free amino acids, was high. A compilation of average coefficients of variation (CV) is shown in Table 2. Fructose and the free amino acids had the highest CV values but whether the laboratory variation explains any of the trends can not be stated. Analytical differences reflected a learning curve for chromatography by the author and natural variation among field grown plants.

TABLE 2. Average coefficients of variation (CV) among laboratory samples.

	SOLUBLE SUGARS[Z]			FREE AMINO ACIDS[Y]
	GLU	FRU	SUC	AMINO ACIDS
TRANS	6.1	21	6.7	11
VEG	2.4	18	0.14	8.8

Z= Average of 2 observations each from 3 sample dates.
Y= Average of 3 observations each from 3 sample dates.

One conspicuous difference between the transitional and vegetative shoot tips was in the quantities of individual sugars. For the two dates in common, DAP 79 and 87, the glucose values for the transitional plants was, at each date, approximately 10 fold greater than the values found in the vegetative shoot tips. Much higher values for the other two sugars, fructose and sucrose, were also found for transitional plants on those two dates. The smooth progression of upwards values for all the transitional biochemicals may reflect the consistent selection technique of choosing plants with the smallest visible stem extension thus insuring the homogeneity of the sample apices. Additionally, since the number of selected transitional plants declined over time, from 13 to 5 to 2, this may have also led to some uniformity of tissue. However, these elements of explanation are balanced by the overall higher CV values found for all the

measurements of the transitional plants.

In rapidly growing tissues, as shoot tips are, high levels of hexoses relative to sucrose are often found, as indicated by invertase activity (Morris and Arthur, 1985). Although a number of reports correlate the availability of soluble carbohydrates to floral transition (Bodson, 1977; Raper et al., 1988; Sadik and Ozbun, 1968) these authors do not separate the reducing sugars from sucrose, claiming only that soluble carbohydrates are involved.

Jones (1990) found glucose and fructose levels at only about 10% of the level found for sucrose in apices of clover, *Trifolium pratense,* and the total soluble sugars increased upon induction to flowering. King and Evans (1991) report no appreciable pool of free glucose or fructose in shoot apices of *Lolium temulentum* L., whether vegetative or induced to flower. Lejuene et al. (1993) report only sucrose is found in the phloem sap of florally induced plants.

One possible explanation for the sharp amino acid peak of the vegetative plants at DAP 79 is that of a metabolic 'pulse' or signal, especially when viewed with the steep decline of sucrose. Consider the case that five vegetative plants were chosen on that day, and that all vegetative plants had been selected based on the absence of any visible stem extension, and knowing that stem extension is one of the later consequences of floral transition, perhaps on that harvest day the chosen plants had recently become large enough (no visible signs) and had just received the floral signal. If the floral signal was, as Raper et al. (1988) and Rideout et al. (1992) suggested, a shift in relative values of soluble sugars and

free amino acids at the meristem, then the biochemical shifts found in the chicory shoot tips may be indicators of such a floral signal. If the sharp fluctuations of amino acids and sucrose found at DAP 79 of the vegetative plants are components of a floral signal, it may also be the case that the signal components, once received, are changed to new values.

To isolate such a pulse or signal would take a highly uniform population of plants known to respond to artificial inducements where the timing of the photoperiodic signal could be precisely controlled. Field grown chicory plants did not meet these criteria. Additionally, the size of the tissue sampled, one cubic mm, was at least 10X larger than the desired mass of dividing meristematic cells; the rest of the tissue being leaf and pith cells, already differentiated (Medford, 1992). Apical meristems are a component of apical shoot tips, which also contain leaf primordia and rib pith cells. These nearby tissues may play no role in floral transition yet here they have provided perhaps the majority of tissue sampled.

The questions revolving around the impact of soluble sugars at the meristem will probably remain until gene expression modulation can be achieved by floral mutants or other direct approaches. Koch (1996), in a recent review article, outlines the known facts regarding the activities of sugars in the plant beyond their effects as substrates and concludes, among other ideas, that since sugars are known to have direct gene effects in microbes, it is only a matter of time until their signaling nature in higher plants will be revealed. Haughn et al. (1995) speculated that many of the questions regarding the mechanisms

underlying floral morphogenesis will be effectively addressed through mutants and molecular techniques and mostly answered within the upcoming decade.

CONCLUSIONS

This research has shown that 'Daliva' chicory will flower within 90 days after planting in late May at Knoxville and that the apical meristem region is much like other Cichorieae of the Asteraceae in morphology, both before and after floral transition.

Soluble biochemicals leached from apical shoot tips of florally transitional chicory plants showed a consistent upwards trend over a 13 day harvest period. The same biochemicals leached from vegetative shoot tips expressed a different trend over 27 days, with sucrose sharply dropping and total free amino acids peaking at the middle harvest date.

Glucose values for transitional plants were the highest of any sugar measured, indicative of high metabolic activity associated with floral development at the shoot tip. Total free amino acids in those plants followed a trend similar in appearance to glucose and more than doubled in value over the period, from 15 to 35 mg per g DW.

It is speculated that the rise in amino acids found in the vegetative plants may represent, or be a component of, a biochemical 'pulse' or metabolic signal from the leaves to the meristem which initiates the transition to the floral state. The identification of purely vegetative plants as the season progressed may have been unaccounted for in the design.

All plants increased in leaf number and root diameter size over the entire

period but those identified as vegetative grew faster but started at a smaller initial size. Novel laboratory analyses and field grown plants contributed to variation.

The objectives of this experiment were successfully concluded in recording the morphological appearance of chicory shoot tips in the vegetative and induced states and identifying possible variation in soluble biochemicals at the apical shoot tips of both vegetative and florally transitional plants over the course of 27 days in August.

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PART IV

CHICORY GROWTH RESPONSES AND BOLTING WITH STRAW MULCH TREATMENT

INTRODUCTION

The successful field culture of vegetable crops requires a knowledge of the growth habits of the crop under local conditions, especially if production is sought outside the established range. Chicory (*Cichorium intybus* L.) is a potential alternative vegetable crop for Tennessee with possible markets for its pale, forced chicons (heads, also known as witloof, Belgian endive or French endive). Chicons are the etiolated and elongated spring buds composed of overlapping, young and tender, yet slightly bitter, lettuce-like leaves. Chicon production from roots is labor intensive and nearly all commercially available chicons in this country are imported from Belgium and Holland (Daemen, 1987) where it is an important salad vegetable (Ryder, 1979; Yamaguchi, 1983). In 1989, 2600 metric tons were imported into this country from Europe (Pearrow, 1992). European labor and air transportation costs combine to make Belgium endive relatively expensive in North America.

Few states currently grow chicory for chicon production although in decades past thousands of acres were grown in Michigan (Martin and Leonard, 1967), principally for the dried and roasted roots often used in New Orleans coffee and other beverages (Mitich, 1988; Steiner, 1983). Research in field production of roots as a feedstock for high fructose syrups has been conducted in Canada (Chubey and Dorrell, 1978, 1977) and recent New Zealand research indicated chicory root to be an easily fermentable biofuel with good root yields

and sugar percentage (Douglas and Poll, 1993). One chicory cultivar, 'Grasslands Puna', introduced to the U.S. from New Zealand is regarded as a highly palatable and nutritious forage and silage crop (Volesky, 1996). Only approximately 500 hectares are currently grown for forcing witloof in North America, including some hectares along both Canadian coasts (Daeman, 1987). Recent research indicates interest in this crop from Connecticut (Hill, 1987, 1988), Massachusetts (Tan and Corey, 1990), Virginia (Mersie and Elliott, 1993; Sterrett and Savage, 1989) and California (Myers, 1991).

Chicory is a perennial member of the Asteraceae (Tribe Cichorieae) but is grown as a biennial (Kalloo, 1988) and is generally regarded as a cool weather crop which thrives best where the average temperature during the season does not exceed 21° C (Martin and Leonard, 1967). For chicory to have any success in east Tennessee, where the average summer temperature (June, July, August and September) in Knoxville is about 24 °C (Logan and Fribourg, 1989), certain cultural measures need to be investigated. Chicory is believed to grow poorly where night temperatures are high (Myers, 1991; Sterrett, personal communication). Chicory root production is much like sugarbeet (*Beta vulgaris)* culture in that both thrive under cool weather, especially during the autumn when carbohydrate storage is reaching the maximum rate (Martin and Leonard, 1967)

One method widely employed to reduce the radiation reaching the soil and thus lower the soil temperature is straw mulch (Martin and Leonard, 1967; Swiader et al., 1993). Straw mulch has been shown to have numerous beneficial effects on crops grown in the southeast US and the tropics where the solar

intensity is great (Manrique, 1995; Skroch et al., 1992). Organic mulches have been shown to reduce soil temperatures, conserve soil moisture, reduce some pests and diseases, and reduce tuber sunburn in potatoes. In Australia, a decomposing mulch treatment provided a less dense and wetter soil and was more likely to contain biopore zones of low root impedance (e. g. worm holes) which improved root growth (Strizaker et al., 1993). In Georgia, Tindall et al. (1991) found that the soil under straw mulched tomatoes (*Lycopersicon esculentum* Mill.) had greater water infiltration rates, lower pH, lower bulk density and lower soil temperatures than either bare soil or plastic mulched soil.

Production recommendations for chicory include avoiding excessive soil nitrogen which promotes the growth of leaves rather than roots, avoiding heavy clay soils which interfere with harvest, avoiding sandy soils if irrigation is not available, especially during the germination and seedling stages, and avoiding planting into cold soil (below 12° C) which encourages bolting (Nunhems, 1994). Bolting of chicory is a problem in every production region and most literature sources maintain that planting into a cold soil or permitting the seedling to experience cold temperatures is the direct cause of premature flower stalk elongation and subsequent early flowering (Anon, 1984; Hill, 1987, 1988; Kalloo, 1988; Rappoport and Witwer, 1956). After many biennial vegetable crops including celery (*Apium graveolens*) , beet, many Brassicaceae, onion (*Allium cepa*) and others (Swiader et al., 1993) reach a certain size they can be influenced to bolt by a cold treatment known as vernalization. These plants are

then induced to flower before economically desirable. The stimulation of bolting and flowering before the plant has reached an acceptable harvest size can result in severe economic loss, since these plants are harvested for their vegetative parts, (leaves, including petioles, bulbs or roots) not the reproductive parts (fruits or seeds) and the appearance of the stem, however slight, can cause a major reduction in quality. Other rosette plants also undergo bolting (rapid stem elongation) prior to flowering (Sachs, 1965; W aycott, 1989).

In Connecticut, Hill (1988) found that planting chicory in early May resulted in many bolting plants, especially among those rated as early and extra early maturing. By sowing in June he was able to avoid the cold soil but still experienced some bolting plants, but only among the extra early maturing cultivars. Hill concluded that "bolting is primarily related to soil temperature at the time of drilling the seed. Cool soil temperatures vernalize the seed causing bolting and floral induction" (Hill, personal communication). Lang, however, maintains that thermoinduction (flower induction by cold) of a biennial seed is not possible but chilling treatments to the seedling may be sufficient for the plant to reach an early stage of sensitivity (Lang, 1956). In Tennessee, similar results have been reported (MacDermott et al., 1993) for extra early cultivars with increasing incidence of plant bolting for seeds sown on the first of June compared to those sown a month later.

Not all chicory cultivars require a cold treatment to flower. Such a cold period (usually winter) when combined with the increasing daylength of spring, would be the natural course of events of a biennial plant with a storage root.

Normal chicory culture requires field growing, lifting and storing the root in cold rooms for specific periods of time and then forcing the dormant vegetative bud in dark rooms of moderate temperatures and finally harvesting the chicon. The length of time in cold storage is critical for each cultivar as the biochemical changes in the root during the cold treatment are directly related to the quality of the resulting chicons (Rutherford and Phillips, 1975). Early cultivars need less cold storage time, late cultivars need a longer time.

Chicory cultivars have been selected and bred to take advantage of this habit in order to keep chicon production houses fully occupied during the forcing season, i.e., winter and spring. These early and late forcing cultivars also experience similar flowering habits in the field during the season. The early cultivars often flower much more readily the first season whereas the late ones seldom flower without a cold period. Paulet (1985) presents a summary of differences between early and late varieties:

'1. The so-called "late" varieties, meaning those that are late to form their "chicory" have an absolute cold requirement.

2. The so-called "early" varieties, meaning those that are early to form their "chicory" can flower without prior cold treatment.'

In this context, the 'chicory' refers to the compressed, overlapping leaves of the vegetative bud as obtained through either forcing or natural conditions. The literature suggests that the maturation of the apical meristem from juvenile to mature is closely related to and must precede the induction of the floral state. When Paulet (1985) speaks of the differences between the early and late

varieties he may be referring to the ease or speed of transition of the apical meristem from juvenile to mature.

Early and late strains of flowering annuals are well known and usually the late types require more floral inductive cycles than early ones or have a longer juvenile phase. In four lines of *Chrysanthemum morifolium* with varying degrees of earliness, Cockshull (1976) found the difference in number of leaves prior to floral initiation ranged from 18 to 57. Once the critical number of leaves is achieved, floral induction can commence. Some strains of sugarbeets bolt more readily than others or at higher temperatures, even though all are very similar in many phenotypic characters (Martin and Leonard, 1967).

In addition to herbaceous perennials, strains or cultivars of woody plants are known to vary significantly in earliness to flower. Apple (*Pyrus malus*) and pear (*Pyrus communis*) trees are known to carry inheritance of flowering where large differences in age to first flower can be found in seedlings of early and late parents (Visser and deVries, 1970). Earliness, or precocious flowering, is an inherited characteristic which has been extensively studied in fruit and forest trees because of their economic value and often extended length of a juvenile phase which can impede selection of improved cultivars (Hackett, 1985). Visser (1965) showed that in apple and pear a good correlation exists between the length of the vegetative phase of a cultivar and the contribution it makes to the length of the juvenile period of its progeny. Visser (1965) also suggested that by selecting for more vigorous seedlings the breeder is also selecting for a shorter

juvenile period since there is considerable evidence to show that seedlings must attain a certain size before they can flower and the most vigorous seedlings can attain that size in the shortest time.

A second possible notion regarding the maturation of the apical bud resides in its ability to withstand the cold, a typical requirement for rosette perennials and biennials which need to successfully overwinter. The ability to avoid damage by the cold is a condition related to the various storage forms of carbohydrates in the roots. It is known that as chicory matures the relative composition of the root carbohydrates shifts from sucrose, a disaccharide, towards the higher weight glucofructosans, notably inulin. Inulin is a long chain fructose polymer composed of 10 to 30 fructose units with a single glucose unit on one end (Bhatia et al., 1974). Fructans and inulin are widely found in the plant kingdom (Pollock, 1986) and have the ability to act as cryoprotectants or osmoregulants, which partly explains their maximal root storage levels prior to winter (Pollock and Jones, 1979). Fructans, including inulin, accumulate in the tubers of Jerusalem artichoke *(Helianthus tuberosous)* and Dahlia *(Dahlia* spp.) and in the storage roots of dandelion (*Taraxacum officianalis*) in addition to other Asteraceae (Pontis, 1989). According to Heilmeier et al. (1986), no member of this vast family, the Asteraceae, stores starch, a glucose polymer, in the root .

The 'late' and 'early' chicory designations are used industry-wide to identify those cultivars which require either more or less time in cold storage prior to forcing. Early cultivars of chicory can be grown for approximately 18 weeks in the field, lifted, stored at 0° C for one or two weeks, then placed in the forcing

beds (Anon., 1984). Late varieties need 24 weeks in the field and six to eight weeks in cold storage but can be kept in cold storage for many months. By the suitable selection of early or late hybrid cultivars a grower may be able to maintain a long and timely harvest and storage of roots and ultimately, chicons. Roots of various cultivars develop slightly differing chicons, principally in density, color, disease resistance, length, width, total weight, and length/width ratio (Nunhems, 1994).

Plants generally do not reach a capacity for sexual reproduction until a certain size or age has been attained. This transition from the juvenile state to the adult is called a 'phase change' in woody plants and is accompanied by a number of observable differences, notably in leaf shape, rooting ability of cuttings, thorniness, pigmentation and growth rate (Hackett, 1985; Zimmerman, 1972). The growth rate of juvenile woody plants is often much greater that the rate of the adult of the same species. The juvenile phase may be one day in some annuals (Cumming, 1959) or 30 to 40 years in certain forest trees (Hackett, 1985). In many herbaceous plants the juvenile phase is very short and when enough leaf area has been attained to support the flowers and fruits, the transition takes place.

The idea of a minimum photosynthetic apparatus needed to ensure the development and maturation of seeds is wholly in keeping with a survival mechanism and it is often stated that flowering is induced when conditions for survival are developing e.g., heat or water stress, but almost never light stress as studies repeatedly show that shade or reduced PAR (Photosynthetically Active

Radiation) will delay or prevent flowering (Schwabe, 1976). The conclusion that size itself is the primary factor determining when the phase change, or adult transition, occurs has been rejected by Robinson and Wareing (1959) who maintained that the change can happen only after the meristem has undergone a certain number of mitotic divisions, which are correlated with but not determined by a particular size. Some degree of vegetative growth usually precedes floral induction as the apical meristem produces leaf primordium before the earliest flower. There is no evidence that the number of leaves in itself is a determinant of floral induction (Gott et al., 1955). However, the number of nodes produced by the meristem prior to the first flower may be used as a measure of the duration of the juvenile period (Bernier et al., 1981).

The environmental or physiological conditions promoting the transition, and in particular, predicting the end of the juvenile stage, have been of considerable interest. In an effort to quantify the pre-flowering phase, Purvis (1934) and Purvis and Gregory (1937) working in winter cereal crops, postulated that the apex meristem needs to initiate a minimum number of leaf primordia before becoming capable of floral transition. Once the minimum number of leaves had been achieved the plant was able to respond to those environmental cues which initiated the process towards meristematic transition. They found however, that such a number was not easily defined and was subject to modification by environmental factors.

Of the several factors put forward as explanations for the juvenile condition, the one of insufficient leaf area is often disregarded as experiments

have shown that very little leaf tissue is sufficient to activate the flowering response in some plants. As little as a few square centimeters from one sensitive leaf of *Xanthium* (Khudairi and Hamnar, 1954) or a single cotyledon of *Brassica* (Friend, 1968) is size enough to initiate the response, indicating that leaf area alone is not the sole factor involved. However, high leaf numbers and area, especially when produced in a short time during the early stages of growth, and often as a response to high photosynthetic rates, are known to shorten the juvenile phase in many species, including the biennials *Oenothera*, and *Lunaria* (Bernier et al., 1981). In fruit trees, at least, the most effective way to shorten the time of appearance of first flowers is to grow the seedlings so that they attain as large a size as rapidly as possible (Zimmerman, 1972). The above instances of rapid leaf production are, of course, also indirect measures of the size of the stem producing them as each new node (a lateral leaf primordia and axillary bud) and internode always occupy a certain minimum space. As the number of leaves increases along the stem the distance between the newest and the oldest leaves also increases, as does the distance between the apical meristem (the source of new leaves) and the root system.

The length of the stem, or relative closeness of the roots to the apex, has been put forward as an explanation of the juvenile condition. This size factor was explored in black currant (*Ribes nigrum)* by Schwabe and Al-Doori (1973) who found that a minimum of 20 nodes between the roots and apex was needed before any induction of flowers was possible. They also observed that any aerial

rooting on the adult shoots totally prevented floral initiation. In the same study, Schwabe and al-Doorhi also measured gibberellic acid (GA) activity from root to shoot tip and tentatively concluded that the lack of GA found at the tips of the flowering shoots, those that are the longest, may be responsible in determining the end of juvenility or 'ripeness-to-flower'. Frydman and Wareing (1973) found more GA in the juvenile buds of English ivy (*Hedera helix* L.) than in the adult buds suggesting that GA at the apical meristem may be an inhibitor of flowering or sort of 'juvenile stabilizer'. Once the GA levels at the shoot tip are reduced, conditions may permit a change to the adult condition. However, tree grafting experiments using juvenile scions grafted onto mature stocks did not result in flowers and Wareing and Frydmann (1976) concluded that low gibberellin levels may be a necessary but an insufficient condition to effect the shift to maturity. Similar results were obtained with the perennial hop (*Humulus lupulus* L.) by Thomas and Schwabe (1969) who found that, according to cultivar, flowering cannot be induced on bines (flexible shoots) of fewer than about 20 differentiated nodes even when plants were grown under optimum photoperiods for flower induction. The influence of the root system in modifying or controlling flowering at the apex is well known from dwarfing rootstocks of apple (Janick, 1973). The roots as a source of GA are documented by Carr (1964) and more recently by Butcher et al. (1987) and Sponsel (1995).

The effects of exogenous GA on rosette plants in promoting bolting are well documented (Cleland and Zeevart, 1970; Metzger, 1985; Zeevaart, 1971) and often dramatic. A photograph of an application of a minute amount of active

GA on cabbage heads which results in the flower stalk shooting up beyond the reach of the gardener appears in many horticultural books (Janick, 1973). Rosette plants often require vernalization and long days to initiate flowering, but not necessarily to bolt. Bolting is a completely separate though closely related developmental process in time and function (exposure of the flowers to insects and possibly, enhanced seed dispersal). Personal observation of bolting chicory plants totally devoid of flowers late in the season can confirm the separation of these processes. The application of exogenous GA on rosette plants to induce bolting suggests that inductive treatments (cold, long days) may enhance GA production and subsequent bolting.

The objectives of this study were:

1) to quantify the change in growth and flowering habit by applying a straw mulch to the soil beneath the leaves of 'Daliva' chicory,

2) to measure rate of growth of 'Daliva' under local conditions of high summer heat, and

3) to draw correlations between bolting and various plant size parameters.

MATERIALS AND METHODS

PLANT CULTURE

Seeds of chicory F1 hybrid 'Daliva' (Nunhems Seed Co., Lewisville, ID) were sown on 19 June 1995 at the Knoxville Plant Science Farm (35° 53' N and 83° 57' W) into two rows 45 cm apart. The soil (Etowah clay loam, a typic Paleudult) had been rotovated three days prior and hand raked into a fine seedbed, 1 m x 33 m. The soil temperature at planting was 28° C at 1100 hours, four cm deep. On 11 July, 22 DAP (Days After Planting) both rows were thinned to achieve a density approaching one plant per 16 cm of row. Thirty-five DAP a wheat straw treatment was applied as a soil mulch to four randomly chosen segments of each row. The straw was pushed under the leaves of the small chicory plants and applied about ten cm deep from the outer edge to the middle of each row. Segments varied in length but all were between 3.5-4.5 meters. Each row contained four segments of straw mulched plants and four of non-mulched or plants in bare soil. Irrigation was as needed through the summer, usually one deep irrigation weekly by directed garden hose was ample. No signs of water stress were observed. Weeds were hand controlled in each treatment. For analysis purposes this was a completely random design with two treatments, mulched and bare soil.

PLANT HARVESTS

Harvest of plants began two weeks after emergence, 5 July, and continued weekly till 1 October, a total of 14 weeks. Prior to treatment imposition (week five) ten plants were chosen randomly from each row for harvest. After week five, approximately ten plants from each treatment per row were chosen. Whole plants were dug, often two or three together, as digging individual plants resulted in considerable disturbance of neighboring roots and often serious root breakage. Plants were rinsed in the field, stored in plastic bags and transported to the campus lab for data collection. In the lab, plants were examined and the five most uniform in root size were chosen as representative of that treatment for that day. Figure 1 is a diagram showing the relationships of the various parts near the top of a chicory plant.

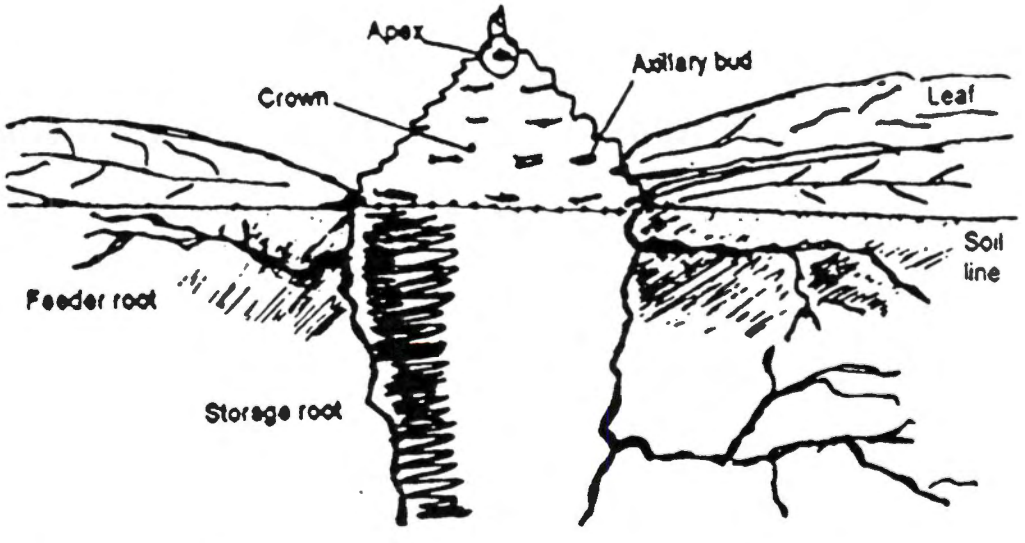

FIGURE 1. Diagram of typical chicory plant showing relationship of crown, apex, leaves, axillary buds and roots.

The following data were collected from each plant: leaf number, leaf dry weight, total leaf area, root diameter, root length, root dry weight, crown diameter, crown length and crown dry weight. After six weeks of growth a subjective measure of a spot of pink or red color found in the interior of the crown was added (Figure 2). The 'crown' is a term describing the highly compressed stem of a certain class of plants and is comparable to more typical stems but with extremely shortened internodes. This compression leads to a clustering of leaves and axillary meristems around the exterior of the crown resulting in very low growing plants, known as rosettes.

Based on prior experience, this spot of color was believed to be related to the age or size of the crown and so might have a correlation with bolting. After the first observable bolting plant, eight weeks after planting, this category (Bolt/NoBolt) was also added. Three of the variables measured, root length, crown length and crown dry weight proved unreliable and will not be discussed.

For each plant all fresh leaves longer than five mm were pulled from the crown, counted and placed on the Delta 'T' areameter screen for area determination (Delta T Devices Ltd., Cambridge, UK). Leaves were then chopped, put in kraft paper bags, stapled shut and placed in forced hot air ovens until fully dry and then weighed. Root diameter was taken at a point about two cm below the crown-root junction with a micrometer (Mitutoyu dial caliper).

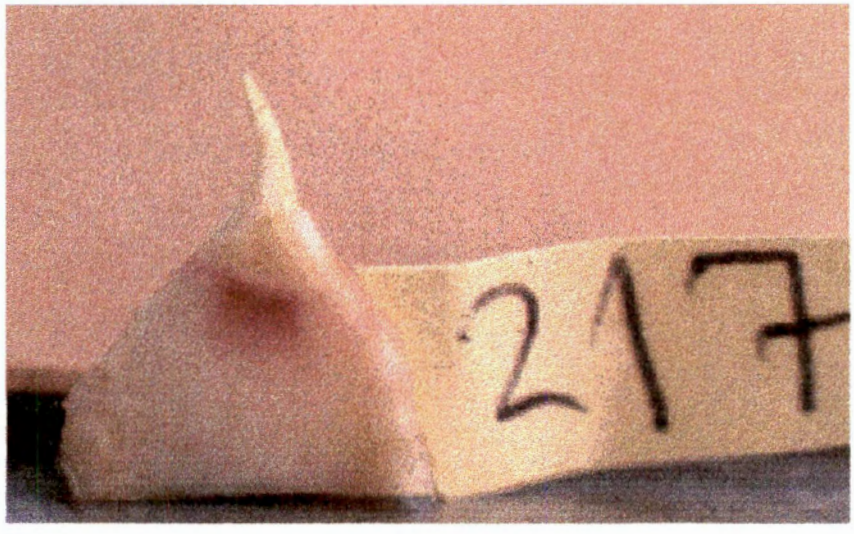

FIGURE 2. Pink color in chicory apical region.

Photograph of a longitudinal section of a vegetative chicory crown showing the spot of pink color residing just below the apical meristem. This spot rated 4 out of a maximum of 6 in color.

The crown was severed from the root about five mm below the crown-root junction. The root was then chopped, bagged, hot air oven dried and weighed.

The maximum crown diameter (actually only the distance between the vascular strands, the pith) was measured with a micrometer at the widest place (Hopkinson and Hannam, 1969). The spot of color in the crown was subjectively rated between zero (no color) and six (maximum color). A plant was judged bolting if it exhibited a macroscopically visible crown extension, often only apparent upon leaf removal. If no stem extension was seen the plant was rated non-bolting.

Soil temperatures at various dates and times were taken with a dial thermometer inserted into the soil at four cm and six cm for three minutes at each location. Two readings from each treatment were taken at each opportunity, two or three times weekly, and the means are presented.

STATISTICAL COMPARISONS

Statistical analysis was carried out on SAS, version 6.11. Graphing analysis were carried out on SigmaPlot, version 3.0. Dependent variables were analyzed using analysis of variance procedures for each treatment at each date by SAS Proc GLM, and means of variables found significantly different were separated by Duncan's Multiple Range Test. Regression analysis, both linear and non-linear over time for each treatment and variable, and finally, correlations with bolting will also be discussed.

CALCULATION OF PLANT GROWTH CURVES

Plants do not in general have a definite maximum size and are said to be indeterminate in growth, unlike animals which reach an adult size and maintain that size for extended periods. For any pattern of biological growth a mathematical function giving rise to a sigmoid curve (i.e. a function that is bounded by two horizontal asymptotes and having everywhere a positive first derivative) can empirically describe growth, since even indeterminate growth will cease at some stage (Causton and Venus, 1981). All sigmoid plots in this thesis were constructed using the Richards equation (Richards, 1959) which was especially developed to describe the growth of whole plants (and animals) and individual parts, such as stems (Karlsson and Heins, 1994) and leaves (Dennett and Auld, 1980). Its use is fully documented in many sources (Causton and Venus, 1981; Hunt, 1982; Leopold and Kriedemann, 1975). The Richards equation is especially applicable since its parameters are all viewed as completely related to the biological meaning.

The Richards equation, $\mathbf{Y= a(1+ b\ e^{-cT})^{-1/d}}$, has four parameters, viz., a=upper asymptote or maximum level of the plant variable in question, b=starting point or lower asymptote, c=growth rate or curve shape and d= curve inflexion point; e= base of natural logs and T=time. Initially, SigmaPlot graphed the mean data points for each treatment variable, processed the equation and produced a set of fitted data points. SAS Proc NLIN was used to generate sums of squares and confidence intervals. Two approaches for comparing two sigmoid curves

were employed: 1) a comparison of treatment means at each week, and 2) a comparison of confidence intervals for some or all of the equation parameters.

CALCULATION OF NET ASSIMILATION RATE

In addition to the growth rate estimations obtained through the Richards equation, mean net assimilation rates (NAR) was calculated for plants in each treatment. The NAR (also called 'unit leaf rate') is a measure of the efficiency of the leaves in producing new growth (Chiariello et al., 1989). The instantaneous NAR is given by: NAR=(1/A)*dW/dA, which after integrating and substituting becomes the useful equation for the mean over time:

NAR=$\{(W_2-W_1) / (A_2-A_1)\}^* \{(\log e\ A_2 - \log e\ A_1) / (t_2 - t_1)\}$, where W= dry weight, A=leaf area, t=time (Radford, 1967).

SHOOT/ROOT RATIO

The shoot/root ratio was also calculated for each treatment at those dates after the treatment imposition, weeks five to 14. The ratio of shoots to roots is an indicator of where the carbon resources of the plant are directed; this may be to new leaf growth or to storage of carbohydrates, generally in the roots. This ratio was included to learn if the straw mulch had any effect on the partitioning of carbohydrates over time.

RESULTS

PLANT GROWTH MEASUREMENTS OVER TIME

Leaf Number

As the season progressed beyond the treatment imposition date, week five, the harvests tended to reflect the effects the straw mulch had on the overall growth of the plants. However, over the course of the entire experiment no significant differences in mean leaf number were seen at any harvest date. Figure 3 displays the number of leaves for both treatments over the harvest period of 14 weeks. The maximum leaf number of 33 leaves, as noted in the mulched treatment, was achieved about nine weeks after planting. The bare soil treatment produced new leaves at a slower, eventually declining rate. The graph shows the plants in the bare soil treatment approaching the asymptote of 33 leaves.

Total Leaf Area

The straw mulch had a significant effect on the mean leaf area over the harvest period, weeks 6-14, as shown in Figure 4. The graph displays the non-linear curves as fitted from the Richards equation. Treatment means differed at the 8th, 9th, 10th, 11th and 13th weeks ($P<0.05$). In all cases, the plants grown in the mulched treatment had greater leaf area. In addition, the mulched curve rose faster ('c' parameter 1.1 vs. 0.39) and reached to ceiling or upper asymptote

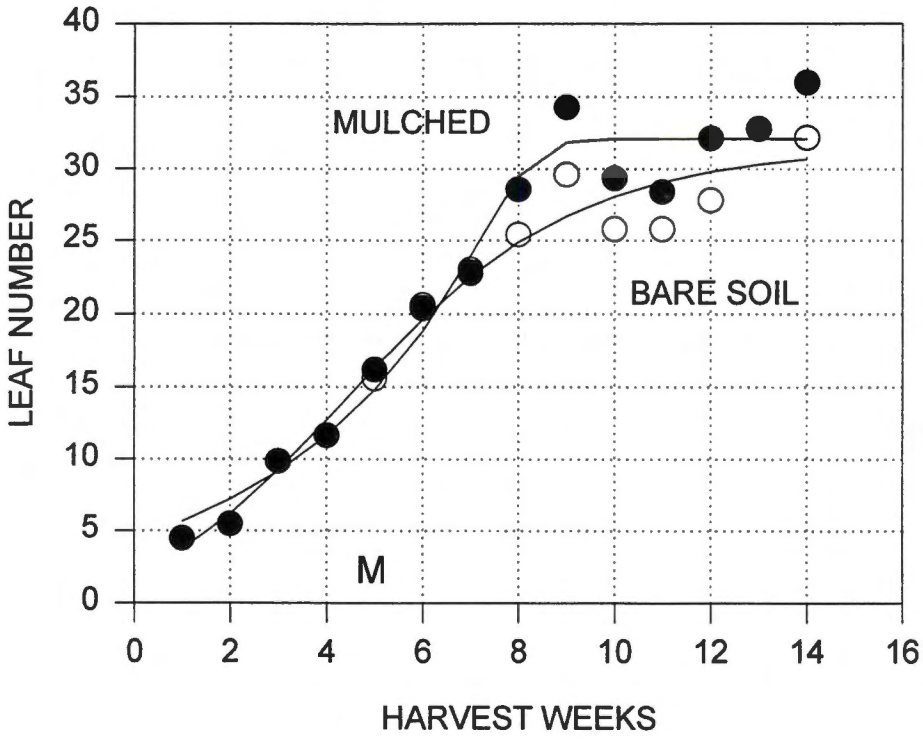

FIGURE 3. Relationship between chicory leaf number and harvest time.

Sigmoid regression over time showing variation in curves between mulched and bare soil treatments. Straw mulch (M) was applied on the 5th week. Data points are the mean of 5 plants. No significant differences were found between curves or weekly means.

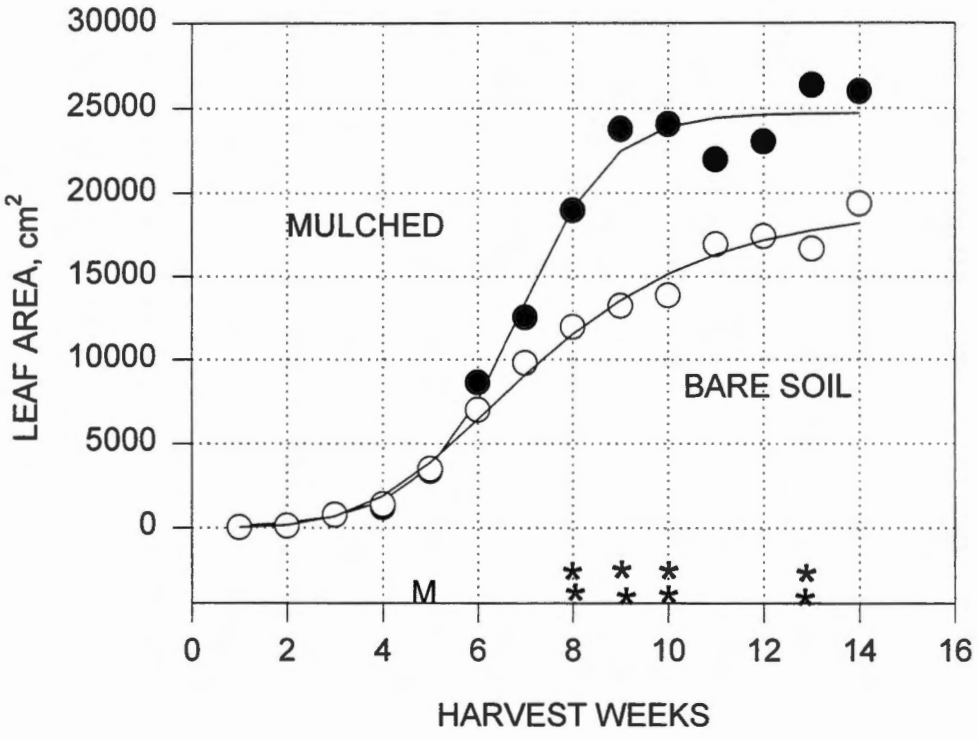

FIGURE 4. Relationship between chicory leaf area and harvest time.

Sigmoid regression over time showing variation in leaf area curves between mulched and bare soil treatments. Straw mulch (M) was applied on the 5th week. Data points are the mean of 5 plants. Treatment means differed at four weeks ($P<0.01$), **.

sooner, at week ten. For the mulched treatment the maximum leaf area per 33 leaves resulted in a mean area per leaf of 25,000/33 lvs= 758 cm^2 per leaf compared to the bare soil maximum (achieved at final harvest) of 18,000/31 lvs = 580 cm^2 per leaf, a 24% difference in mean leaf area.

Table 1 shows the 95% confidence intervals for the upper asymptote for the two treatments. No overlap between the levels is found showing that the mulch did exert a significant effect on the mean leaf area over time.

Table 1. Confidence intervals of the mean leaf area upper asymptote.

	Leaf Area, cm^2.	
	Upper Limit	Lower Limit
Treatment		
Mulched	26253	23162
Bare Soil	21649	16251

Leaf Dry Weight

As expected, the leaf dry weight, as shown in Figure 5, very closely follows the sigmoid pattern of the leaf area (Figure 4) as size and weight are closely related for most objects, including leaves. The dry weight increases at a rapid rate after the short lag phase. Treatment means differed at the 8th, 9th, 10th, 11th and 13th weeks ($P<0.05$).

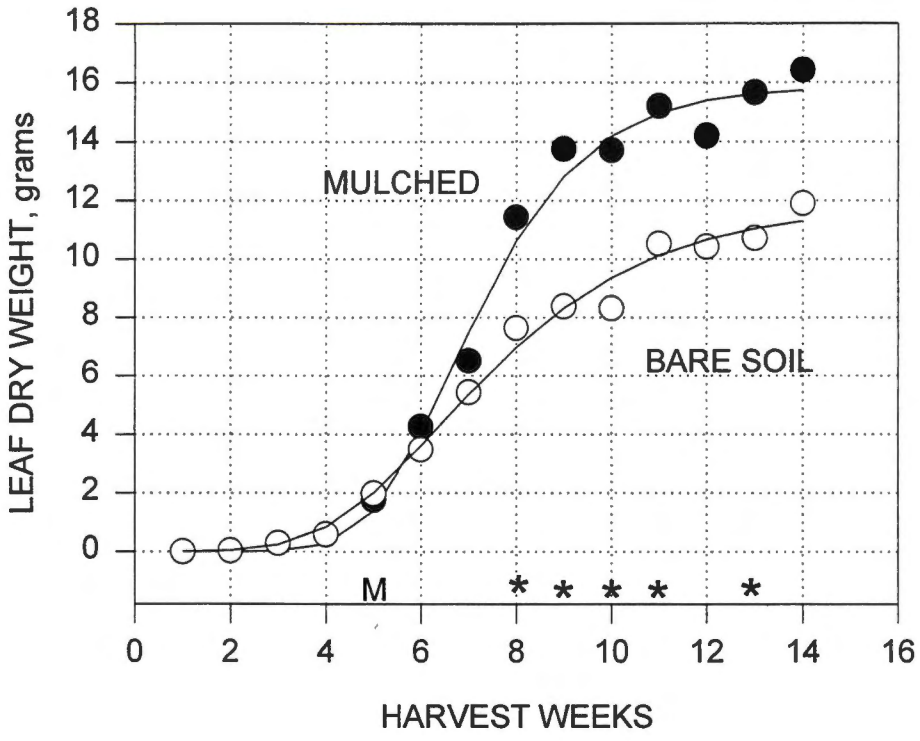

FIGURE 5. Relationship between chicory leaf dry weight and harvest time.

Sigmoid regression over time showing variation in leaf DW curves between mulched and bare soil treatments. Straw mulch (M) was applied on the 5th week. Data points are the mean of 5 plants. Treatment means differed at five weeks ($P<0.05$), *.

For the 'a' parameter, which is the maximum or upper asymptote, the 95% confidence intervals do not overlap (Table 2), thus the leaf dry weight differs between treatments ($P<0.05$).

Table 2. Confidence intervals of the mean leaf dry weight upper asymptote.

	Leaf Dry Weight, g	
	Upper Limit	Lower Limit
Treatment		
Mulched	16.5	14.5
Bare Soil	12.6	10.2

Crown Diameter

Figure 6 shows the effect of treatments on crown diameter over the 14 harvest dates. Prior to treatment imposition both curves are identical but by week seven, two weeks after the mulch application, the treatment means differed ($P<0.05$); differences were also noted at the 10th and 12th week ($P<0.01$). No statistical difference in curve parameters or curve confidence intervals between treatments was identified.

Root Diameter

Figure 7 shows the linear response of root diameter over time for the two treatments and the 95% confidence intervals for the slopes. Treatment means differed at the 8th and 14th weeks ($P<0.05$); differences were also noted at the 10th and 11th week ($P<0.01$). The greater slope (1.86 vs 1.39) given by the

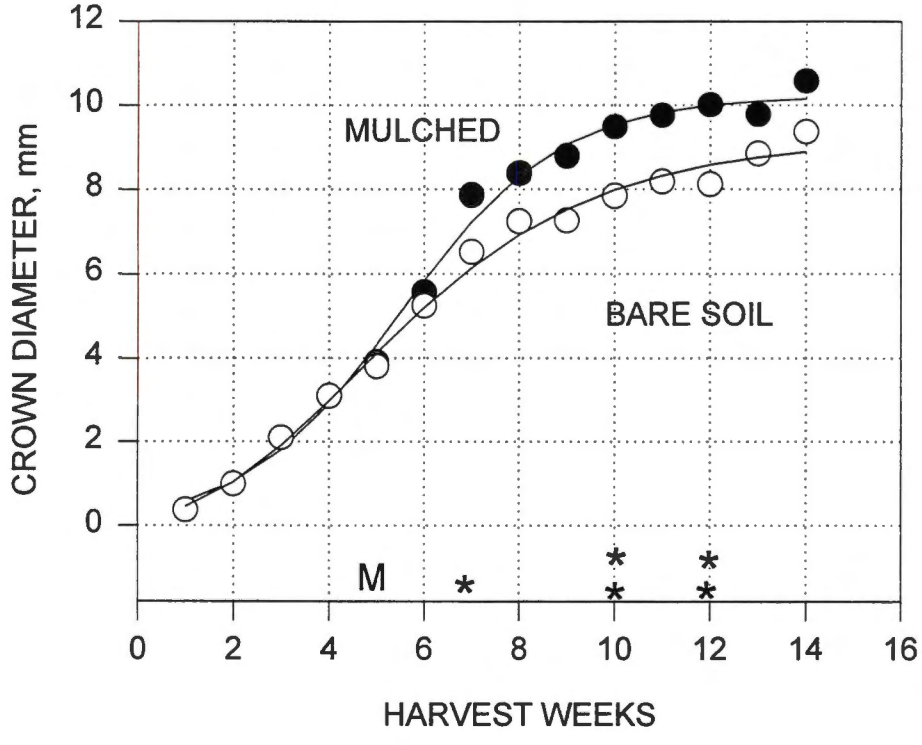

FIGURE 6. Relationship between chicory crown diameter and harvest time.

Sigmoid regression over time showing variation in crown diameter curves between mulched and bare soil treatments. Straw mulch (M) was applied on the 5th week. Data points are the mean of 5 plants. Treatment means differed at three weeks ($P<0.05$), *, or ($P<0.01$), **.

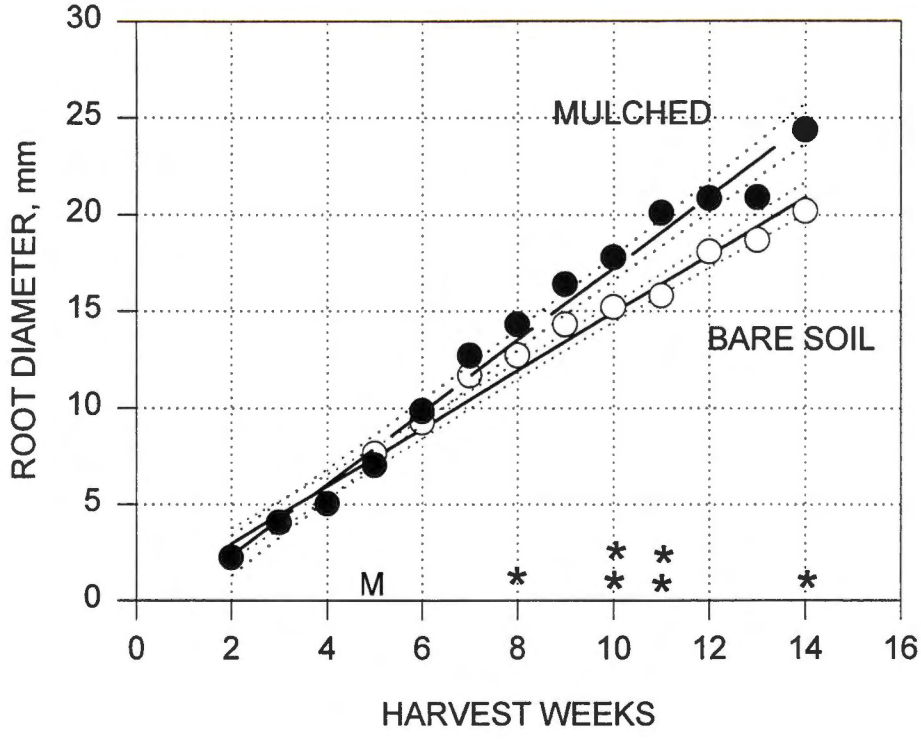

FIGURE 7. Linear regression between chicory root diameter and harvest time.

Linear regression over time showing variation in root diameter between mulched and bare soil treatments. Straw mulch (M) was applied on the 5th week. Data points are the mean of 5 plants. Treatment means differed at four harvest dates ($P<0.05$), *, or ($P<0.01$), **. Regression equation for mulched line: $Y= 1.86X - 1.39$; regression equation for bare soil line: $Y= 1.49X - 0.047$. Small dotted lines represent the 95% confidence limits for the two lines.

regression equation for the mulched treatment identifies a faster rate of root size increase, compared to the bare soil treatment, over the period of 14 weeks. Since these data points did not follow a sigmoid pattern, that is having upper and lower limits of growth, the Richards equation would have been inappropriate for use in this case.

Root Dry Weight

Mean root dry weights did not show any decline in values over time, an indicator of a sigmoid curve, and the best fit to this data was quadratic. Figure 8 shows the quadratic regression over time for the root dry weight by treatments. The plants in the mulched treatment were heavier ($P<0.05$) in root weight by the 10th week and this trend continues. Treatment means also differed at the 12th and 14th weeks ($P<0.05$); differences were also noted at the 13th week ($P<0.01$).

Total Plant Dry Weight

Figure 9 shows the linear increases in total dry weight over time for the two sets of plants. The regression equations were: mulched, $Y= 4.13X - 17.4$ and bare soil, $Y=2.64X - 9.9$. A comparison of equality between regression coefficients , the 'Chow test' (Kmenta, 1986), was performed and the results indicated that the slopes differed ($P<0.01$). Treatment means differed at the 8th, 11th and 14th weeks ($P<0.05$); also at the 10th and 13th weeks ($P<0.01$).

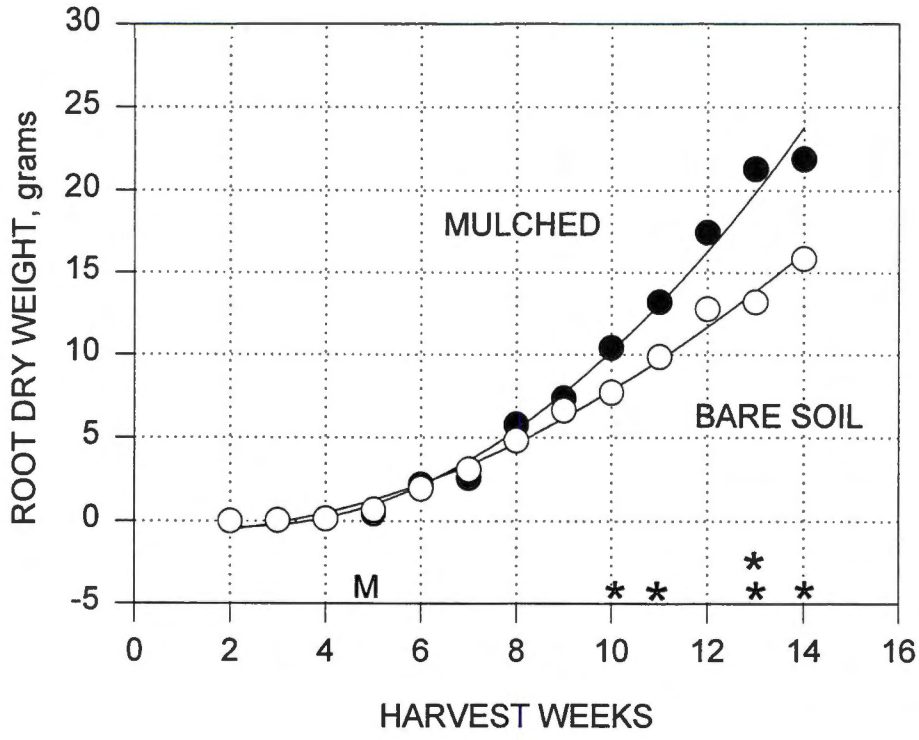

FIGURE 8. Quadratic regression between chicory root dry weight and harvest time.

Quadratic regression over time showing variation in root dry weight between mulched and bare soil treatments. Straw mulch (M) was applied on the 5th week. Data points are the mean of 5 plants. Treatment means differed at four harvest dates ($P<0.05$), *, or ($P<0.01$), **.

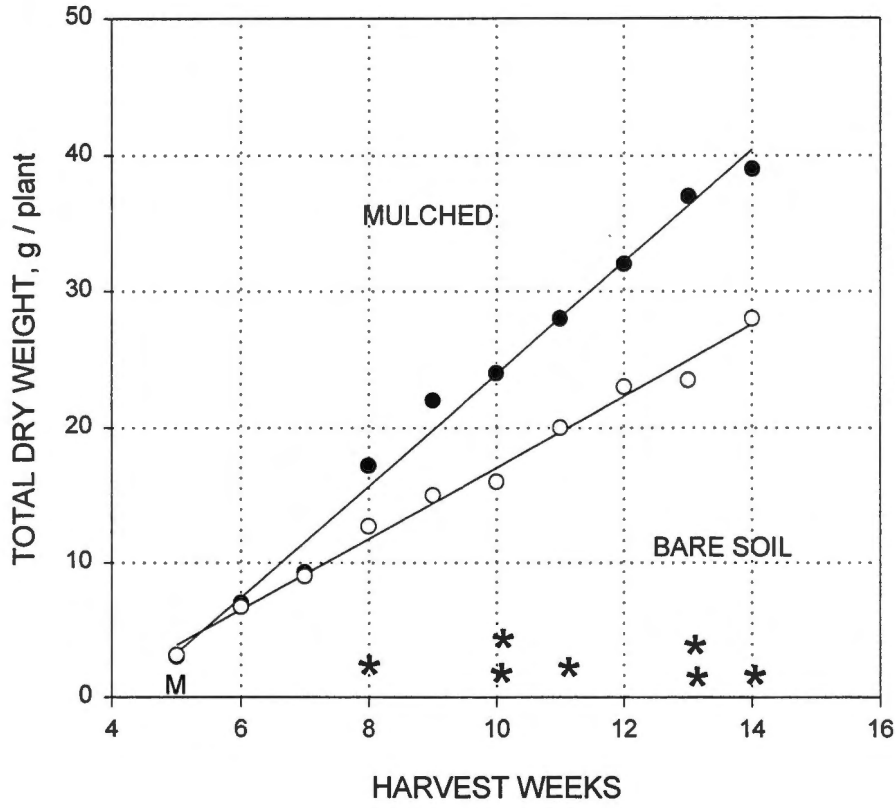

FIGURE 9. Linear regressions between total chicory plant dry weight and harvest weeks.

Linear relationships in total dry weight (leaf plus root) over time by treatment. Straw mulch was applied on the fifth week (M). Values are means of five plants. A comparison of slopes, 4.1 vs. 2.6 shows they differ ($P<0.01$). Treatment means differed at six harvest weeks ($P<0.05$), *, or ($P<0.01$), **.

Mean Net Assimilation Rate

The mean net assimilation rate for the mulched plants was 1.05 g per day over the length of 104 days, the duration of the experiment. The rate for the bare soil treatments was barely less, 0.95 g per day. No statistical evidence of difference is offered.

The Shoot/Root Ratio

Figure 10 shows the lack of variation between the treatments in regards the balance of leaf dry weight and root weight over the harvest season. Early in the season the leaf dry weight greatly exceeds the root weight and the ratio is well above unity. As the season progresses, however, both sets of plants slowly modify the partitioning of available carbohydrates as the leaves stabilize in growth and weight and the roots continue in increase in size. The ratio slowly drops through the season till late September when the roots have exceeded in weight the total of the leaves and the ratio drops below unity. The mulched plants did not differ in this fundamental aspect of carbohydrate storage by perennial roots which suggests that whatever stresses were avoided by the mulched plants did not tend to support a shift in carbohydrate partitioning.

BOLTING OBSERVATIONS

Environmental

Table 3 lists the number of bolting plants, by treatment and harvest week in addition to the daylength for each week. The first bolting plant was observed on the eighth week after planting, 70 DAP. Only harvest week 14 had days less

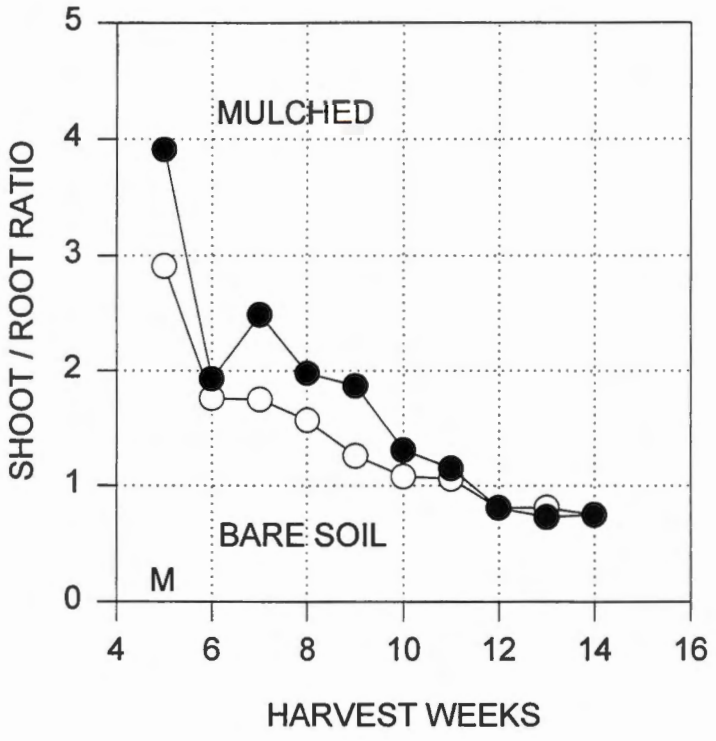

FIGURE 10. Chicory shoot/root ratio over harvest weeks.

Graph depicting variation in shoot/root ratio over time between mulched and bare soil treatments. Straw mulch (M) was applied on the 5th week. Data points are the mean of 5 plants.

Table 3. Effect of mulch treatment on bolting plants by harvest week.

HARVEST WEEK	DAY LENGTH HOURS [Z]	NUMBER OF BOLTING PLANTS MULCHED	BARE SOIL
8	13.3	1	0
9	13	3	2
10	12.8	0	0
11	12.5	1	0
12	12.3	1	0
13	12	0	0
14	11.8	0	1
	TOTALS	6	3

Z= MEASURED AT MID-WEEK

than 12 hours in length, but even then, at least one bolting plant was found. Harvest weeks eight and nine had the longest daylength and also the greatest number of bolting plants. A total of nine bolting plants, six from the mulch treatments and three from the bare soil, from a total of 71 harvested plants, was found over the course of seven weeks.

Effects of Mulch Treatment on Bolting

A chi-square test was constructed over the period covering the harvest weeks 8 to 14 (Table 4).

Table 4. Frequency table and chi-square value of Bolting Condition vs Treatments.

	NUMBER OF PLANTS	
	NO BOLT	BOLT
Mulched	30	6
Bare Soil	32	3
CHI-SQ= 1.05 DF=1, P>0.30		

No significant association between bolting and mulch treatment was found, even though the mulched treatment contributed six of the nine bolting plants. This percentage of bolting plants, 9 / 71, or 12.6% is comparable to other reports of chicory bolting during the seedling year (Hill, 1987; Volesky, 1996), regardless of cultivar.

Effects of Plant Size on Bolting

Table 5 shows the effects of various plant variables on bolting. Only one plant variable, leaf number, differed between the bolting and non-bolting plants ($P<0.01$). Table 5 displays the statistical evidence that only the leaf number was different between the vegetative and bolting plants, all other variables were found not different. Although not all bolting plants had a leaf count greater than non-bolting plants, on average, the bolting plants had in excess of 33 leaves.

Table 5. Changes in means due to bolting condition.

		Plant Variable					
	N	Leaves	LfArea	LfDW	RtDia	RtDW	CrnDia
Condition							
BOLT	9	34.0 a	22278 a	11.8 a	17.9 a	12.2 a	8.9 a
NO-BOLT	62	28.1 b	19215 a	13.6 a	17.9 a	11.2 a	9.3 a

Treatment means within a column followed by different letters differ (P<0.01).

Figure 11 is a photograph of various examples of chicory plants with the leaves stripped off showing a progression of bolting stalks from vegetative through bolting.

Correlation of Bolting and Spot of Color in Crown

The spot of color (Figure 2) found in the crown was not significantly correlated with bolting plants, nor any measured plant parameter (data not shown). The meaning of this zone of coloration, probably anthocyanin pigments, as they have been reported in the leaves of certain cultivars of chicory (Ryder, 1975), is not known. Additionally, the intensity of color was not found correlated with time of harvest since darkly colored spots appeared early in the season on some plants and many large plants had no color late in the season. Contrary to expectations it now seems unlikely that this coloration can be used to estimate plant maturity or 'ripeness-to-flower'.

FIGURE 11. Bolting progression in chicory plants.

Photograph of 'Daliva' chicory plants with leaves stripped off showing a progression of bolting. A vegetative plant is on the far left and a plant with an extended flower stalk is on the right.

Soil Temperatures

Figures 12 shows the variation in soil temperatures, at four cm deep, as affected by the straw mulch treatments. The mulch kept the soil cooler nearly all day but especially during midday as the sun approached and passed its daily zenith. The straw provided only marginal, in any, soil heat containment during the night. No temperatures were taken at night but early morning readings suggest a common stable nighttime temperature. The maximum temperature on sunny days was above 40° C in the bare soil but seldom above 35° C under the mulch. During the hottest part of the day the mulch kept the soil about 10° C cooler.

At six cm deep the soil temperatures were slightly less as the bare soil readings never exceeded 40° C and the soil beneath the mulch only occasionally exceeded 30° C (Figure 13). At this depth the mulched soil was about 8 degrees cooler at midday.

Table 6 is a paired 't' test comparing means of soil temperature at both depths by treatments. The results indicate that at four cm the mulched soil was, on average, 28.7° C compared to the bare soil treatment at 32.5° C ($P < 0.001$). At six cm the temperatures were slightly lower, the mulched treatment 28.3° C while the bare soil was 31.9° C degrees ($P<0.001$).

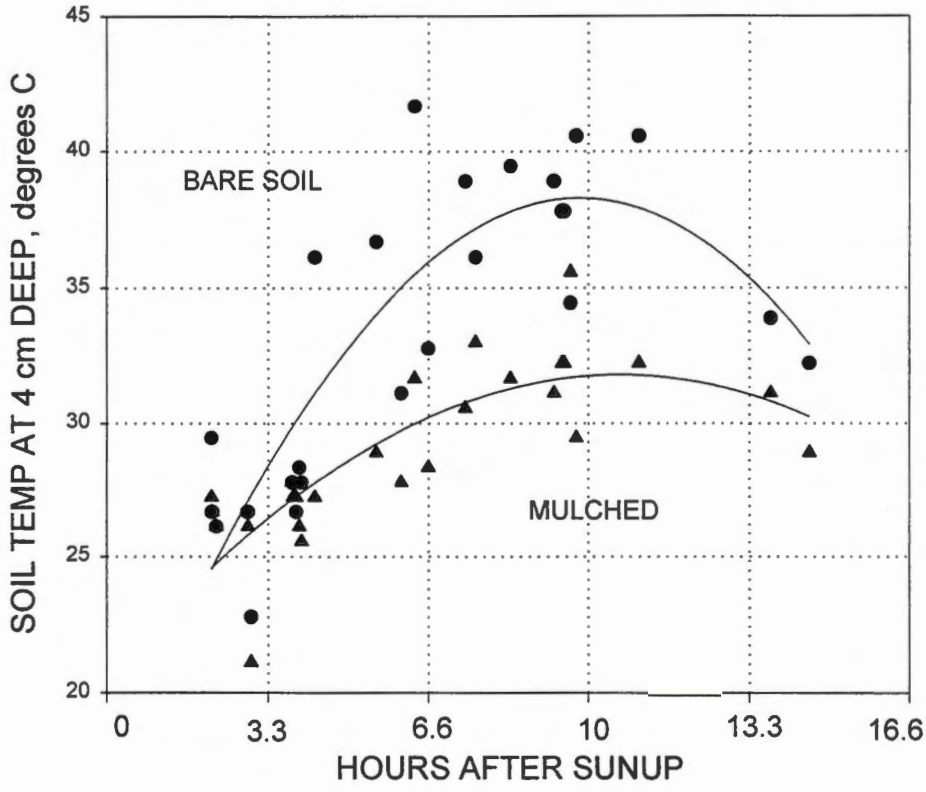

FIGURE 12. Quadratic regressions of soil temperatures at 4 cm depth.

Quadratic regressions of soil temperatures over time after sunup from mid-June to mid-September at four cm depth under straw mulched and bare soil conditions at Knoxville. Data points are the mean of two observations. Regression equation for bare soil treatment: $Y= (-6.52 * 10^{-5})X^2 + 0.08X + 15.6$; $r^2= 0.72$. Regression equation for mulched treatment: $Y= (-2.96 * 10^{-5})X^2 + 0.04X + 20.2$; $r^2= 0.64$.

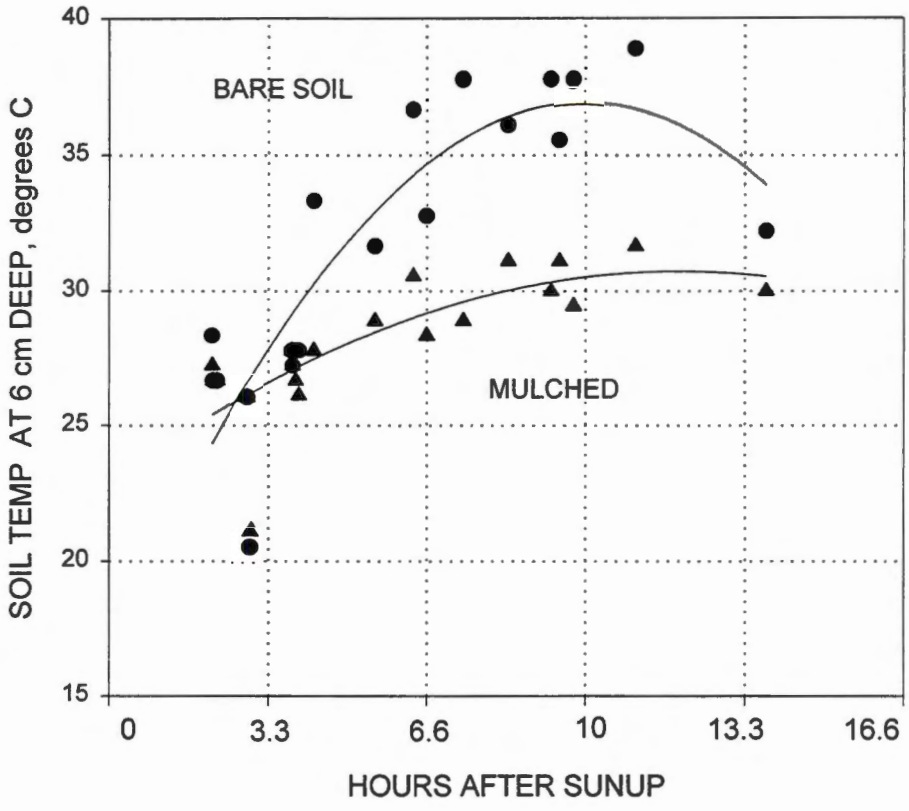

FIGURE 13. Quadratic regressions of soil temperatures at 6 cm depth.

Quadratic regressions of soil temperatures over time after sunup from mid-June to mid-September at six cm depth under straw mulched and bare soil conditions at Knoxville. Data points are the mean of two observations. Regression equation for bare soil treatment: $Y= (-5.76 * 10^{-5})X^2 + 0.07X + 16.35$; $r^2= 0.78$. Regression equation for mulched treatment: $Y= (-1.55 * 10^{-5})X^2 + 0.02X + 22.7$; $r^2= 0.62$.

Table 6. Mean soil temperatures (° C) at two soil depths by treatment.

	SOIL DEPTH	
	4 CM	6 CM
TREATMENT		
Mulched	28.7 a	28.3 a
Bare Soil	32.5 b	31.9 b

Means followed by different letters in the same column differ ($P<0.001$).

DISCUSSION

The application of soil applied straw mulch to chicory plants resulted in cooler soil temperatures ($P<0.001$) over the course of the summer with an average difference of 3° C. At midday however, the temperatures beneath the straw mulch were frequently ten degrees cooler. This is in agreement with other workers who have found organic mulches tend to stabilize daily fluctuations and minimize soil heat absorption, conserve soil moisture and reduce crusting. Other effects have been reported on the benefits of straw mulch, such as enhanced nutrient absorption due to better surface feeding roots and incorporation of additional organic matter in the soil (Russell, 1973), none of these data were collected in this study.

In addition to direct and indirect soil modifications by an organic mulch the altered spectra from sunlight reflected off straw mulch has been reported to act through the photomorphogenic pigments in the leaves of plants above the straw to modify the ratio of red/far red light and PAR (Photosynthetically Active Radiation) which subsequently may influence the carbohydrate partitioning and shoot/root ratios (Kasperbauer and Hunt, 1992; Matheny et al., 1992). Besides the data on soil temperatures the only other observed feature was a crusty soil surface on the bare soil surface and occasional puddling, rather than rapid infiltration, after the hand delivered sprinkler irrigation. No provisions had been made to isolate treatments from lateral soil water movement so presumably all

plants tapped the same soil moisture bank.

Evaporation of moisture from the surface and maximal heat absorption near the surface may have resulted in fewer or less active surface feeding roots on those plants in the bare soil treatment. As the season progressed however, the size of the rosette increased in all plants due to increased leaf number and individual leaf expansion. This increased leaf area provided a relatively complete circle of leaves emanating from the crown which provided shade for the soil near the plant. These leaves may have reduced the soil heat absorption near the plant and decreased the humidity gradient. No soil temperatures were taken under chicory leaves.

In addition to cooling the soil, the effects of the straw mulch were measurable in the growth of the chicory plants. Compared to the bare soil treatments the mulched plants had a significantly greater leaf area and reached the maximum leaf area far sooner than did the bare soil treatments. Geiger (1966) found that cooling a sugarbeet root decreased the transport of sucrose to the root and Rapoport and Loomis (1985) reported that cooling of sugarbeet roots resulted in less root growth and greater leaf growth. Whether the magnitude or fluctuations of cooling in the field was sufficient to cause the changes noted remains uncertain.

The effects of the mulch treatment on the growth of the chicory plants were summarized by the difference in total dry weight ($P<0.01$), which is the best overall measure of comparative plant performance (Lord et al., 1971). Although the effects of the mulch can be measured in the performance of the plants, why

the plants performed as found must be inferred from the above mentioned research on soil heat absorption, moisture retention, soil nutrients and microbial action, feeder root proliferation in zones of low soil impedance, and slow or low water infiltration due to soil crusting. These factors may have played a role in plant response.

The only parameter measured which did not significantly increase due to the mulch treatment was leaf number. The maximum leaf number of 33 leaves was reached sooner in the mulch treatment, by week nine, but that number remained constant over the next month, although the individual leaves expanded in area and the mulched leaves were on average 24% larger. The chicory plants in the bare soil continued to develop new leaves but the average number was never significantly different from the mulched treatment and never exceeded 33 leaves. This number of leaves may represent a genetic optimum or critical number of leaves or axillary nodes on the crown which must precede the transition from the juvenile to the adult reproductive stage.

Since the experiment was terminated as the plants were in autumnal decline, 1 October, little additional growth of the leaves was expected since the rate of leaf production is very sensitive to temperature (Robson, 1972). Possibly more root size could be gained as storage of carbohydrates continues through the cool weather, partly due to the dynamics of fructan biosynthesis in the leaves and roots, especially in the Asteraceae (Cyr et al., 1980)

Mulched plants grew bigger and faster but did not bolt at any greater rate. From the initial observation of a bolting plant during harvest week eight till the

final harvest seven weeks later, a total of 71 plants were harvested, yet only 13% of these or 9 plants were found bolting.

The question of why such a low percentage of plants flower remains unknown. It does suggest a highly variable population in regards this feature of premature bolting, especially since this cultivar is sold as an F-1 hybrid (Nunhems, 1994). The flowers of chicory are known to be self-incompatible due to the inability of the pollen grain to germinate on the stigma of the same flower (Paulet, 1985). This results in low rates of inbreeding and a lack of pure inbred lines which are needed to form the F-1 hybrids. Due to the self-incompatibility of chicory flowers the parents of F-1 hybrids have varying amounts of residual heterozygosity. Ryder (1979) reported that chicory cultivars labeled F-1 hybrids are not strictly so, due to this residual heterozygosity in the parents. Such a variation in parental stock may be the cause of the bolting variation of the plants in the field, only 13% of which flowered during the course of the experiment.

More than 65% of the bolting plants (6/9) came from the mulched treatment suggesting that those plants which grew best had the greatest opportunity for bolting. This is fully in agreement with the theory proposed by Harper and Ogden (1970) that the change to the flowering habit is related to the accumulation of a certain amount of stored food reserves. This concept has been reinforced by others (Hirose and Kachi, 1982; Werner, 1975) who have found that in biennials especially, a critical plant size is necessary before attainment of the flowering habit can be achieved. Biennials are generally single flowering (monocarpic or big-bang) and are thought to allocate food reserves in

order to maximize seed production output. Thus plants grown in a fertile soil environment can attain a critical size earlier and produce a larger number of seeds than plants grown in a less fertile environment. Werner (1975) found that a critical rosette size, (highly correlated with dry weight), independent of age, was needed prior to flowering in teasel (*Dipsacus fullonum* L.), a biennial. How the biennial plants of agriculture, which have been selected and bred for many years, can be modeled after their wild counterparts remains unclear.

The Net Assimilation Rate (NAR) expresses a plants' capacity to increase dry weight in terms of the area of its assimilatory surface. Herbaceous plants generally have higher growth rates than perennials or woody plants. This rate of increase in a whole plant dry weight per unit leaf area, also called 'unit leaf rate' (Chiariello et al., 1989) may represent the photosynthetic efficiency in an overall sense (Leopold and Kriedemann, 1975). The NAR is a measure of net result which is [total carbohydrate gain - total respiration loss] and may vary substantially according to respiration losses. NAR is not necessarily a measure of economic yield which is subject to various controls besides NAR (Watson, 1983). Various NAR's have been reported and usually are the highest by C4 plants over early growth when very little area produces photosynthates most of which are used to support growth and little allocated to storage. The highest NAR belongs to corn (*Zea mays*), producing 21 grams dry weight per meter square leaf per day, as listed in Leopold and Kriedemann (1975). Recent work listed oil palm (*Elaeis guineensis* Jacq.) at 0.43 g m^{-2} day^{-1} over 105 days (Lal

and Nor, 1994), bananas (*Musa* AAA; Cavendish sub-group) at about 5.7 g m^{-2} day^{-1} over 400 days (Eckstein et al., 1995) and summer squash (*Cucurbita pepo* L.) at about 10 g m^{-2} day^{-1} for only 30 days (NeSmith, 1993). The chicory NAR of about 1 g m^{-2} day^{-1} over the 105 days, regardless of treatment, indicates the straw mulch had little effect on the efficiency of the leaves to produce carbohydrates or the respiration of carbohydrates by the roots.

The Shoot/Root ratio (S/R) is a good guide to the stressfulness of the environment (Fitter and Hay, 1987). Plants in stressful conditions frequently allocate a larger percentage of their photosynthate to storage, often underground, thus decreasing the S/R ratio. The closeness of the S/R ratios between the treatments, throughout the season, and especially towards the end of the season, suggest no difference in partitioning of the carbohydrates by the chicory plants was afforded by the straw mulch.

Plants which were identified as bolting had a greater ($P<0.01$) number of leaves than non-bolting plants, 34 vs 28 leaves, but all other variables measured were not significantly different. Although the absolute number of leaves or the area they obtain are not in themselves thought to be the prime factor of floral induction, research does support the value of the photosynthetic contribution those leaves do make to the attainment of ripeness to flower. Bernier et al. (1981) list a number of plants where a high photosynthetic output, high light intensity and/or long days, especially during the early stages of growth shorten the juvenile stage. They attribute this response to the rapid increase in leaf number and area available to provide carbohydrates which support growth in the

roots, meristems, etc. Recently researchers have systematically removed the leaves from the apex of some plants thereby leaving only the rosette stem and shoot apex intact, and yet, even without the leaves, flowering occurred, indicating a specific node count may affect floral induction in long days (Lyons and Booze-Daniels, 1986).

Thomas and Schwabe (1969) reported that hops needed to initiate about 30-32 nodes before flowering. Schwabe and al-Doorhi (1973) found in black currants that the failure to respond to flower inducing treatments was due to the proximity of the roots to the apical growing points. Schawabe and al-Doorhi (1973) also found that at least 20 nodes were needed on the stem before flowering could occur. The closer the roots were to the apical meristem the less chance for flowering. Gebhardt and McDaniel (1991) found that in tobacco (*Nicotiana tabacum* L.), floral initiation is regulated by at least two inputs, a stimulatory signal from the leaves and an inhibitory signal from the roots. They also found the leaves increase in their signal output with age and size and can eventually override the inhibition of the roots. McDaniel (1980) found that in day neutral Wis 38 tobacco the close proximity of the roots to the apex is sufficient to prevent flowering. As the plant initiates new leaves more nodes separate the shoot meristem from the roots, and this distance can apparently reduce the root influence.

Larger apical juvenile bud size is typically associated with a longer distance from root to shoot meristem. Khait (1986) proposed three mechanisms by which this greater size could contribute to a lower concentration of a potential

inhibitory chemical mediator at the apical meristem. First, more axillary buds are present in the larger crown or stem; they may act as sinks, drawing off the mediator until finally the chemical concentration is too little to make a difference. Second, the mediator may undergo a chemical change as it passes through the crown. Third, either the dilution or chemical change may not be so much the direct result of the distance traveled, but rather of the time required to travel that distance.

Schwabe and al-Doorhi (1973) found the activity of GA's declined as the distance from the roots to the shoot increased and they speculated that reproductive activity was possible only when the apical meristem was sufficiently far from a source of the gibberellins, the roots. The roots are known as a source of enough GA to supply the entire plant (Carr, et al.,1964). Stem elongation and bolting can be induced with GA in some plants (Sachs, et al., 1959).

CONCLUSIONS

This research has shown that straw mulch effectively reduced the soil temperature at four and six cm by as much as 10° C at midday, and on average, 3.5° C. The effects of the straw mulch on the chicory plants over the harvest period were seen in the increased rate of growth of leaves and roots for those plants growing in the mulched soil.

The objectives relating to the growth rate of chicory in Knoxville under local summer conditions were met by measuring plant size parameters over time. A comparison of total dry weight increase over time gave an overall measure of how effective the mulch was in elevating the growth rate.

The one plant variable unaffected by the straw was the number of leaves per plant. Also unaffected by the straw mulch was the number of bolting plants. Although the mulched plants grew faster and larger they did not have more leaves nor did they show an increased tendency to bolt.

A positive association between bolting and leaf number per plant was found. Regardless of treatment, plants which exceeded approximately 33 leaves per plant had a greater ($P<0.01$) tendency to bolt. No other plant variable was correlated with bolting.

'Daliva' chicory culture in Knoxville is possible during the summer with irrigation necessary some years. Mulched plants grew fast and some reached a minimum marketable size, three cm, in the 14 weeks, though some plants were

bolting within that time. Since chicory is able to effectively utilize the cooler weather of autumn to store carbohydrates it may be instructive to continue these experiments by planting into mid-July, thus investigating second crop potential. Such a late planting may reduce the number of bolting plants by allowing the attainment of the adult vegetative stage after the long days of summer.

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SUMMARY CONCLUSIONS

These experiments were designed to identify and measure some of the factors associated with premature floral induction of 'Daliva' chicory. 'Daliva' is an extra early cultivar known to have a low requirement for winter temperatures prior to spring growth. Under certain conditions 'Daliva' can bolt the first year.

Although classified as a long day plant, pot grown 'Daliva' chicory with 22 or fewer leaves did not form any floral meristems in response to photoperiods greater than 14 hours. One day after the imposition of long photoperiods the total free amino acids leached from vegetative shoot tips appeared to rise sharply. Such a response to long photoperiods may indicate an involvement of free amino acids in the floral signal.

This research showed that by planting in early summer, bolting of 'Daliva' can begin within 90 days of planting in Knoxville. Some of this response may have been due to location as earlier research showed that plants in Crossville did not bolt when those in Knoxville did. Bolting plants from the field averaged more than 30 leaves, significantly more than the number found in vegetative plants (28). In many plants, a minimum number of leaves or nodes is associated with the conclusion of the juvenile state. This minimum number in 'Daliva' chicory is probably very close to 30 leaves. After the chicory plants attained 30 leaves the environmental cues were able to induce floral transition. The

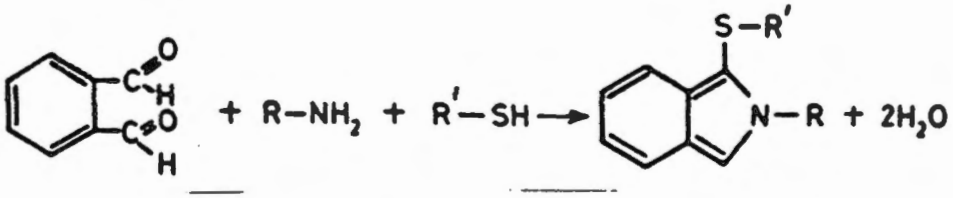
vegetative meristem in chicory was shown to be a flattened region surrounded by leaf primordia. Once induced to flower, however, the meristem changed to a domed shape.

Straw mulch beneath the chicory plants effectively cooled the soil and promoted vegetative growth. The mulch treatment had a two fold increase in bolting, but this was not significant due to low numbers.

APPENDICES

APPENDIX A: AMINO ACIDS

The di-aldehyde, *o*-phthlalaldehyde, (OPA) in the presence of free amino groups and a strong reducing agent, e.g. 2- mercaptoethanol, forms a planar fluorescent molecule which absorbs best at wavelength 334 nm and emits at wavelength 445 nm. The mobile carrier through the fluorimeter was HPLC water at a flow rate of 0.5 ml per minute.



OPA + Amino Acid + mercaptoethanol –>> OPA-AA + water

"Fluorescence refers to the secondary emission of light, generally for a period of 10^{-9} sec, by a compound after it has been 'excited' by the absorption of light of an appropriate wavelength. The absorption of photons of visible or ultraviolet radiation can raise the electrons of certain compounds from their stable, ground state to higher excited states. The return of these excited electrons to their original ground state is accompanied by dissipation of some of this excess energy via the emission of radiant energy (usually of a longer wavelength than that of the excitation radiation). Thus, in fluorescence detection a given substance is usually excited by a particular monochromatic light and the emitted wavelength is then analyzed by a fluorimeter."

Source: Rosenthal, G.A. 1985. Colorimetric and fluorimetric detection of amino acids. In: G. C. Barrett (ed.). Chemistry and biochemistry of the amino acids. Chapman and Hall, New York.

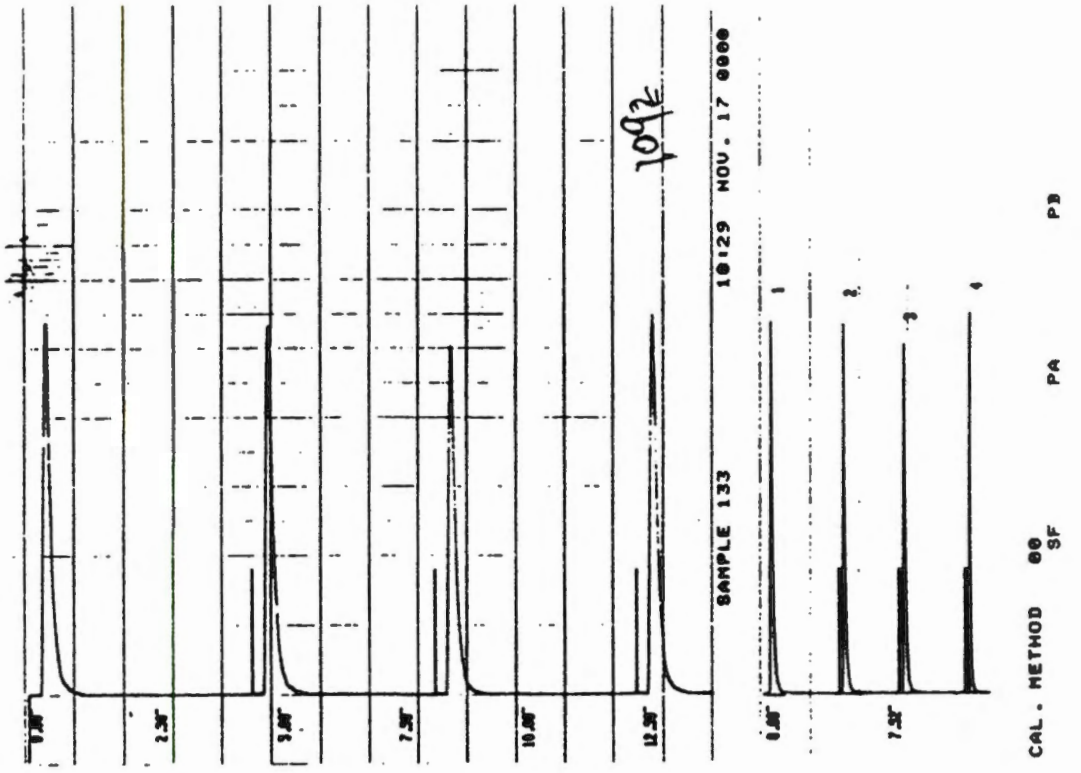


FIGURE 1. Sample chromatogram of fluorescent response of OPA derivatized amino acids.

Chromatogram showing response (area under the peak) of three separate samples, peaks 1,2, and 3, of leached liquor from lyophilized apical tissue from 'Daliva' chicory plants. Peak 4 is a reference peak of known amino acid amounts prepared exactly as the unknowns.

Amino Acid Calibration

An example: Start with 6 mg dry tissue. Leach tissue with 1200 ul methanol/water for 48 hrs. Dilute an aliquot 1 to 10. Take 10 ul this dilution, add 20 ul of OPA, add 50 ul of water. Fill a 50 ul loop and inject this volume into a line leading to the detector. The area of resultant peak is 3,600,000 units. A standard curve relates 437 picomoles free amino acids/10 ul to 2,500,000 area units. Therefore, the peak of the unknown quantity represents 629 picomoles/10 ul.

Since the injection of the standard was 50/80 x 100 = 62.5%, the total reaching the detector was 62.5% of the 437 picomoles or 273 picomoles. And the unknown will be 62.5% of 629 picomoles = 393 pmol per 10ul. But considering the dilution factor we increase by 10 fold the quantity to 3930 picomoles per 10 ul of initial leachate.

If 10 ul of leachate contains 3930 pmoles then the total for 1200 ul will be 471,600 pmol per .006 g. And the total is: 78,600,000 pmoles of free amino acids per gram of dry wt. Or 78.6 umol/g.

But since SI units require mixtures (as these readings represent) to be reported in *mass*, a conversion would be : The average mass of 20 amino acids is 137, therefore (78.6 umoles*137 grams) =108 ug/ g DW.

APPENDIX B: SUGARS

Neutral carbohydrates have pKa values around 12, e.g. glucose, 12.28; fructose 12.03, which shows that they are in fact, weak acids and at high pH values will be either partially or completely ionized and thus can be separated by anion exchange mechanisms. Classical silica based columns typically break down at high pH and the use of anion exchange to separate carbohydrates became possible only with the development of polymeric, non-porous pellicular resins, the packing material in PA-1 columns.

Pulsed amperometric detection (PAD) utilizes a repeating sequence of 3 applied electrical potentials, applied for specific durations. Cyclic voltametry has been used to determine the potentials of choice with the results that: 1) the same detector settings can be used for all carbohydrates and 2) PAD is equally efficient at detecting reducing and non-reducing carbohydrates. In practice, as the basic mobile phase carrying the sugar passes over a gold electrode, the time for oxidation to gold oxide with the positive current and the subsequent reduction back to gold upon current reversal is compared to the time for these reactions in the mobile phase without the sugar. This method can detect low picomoles of sugars. In this system the mobile phase was 40% HPLC water and 60% of a 200 mM solution of NaOH which supplied a 160 mM NaOH solution, pH 12.6, at the pumphead. The flow rate was 0.5ml per minute at 1250 psi. Source: Dionex Technical Note, TN 20, March 1989.

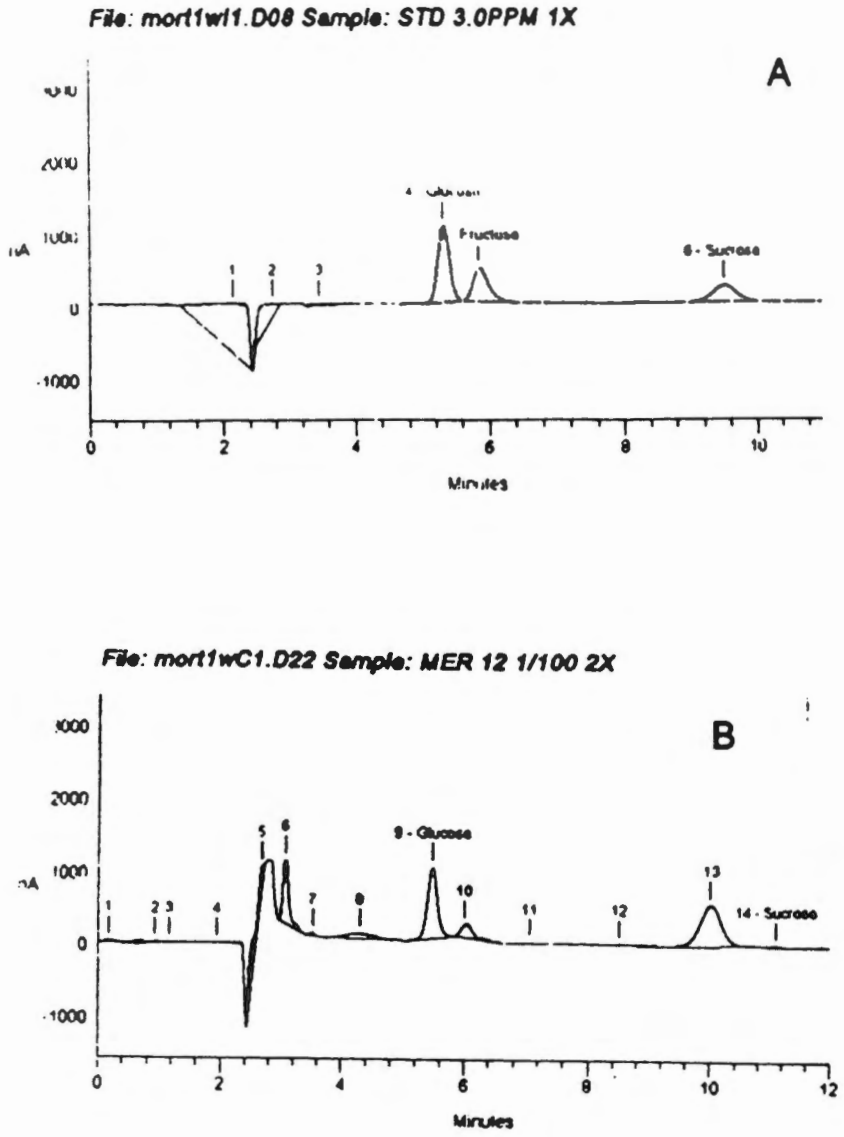
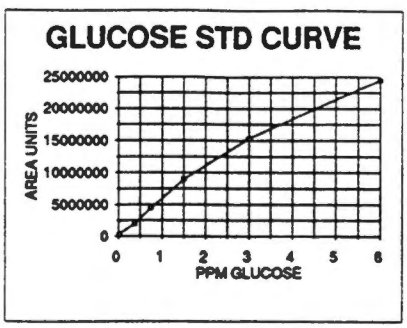


FIGURE 2. Sample chromatograms of soluble sugars separation.

Figure 2A shows peaks and retention times of known standards, at 3 ppm, of named sugars. The ordinate value is given in nanoamps. The labeling of peaks is operator directed and corresponds to time of appearance, + or - 5%.
Figure 2B shows an actual chromatogram of the leached liquors after dilution with water only, from the apical shoot tissue of 'Daliva' chicory plants. Note peak labeling in error as peaks 9, 10, and 13 are the sugars in question but have slightly modified retention times.

Regression Output:

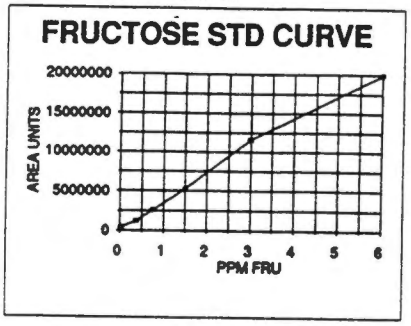
Constant	1360827
Std Err of Y Est	1498813
R Squared	0.978782
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	4046607
Std Err of Coef.	297896.5



REGRESSION EQUATION
Y = 4046607 X +1360827

Regression Output:

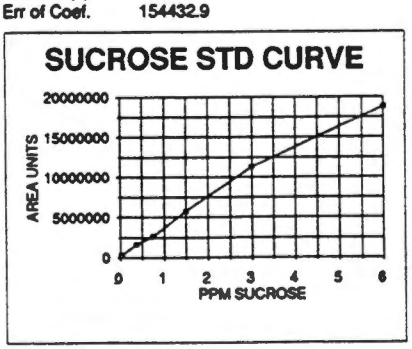
Constant	294788.8
Std Err of Y Est	692381.3
R Squared	0.993313
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	3354449
Std Err of Coef.	137614.2



REGRESSION EQUATION
Y= 3354448 X + 294788.8

Regression Output:

Constant	635001.4
Std Err of Y Est	777001.4
R Squared	0.99039
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	3135549
Std Err of Coef.	154432.9



REGRESSION EQUATION

Y = 3135549 X + 635001.4

FIGURE 3. Standard sugar curves.

Sugars Calibration

An example: Start with 6 mg dry tissue. Leach tissue with 1200 ul methanol/water for 48 hrs. Dilute an aliquot 1/100. Inject 25 ul of this dilution into the ion chromatograph. The area of the resultant glucose peak is 8,500,000 units. The standard curve relates 1.5 ppm glucose to 12,000,000 units. Therefore the unknown contains 1.06 ppm glucose. Considering the dilution factor, we multiply by 100 to get 106 ppm glucose per 6 mg dry tissue.

If 6 mg dry tissue contains 106 ppm then 1 gram contains 17,708 ppm glucose. To convert ppm glucose to mg glucose use the formula: 180 mg glucose / g H_2O = 180,000 ppm. Hence 17,708 ppm is equivalent to 17.7 mg glucose/ g dry wt.

To report pure substances in SI units one uses 'moles of glucose'. To convert 17.7 mg/g to moles use 180 g = 1 mole glucose and multiply out giving $9.83 * 10^{-5}$ moles/g DW. Or 98.3 umol glucose/g DW.

VITA

Jomo MacDermott was born in Chicago on 1 February, 1944. He attended Catholic schools in the city. After one semester of college he joined his cousin, a missionary priest, in the mountains of Papua New Guinea for nearly two years. He returned home and restarted his education. He received a B.S. from the University of Minnesota and a M.S. from Ohio State University, both in applied plant sciences.