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ETHYLENE SYNTHESIS AND SENSITIVITY IN CROP PLANTS

by

Joseph F. Romagnano

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOHPY

in

Plant Science

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Logan, Utah

2008

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ABSTRACT

Ethylene Synthesis and Sensitivity in Crop Plants

by

Joseph F. Romagnano, Master of Science

Utah State University, 2008

Major Professor: Dr. Bruce G. Bugbee
Department: Plants, Soils, and Climate

The gaseous plant hormone ethylene is a small molecule that regulates developmental change. Research was conducted in three areas: sensitivity, synthesis, and alterations to synthesis. Vegetative pea plants were more sensitive than radish plants to atmospheric ethylene. Light intensity did not affect ethylene sensitivity. Ethylene synthesis rates were measured for unstressed cotton, corn, soybean, and tomato plants. The per-plant ethylene synthesis rate ranged from 0.1-80 pmol $plant^{-1}$ s^{-1}. However, when normalized to net photosynthetic rate, this range was 1-4 µmol of ethylene synthesis per mol of CO_2 uptake. Diurnal cycles in ethylene synthesis were present in all crops studied. These cycles were disrupted by drought stress and were attenuated when synthesis rates underwent large changes. Drought stress decreased synthesis in cotton. Flooded corn and soybean had increased synthesis. Blocked perception had no effect on ethylene synthesis or net photosynthetic rate in healthy unstressed plants. (192 pages)

DEDICATION

To my mother and my sister
Prosapia est animus vita.

and

for my grandfather

ACKNOWLEDGMENTS

No man is an island unto himself. The successful completion of this journey is due to the combined efforts of many people. All of you who have helped me along the way, you have my gratitude. This work would not have been finished without your help.

First thanks, as always, go to my family who have always supported me. You all mean the world to me, and I wouldn't have been able to do this without your support. I would also like to acknowledge the passing of my grandfather before this dissertation was complete. He never saw it, but he always believed.

Special thanks go to my major professor, Dr. Bruce Bugbee. Your guidance has been invaluable. Thank you for your patience, understanding, excellent discussions, and challenging me every day. Thank you to all of my committee members, Ray Wheeler, Gail Bingham, Heidi Kratsch, John Carman, and Yajun Wu, who have taken the time to teach me new and different ways of looking at the world of plants.

Finally, I must acknowledge the help of all the members of St. Jerome Newman Center, my second family. To Jake for putting up with endless babble about plants in the middle of the night. Marty, for helping with the preparation of foil hats and foiled plants in the dead of night. Amanda, Eamonn, Sarah, Amy, Aldo, and Joe for acting as sanity checkers and safety valves. Ann for finding me a home and then selling it with many adventures in between. Mark, Austin, John, and Kelley for friendship and caring. Terri and Gladys deserve special thanks for acting as surrogate "moms" when all else was going haywire. And, most

especially, thanks to Fr. JJ Schwall for all the good meals together, the guidance, and the good swift kicks in the butt when needed.

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Joseph Romagnano

FOREWORD

Agriculture is a cornerstone of our global civilization. The technical laborers and city-dwellers of our society all depend on crops, often grown hundreds, if not thousands, of miles from their location, to fulfill basic nutritional requirements. The advent of the “green revolution,” triggered by advances in the field of crop science, has allowed more people to survive per hectare of arable land than ever before. Advances in plant nutrition, crop breeding, and hormone application have all contributed to the increased yields. Also, advances in greenhouse management, plant propagation techniques and commercial automation have led to a boom in the floriculture industry. All of these operations are subject to the effects of drought, flood, and other biotic and abiotic stresses.

Ethylene gas is a plant hormone responsible for the regulation of developmental change and the perception of stress. Although its identity was unknown, for thousands of years, ethylene was used to promote uniform fruit ripening. Since its discovery at the end of the 19th century as the active agent in illumination gas, much has been learned about the effects of ethylene on plant growth. The culmination of this work has been the elucidation of the complete ethylene synthesis pathway and a near complete picture of the ethylene perception pathway. This knowledge, coupled with advances in ethylene measurement, paves the way for studies that further enhance our ability to control ethylene synthesis and perception. These controls will have a widespread commercial impact that can lead to an improved quality of life.

There have been many experiments demonstrating the effects of ethylene at high concentrations (>0.05 μmol mol^{-1}, 0.05 ppm). In the past, our lab has focused on the effects of chronic long-term, low-dose ethylene exposure (<0.05 ppm). The impetus for this research was the need to develop a system capable of maintaining healthy plant growth in the controlled environments of spacecraft. The data could further be applied to understanding the effects of ethylene in other controlled environments also experiencing poor air exchange. Examples include large commercial greenhouses with hydrocarbon-based heaters that have poor combustion or forklifts that generate ethylene as a by-product.

Plants constitutively produce ethylene. In most controlled environments, even unstressed plants are the chief source of ethylene. In nearly all cases, it is a change in the rate of ethylene synthesis that signals a stress state or developmental change. The techniques used to study ethylene synthesis to date have been problematic at best, and there is a lack of clear data for multiple crops using the same technique. The studies in this dissertation quantified rates of ethylene synthesis for four crop plants under normal and stressed conditions, thus providing a cohesive data set for future research into ethylene physiology.

The completed studies supplement this body of work in three key areas. First, the physiological effect of completely blocking ethylene perception through the application of 1-methylcyclopropene (1-MCP) was examined. The effect of light intensity on ethylene sensitivity was also examined. These are two simple techniques that could alter plant responses to stress. Third, acute water deficit and flood stress ethylene synthesis rates were obtained using intact plants in a

steady-state flow-through system. These studies will help determine the validity of prior work conducted using closed chambers and detached plant tissue.

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CHAPTER 1

LITERATURE REVIEW

Ethylene in History

Ethylene gas played a role in history and agriculture long before it was recognized as a plant hormone. Some of the first documented historical techniques used to promote fruit ripening are ancient Egyptians cutting sycamore figs and the Chinese burning incense to ripen pears (Wright, 1976; Chaves and Mello-Farias, 2006). Although unknown to the practitioners of the time, these practices released ethylene gas, which promoted fruit ripening. Ethylene gas emitted from a rock fissure may also have been responsible for the trance states the oracle of Delphi would enter before prophesying (Spiller et al., 2002). It was in 1795 that ethylene was combined with chlorine gas to produce oil of the Dutch chemists. The name is due to the Society of Hollandish Chemists (a loose affiliation of four friends). For its part in the process, ethylene was known as olefiant gas – or oil-making gas (Snelders, 1980), and it became a compound of commercial interest. Later, with the introduction of a standard nomenclature system, olefiant gas was named ethylene.

Ancient agricultural practices notwithstanding, several astute observations in the mid to late 19th century led to the identification of ethylene as a modifier of plant growth and development. The chronology of events has been conveyed in great detail in the works of Abeles et al. (1992) and Chaves and Mello-Farias (2006). In brief, the use of gas generated from coal (i.e., illuminating gas) for lighting purposes was popular throughout the 19th century. It was noticed that

trees and plants growing near buried gas lines and gas lights were often stunted and injured (Girardin, 1864; Crocker and Knight, 1908). These observations and experiments were validated by the seminal work of Neljubow (1901), who showed that 1 part ethylene per 1,000,000 in air (1 μmol mol^{-1} or 1 part per million, ppm) was able to generate the same response in etiolated pea seedlings as exposure to illumination-gas tainted air. From that point on, ethylene research has catapulted forward to an age in which we now know both the molecular underpinnings of ethylene synthesis and perception, and quantification of ethylene is an automated routine procedure. These techniques have been made possible due to advances in molecular biology and analytical chemistry. It is now possible to observe responses, not only in isolated plant tissues or detached organs, but also in whole plants under conditions that can be carefully controlled and monitored.

Ethylene Measurement

The largest contribution to ethylene research has been the development of a rapid means of quantifying ethylene from gas samples. Prior to the 1950's, ethylene researchers had to rely upon time consuming wet chemistry techniques. For example, the technique used by Crocker and Knight (1908) in their experiments with carnations relied upon bubbling illumination gas through a special ice-packed absorption chamber containing a solution of bromin (bromine) and water. Ethylene would form ethylene dibromid (now called ethylene dibromid<u>e</u>) and, with the other compounds in the illuminating gas, would form oil in the solvent. This oil was then washed, fractionated, and distilled. The end

result of the process was the mass of ethylene dibromid formed by the ethylene contained within the illuminating gas. Researchers could then back-calculate the amount of ethylene needed to form the oil and, thus, arrive at the concentration of ethylene in the illuminating gas. However, as Crocker and Knight (1908) noted, there were serious drawbacks to this technique. Foremost is loss of ethylene dibromid at every fractionation and distillation step, and second is interference due to other oils with a boiling point similar to ethylene dibromid. Their technique was able to measure 3.2% ethylene content in illumination gas samples, although this was represented as an underestimate.

Forty-four years later, Young et al. (1952) introduced a refined version of the wet chemistry techniques used by Crocker and Knight. Their manometric technique claimed an accuracy of ±5% for an ethylene concentration range 0.5 ppm and higher with a maximum loss of 0.05 ppm. Similar to the technique used by Crocker and Knight, ethylene-containing gas samples were instead passed over a solution of mercury perchlorate as opposed to a bromine solution. This created an ethylene-mercury complex that would later be broken by the release of hydrochloric acid into the solution. The released ethylene gas could then be collected in a manometric cylinder and the volume measured. Although it was still a time-consuming process, this technique had the advantage of being specific to ethylene when used to measure plant emissions. Further explanations and evaluations of other period techniques can be found in the review by Burg (1962).

The introduction of the flame ionization detector (FID) in 1958 (Ettre, 2002) paved the way for rapid, direct, analysis of ethylene in minute quantities. The hallmarks of the detector, high sensitivity, predictable response, and extended linear range, have made it nearly universal in gas chromatography applications (Ettre, 2002). Although refinements to electronics and subsequent automation by computer have occurred, the basic design of the FID has remained unchanged. In essence, an FID consists of a stable flame fueled by a hydrogen/air mixture that is ionized by the placement of electrodes at the base of the flame. A detector, consisting of a second pair of electrodes or a wire mesh, is then placed above the flame. By the end of the 1950's, Burg and Stolwijk (1959) had used the new detector to measure nanomolar ethylene production rates from apple tissue slices. Although theoretical limits of detection have not improved (lowest is 10^{-11} moles in Burg, 1962), advances in sample concentration, column packing materials and automated sampling techniques allow for the near-real-time measurement of picomolar gas concentrations from concentrated gas samples (see discussion in Materials and Methods section of Ch. 2).

Laser photoacoustic spectroscopy is an alternative technique that, in principle, also permits rapid quantification of non-concentrated samples. This technique relies upon the absorption of infrared energy by the molecule of interest. The energy, usually provided by an infrared laser, excites the molecule to a higher kinetic state. When this occurs in a static vessel of known volume and the absorbed energy is released, temperature is increased which also

increases the pressure within the chamber. A sensitive microphone can detect induced pressure waves when the energy source is modulated at acoustic frequencies (Woltering et al., 1988). Woltering et al. (1988) reported a sensitivity of 0.05 nmol mol^{-1} (parts per billion, ppb which is equivalent to 0.00005 ppm) in such a system. However, their sampling times were limited to 45 minutes per sample. Although these instruments have great potential for improvement in sensitivity and speed, the technique is not widespread, and there are no available commercial instruments.

Ethylene Synthesis Biochemistry

Chaves and Mello-Farias (2006) provide a thorough review of the ethylene synthesis pathway. In brief, the end of the ethylene synthesis pathway involves three enzymes to convert methionine into ethylene (Fig. 1-1). Two of these enzymes are involved in the formation and oxidation of the immediate precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC). ACC-synthase converts S-Adenosylmethionine (SAM) into ACC and is the rate-limiting step in the pathway. ACC-oxidase catalyzes the conversion of ACC to ethylene. The final conversion of ACC to ethylene is oxygen dependent (Kende, 1993). Ethylene synthesis inhibitors disrupt the pathway by targeting either ACC-synthase or ACC-oxidase. There are four chemical inhibitors of ethylene synthesis: aminoethoxyvinylglycine (AVG) and aminoethoxycetic acid (AOA) disrupt ACC synthase; cobalt (Co^{2+}) and α-aminois-butyric acid (AIBA) disrupt ACC oxidase. Yang and Hoffman (1984) reviewed these compounds and their inhibition mechanisms. AOA, by virtue of being in the same chemical family as

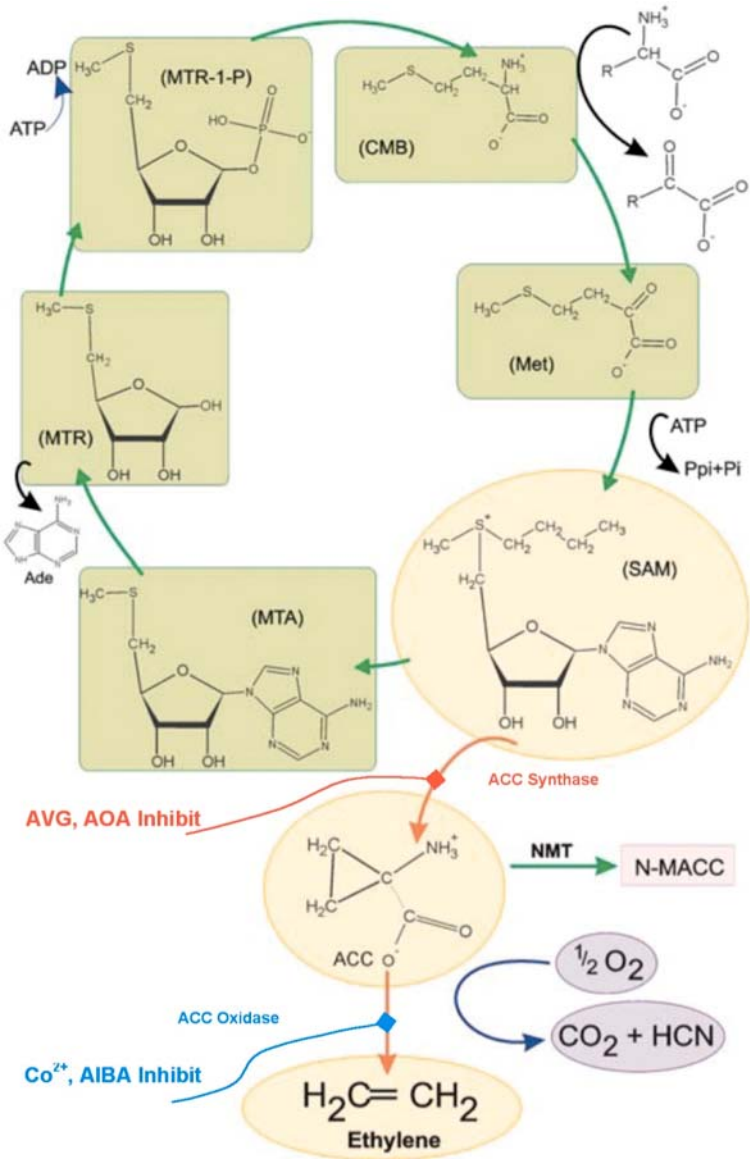

Figure 1-1. The ethylene synthesis pathway (modified from Chaves and Mello-Farias, 2006). Aminoethoxyvinylglycine (AVG) and Aminoethoxycetic acid (AOA) disrupt ACC Synthase and Cobalt (Co^{2+}) and α-aminois-butyric acid (AIBA) disrupt ACC Oxidase.

AVG reacts in a similar manner (Yang and Hoffman, 1984). Ions of Co^{2+} were first shown to interfere with ethylene synthesis in plugs of apple tissue (Lau and Yang, 1976). Later, it was proposed that Co^{2+} acts by complexing with sulfhydryl protein groups (Yang and Hoffman, 1984). The data, however, were

inconclusive. AIBA is structurally similar to ACC and, therefore, acts as a competitor for the binding site of ACC oxidase (Satoh and Esashi, 1980; Liu et al., 1984).

The primary advantages of these chemicals in the context of controlled environment plant growth is the ability to reduce atmospheric ethylene concentrations without resorting to the use of bulky filter material or other scrubbing apparatus. Also, the ability to time when the chemicals are applied allows for a targeted removal of ethylene and for experiments that look at ethylene-critical development stages. The primary disadvantage of AVG, AOA, and Co^{2+} is that by their mechanism of action, they are inherently nonspecific to the ethylene synthesis pathway (Jackson, 1985). Thus, there is an elevated risk of secondary effects associated with using these compounds, although no severe effects have been documented. Since it is competitively binding to ACC oxidase, AIBA is thus more specific to the ethylene synthesis pathway. Possible contamination of a controlled environment due to external application of compounds and the fact that the effects induced by these inhibitors last only as long as the supply in the plant are two primary disadvantages. Thorough cleaning and proper disposal of the waste is required between experimental trials for the former. For the latter, a continuous-dosing requirement is imposed in order for the effect to remain for a long duration study.

The different enzymes these compounds act on allow for multiple combinations and applications to experiments. Thus, through careful timing and application, control over ethylene synthesis can be achieved. For example, in an

experiment designed to only slow down synthesis but not completely disable it, applications of a low concentration of any of these compounds would work. For stronger synthesis inhibition, applications of two or more of these compounds at a higher concentration would work to block the activities of both enzymes. If an experimenter were interested in controlling the rate of ethylene synthesis through the use of ACC, AVG or AOA should also be supplied so that only the ACC provided would be converted to ethylene.

The rise of molecular biology and the genetic techniques developed from it brought new tools to the study of ethylene physiology. Antisense techniques, for example, can permanently reduce the amount of functional synthesis enzyme. An alternative method inserts a gene that encodes an enzyme (ACC deaminase) capable of removing the substrate required for ethylene synthesis.

Over the past 15 years, transgene and antisense methods have been developed to permanently modify ethylene synthesis in crop plants. Antisense methods control gene expression by exploiting base-pair complementarity to regulate the level of a transcripted target RNA strand. This is accomplished by inserting a constructed gene that generates an mRNA that is complementary to the target gene mRNA. Thus, copies of the anti-sense gene mRNA will bind to the mRNA of the target gene, preventing translation. Transgene techniques differ from antisense since it is often the end-product of an imported gene that is used to control the target gene. For example, ACC deaminase proteins from bacteria can lower the pool of available ACC in the plant cell, decreasing ethylene synthesis.

Klee et al. (1991) was one of the first to report transgenic control of ethylene synthesis by the insertion and expression of a bacterial ACC deaminase gene into tomato plants. The ACC deaminase produced by the transgene degraded enough ACC such that ethylene synthesis was decreased, time to ripening was significantly delayed, and mature fruits were firm six weeks longer than their nontransgenic counterparts. Since that initial work, subsequent researchers have used molecular techniques to regulate other steps in ethylene synthesis. Ayub et al. (1996) used antisense techniques to reduce ACC oxidase levels in cantaloupe fruits. Good et al. (1994) inserted a transgene that expresses S-adenosylmethionine hydrolase (SAMase) into tomato plants. Similar to ACC deaminase, this protein affects ethylene synthesis by decreasing the pool of available SAM. These represent a small sample of the applications of these techniques for ethylene synthesis control. Further discussion can be found in Stearns and Glick (2003).

Despite the extensive literature on biological ethylene production, rates of whole plant synthesis are not well characterized. Klassen and Bugbee (2004) summarized the literature on ethylene production by crop plants. Rates of synthesis range 200-fold from 0.01 to 2.0 nmol $kg_{(Dry)}^{-1}$ s^{-1} in roots and shoots of healthy plants, and production rates are 2 to 10 times higher in stressed plants. The majority of these studies measured ethylene synthesis from excised tissues in closed containers. The techniques used were consistent with the detection limits of instruments available to researchers at that time. It was often necessary for ethylene to accumulate in sealed containers for a considerable period of time

before measurements so that detectable levels could be obtained. It is well known that mechanical perturbations and excision promote wound ethylene production. Accumulation times, often hours long, can also induce artifacts as the excised tissue desiccates or is depleted of necessary metabolites. As a result, many studies may predict artificially high estimates of production rates in intact plants (Abeles et al. 1992; Morgan and Drew, 1997). Also, quantification of wound ethylene contribution to the synthesis is often overlooked. Instead, most techniques rely on a waiting period post-detachment for the wound-ethylene to subside before making their measurements (Abeles et al. 1992). Rates of ethylene production also vary with environmental conditions.

Ethylene Perception Biochemistry

Ethylene perception is a two-component system for signal transduction that is regulated by negative feedback (Urao et al., 2000). Negative feedback occurs when the product of an enzymatic pathway is able to influence the pathway in such a manner as to decrease the formation of the end product. In the case of a response pathway, such as the response pathway for ethylene, this definition is altered to reflect how a signal from a receptor protein is modulated in response to the binding of a signal molecule. For a negatively regulated response pathway, the signal molecule inactivates a constitutive signal (or interaction in this case) transmitted by the activity of the receptor protein (Urao et al., 2000). As reviewed by Bleecker and Kende (2000), Alonso and Stepanova (2004), and summarized in Chaves and Mello-Farias (2006), the ethylene receptor proteins interact with CTR1 which, through a not yet fully defined

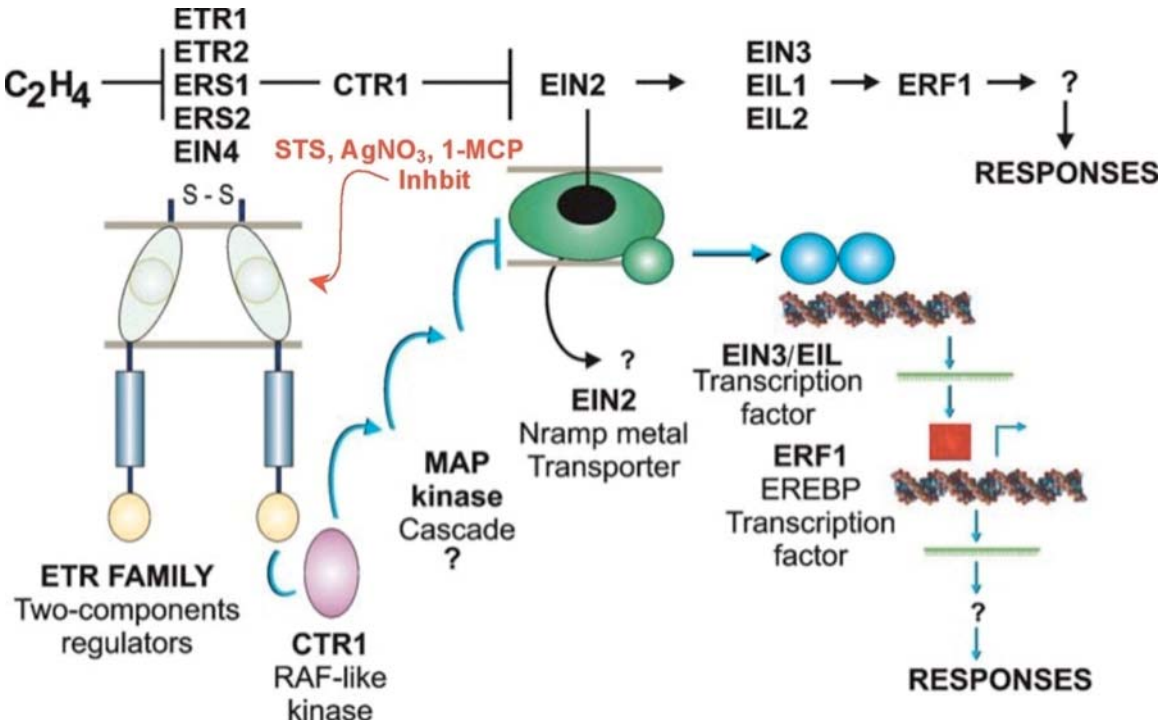

Figure 1-2. Components of the ethylene perception pathway as currently understood. Figure modified from Bleecker and Kende (2002) and Chaves and Mello-Farias (2006).

mechanism, inactivates EIN2 which, in turn, suppresses the subsequent genetic responses (Fig. 1-2). Thus, when an ethylene molecule binds to a receptor protein, the activation of CTR1 by the receptor proteins is stopped, and subsequent pathway responses begin. Detailed discussion of how these mechanisms were elucidated in *Arabidopsis* plants can be found in Hua and Meyerowitz (1998). It is important to note that many other signals of abiotic stress also take advantage of MAPK signal cascades and that cross-talk between response systems likely occurs *in planta* (Knight and Knight, 2001).

The practical consequence of this mechanism directly relates to the types of compounds that would be suitable for use as ethylene perception inhibitors. Specific factors to consider for such a compound would be: Where in the

perception pathway does it act? How specific to the ethylene pathway is it? What is the proposed action mechanism? The three compounds typically used to block ethylene perception (silver thiosulfate, silver nitrate, 1-methylcyclopropene) all act upon the ethylene receptor proteins and not later portions of the pathway. Dissociated silver ions from silver thiosulfate (STS) and sliver nitrate ($AgNO_3$) displace the copper cofactors used in the binding sites of receptor proteins. 1-MCP binds to the protein and physically occludes the binding site, blocking ethylene. For both mechanisms, the conformation of the CTR1 interaction portion of the protein is unaltered. Thus, the CTR1 suppression of EIN2 remains, and plant responses to ethylene are terminated.

Since increases in ethylene synthesis serve as a signal for stress, blocking ethylene perception has the potential to mitigate the effects of abiotic stressors experienced by plants and plant products. Common stressors include: elevated ethylene in atmospheres with poor gas exchange, drought, and flood-induced hypoxia. The acute effects of these stresses lead to crop damage, and loss of potential yields. Obtaining the ability to block these effects in a reversible, consistent manner is of great value.

Chemical control of ethylene synthesis has been achieved with aminovinylglycine (AVG), aminooxyacetic acid (AOA), aminoisobutyric acid (AIBA), and Co^{2+} (see discussion above). Although these compounds have been used with success, they must be dissolved and sprayed onto the plant, which means that uptake is variable. Also, several of these compounds are toxic to humans.

1-MCP is a nontoxic alternative that can be homogeneously applied as a gas. Most studies of MCP have focused on its effects in post-harvest physiology (Blankenship and Dole, 2003). MCP appears to decrease both ethylene synthesis and respiration of climacteric fruit. Since the ethylene signal is blocked, autocatalytic ethylene production cannot occur. Subsequent fruit-ripening steps, requiring increased respiration (conversion of starches to sugars, softening of cell walls, etc.) are not initiated.

Limited information on nonclimacteric fruits indicates that the effect of 1-MCP is inconsistent and needs to be evaluated on a case-by-case basis (Lurie, 2005). For example, ethylene synthesis increased in citrus fruits was unaffected in strawberries (Lurie, 2005), and decreased in grapes (Chervin et al., 2005). Although the effects of 1-MCP on harvested organs are of importance for increasing shelf life and storage, there is sparse information for the effect of 1-MCP in whole plant physiology.

Faust and Lewis (2004) examined the effect of 1-MCP in unrooted Poinsettia cuttings and found it caused an increase in ethylene accumulation in their sealed containers. However, Faust and Lewis did not measure the accumulation of carbon dioxide in their containers. The increased ethylene synthesis may be the result of increased respiration due to increased temperature. This may be the case since the ethylene accumulation did not occur at lower temperatures. Although ethylene increased, leaf abscission post-storage decreased.

Atmospheric Ethylene Sensitivity

Elevated levels of atmospheric ethylene cause a variety of abnormal responses, including inhibited root and hypocotyl elongation, leaf epinasty, reduced growth, premature leaf senescence, and sterility (Morison and Gifford, 1984; Mattoo and Suttle, 1991; Abeles et al., 1992; Smalle and Van Der Straeten, 1997; Klassen and Bugbee, 2002, 2004;). Plants are the primary source of the elevated ethylene that accumulates in controlled environments with inadequate air exchange, such as sealed plant growth chambers (Wheeler et al., 1996, 2004), the space station (2003Campbell et al., 2001; Perry and Peterson,), and large commercial greenhouses. Ethylene gas is also generated in greenhouse environments as a byproduct from combustion powered equipment, such as heaters and forklifts (Sargent, 2001).

The sensitivity of flowers to ethylene at concentrations as low as 20 nmol mol^{-1} (ppb) during anthesis has been well documented and is a primary cause of yield loss in flowering crop plants (Payton et al., 1996; Oráez, et al., 1999; Klassen and Bugbee, 2002). Vegetative tissue generally has a higher tolerance to elevated ethylene. Eraso et al. (2002) demonstrated that ethylene greater than 50 ppb was required to reduce leaf area and total biomass in vegetative radish crops. Klassen and Bugbee (2002) found that vegetative biomass of wheat and rice was not significantly decreased at 1000 ppb whereas yield of both crops was significantly reduced by 200 ppb. Thus, reproductive organs appear to be more sensitive to elevated ethylene.

Ethylene Synthesis under Hypoxia Induced by Flooding

Flooding is a common cause of stress both in the field and in controlled environments. The engineering challenges associated with uniform distribution of water and air throughout the root zone has made inadequate root-zone aeration a common stress in microgravity (Monje et al., 2003; Porterfield et al., 2003). Heavy rains or a malfunctioning watering system can also trigger flood-induced hypoxic conditions in the root zone, resulting in crop damage or loss (Drew, 1997; Fukao and Bailey-Serres, 2004). The chief role of ethylene in flood conditions is to trigger the development of aerenchyma tissue to allow for the low-resistance transport of oxygen to sites of active root growth (Colmer, 2003). Indeed, one of the primary indicators of crop sensitivity to flood stress is the ability to form aerenchyma tissues (Justin and Armstrong, 1987; Abeles et al., 1992; Colmer, 2003). Justin and Armstrong (1987), for example, studied the characteristics of flooded roots for ninety-one plant species. From their table that includes data on both pre- and post-flood root porosity, inferences can be made about relative tolerance to flood stress. For example peas, a nontolerant crop, had a maximum root porosity of only 4% when flooded, whereas corn (an intermediate crop) had 13%, and rice (highly tolerant) had up to 30% porosity (Justin and Armstrong, 1987; Colmer, 2003).

The conversion of ACC to ethylene is oxygen dependent. Hypoxia induced by flooding promoted the synthesis of ACC in the roots of tomato. In turn, ACC is transported to the shoots and rapidly oxidized to ethylene (Bradford and Yang, 1980). Hypoxia increased ethylene production in both roots and

leaves of tomato resulting in leaf epinasty and chlorosis (Bradford and Dilley, 1978; Morgan and Drew, 1997). It has been suggested that ethylene acts as part of a signal pathway indicating hypoxia in roots (Drew, 1997). Under hypoxic root-zone conditions small amounts of ethylene build-up in root tissue. This build-up is due to floodwater acting as a diffusion barrier at the surface of the root (Jackson, 1985). Such build-up stimulates cellulase and pectinase resulting in the breakdown of cell walls and the formation of aerenchyma tissue (Moore et al., 1998). This build-up occurs in the tissues of many crops, including wheat, maize, rice, and radish (Kawase, 1978; Atwell et al., 1988; Tonutti and Ramina, 1991). This response can be rapid. The ethylene production rate of wheat leaves doubled within two hours of exposure to 10% O_2 in the root-zone (Tonutti and Ramina, 1991). Changes in production rates can be dramatic. Hypoxia increased ethylene synthesis up to 8-fold in roots and 15-fold in shoots (Atwell et al., 1988; Tonutti and Ramina, 1991).

Soybeans are considered a flood sensitive crop (Bacanamwo and Purcell, 1999). Oosterhuis et al. (1990) examined the effect of flood stress on two soybean cultivars. They found that photosynthesis decreased by 16-32% 48 h after flooding. The effect was apparent 24 h after flooding. These effects were mirrored by similar decreases in stomatal conductance. Given the observed decrease in photosynthesis, we hypothesized that ethylene synthesis would also decrease.

Hypoxic conditions should not be confused with growing plants under hypobaric conditions. Under such conditions, the overall pressure of the system

can be lowered to 1/3 that of ambient pressure (30 kPa) while still maintaining a high partial pressure of oxygen. Growth while under hypobaric conditions, reduced ethylene biosynthesis in wheat and lettuce plants by 65% while increasing plant growth (He et al., 2003).

Although much work has been done with the molecular effects of flood stress (Grichko and Glick, 2001), little has been done to quantify the ethylene produced as a result. In combination with ethylene synthesis and/or perception modifications, it may be possible to diminish or eliminate plant response to temporary flood stress. Further discussion of ethylene movement through waterlogged soils can be found in Appendix A.

Ethylene Synthesis During Water Deficit Stress

Inconsistencies in the literature on the effect of water stress on ethylene production provide a clear example of inadequate experimental methods in ethylene research. Studies that involved desiccation of detached leaves suggest water stress increases ethylene production, but studies of intact plants subject to water stress suggest decreased ethylene synthesis (Morgan et al., 1990; Narayana et al., 1991). Ethylene synthesis rates were unaffected in maize mutants with variable internal concentrations of abscisic acid (Voisin et al., 2006). Sobeih et al. (2004) subjected the split root zones (one-half in a watered column, one-half in water stressed conditions) of tomato plants to water deficit stress. Unlike maize, they found increased ethylene synthesis as a result of water stress. Also, a mutant with low ethylene production was unaffected by the stress.

However, the technique used to measure ethylene in both studies, detached leaf tissue from the plant and placed it in a sealed vessel for an extended incubation period. Thus, ethylene synthesis measured was not from the whole plant.

The current understanding is that the effect of water stress on ethylene synthesis depends on the rate at which the plants are stressed. Rapid induction of water stress should promote ethylene production, and slow induction should inhibit production (Xu and Qi, 1993; Morgan and Drew, 1997). Despite a lack of consistency in the technique used for whole-plant measurements, molecular techniques suggest that abscisic acid (ABA) influences ethylene effects in plant organs leading to a decrease in synthesis (Chaves et al., 2003). Indeed, several transcription factors that link ABA levels and ethylene production have been identified (Manavella et al., 2006). Members of this same family have also been influenced by light (Manavella et al., 2006). Reduced ethylene production is expected in the field since drought stress typically occurs slowly. However, water deficit stress occurs rapidly in highly porous media, especially when the root-zone volume is restricted (Morgan and Drew, 1997). Given prior observations made with different techniques and the molecular data, we expect ethylene synthesis to decrease as a result of water deficit stress.

Ethylene Synthesis Affected by Light

Plants grown under low light levels are typically etiolated and less robust than plants grown under higher light. Indeed, the effects of ethylene were first characterized by studies on etiolated pea seedlings (see review by Eisinger, 1983). Light quantity and quality have been shown to alter ethylene synthesis.

Jiao et al. (1987) found interactions between light and ethylene synthesis. They observed that ethylene synthesis in dark grown wheat leaves had decreased after exposure to white light. Their results also showed that red and far-red light altered ethylene synthesis, suggesting that phytochrome may regulate ethylene synthesis. Subsequent work using leaf discs of Begonia (Rudnicki et al., 1993) demonstrated that white, blue, green, and red light inhibited ethylene synthesis, but far-red light stimulated production. Vandenbussche et al. (2003) studied shade-avoidance in *Arabidopsis* and reported a decrease in ethylene synthesis with increased light in short-term studies (hours). The uptake of CO_2 was higher in the light, but ethylene synthesis was less.

First observed in young cotton seedlings, ethylene synthesis follows circadian rhythms (Rikin et al., 1984; Jasoni et al., 2000). Subsequent work with *Stellaria longipes* demonstrated circadian rhythmicity in the abundance and activity of mRNA associated with ACC oxidase (Kathiresan et al., 1996). Light / dark cycles had a greater entraining effect than temperature cycling. A red light pulse in darkness was capable of resetting the rhythm (Kathiresan et al., 1996). The CAM plants *Tillandsia usneoides* (Spanish moss) were studied to determine if CO_2 availability played a role in the circadian rhythmicity (Beβler et al., 1998). Ethylene synthesis increased in response to light, a time when internal CO_2 concentrations were lowest (Beβler et al., 1998). Ethylene emissions from ACC-solution-soaked plants monitored in the dark demonstrated that ACC-oxidase was not light regulated (Beβler et al., 1998). Later work with sorghum showed that phytochrome B mutants exhibited severe overproduction although circadian

rhythms were still present (Finlayson et al., 1998, 1999). Contrary to the work in *Tillandsia*, work with sorghum demonstrated a circadian rhythm independent of constant light, constant dark, and isothermal conditions (Finlayson et al., 1998, 1999). Foo et al. (2006) recently demonstrated phytochrome A and B regulation of ethylene in pea plants by showing that plants lacking both phytochromes overproduced ethylene.

Molecular techniques illuminated the inner workings of the circadian clock for *Arabidopsis* plants (McClung, 2000; McClung et al., 2002). As a result, the interactions of the oscillation mechanisms uncovered with ethylene synthesis were explored using *Arabidopsis* plants with various mutations in their ethylene synthesis and perception pathways (Thain et al., 2004). The following was found: The rhythm was light entrained and was persistent. The circadian rhythm was not dependent upon ethylene signaling. Two components of the circadian clock, TOC1 and CCA1, were found to control the rhythm of ethylene production. In agreement with the *Stellaria* data, some ACC synthase and ACC oxidase genes followed the circadian rhythm and dictated the release of ethylene. Finally, ethylene perception mutants exhibited increased ethylene synthesis when compared to wildtype (20x higher in one case) while still maintaining a circadian rhythm. This suggested that ethylene-mediated stress signals should not have an effect on circadian ethylene synthesis (Thain et al., 2004). Indeed, in his minireview, McClung (2000) suggested that the complication of circadian rhythm could no longer be ignored in hormone research. Although a great deal of good science has been done using trap-and-accumulate techniques for ethylene

measurement, it is clear that continuous measurement is necessary in order tease out the effects of a stress signal from the normal oscillation. Also, the presence of a circadian cycle gives the researcher two new tools to define a stress signal; changes in amplitude and period can also potentially carry a signal of ethylene stress.

Rapid leaf expansion allows a plant in a crop community to capture as much incoming radiant energy as possible to drive plant growth. A decrease in the leaf expansion rate reduces the overall amount of energy a plant has to grow. Endogenous ethylene in *unstressed* terrestrial plants does not appear to inhibit leaf expansion. Although Bleeker et al. (1988) found that leaves of ethylene insensitive *Arabidopsis* plants were larger than their wildtype counterparts; when Tholen et al. (2004) replicated the study and controlled for accumulated ethylene in the atmosphere of the petri dishes used for the experiment, they found that wildtype and ethylene-insensitive mutants had equal leaf expansion rates. Thus, they concluded that endogenous ethylene levels do not affect leaf expansion in unstressed plants. This is consistent with previously reported data that shows an ethylene threshold for leaf expansion (Klassen and Bugbee, 2004). These papers uphold the paradigm that ethylene levels are elevated from a background production rate in order to signal stress.

Elevated ethylene above the endogenous rate of production reduces leaf expansion rate and increases leaf epinasty (Abeles et al., 1992). This decreases overall radiation capture and leads to the appearance of a decreased photosynthetic rate. Woodrow et al. (1988, 1989) and Woodrow and Grodzinski

(1993) demonstrated that photosynthesis was not affected by ethylene when epinastic leaves were straightened to allow for original rates of radiation capture. Taylor and Gunderson (1988) found that acute exposure to extremely high ethylene concentrations (10,000 ppb) reduced quantum yield in soybean leaves, but this high level is not representative of the chronic low levels that accumulate in a contaminated environment. In an earlier paper (1986), Taylor and Gunderson documented this effect in *Arachis hypogaea, Gossypium hirsutum, Glycine max, Cucurbita pepo, Phaseolus vulgaris, Setaria viridis,* and *Raphanus sativus*. However, they did not document the final concentrations of ethylene in their experimental system. It is probable that their concentrations were as high, or higher, than the 10,000 ppb concentration used in their subsequent paper. The general consensus is that low chronic exposure to ethylene has a minimal effect on quantum yield and photosynthetic apparatus (Abeles et al., 1992).

Given the sensitivity of etiolated plants to ethylene, the circadian nature of ethylene production and the effect of light quantity and quality on ethylene synthesis, we hypothesized that ethylene sensitivity would increase in low photosynthetic photon flux (PPF). This hypothesis is particularly important for the closed plant growth chambers on the space station, where ethylene routinely accumulates and where the light levels are low. The objective of these experiments was to determine if the sensitivity of either vegetative (radish) or reproductive (pea) plants was increased in low light.

To supplement the discussion found in this dissertation, the following appendices are included: Appendix B provides additional information on the

selction of Earligreen pea; Appendix C provides an in-depth discussion of the affect of helium quality on thermal desorber calibration; Appendix D provides an overview of the validating controlled environment chambers: Appendix E provides a discussion of the effects of photoperiod and light integral on plant growth; and Appendix F provides additional discussion on dwarf crop responses to multiple photoperiod regimes.

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CHAPTER 2

HIGH LIGHT DOES NOT DECREASE ETHYLENE SENSITIVITY IN RADISH AND PEA

Abstract

Ethylene accumulation due to inadequate air exchange occurs in a variety of controlled environments used for plant production and research. In some instances, such as chambers used in the International Space Station or a greenhouse in winter, low photosynthetic photo flux (PPF) is also a stress factor. Ethylene synthesis rates can be altered by light. We hypothesized that ethylene sensitivity may increase in low light. Ethylene sensitivity of radish and pea plants was evaluated. Plants were grown under 50 or 70, 200, and 400 µmol m^{-2} s^{-1} PPF and an ethylene concentration high enough to affect plant growth (200 ppb for radish, 50 ppb for pea). There was no interaction between ethylene sensitivity and PPF. This suggests that increasing PPF cannot mitigate the detrimental effects of chronic long-term ethylene exposure.

Introduction

Elevated levels of atmospheric ethylene cause a variety of abnormal responses, including inhibited root and hypocotyl elongation, leaf epinasty, reduced growth, premature leaf senescence, and sterility (Morison and Gifford, 1984; Mattoo and Suttle, 1991; Abeles et al., 1992; Smalle and Van Der Straeten, 1997; Klassen and Bugbee, 2002, 2004). Plants are the primary source of atmospheric ethylene that accumulates in controlled environments with

inadequate air exchange, such as sealed plant growth chambers (Wheeler et al., 1996, 2004), the space station (Campbell et al., 2001; Perry and Peterson, 2003), and large commercial greenhouses. Ethylene gas is also generated in greenhouse environments as a byproduct from combustion powered equipment such as heaters and forklifts (Sargent, 2001).

The sensitivity of flowers to ethylene at levels as low as 20 nmol mol^{-1} (ppb) during anthesis has been well documented and is a primary cause of yield loss in flowering crop plants (Payton et al., 1996; Oráez et al., 1999; Klassen and Bugbee, 2002). Vegetative tissue generally has a higher tolerance to elevated ethylene. Eraso et al. (2002) demonstrated that ethylene greater than 50 ppb was required to reduce leaf area and total biomass in vegetative radish crops. Klassen and Bugbee (2002) found that vegetative biomass of wheat and rice was not significantly decreased at 1000 ppb whereas yield of both crops was significantly reduced by 200 ppb. Thus, reproductive organs appear to be more sensitive to elevated ethylene.

Elevated ethylene also reduces leaf expansion rate and increases leaf epinasty (Abeles et al., 1992), which decrease radiation capture. Woodrow et al. (1988, 1989) and Woodrow and Grodzinski (1993) demonstrated that photosynthesis was not affected by ethylene when epinastic leaves were straightened to allow for original rates of radiation capture. Taylor and Gunderson (1988) found that acute exposure to extremely high ethylene concentrations (10,000 ppb) reduced quantum yield in soybean leaves, but this high level is not representative of the chronic low levels that accumulate in a contaminated

environment. The general consensus is that low chronic exposure to ethylene has a minimal effect on quantum yield and photosynthetic apparatus (Abeles, 1992).

Endogenous ethylene in *unstressed* terrestrial plants does not appear to inhibit leaf expansion. Although Bleeker et al. (1988) found that leaves of ethylene insensitive *Arabidopsis* plants were larger than their wildtype counterparts, when Tholen et al. (2004) replicated the study by Bleeker et al. and controlled for ethylene build-up in the atmosphere of the petri dishes, they found that wildtype and ethylene-insensitive mutants had equal leaf expansion rates. Thus, they concluded that endogenous ethylene levels do not affect leaf expansion in *unstressed* plants. This agrees with previously reported data that shows an ethylene threshold for leaf expansion (Klassen and Bugbee, 2004). Together, these two papers uphold the paradigm that altered ethylene synthesis is a signal of stress conditions.

Plants grown under low light levels are typically etiolated and less robust than plants grown under higher light. Etiolated pea seedlings are a model for studying the effect of ethylene on internode elongation (see review by Eisinger, 1983). Light quantity and quality alter ethylene synthesis. Jiao et al. (1987) are among the first to show interactions between light quality and ethylene synthesis. They observed that dark grown wheat leaves had decreased ethylene synthesis after exposure to white light. Their results also showed that red and far-red light altered ethylene synthesis, suggesting that phytochrome may regulate ethylene synthesis. Subsequent work using leaf discs of Begonia (Rudnicki et al., 1993)

demonstrated that white, blue, green, and red light inhibited ethylene synthesis, but far-red light stimulated production. Vandenbussche et al. (2003) studied shade-avoidance in *Arabidopsis* and reported a decrease in ethylene synthesis with increased light in short-term studies (hours). The uptake of CO_2 was higher in the light, but ethylene synthesis was less. Foo et al. (2006) recently demonstrated phytochrome A and B regulation of ethylene in pea plants by showing that plants lacking both phytochromes overproduced ethylene.

Given the sensitivity of etiolated plants to ethylene and the effect of light quantity and quality on ethylene synthesis, we hypothesized that ethylene sensitivity would increase in low PPF. This hypothesis is particularly important for the closed plant growth chambers on the space station, where ethylene routinely accumulates and where the light levels are low. The objective of this study was to determine if the sensitivity of either vegetative (radish) or reproductive (pea) plants was increased in low light.

Materials and Methods

Plant Growth Chambers for Radish Ethylene Sensitivity and Ethylene-PPF Interaction Experiments

Plants were grown in 30 cm diameter containers with a root zone depth of 21 cm filled with 1:1 peat:perlite media (Fig. 2-1). Clear polycarbonate chambers (60 cm tall) enclosed each container. Each chamber was independently supplied with air or an air/ethylene mix at 15 L min^{-1}. A complete description of the ethylene dilution and distribution system and chambers can be found in Klassen and Bugbee (2002). Each chamber was maintained at a 25/20˚C day/night temperature. Nutrients were provided by watering 3x daily with Peters 5-

Figure 2-1. Example of radish plants in pots used for radish sensitivity and both PPF interaction trials. The front-center polycarbonate chamber has been removed for the photo. Blended-gas supply lines feed into the top of chamber directly in front of fan. Photo has been color corrected to remove orange cast of HPS lamps.

11-26 Hydrosol supplemented with 10 µM Fe EDDHA, 1.4 mM $CaNO_3$, and 10 µM Na_2SiO_3. Plants for all experiments, except the ethylene-PPF interaction study, were grown at a photosynthetic photon flux (PPF) of 400 µmol m^{-2} s^{-1} from high pressure sodium (HPS) lamps with a 16-h photoperiod.

For the ethylene-PPF interaction experiment, plants were grown at a PPF of 50 or 70, 200 and 400 µmol m^{-2} s^{-1}. For dose-response studies, radish plants were grown at 0, 80, 120 and 160 ppb ethylene. Radish plants were grown until 20 days post emergence (DPE). For ethylene-PPF interaction studies, plants were grown at 0 ppb or 200 ppb (radish), and 0 or 50 ppb (pea) ethylene. Radish plants were grown for 22 DPE, pea plants for 14 DPE.

Plant Growth Chambers for Pea Ethylene Sensitivity Studies

Figure 2-2. Pea plants growing in greenhouse system. Each chamber was individually controlled for temperature and ethylene mix. Lighting was controlled by screening each chamber. Each row was treated as a block in a randomized complete block experiment design. Photo has been color corrected.

Peas (cv. *Earligreen*) were planted in replicate greenhouse chambers using a randomized complete block design and a density of 40 plants m^{-2} (8 plants per chamber; Fig. 2-2). Supplemental lighting with HPS lamps was provided for a 16 h photoperiod. Plants were watered with the same nutrient solution described above. Ethylene concentrations were maintained at 0, 10, 20, 40, 70 and 120 ppb. Plants were harvested at 53 DPE.

Plant Growth Chambers for Pea Vegetation Response

Individual plants were grown in replicate 1 L pots placed in chambers identical to the ones described above. HPS lamps were the sole light source at a PPF of 600 µmol m^{-2} s^{-1}. Nutrient solution was provided as described above for radishes. Ethylene levels were 0, 30, 60, 120, and 200 ppb. Plants were harvested 33 days post planting before set pods in the controls could fill. Dry mass was taken for the vegetative portion of the plants, including unfilled pods.

Quantification of Plant Size via Digital Photography

Digital images of plants were captured using a Nikon Coolpix 4500 camera with the lens height kept at a constant height above the media surface. Images were imported into Adobe Photoshop CS2® for the Macintosh operating system. The extract filter was used to improve separation of the plants from the background. Once plants were separated from the original background, 15% grey was placed as the new background. The “magic wand” tool with the tolerance set from 1-10 and set to highlight contiguous pixels only was used to select the grey background. The “inverse selection” command was then used to select for the plants. The histogram palette was used to obtain the total number of pixels for all plants in the container. The number of pixels per plant was then calculated as an average of all plants in a chamber. Further techniques and discussion can be found in Klassen et al. (2003).

Ethylene Measurement

Ethylene was measured using an automated Shimadzu GC17a v. 3.4 equipped with a flame ionization detector. An 1/8 in diameter x 2 m Porapak® Q column at 120˚C oven temperature and 70 mL min^{-1} helium carrier flow was used to separate ethylene contained in samples loaded via 5 ml sample loop. Ethylene was retained for approximately 0.83 min with a 5 ppb detection threshold. The system was equipped with two common-outlet 16 port sample valves (VICI Valves, Houston, TX) which allowed for the continuous monitoring of ethylene from 31 separate locations.

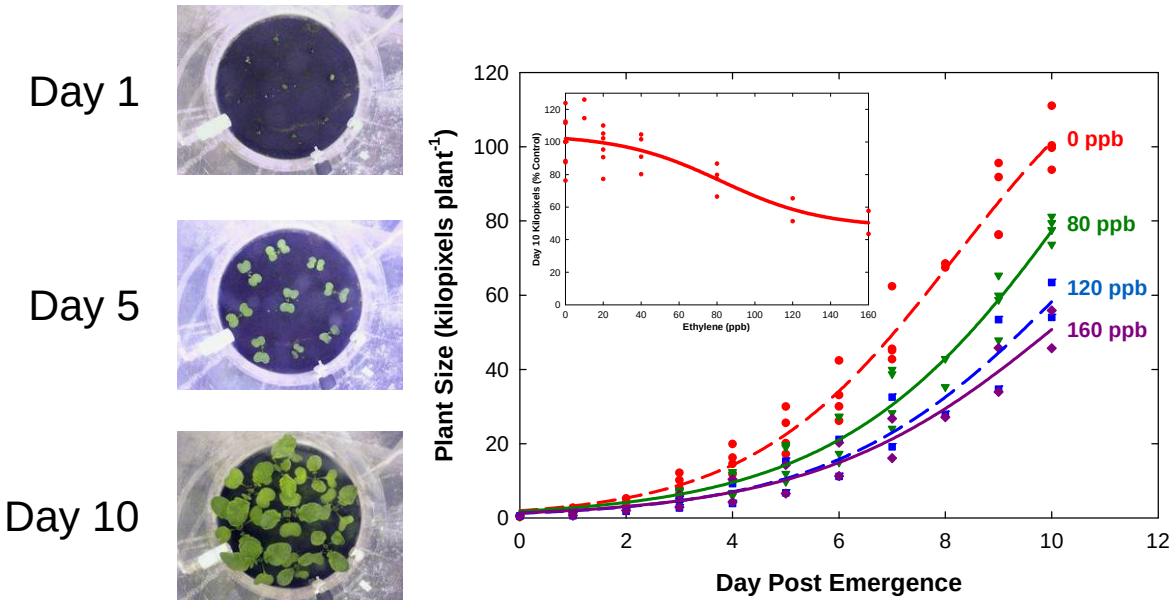

Figure 2-3. Pixel data for *Cherry belle* radish sensitivity to ethylene. Photos on the left are control (0 ppb) plants on days 1, 5 and 10 post emergence. Data points in the graph represent average pixels per plant from individual chambers in two experimental trials. The equation for a sigmoid growth curve was used to fit regression lines to the data. The inset shows pixel data from day 10 post emergence from four independent trials as a percent of control; 160 ppb reduced plant size by 40%.

Results

Sensitivity of Radish and Pea to Ethylene Quantified Through Digital Photography

Elevated ethylene decreased green pixel area (Fig. 2-3). Affected plants that were small at emergence remained comparably small throughout the life cycle. By 10 DPE when the canopy started to close, plants grown at 160 ppb were 35-40% the size of controls (Fig. 2-3, inset). The effect of ethylene on pea was more severe than radish (Fig. 2-4). Similar to radish plants, the effect on

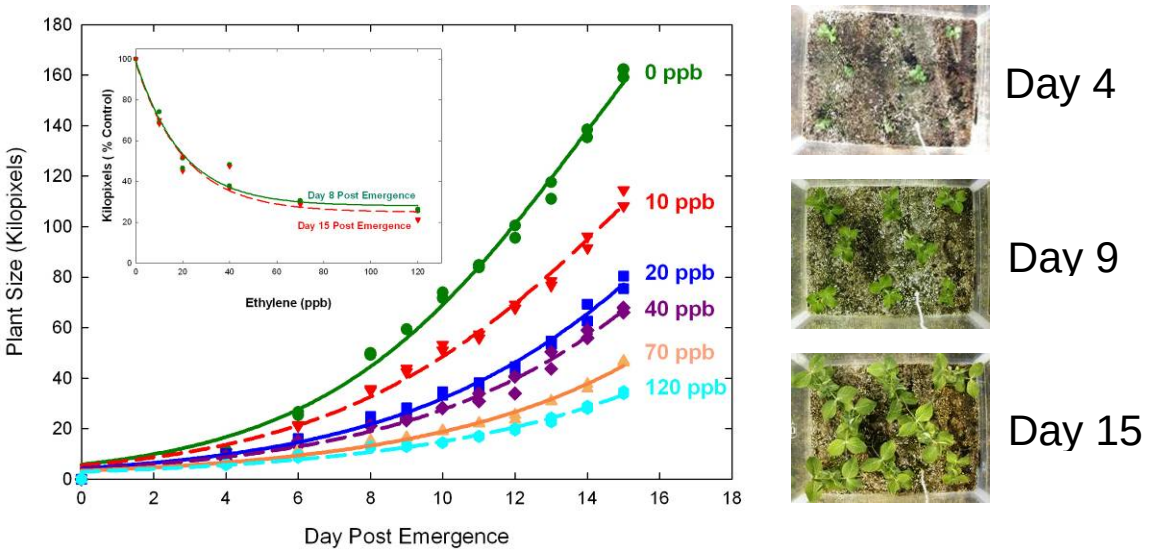

Figure 2-4. Pixel data for *Earligreen* pea sensitivity to ethylene. Photos on the right are control (0 ppb) plants on days 4, 9 and 15 post emergence. Data points in the graph represent average pixels per plant from replicate chambers in a randomized complete block experiment. The equation for a sigmoid growth curve was used to fit regression lines to the data. The inset shows pixel data from days 8 and 15 post emergence as a percent of control; 20 ppb reduced plant size by 50%. Sensitivity to ethylene was constant over time (inset). Data have been normalized to remove the effect of blocks.

plant size was apparent at emergence and remained throughout the life cycle. Plant size was reduced by 30% at 10 ppb; this is a lower sensitivity threshold than radish (Fig. 2-4, inset). The effect on plant size was constant at days 8 and 15 post emergence (Fig. 2-4, inset). The fact that digital photograph measurements are ineffective once the canopy begins to close demonstrates an effect of ethylene on leaf expansion, not reproductive growth since *Earligreen* peas typically flower 20-22 DPE. Vegetative dry mass of peas grown under electric lights and harvested 33 days post planting (DPP) showed a similar decrease (see Discussion and Fig. 2-12). This demonstrates that both the

reproductive and vegetative organs of pea plants were equally affected by ethylene.

Yield

Both root and shoot dry mass of radish decreased in response to ethylene (Fig. 2-5). As predicted by digital pixel counts, shoot and root dry masses were also 35-40% of controls at 160 ppb ethylene. Both shoot and root percent dry mass showed a slight, but not significant, increase (Fig. 2-5). Harvest index increased in the first trial and decreased in the second and third (Fig. 2-5). Combined, this suggests that carbon partitioning into the radish root was not greatly affected by ethylene.

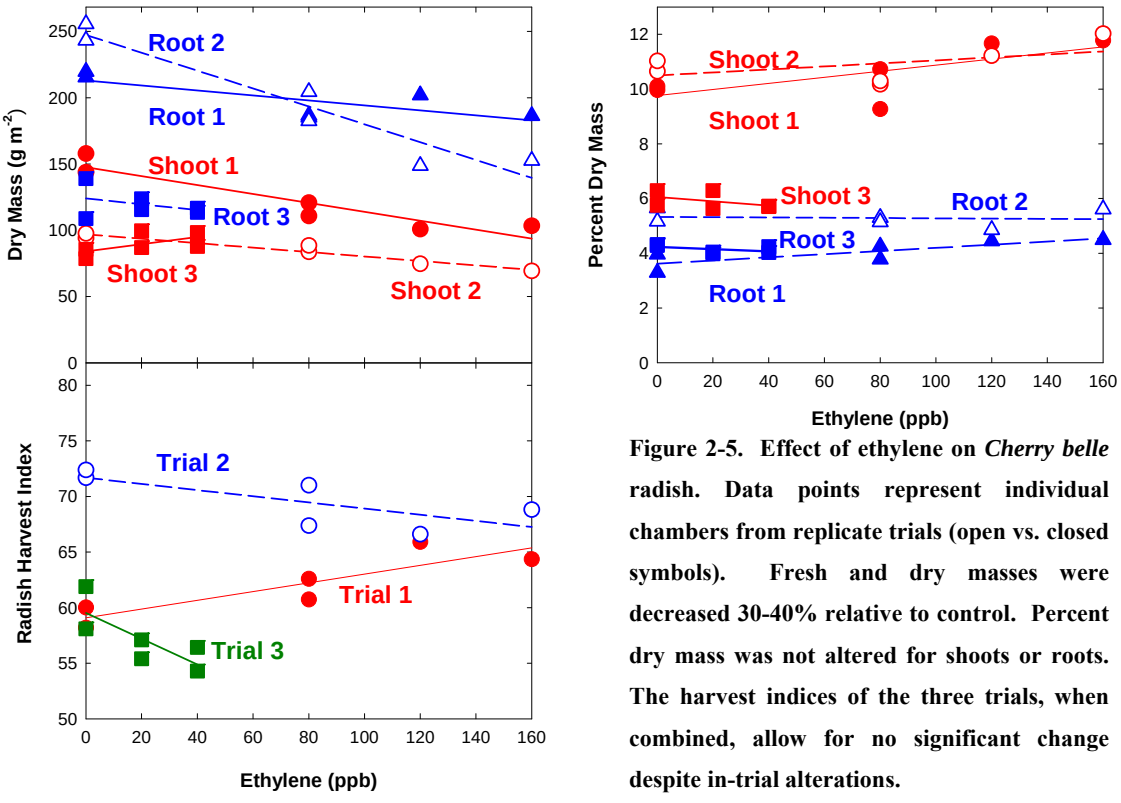

Figure 2-5. Effect of ethylene on *Cherry belle* radish. Data points represent individual chambers from replicate trials (open vs. closed symbols). Fresh and dry masses were decreased 30-40% relative to control. Percent dry mass was not altered for shoots or roots. The harvest indices of the three trials, when combined, allow for no significant change despite in-trial alterations.

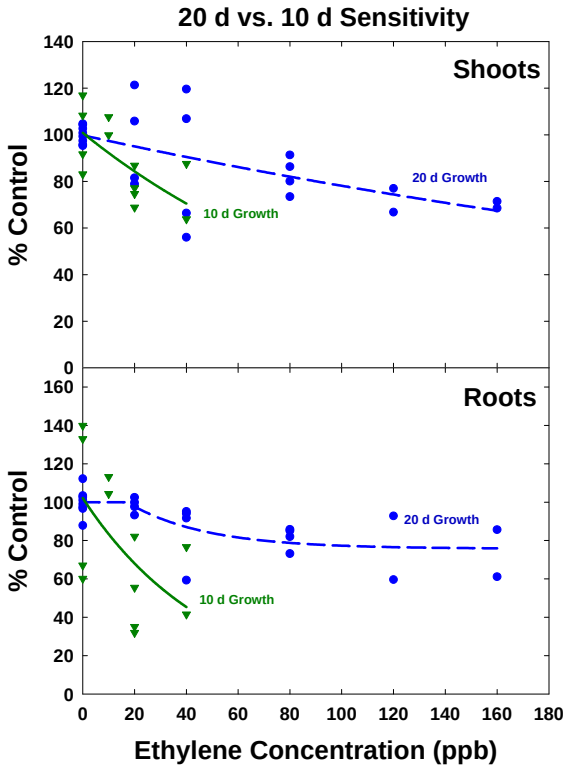

Figure. 2-6. Sensitivity of radish shoot and root dry mass expressed as a percentage of control to ethylene when harvested at 10 or 20 DPE. Radish growth at 10 DPE (time of canopy closure) was more affected by ethylene. However, by 20 DPE the effect of ethylene on vegetative growth was decreased. This suggests that post canopy closure the capture of radiant energy was a larger driving force than ethylene sensitivity.

Radish shoot and root dry mass from plants grown until 20 DPE were both about 80% of controls at 160 ppb of ethylene (Fig. 2-6). Radish shoot and root dry mass from plants grown until 10 DPE were 60% of controls at only 40 ppb (Fig. 2-6). This suggests that once the canopy closed, the effect of radiation capture was greater than that of ethylene.

Ethylene exponentially decreased pea yield (Fig. 2-7). Yield decreased 35-40% at 10 ppb ethylene, similar to pixel count predictions. Shoot fresh and dry mass, pod fresh and dry mass, number of seeds per pod, shoot height, internodal length, and number of pods per plant all followed similar trends (data not shown). Harvest index for both blocks decreased, demonstrating an alteration in carbon partitioning away from reproductive growth (Fig. 2-7).

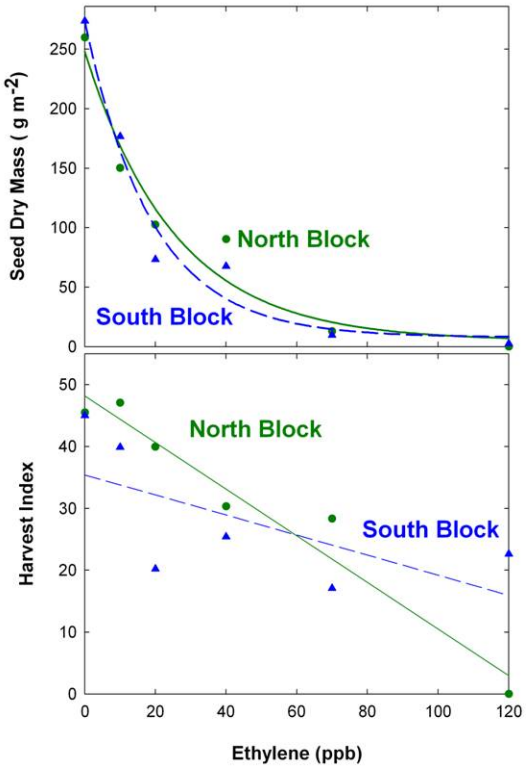

Figure 2-7. Effect of ethylene on *Earligreen* pea seed yield and harvest index. Seed yield was decreased 30-40% with 10 ppb ethylene. Harvest index decreased in both blocks, indicating a decrease in carbon partitioning to reproductive structures.

PPF Interaction with Ethylene

Low PPF decreased plant size and altered the morphology of both radish and pea plants (Figs. 2-8 & 2-9). At 50 µmol m^{-2} s^{-1} PPF, 200 ppb of ethylene was able to lessen the epinastic response of radish shoots (Fig. 2-8). Epinasty was not seen in pea plants grown under 70 µmol m^{-2} s^{-1} PPF (Fig. 2-9). As expected, higher PPF corresponded with greater yield. Still, ethylene significantly decreased radish root and pea shoot fresh mass (Figs. 2-10 & 2-11). When plotted as percent control vs. PPF, there was no significant effect of PPF on ethylene sensitivity. Treated plants were decreased in size by the same amount regardless of PPF level (Figs. 2-10 & 2-11).

Discussion

Consistent with previous data (Fig. 2-12; modified from Klassen and Bugbee, 2002) radish (cv. *Cherry belle*) shoots were among the least affected of the crop species tested. Radish roots, however, were more sensitive. This demonstrates the link between the sensitivity of one organ and its affect on other

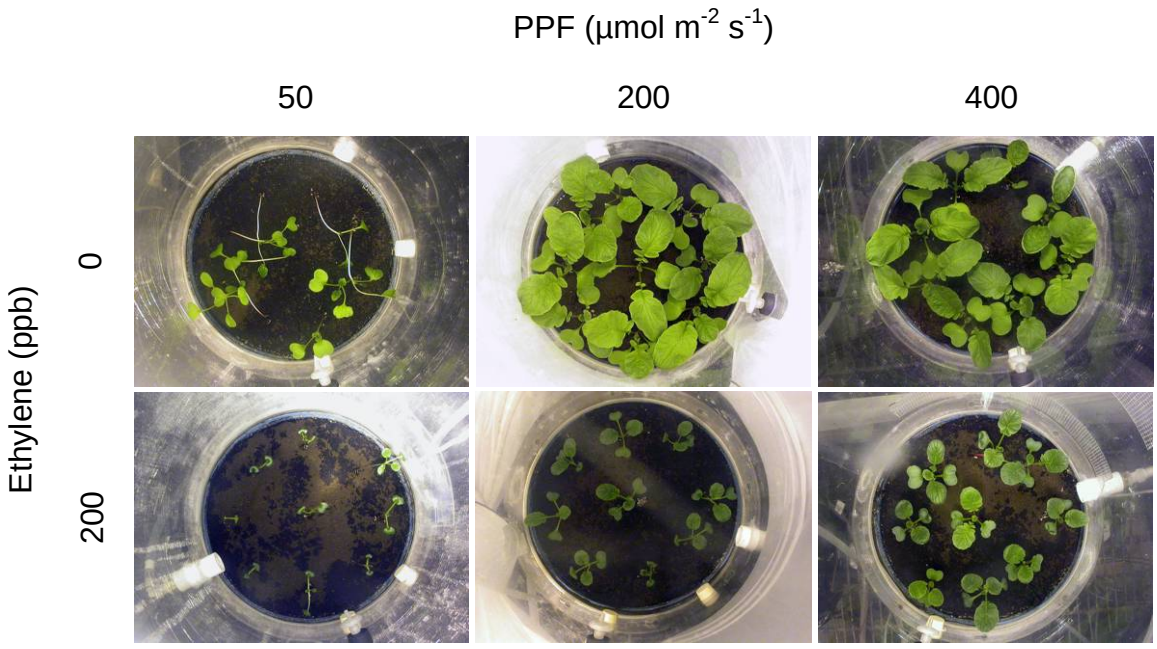

Figure 2- 8. Radish plants from the ethylene sensitivity–light interaction trial. Increased light levels did not decrease sensitivity to ethylene.

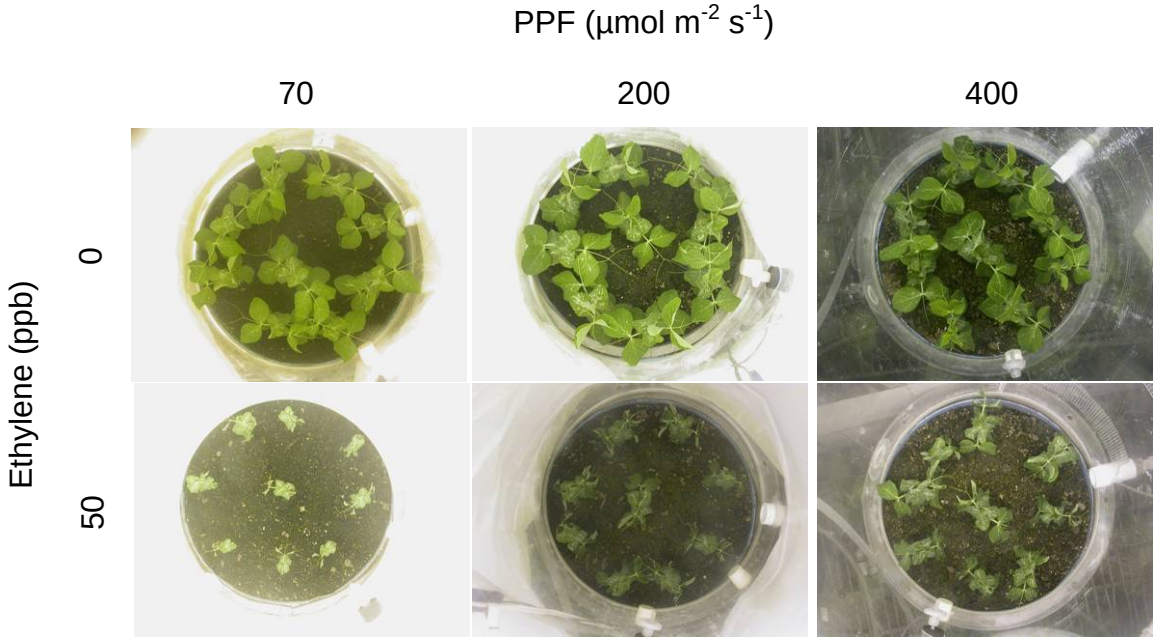

Figure 2-9. Pea plants from the ethylene sensitivity–light interaction trial. Increased light levels did not decrease sensitivity to ethylene.

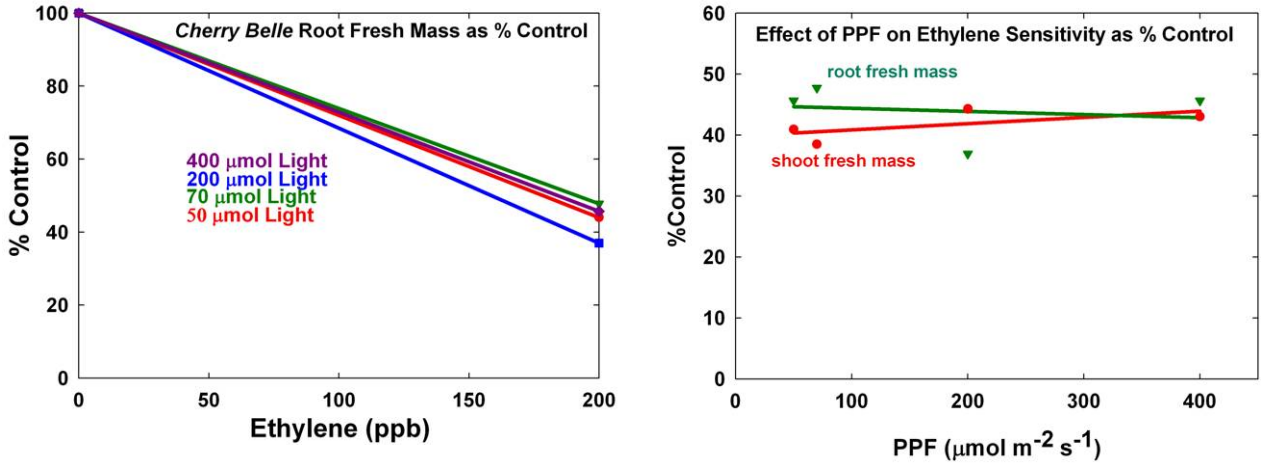

Figure 2-10. ***Effect of ethylene on*** **Cherry belle** ***radish grown under different PPF levels.*** **A. Root fresh mass significantly decreased as a result of ethylene treatment. B. Effect of PPF on root fresh mass from plants grown at 200 ppb ethylene. Increased light levels did not decrease sensitivity. All plants were approximately 45% of controls.**

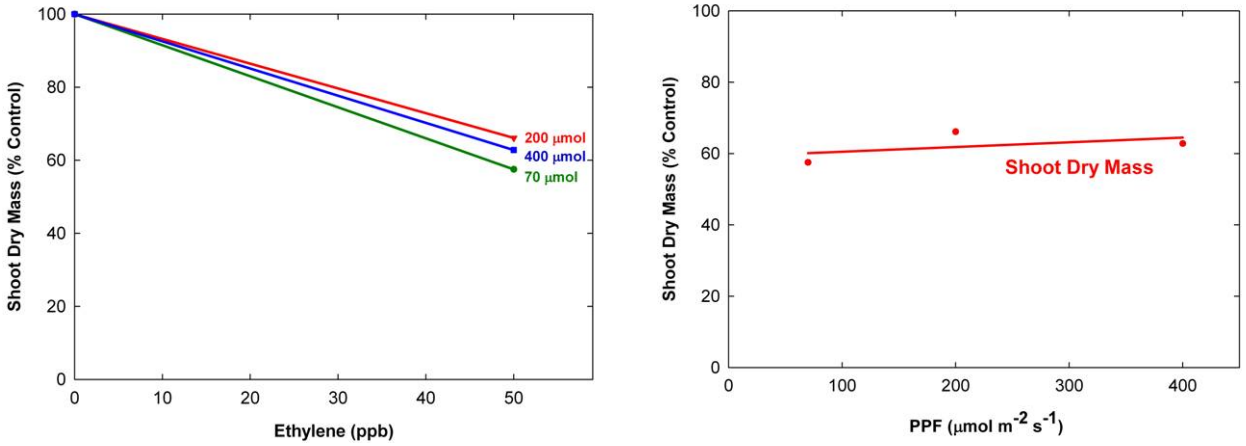

Figure 2-11. ***Effect of ethylene on*** **Earligreen** ***pea grown under different PPF levels.*** **A. Shoot dry mass significantly decreased as a result of ethylene treatment. B. Effect of PPF on shoot dry mass from plants grown at 50 ppb ethylene. Increased light levels did not decrease sensitivity. All plants were approximately 65% of controls.**

parts of the plant. Although radish roots are of horticultural interest since they are consumed (which is why radish flowering and seed yield were not examined), there is an important physiological difference since photosynthate must be transported to the roots from the leaf tissue. Thus, it is possible that the decrease in root mass was the result of an ethylene effect limited to the leaf tissue. A decrease in radiation capture by the leaves, due to decreased leaf size, leads to a decrease in photosynthate available for transport to the storage root. This is borne out in that both roots and shoots of radish plants showed a greater sensitivity earlier in their life cycle (Fig. 2-6). By the time of canopy closure, the ethylene effect on leaf expansion is diminished since there is a finite area with which to capture light. As time went on, the ethylene affected plants were, in essence, able to catch up with the control plants. This hypothesis is further bolstered by pixel data.

Based on pixel counts, the decrease in vegetative growth was apparent at the time of the first photograph (day 2 to 4; Figs. 2-3 & 2-4). Sigmoid curve regression lines fitted to the pixel data indicate that the effect of ethylene on the shoot was apparent starting at the day of emergence. This suggests that ethylene decreased cell expansion or cell number starting shortly after germination. This resulted in decreased radiation capture and led to decreased growth rate. This relative effect of ethylene on pixel count and leaf area was constant throughout the study (Fig. 2-4, inset).

As outlined in Klassen et al. (2003), pixel counts can accurately predict both plant size and ground cover. The accuracy of the counts, however, are

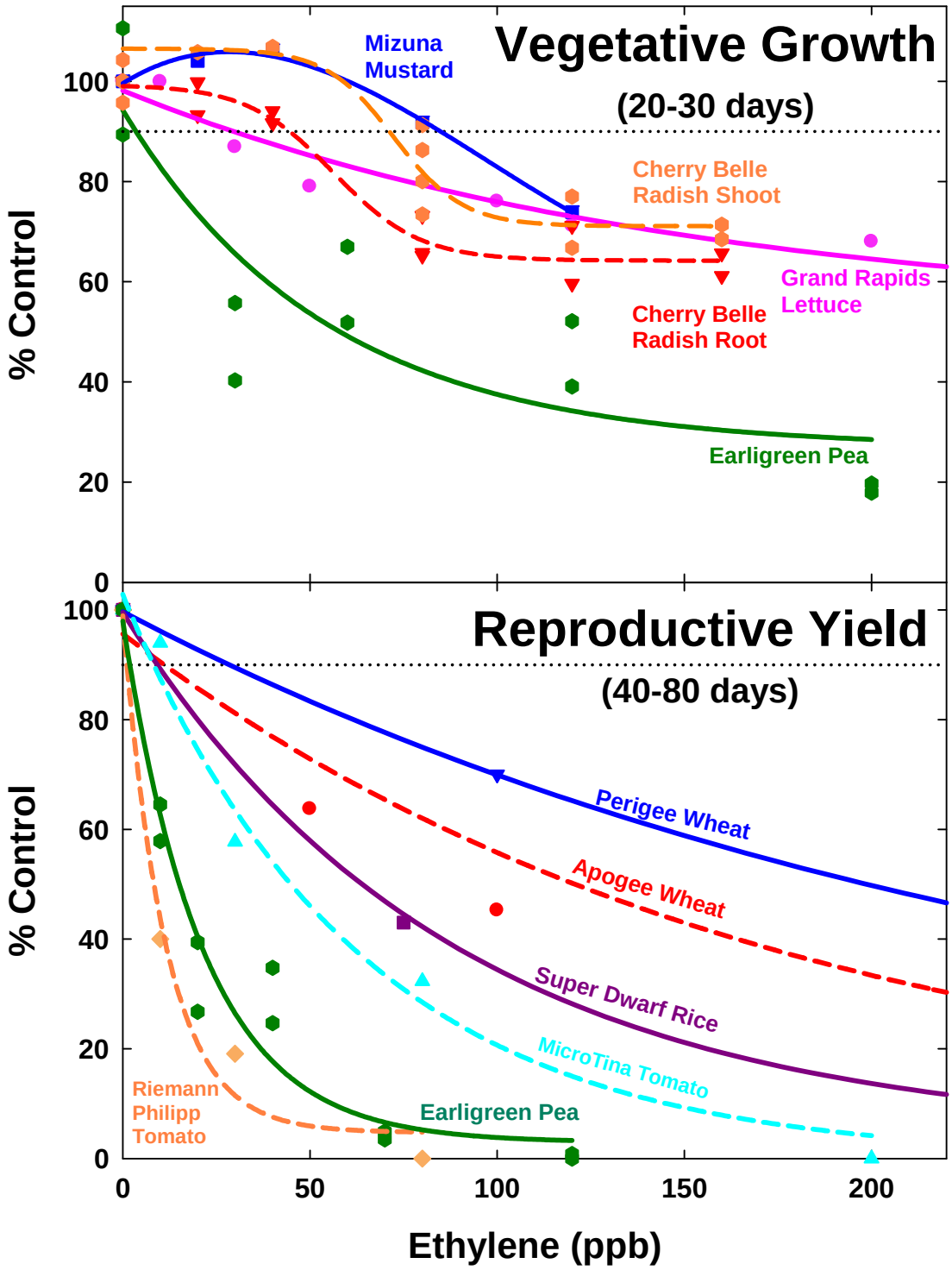

Figure 2-12. Ethylene sensitivity curves for vegetative and reproductive crop plants. Vegetative crops are, in general, less sensitive to elevated ethylene than reproductive crops. Radish plants were not as sensitive as lettuce or mustard. Pea plants are one of the most sensitive crops tested. Dotted reference lines indicate a 10% loss in potential yield. Except for pea and radish data, all data are modified from Klassen and Bugbee (2002).

constrained by several factors. Foremost, altered leaf angle to the camera can lead to an underestimation of plant size. Ethylene can have an effect on leaf angle. If light is provided from a single direction and side lighting is minimized, then a decrease in pixel count due to leaf-angle change is representative of decreased radiation capture potential, assuming that actual leaf area has not changed.

Neither radish nor pea plants exhibited noticeable changes to leaf angle. Alterations to leaf size, as reflected by pixel counts, caused the greatest differences between treatments. Indeed, in this study pixel counts were accurate in predicting yield loss at time of harvest. The effect on cell expansion or cell number differs from the epinastic response described by Woodrow et al. (1988, 1989) and Woodrow and Grodzinski (1993). Instead of a restoration of radiation capture leading to further growth, there is no leaf area to support increased capture. Indeed, as would be expected, plants grown under higher light at the same ethylene concentration were larger (Figs. 2-8 & 2-9). However, simply increasing the light level did not mitigate the effects of ethylene (Figs. 2-10, & 2-11) since PPF did not significantly affect the ethylene response. This demonstrates that although increased light could be used as a tool in an already-stressed environment, PPF levels do not directly affect the mechanisms behind loss of potential yield due to ethylene.

Reproductive structures are particularly sensitive to ethylene (Figs. 2-7, 2-12). Peas differ from wheat and rice (Klassen and Bugbee, 2002) in that the leaf area (pixels) and vegetative biomass are significantly reduced by low ethylene.

Hence, peas do not appear to catch up at the same rate as their radish or mustard counterparts. This also explains the lack of a vegetative effect of ethylene on wheat and rice (Klassen and Bugbee, 2002) and on tomato plants (Hudelson, 2006). By the time the plants had reached reproductive maturity, the canopy had closed. Thus, the effect of ethylene was limited to reproductive tissue. The lack of an ethylene-PPF interaction indicates that the loss of potential radiation capture compounded the ethylene problem only during the early stages of crop growth before the canopy has a chance to close.

If this is so, then why did the *Arabidopsis* plants the Vandenbussche et al. (2003) experiment not respond opposite to that which was reported? Plants in low light should produce minimal ethylene so that leaf and stem expansion are as rapid as possible. Once the plants have adequate light, ethylene synthesis should increase, triggering reproductive development (a movement of carbon away from shoots and leaves). The work of Foo et al. (2006) also supports the observations of Vandenbussche et al. (2003), suggesting that in this case of chronic exposure, photoreceptor regulation is not affecting the chronic ethylene response. This highlights that it may not be possible to predict the ethylene synthesis or sensitivity of a plant if only one of the factors is known. More studies that examine synthesis-light interactions during long-term plant growth are required. Although ethylene sensitivity does not appear to be affected, PPF adjustments might potentially be used to manipulate synthesis, thus skirting sensitivity.

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CHAPTER 3

ETHYLENE SYNTHESIS FOLLOWING DROUGHT AND FLOOD STRESS IN COTTON, SOYBEAN, AND CORN

Abstract

Extended exploration missions to the moon and Mars require the development of closed-loop life support systems. Crop plants will form an integral part of these systems. Minute (nmol mol^{-1} or ppb) accumulated quantities of the gaseous plant hormone ethylene decrease yield and alter plant growth at concentrations that are not toxic to humans but are difficult to remove from the atmosphere. Plants are the primary source of ethylene. Cotton and soybean plants were found to have ethylene synthesis rates from 0.01-80 pmol $plant^{-1}$ s^{-1}. Water deficit decreased ethylene synthesis in cotton plants. Flood stress did not significantly affect ethylene synthesis or photosynthesis in soybean.

Introduction

Crops and Life Support

Human exploration is the core of the NASA “Vision for Space Exploration” in the 21st century (NASA, 2004). The vision calls for crewed expeditions to both the moon and Mars. By necessity, these missions will be anywhere from a month to several years in duration and will in time require a closed-loop life support system (Myers, 1963; Taub, 1974; Schwartzkopf, 1992; Mendell, 2005). Early attempts to use algae photosynthesis as the foundation for such a system (Taub, 1974) paved the way for the use of higher crop plants. Since the 1960’s,

numerous steps have been made toward the development of suitable hardware for the growth of plants in the spaceflight environment (Porterfield et al., 2003).

Air quality in cabin and plant growth chamber atmospheres must be free of contaminants that would endanger human health and life support system stability. In particular, the presence of volatile organic chemicals (VOCs) has the potential to impact plant health. Stutte et al. (2006) reviewed the current standards for VOC exposure and evaluated the bioactivity of several compounds found in spaceflight atmospheres. For their most active compound, t-butanol, a threshold of 40 µmol mol^{-1} (parts per million, ppm) was sufficient to reduce radish seedling growth by 10%. In contrast, ethylene levels of just 10 nmol mol^{-1} (parts per billion, ppb), a difference of 3 orders of magnitude, are enough to reduce yields in tomato plants by a similar amount (Klassen and Bugbee, 2004).

Elevated levels of the plant hormone ethylene in the atmosphere of growth chambers used in space caused numerous problems in plant growth (Salisbury, 1997; Monje et al., 2003). Although there is thorough documentation of the effects of elevated ethylene on plant growth (Klassen and Bugbee, 2004), there is a paucity of literature that describes ethylene synthesis rates in intact plants under steady-state non-accumulating conditions. Although ethylene is nontoxic to people, in quantities that harm plants it is difficult to remove from the atmosphere. This is important since plants are the primary source of ethylene in controlled environment systems (Perry and Peterson, 2003; Wheeler et al., 2004). To this end, we designed and built systems suitable for measuring ethylene synthesis from various crop plants under normal, water deficit, and flood

conditions. Such data is useful not only to plant physiologists seeking to understand responses to these stresses, but also to life support system engineers who can use these rates as guidelines for the development of ethylene removal apparatus.

Flood Stress

Flooding is a common cause of stress both in the field and in controlled environments. The engineering challenges associated with uniform distribution of water and air throughout the root zone has made inadequate root-zone aeration a common stress in microgravity (Porterfield et al., 1997; Monje et al., 2003). Heavy rains or a malfunctioning watering system can trigger flood-induced hypoxic conditions in the root zone, resulting in crop damage or loss (Drew, 1997; Fukao and Bailey-Serres, 2004). Although there is a great deal of literature detailing the molecular aspects of flood stress in plants (Grichko and Glick, 2001), there is sparse data quantifying the result of these processes for a variety of crops.

What is clear, however, is that ethylene is involved at nearly every level of response to flood stress (Pierik et al., 2007). Examples of two survival strategies that are tied to ethylene are submergence avoidance in rice (Kende et al., 1998) and *rumex* (Rijnders et al., 1997) species, and the formation of aerenchyma tissue in various aquatic and semi-aquatic crops (Colmer, 2003). Indeed, since diffusion of ethylene gas is 10,000 times *less* through water than it is through the air, it is often a build-up of ethylene gas in submerged plant tissues that triggers the flood response strategies (Voesenek et al., 2006). In a unique demonstration

aimed at separating the hypoxia effect from diffusion limitations, Brailsford et al. (1993) sealed intact maize roots into cuvettes and controlled the partial pressure of oxygen flowing through the system. In all treatments below 5 kPa of O_2 pressure, ethylene synthesis increased, and root morphology was similar to flood-stressed plants.

Soybeans are considered a flood sensitive crop (Bacanamwo and Purcell, 1999a). Roots from plants flooded for 21 days had 10-15% porosity whereas there was negligible airspace in nonflooded plants (Bacanamwo and Purcell, 1999b). Oosterhus et al. (1990) examined the effect of flood stress on two soybean cultivars. They found that photosynthesis decreased by 16-32% 48 h after flooding. The effect was apparent 24 h after flooding. These effects were mirrored by similar decreases in stomatal conductance. Given the observed decrease in photosynthesis in soybean, we initially hypothesized that ethylene synthesis would also decrease. However, since soybeans do not have aerenchyma tissue under drained conditions, ethylene synthesis should increase in order to respond to the need for their formation.

Corn, which also has the ability to form aerenchyma tissue, is considered an intermediate-level flood tolerant species (Justin and Armstrong, 1987). Flooded roots were found to have a porosity of 18.5%, which is slightly higher than the 16% reported for nonflooded roots (Justin and Armstrong, 1987). We hypothesized ethylene synthesis to be low for corn plants since they are flood-adapted and porosity does not significantly increase as a result of flood stress application.

Sachs et al. (1996) characterized anaerobically induced genes, identified flooding tolerance genes, and analyzed oxygen deprivation signal transduction in corn plants. Although they highlighted the effect of xyloglucan endotransglycosylase (XET) as a cell-wall softening agent and reiterated the ethylene-cellulase link (Drew, 1992; Grineva and Bragina, 1993; He et al., 1996), they were unable to demonstrate a direct link between ethylene, hypoxia, and these enzymes with the exception of the possible role of calcium signaling. Thus, there is the possibility that ethylene synthesis is increased at the direction of a signaling cascade. This argument is further bolstered by the fact that ethylene synthesis increased under hypoxic conditions when no diffusive limitation was present (Brailsford et al., 1993). To date, no model, other than accumulation due to diffusion limitations, has been put forward to explain a possible signal that would direct increased ethylene synthesis in hypoxic plants. The proposal of such a model would explain the observations of Brailsford et al. (1993) and shed light on the process of flood adaptation and avoidance. Observations of ethylene synthesis under hypoxic conditions for a diverse set of crop plants can help lay the foundations for the development of such a model.

Water Deficit Stress

Inconsistencies in the literature on the effect of water stress on ethylene production provide a clear example of inadequate experimental methods in ethylene research. Studies involving the desiccation of detached leaves suggest water stress increases ethylene production, but studies of intact plants subject to water stress suggest decreased ethylene synthesis (Morgan et al., 1990;

Narayana et al., 1991). Ethylene synthesis rates were unaffected in maize mutants with variable internal concentrations of abscisic acid (Voisin et al., 2006). However, the technique used to measure ethylene was to detach leaf tissue from the plant and place it in a sealed vessel. Thus, ethylene synthesis measured was not from the whole plant. The current understanding is that the effect of water stress on ethylene synthesis depends on the rate at which the plants are stressed. Rapid induction of water stress should promote ethylene production and slow induction should inhibit production (Morgan and Drew, 1997; Xu and Qi, 1993). Despite a lack of consistency in the technique used for whole-plant measurements, molecular techniques suggest that abscisic acid (ABA) influences ethylene effects in plant organs leading to a decrease in synthesis (Chaves et al., 2003). Indeed, several transcription factors that link ABA levels and ethylene production have been identified (Manavella et al., 2006). Members of this same family have also been influenced by light (Manavella et al., 2006). Reduced ethylene production is expected in the field since drought stress typically occurs slowly over the course of weeks. However, water deficit stress occurs rapidly in highly porous media, especially when the root-zone volume is restricted (Morgan and Drew, 1997). Given prior observations made with different techniques and the molecular data, we expect ethylene synthesis to decrease as a result of water deficit stress.

Materials and Methods

Chambers for Ethylene Synthesis Measurements

Figure 3-1. Soybean plants in growth chambers used for ethylene synthesis experiements.

Clear, cast acrylic chambers 54.5 x 54.5 x 175 cm (517 L volume) contained plants for all experiments (Fig. 3-1). Temperature control in each chamber was accomplished by an in-chamber plenum containing heat bars, water-cooled radiator and a fan for in-chamber air circulation. High-pressure sodium and metal halide lamps provided 550 µmol m^{-2} s^{-1} (± 5 %) of light in each chamber. Temperature and photoperiod were tailored to each species studied. Input air was filtered through potassium permanganate saturated beads (Purafil) and supplied at a rate of 7 to 20 L min^{-1} to each chamber. Flow rate into the chamber was determined by carbon dioxide requirements. Dilute nutrient solution (Peters 20-10-20 Peat Lite (final [N] 7.0 mM) supplemented with 10 mM Fe EDDHA) was provided three times daily to ensure adequate nutrition. Plants were grown using a 1:1 peat/perilite substrate. Chambers were validated through repeated testing of filtered input air compared to outside levels and the use of ethylene injections to create volume fraction remaining curves. In all cases, filtered air was lower than outside air. Measured VFR curves matched with predicted values thus demonstrating system stability.

Cotton Growth Conditions

Controlled-environment grown cotton plants (*cv NG2448RR*) with flowers and squares (immature cotton bolls) were transferred into the growth chambers. A 16 h photoperiod and a 30 / 25˚C thermoperiod was used.

Water Deficit Imposition in Cotton

Watering to cotton plants was shut off. Water deficit stress was indicated by both a loss of leaf turgor and when photosynthesis was decreased compared to control plants (24 h post water stoppage). In order to see if rewatering resulted in a transient increase of ethylene synthesis, water was restored to the plants at midnight, when ethylene synthesis was at its lowest level. This technique represents a severe acute water deficit stress that would occur if a nutrient delivery system failed and was subsequently repaired.

Soybean Growth Conditions

Dwarf soybean plants (*cv Hoyt*) with pods were transferred from greenhouse conditions into the controlled environment chambers. A 12 h photoperiod and a 25 / 20˚C thermoperiod was used.

Flood Stress in Soybean

Chambers were opened, and the pots of the soybean plants were placed in larger, plastic-lined pots. The plants were then watered until approx. 2 cm of standing water was present at the top of the pot. This was maintained until the plants were removed from the outer pots and allowed to drain. Flooding was

imposed at 9.75 days post enclosure. Plants were drained at 13.93 days post enclosure.

Corn Growth Conditions

Greenhouse grown vegetative (V6) corn plants (*cv DK-641*) were transferred into the growth chambers. A 16 h photoperiod and a 25 / 20˚ C themoperiod were used. Flood stress was imposed as described for soybeans above one day following enclosure in the chambers.

Ethylene Measurement

An automated thermal desorption system (Perkin-Elmer, *TurboMatrix*) equipped with an on-line sampling accessory concentrated 300 mL (30 mL min^{-1} for 10 min) air samples onto a -30˚C trap containing Carbopak B (Supelco). The trap was heated to 135˚C for 4 minutes as samples were transferred to a gas chromatograph (Shimadzu 17 A) outfitted with a 30 m CARBOXEN-1006 PLOT wide-bore (0.53 mm o.d.) capillary column and flame ionization detector. The column temperature was at 35˚C for 5 minutes before ramping to 135˚C for the remainder of the run. The detection limit for this system was 84 picomoles $mole^{-1}$ (parts per trillion, ppt). Ethylene retention was 10.1 min. The column was baked out at 200˚C for 5 minutes every 3 samples. Total sample-to-sample run time was approximately 23 min. Same-chamber sample cycle time was 4 h.

Carbon Dioxide Measurement and Control

An infrared gas analyzer (LI-COR, LI-6251) tied into a datalogger (Campbell Scientific CR1000) monitored and recorded carbon dioxide input and

growth chamber concentrations. Daytime concentrations were kept at 400 ppm ±5%. Net photosynthetic rate was then calculated. A second analyzer was used to provide continuous measurements of CO_2 into the main air supply. The numbers were then used by a PID algorithm controlled valve to maintain a steady input level into all chambers. Individual flow rates to each chamber were adjusted to maintain an ambient level of 400 ppm ±5% in each chamber.

Ethylene Synthesis to P_{net} Ratio

The ratio of the ethylene synthesis rate to net photosynthesis (P_{net}) was calculated to determine ethylene synthesis as a function of metabolism. This eliminated metabolic rate as a variable and allowed for the comparison of multiple species. Calculating this ratio also allowed us to determine whether ethylene signaling under stress conditions is decoupled from the rate of carbon metabolism. This ratio also eliminates plant size as a variable. Small, rapidly growing plants can produce more ethylene than large, slow growing ones; however, per unit metabolism, they may be identical.

Diurnal Fluctuation in Ethylene

Ethylene synthesis rates were converted from chamber concentrations and expressed as a percentage of the maximum synthesis rate. This served to normalize chamber variability and to highlight the common rhythm expressed by the plants. This also facilitated relative comparisons in amplitude for the cycle.

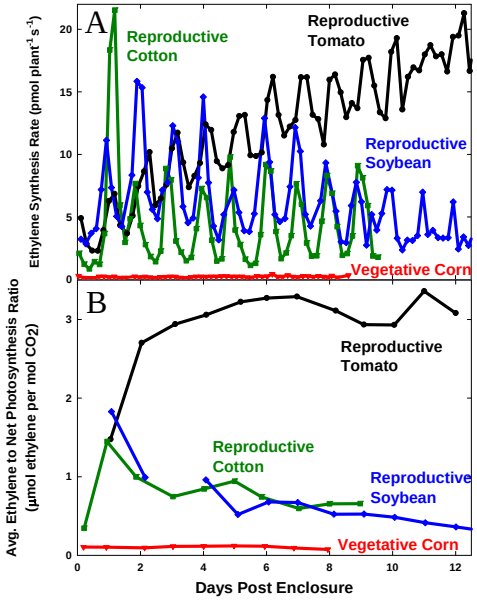

Figure 3-2. Ethylene synthesis from representative tomato, cotton, soybean and corn plants as a per plant rate (A) and per unit net photosynthesis (B).

Results

Ethylene Synthesis

Ethylene synthesis rates per plant, and as a function of net photosynthetic rate, varied with species (Fig. 3-2). Tomato plants (described in Ch. 4) with early green fruits had the highest rate of ethylene synthesis both per plant and per unit net photosynthesis. Cotton, soybeans and corn were all lower than tomato (Fig. 3-2). There are diurnal fluctuations in ethylene synthesis (cotton and soybean are the most noticeable examples). The large increase in ethylene during the first few days of tomato and cotton growth may be an acclimatization period since the plants were transferred from a greenhouse environment into the growth chambers.

Water Deficit in Cotton

Cotton ethylene synthesis, both per plant and per unit carbon uptake, decreased as result of acute water deficit stress (Fig. 3-3). Per-plant ethylene synthesis remained low after the relief of water deficit until the end of the study. Ethylene synthesis per unit carbon uptake, however, returned to control levels one day after watering resumed (Fig 3-3). This suggests that the lower per-plant rate was due to water deficit-induced decrease in plant size. Decrease in plant

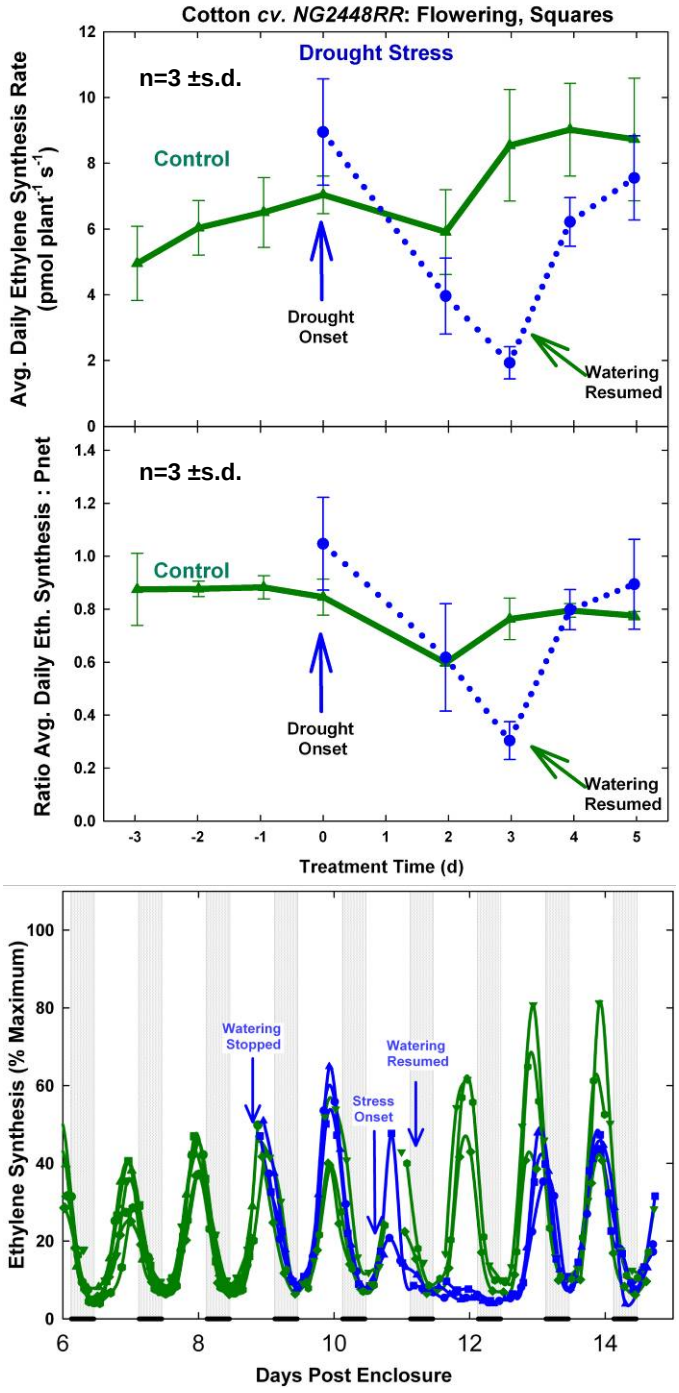

Figure 3-3. Cotton daily average ethylene synthesis, ethylene to net photosynthesis ratio, and ethylene synthesis as a percentage of maximum rate. Drought decreased ethylene synthesis. There was no "burst" of ethylene synthesis upon re-watering. Cyclic ethylene synthesis was disrupted until plant recovery.

size due to water stress was apparent both by a decrease in net photosynthetic rate and visual inspection. The imposition of drought disrupted the diurnal fluctuation in ethylene synthesis for a day following re-watering. Normal cycling was restored the next day and coincided with wilted leaves returning to a normal state.

Flood Stress in Soybeans

Flood stress did not significantly alter ethylene synthesis or photosynthesis in soybean (Fig. 3-4, top, middle). There was a slight increase in ethylene synthesis the day normal conditions were restored; however, that may be due to plant handling rather

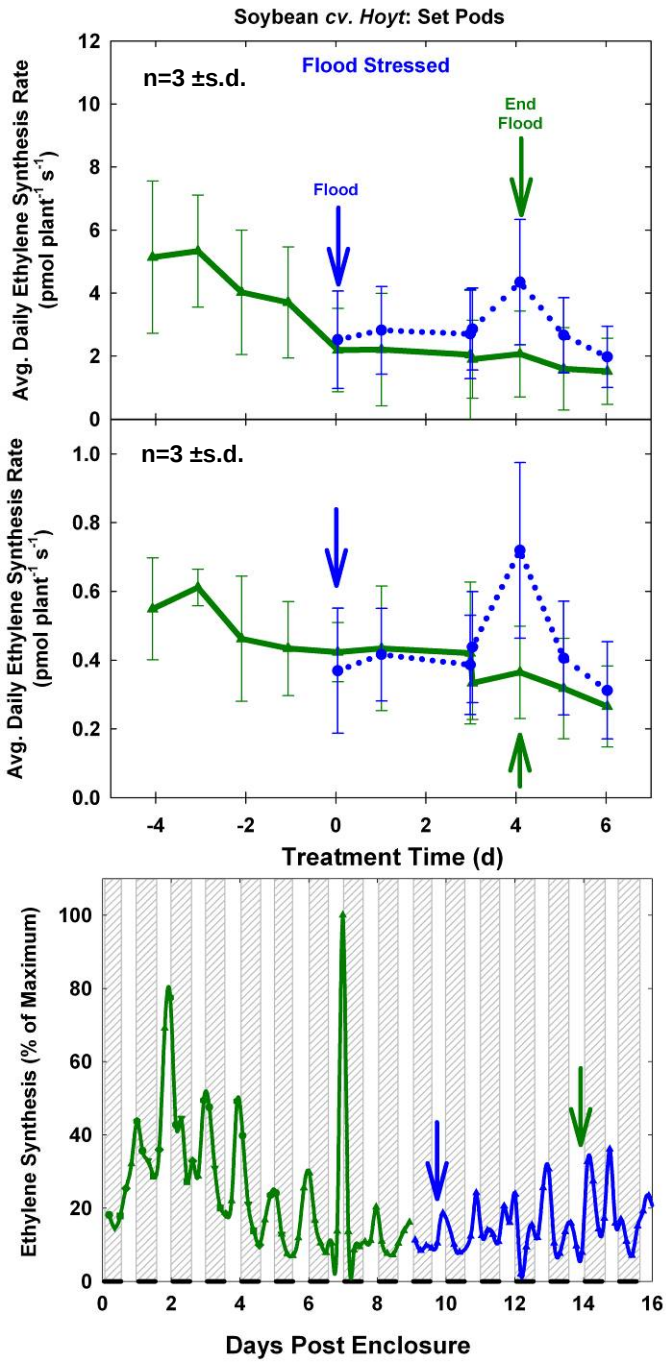

Figure 3-4. Soybean daily average ethylene synthesis, ethylene to net photosynthesis ratio, and synthesis as a percent of maximum rate. Flood stress did not have a significant effect on ethylene synthesis or photosynthesis. Diurnal ethylene synthesis fluctuations were not affected by flood.

than a true effect of flood. Unlike drought stress, flood stress did not affect the diurnal cycling of the ethylene emissions (Fig. 3-4, bottom).

Flood Stress in Corn

Application of flood stress to corn plants caused a dramatic increase in ethylene synthesis (Fig. 3-5, top, middle). This increase was apparent the day following stress application and continued to increase until a new steady-state level was reached. Likewise, the diurnal fluctuation in ethylene synthesis was attenuated throughout the duration of the flood event (Fig. 3-5, bottom).

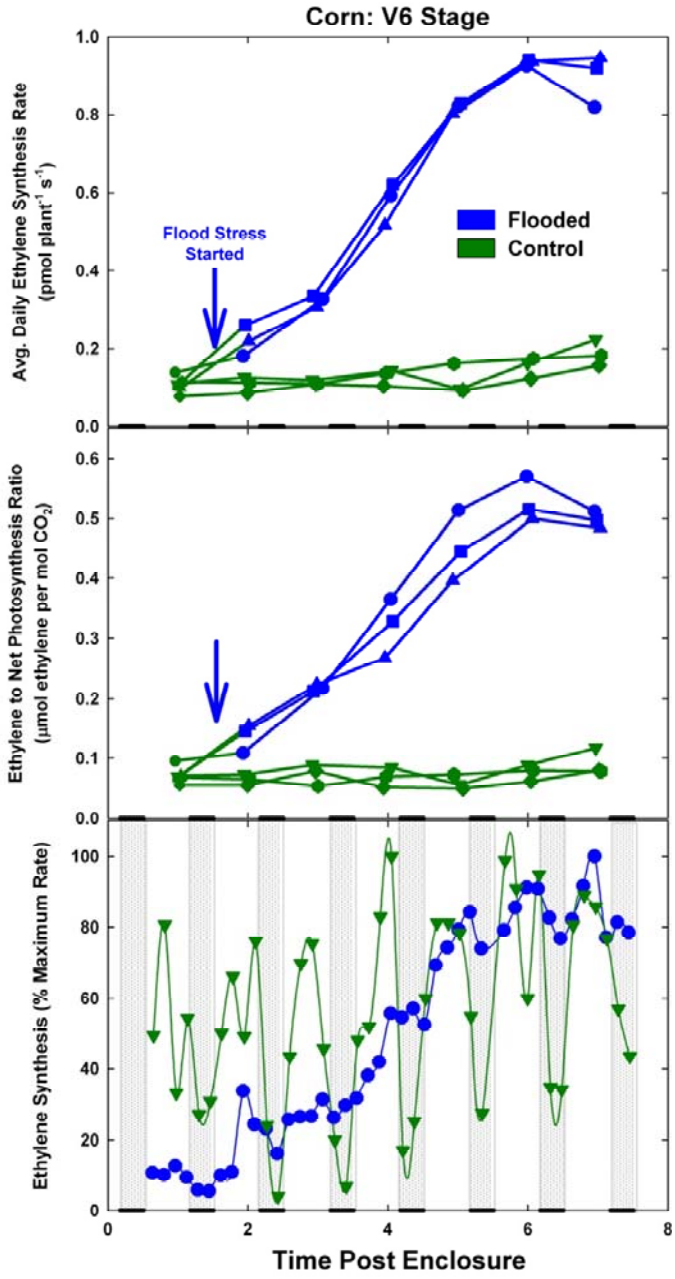

Figure 3-5. Corn daily average ethylene synthesis, ethylene to net photosynthesis ratio, and ethylene synthesis as percent of maximum rate. Flood stress greatly increased ethylene synthesis rate. Diurnal fluctuations were attenuated until a new steady state was reached. Lines represent individual replicate chambers.

Discussion

Ethylene Synthesis

Ethylene synthesis rates both per plant and per unit metabolism were significantly different for each crop. The measurement of ethylene per unit carbon uptake allows for an instantaneous nondestructive measurement that facilitates comparison of plants of different sizes and life cycle stages. All three species tested had similar carbon uptake rates ranging from 1-15 µmol plant^{-1} s^{-1}. Plants grew while in the chambers; hence daily carbon uptake rates increased. The per-plant ethylene synthesis rates varied from 0.25-60 pmol plant^{-1} s^{-1}. Those synthesis rates, coupled with the relative uniformity of carbon uptake, were enough to separate the three

crops from each other even though the rates were normalized per unit carbon uptake. Vegetative corn produced less ethylene than reproductive cotton, which, in turn, is less than reproductive soybean and fruiting tomato. For all control plants, diurnal fluctuations peaked towards the end of the photoperiod each day and then rapidly decreased at the onset of darkness. This suggests that ethylene synthesis per unit metabolism, although different for each species tested, is generally tied to the overall circadian activity of the plant.

For tomato plants, our rates of whole-plant ethylene synthesis (avg. of 30 pmol $plant^{-1}$ s^{-1} or 0.1 nmol kg^{-1} s^{-1} dry mass) are 20x less than the value of 2.1 nmol kg^{-1} s^{-1} dry mass reported by Corey and Barker (1987). One significant difference between the two studies is measurement technique. Corey and Barker (1987) used headspace sampling from a closed chamber which can overestimate production. This does not compare well with our repeated measurements from open flow steady-state chambers.

Sarquis et al. (1991) report ethylene synthesis rates in a flow-through chamber of 0.01 to 0.06 pmol g^{-1} s^{-1} fresh mass for young corn seedlings grown with different impedance pressures. Although the corn plants in this study were considerably larger (about 300 g fresh mass on average) than the seedlings used by Sarquis, our ethylene synthesis rate (0.3 pmol $plant^{-1}$ s^{-1}) is an order of magnitude lower than for their seedlings (.001 pmol g^{-1} s^{-1}). This highlights the need for ethylene researchers to report in units that can be easily compared with each other while the plants are growing. It is possible that our corn plants, which

were more mature, did not have as rapid a metabolism as the seedlings used in the Sarquis study.

The maximum value for cotton seedling ethylene production reported by Jasoni et al. (2000) is approximately 0.36 nmol plant^{-1} h^{-1} or 0.1 pmol plant^{-1} s^{-1}. This is 100x lower than our value of 10 pmol plant^{-1} s^{-1}. However, since we are comparing measurements from seedlings to one from mature reproductive plants, in addition to any changes due to different cultivars, a true comparison of these values cannot be made, again highlighting the need to tie ethylene synthesis to carbon metabolism.

First observed in young cotton seedlings, ethylene synthesis follows circadian rhythms (Rikin et al., 1984; Jasoni et al., 2000). Subsequent work with *Stellaria longipes* demonstrated circadian rhythmicity with the abundance and activity of mRNA associated with ACC oxidase (Kathiresan et al., 1996). Light / dark cycles had a greater entraining effect than temperature cycling. A red light pulse in darkness was capable of resetting the rhythm (Kathiresan et al., 1996). The CAM plants *Tillandsia usneoides* (Spanish moss) were studied to determine if CO_2 availability played a role in the circadian rhythmicity (Beβler et al., 1998). Ethylene synthesis increased in response to light, a time when internal CO_2 concentrations were lowest (Beβler et al., 1998). Ethylene emissions from ACC-solution-soaked plants monitored in the dark demonstrated that ACC-oxidase was not light regulated (Beβler et al., 1998). Later work with sorghum showed that phytochrome B mutants exhibited severe overproduction although circadian rhythms were still present (Finlayson et al., 1998, 1999). Contrary to the work in

Tillandsia, work with sorghum demonstrated a circadian rhythm independent of constant light, constant dark, and isothermal conditions (Finlayson et al., 1998, 1999). Foo et al. (2006) recently demonstrated phytochrome A and B regulation of ethylene in pea plants by showing that plants lacking both phytochromes overproduced ethylene.

Molecular techniques illuminated the inner workings of the circadian clock for *Arabidopsis* plants (McClung, 2000; McClung et al. 2002). As a result, the interactions of the oscillation mechanisms uncovered with ethylene synthesis were explored using *Arabidopsis* plants with various mutations in their ethylene synthesis and perception pathways (Thain et al., 2004). The following was found: The rhythm was light entrained and was persistent. The circadian rhythm was not dependent upon ethylene signaling. Two components of the circadian clock, TOC1 and CCA1, were found to control the rhythm of ethylene production. In agreement with the *Stellaria* data, some ACC synthase and ACC oxidase genes followed the circadian rhythm and dictated the release of ethylene. Finally, ethylene perception mutants exhibited increased ethylene synthesis when compared to wildtype (20x higher in one case) while still maintaining a circadian rhythm. This suggested that ethylene-mediated stress signals should not have an effect on circadian ethylene synthesis (Thain et al., 2004). Indeed, in his minireview, McClung (2000) suggested that the complication of circadian rhythm could no longer be ignored in hormone research. Although a great deal of good science has been done using trap-and-accumulate techniques for ethylene measurement, it is clear that continuous measurement is necessary in order to

tease out the effects of a stress signal from the normal oscillation. Also, the presence of a circadian cycle gives the researcher two new tools to define a stress signal; changes in amplitude and period can also potentially carry a signal of ethylene stress.

Water Deficit Response in Cotton

The water deficit stress simulated in this trial would be similar to that encountered by plants during a failure of the watering system in an advanced life support system plant chamber followed by a restoration of watering. This would be consistent with a severe acute stress since net carbon uptake rate was decreased as a result of the stress. The observed decrease in ethylene synthesis is consistent with the molecular work highlighted in Manavella et al. (2006) and Chaves et al. (2003) and the earlier results of Morgan et al. (1990) and Narayana et al. (1991). However, no burst of ethylene synthesis was observed upon re-watering as summarized in Morgan and Drew (1997). Due to the short length of time over which the water deficit was applied, the data presented here are not fully representative of what would occur in the field over a prolonged period of drought. The fact that the diurnal cycle was repressed for at least a day post re-watering suggests that it is not possible for a “burst” in synthesis to occur for this type of stress event. Perhaps the effects of acute water deficit response are such that the normal diurnal rhythm of the plant is disrupted until full turgor is restored to the plant. Thus, upon rehydration, normal cellular functioning is restored and the rhythm is resumed following an appropriate time to recalibrate the clock.

Flood Stress Response in Soybean and Corn

Although there was no significant response of the soybean plants to flood stress, the overall trend seen in the data agrees with that seen by Oosterhus et al. (1990) (slight decrease in photosynthesis) and predicted by Morgan and Drew (1997) (increase in ethylene synthesis). Oosterhus et al. (1990) note that there was significant difference in the flood tolerance between the two soybean cultivars they tested. The *Forrest* cultivar tested had 10 to 17% more photosynthesis compared to *Essex* 48 h after flood initiation. It is possible that the *Hoyt* cultivar we tested could be more tolerant and that flood stress needed to be applied for a greater period of time for a significant effect.

Contrary to our hypothesis and our soybean plants, corn plants exhibited an almost immediate increase in ethylene production when subjected to flood stress. This production rate, recorded as an emission from intact plants, was over-and-above the ethylene output from the control plants and suggests that trapped ethylene alone cannot be responsible for the increase. This lends support to the hypothesis that there is another factor, at least in corn, responsible for signaling a rise in ethylene production so that flood survival strategies may be engaged. This type of observation, coupled with data on root porosity, could be used to select, categorize, and breed plants that are more tolerant of this stress. However, more study is needed with different plants to determine the magnitude and direction of a response that could be deemed beneficial.

In contrast to the disrupted diurnal fluctuation seen in drought-stressed cotton, the diurnal fluctuation in soybean was not disturbed by flood stress. The

attenuated response of corn demonstrated that the diurnal mechanism could be overridden by flood. This suggests that the response to flood stress is at a tissue-specific rather than general level and that disruption to the diurnal cycle does not necessarily indicate the presence or magnitude of a stress effect.

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CHAPTER 4

BLOCKING ETHYLENE PERCEPTION WITH 1-MCP DOES NOT AFFECT ETHYLENE SYNTHESIS OR PHOTOSYNTHETIC RATE OF CORN, COTTON, SOYBEAN, AND TOMATO

Abstract

1-methylcyclopropene (1-MCP) is an inhibitor of ethylene perception. In harvested climacteric fruit 1-MCP delays the rise in ethylene synthesis and respiration, resulting in delayed ripening and increased shelf life. 1-MCP does not always affect non-climacteric fruit.

We quantified the rate of ethylene synthesis using steady-state flow-through gas exchange chambers and an automated thermal desoprtion gas chromatography system capable of quantifying 84 parts per trillion. This approach allowed whole plant ethylene synthesis to be continuously monitored over multiple days.

1-MCP application doubled the ethylene synthesis rate in both stressed and unstressed tomato plants; treated plants returned to control levels after 4 days. In corn, there was a transient increase in synthesis (3 hours) when a high dose of 1-MCP was applied. 1-MCP had a negligible effect on ethylene synthesis in cotton and soybean plants. Net photosynthesis was unaffected for any crop.

Introduction

Economic loss due to crop damage associated with elevated ethylene levels can occur at any stage of plant growth from in the field to postharvest

processing and shipping. Since ethylene is so pervasive, it is difficult to directly quantify the economic damages associated with elevated ethylene (Abeles et al.,1992).

Since increases in ethylene are thought to serve as a signal for stress, blocking ethylene perception has the potential to mitigate the effects of abiotic stressors experienced by plants and plant products. Common stressors include: elevated ethylene in atmospheres with poor gas exchange (Sargent, 2001; Wheeler et al., 1996, 2004), drought (Morgan and Drew, 1997), and flood-induced hypoxia (Pierik et al., 2006). The effects of these stresses lead to crop damage and subsequent loss of potential yields. Also, blocking plant ethylene perception could reduce the need for complex ethylene scrubbing systems during times of plant stress in those areas where such a system is possible (i.e. post-harvest storage, controlled environment chambers). Thus, obtaining the ability to block harmful ethylene effects in a reversible, consistent manner is of great value.

Chemical control of ethylene synthesis has been achieved with aminovinylglycine (AVG), aminooxyacetic acid (AOA), α–aminoisobutyric acid (AIBA), and Co^{2+}. Yang and Hoffman (1984) reviewed these compounds and their inhibition mechanisms. By virtue of being in the same chemical family as AVG, AOA reacts in a similar manner. Ions of Co^{2+} were first shown to interfere with ethylene synthesis in plugs of apple tissue (Lau and Yang, 1976). Later, it was proposed that Co^{2+} acts by complexing with sulfhydryl protein groups (Yang and Hoffman, 1984). The data, however, were inconclusive due to limitations of

the techniques available at the time. AIBA is structurally similar to ACC and, therefore, acts as a competitor for the binding site of ACC oxidase (Satoh and Esashi, 1980; Liu et al., 1984).

The primary advantages of these chemicals in the context of controlled environment plant growth is the ability to reduce ethylene loads without resorting to the use of bulky filter material or other scrubbing apparatus. Also, the ability to time when the chemicals are applied allows for a targeted removal of ethylene and for experiments that look at ethylene-critical development stages. The primary disadvantage of AVG, AOA, and Co^{2+} is that by their mechanism of action, they are inherently nonspecific to the ethylene synthesis pathway. Thus, there is an elevated risk of secondary effects associated with using these compounds, although no severe effects have been documented. Since it competitively binds to ACC oxidase, AIBA is more specific to the ethylene synthesis pathway. Possible contamination of a controlled environment due to external application of compounds and the fact that the effects induced by these inhibitors last only as long as the supply in the plant are two primary disadvantages. For the former, thorough cleaning and proper disposal of the waste is required between experimental trials. The latter imposes a continuous-dosing requirement in order for the effect to remain for a long study. Although these compounds have been used with success, they must be dissolved and sprayed onto the plant, which means that uptake is variable. None of these compounds block the perception of ethylene gas present due to pollution or

chamber contamination. Also, several of these compounds are potentially toxic to humans.

Dissociated silver ions from silver thiosulfate (STS) and sliver nitrate ($AgNO_3$), chemicals classically used to inhibit ethylene perception, displace the copper cofactors used in the binding sites of ethylene receptor proteins (Abeles et al., 1992). However, toxicity effects have been reported, and the compounds suffer the same limitations as their synthesis-blocking cousins in that they must be applied as a liquid with variable uptake (Abeles et al., 1992).

1-methylcyclopropene (1-MCP) is a potential alternative that can be homogeneously applied as a gas. 1-MCP binds to the protein and physically occludes the binding site, blocking ethylene perception (Sisler and Serek, 1997). Most studies of 1-MCP have focused on its effects in post-harvest physiology. 1-MCP decreases both ethylene synthesis and respiration of climacteric fruit (Blankenship and Dole, 2003). Limited information on non-climacteric fruits indicates that the effect of 1-MCP is inconsistent and needs to be evaluated on a case-by-case basis (Lurie, 2005). For example, ethylene synthesis increased in citrus fruits, was unaffected in strawberries (Lurie, 2005), and decreased in grapes (Chervin et al., 2005). Although the effects of 1-MCP on harvested organs are of importance for increasing shelf life and storage, there is sparse information for the effect of 1-MCP in whole plant physiology especially with respect to effects on ethylene synthesis and net photosynthetic rate.

Faust and Lewis (2004) examined the effect of 1-MCP in unrooted Poinsettia cuttings and found it caused an increase in ethylene accumulation in

their sealed containers. However, Faust and Lewis did not measure the accumulation of carbon dioxide in their containers. The increased ethylene synthesis may be the result of increased respiration due to increased temperature. This may be the case since the ethylene accumulation did not occur at lower temperatures. Although ethylene increased, leaf abscission post-storage decreased.

Hays et al. (2007) examined the effect of 1-MCP application during heat stress on susceptible and nonsusceptible wheat cultivars. The susceptible cultivar tested had a 6x increase in ethylene synthesis in developing kernels and a 12x increase in the flag leaves. This resulted in a significant decrease in grain set per ear and kernel mass. These losses were removed by application of 1-MCP dissolved in an adjuvant solution and applied in a spray. These effects were not seen using the heat-tolerant variety. This suggests that 1-MCP application could allow for a more diverse selection of crops in stress-prone or marginal regions, thus increasing the potential to improve overall yields. This effect also demonstrates that observed 1-MCP application effects will not be universally applicable to all cultivars of a given species.

Mishra et al. (2008) examined the effect of 1-MCP application on the break strength of the abscission zone in cotton leaves. They found that 1-MCP increased the breaking strength of the abscission zone compared to ethylene-treated controls. Also, 1-MCP application significantly reduced cellulose and polygalacturonase activities in ethylene-induced abscission zones. This effect was synergistically increased when coupled with application of IAA and the

compounds were applied prior to ethylene stress. This suggests that plants undergoing drought and heat stress, which are noted for causing organ abscission (Tudela and Primo-Millo 1992; Addicott and Lynch 1955; Zhao et al., 2005), will be protected by application of 1-MCP and IAA in anticipation of or during the stress event.

Figure 4-1. Soybean plants in growth chambers used for ethylene synthesis experiements.

Our objectives were to determine what, if any, effect 1-MCP would have on ethylene production in intact crop plants under steady-state controlled environment conditions.

Materials and Methods

Chambers for Ethylene Synthesis

Clear, cast acrylic chambers 54.5 x 54.5 x 175 cm (517 L volume) contained plants for all experiments (Fig 4-1). Temperature control in each chamber was accomplished by an in-chamber plenum containing heat bars, water-cooled heat exchanger, and fan for in-chamber air circulation. High-pressure sodium and metal halide lamps provided 550 µmol m^{-2} s^{-1} (± 5 %) of light in each chamber. Temperature and photoperiod were tailored to each species studied. Input air was filtered through potassium permanganate saturated beads (Purafil) and supplied at a rate of 7 to 20 L min^{-1} to each chamber. Flow rate into the chamber was determined by carbon dioxide and

ethylene requirements. Dilute nutrient solution (Peters 20-10-20 Peat Lite (final [N] 7.0 mM) supplemented with 10 mM Fe EDDHA) was provided three times daily to ensure adequate nutrition. Chambers were validated through repeated testing of filtered input air compared to outside levels and the use of ethylene injections to create volume fraction remaining curves. In all cases, filtered air was lower than outside air. Measured VFR curves matched with predicted values, thus demonstrating system stability.

Blocking Ethylene Perception

In all studies, 1-MCP tablets (Rohm and Haas, USA) were dissolved in a citric acid solution to obtain either 340 or 680 nmol mol^{-1} (parts per billion, ppb) gas concentrations. In the corn study, 1-MCP powder (Rohm and Haas, USA) was dissolved into deionized water at a rate of 4.7 g L^{-1} (0.179 g active ingredient); no wetting agent was used. Approximately 225 mL of solution was then sprayed onto the plants. If all of the 1-MCP dissolved and converted to gas, a theoretical maximum chamber gas concentration of 120,000 ppb (0.012%) would be obtained. All applications were done with the lights on and airflow to the growth chambers turned off over the course of one or two hours.

Tomato Growth Conditions

Greenhouse grown *Florida 47* tomato plants with flowers and early green fruit were transferred into the growth chambers with a 12 h photoperiod and a 26 / 16° C thermoperiod. Relative humidity was at 75-80% for all chambers. Plants were gassed with 1-MCP at 680 ppb for 2 h at 4.7 d post enclosure. Under these

conditions, the tomatoes had clear signs of intumescence injury. Subsequent trials were conducted using the environmental parameters for *Beefsteak* tomatoes described below.

Greenhouse-grown *Beefsteak* tomato plants with flowers and no fruit were transferred into the growth chambers with a 12 h photoperiod and a 25 / 20˚ C thermoperiod. Relative humidity was at 45-50% for all chambers. In order to mitigate intumesence injury, UV lights were kept on for 24 h a day for the duration of the experiment. Although UV light between the range of 100-190 nm will degrade ethylene (Calvert and Pitts, 1966), Maneerat et al. (2003) demonstrated that common “blacklight” bulbs that emit a wavelength range of 300-400 nm do not photodegrade ethylene.

Corn Growth Conditions

Greenhouse-grown vegetative (V6) corn plants (*cv DK-641*) were transferred into the growth chambers. A 16 h photoperiod and a 26 / 16˚ C themoperiod were used. Plants were gassed with 340 ppb 1-MCP at 2.9 days post enclosure. Plants were later sprayed as described above at 6.1 and 8.0 days post enclosure.

Cotton Growth Conditions

Controlled-environment grown cotton plants (*cv NG2448RR*) with flowers and squares were transferred into the growth chambers. A 16 h photoperiod and a 30 / 25˚C thermoperiod was used.

Soybean Growth Conditions

Dwarf soybean plants (*cv Hoyt*) with pods were transferred from greenhouse conditions into the controlled environment chambers. A 12 h photoperiod and a 25 / 20˚C thermoperiod was used.

Ethylene Measurement

An automated thermal desorption system (Perkin-Elmer, *TurboMatrix*) equipped with an on-line sampling accessory concentrated 300 mL (30 mL min^{-1} for 10 min) air samples onto -30˚C trap containing Carbopak B (Supelco). The trap was heated to 135˚C for 4 min as samples were transferred to a gas chromatograph (Shimadzu 17 A) outfitted with a 30 m CARBOXEN-1006 PLOT wide-bore (0.53 mm o.d.) capillary column and flame ionization detector. The column temperature was at 35˚C for 5 min before ramping to 135˚C for the remainder of the run. Detection limits were 84 picomoles $mole^{-1}$ (parts per trillion, ppt). Ethylene retention was 10.1 min. The column was baked out at 200˚C for 5 min every 3 samples. Total sample-to-sample run time was approximately 23 min. Same-chamber sample cycle time was 4 h.

Carbon Dioxide Measurement and Control

An infrared gas analyzer (LI-COR, LI-6251) tied into a datalogger (Campbell Scientific CR1000) monitored and recorded carbon dioxide input and growth chamber concentrations. Daytime concentrations were kept at 400 ppm ± 5%. Photosynthetic rate and daily net carbon gain were then calculated. A second analyzer was used to provide continuous measurements of CO_2 into the

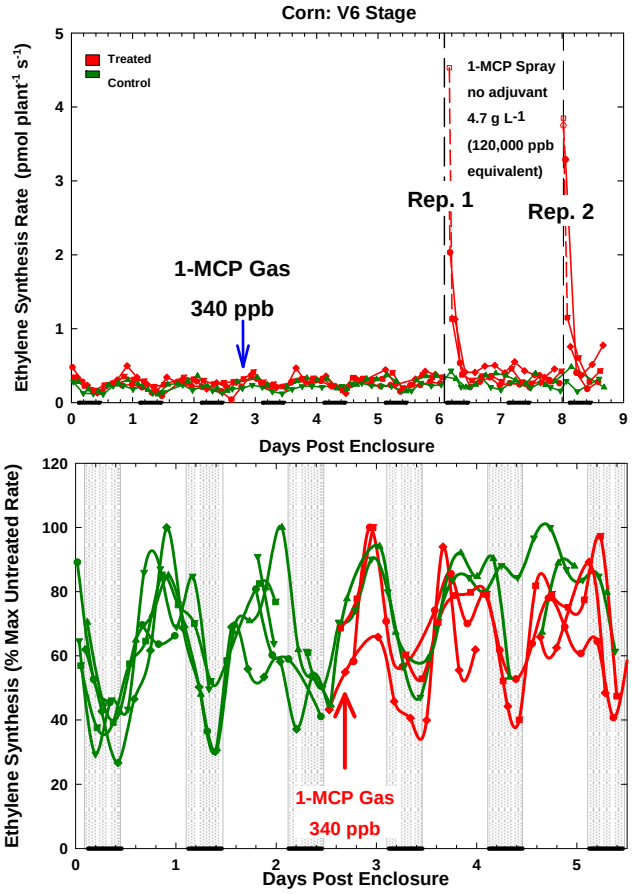

Figure 4-2. The effect of 1-MCP gas and spray on corn ethylene synthesis. 1-MCP applied at 340 ppb had a negligible effect on ethylene synthesis (top). Diurnal fluctuations in synthesis were also not effected (bottom). When applied as a spray (120,000 ppb equivalent), ethylene synthesis showed a brief increase lasting less than 3 h (top). Dotted lines and open symbols indicate points back-calculated using the volume fraction remaining equation.

main air supply. The numbers were then used by a PID algorithm controlled valve to maintain a steady input level into all chambers. Individual flow rates to each chamber were then adjusted to maintain an ambient level of 400 ppm ± 5% in each chamber.

Results

The application of 1-MCP as a gas did not affect corn (Fig. 4-2), cotton (Fig. 4-3), or soybean (Fig. 4-4) ethylene synthesis. All ethylene synthesis data, with the exception of corn, represents an average and standard deviation of three independent replicate chambers. Corn ethylene synthesis and diurnal cycling data is presented either as individual chambers or as a representative example.

Ethylene synthesis in corn increased for a brief period (<4 h) when 1-MCP was applied as a spray (Fig 4-2). Open symbols connected to dashed lines in

Figure 4-2 represent projected synthesis rates calculated using the volume fraction remaining equation. This was necessary due to the constraints imposed by the instrument sampling times (Fig. 4-2). The concentration of 1-MCP gas in the chambers far exceeds the amount that would be present in a normal field application.

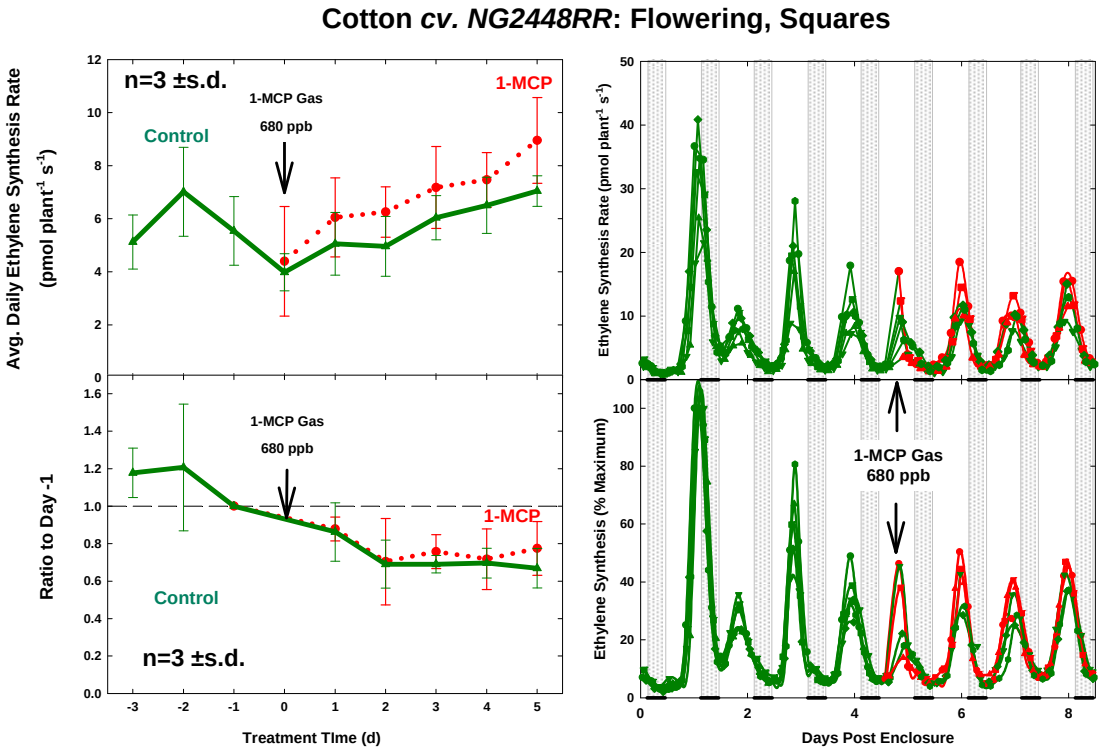

Figure 4-3. Average daily ethylene synthesis rate, ethylene synthesis rate, normalized rate of synthesis and ethylene synthesis expressed as a percent of maximum rate for cotton plants. The effect of 1-MCP gas at 680 ppb on cotton ethylene synthesis was not significant when individual days were analyzed. When analyzed as an aggregate over the six treatment days, there is an almost significant trend towards a slight increase in synthesis. Diurnal fluctuations in ethylene synthesis were not affected by 1-MCP application. The apparent decrease in synthesis on the day of application can be accounted for by data points eliminated from the analysis during the time of compound application. Thus, the peak rate for that day may not have been captured.

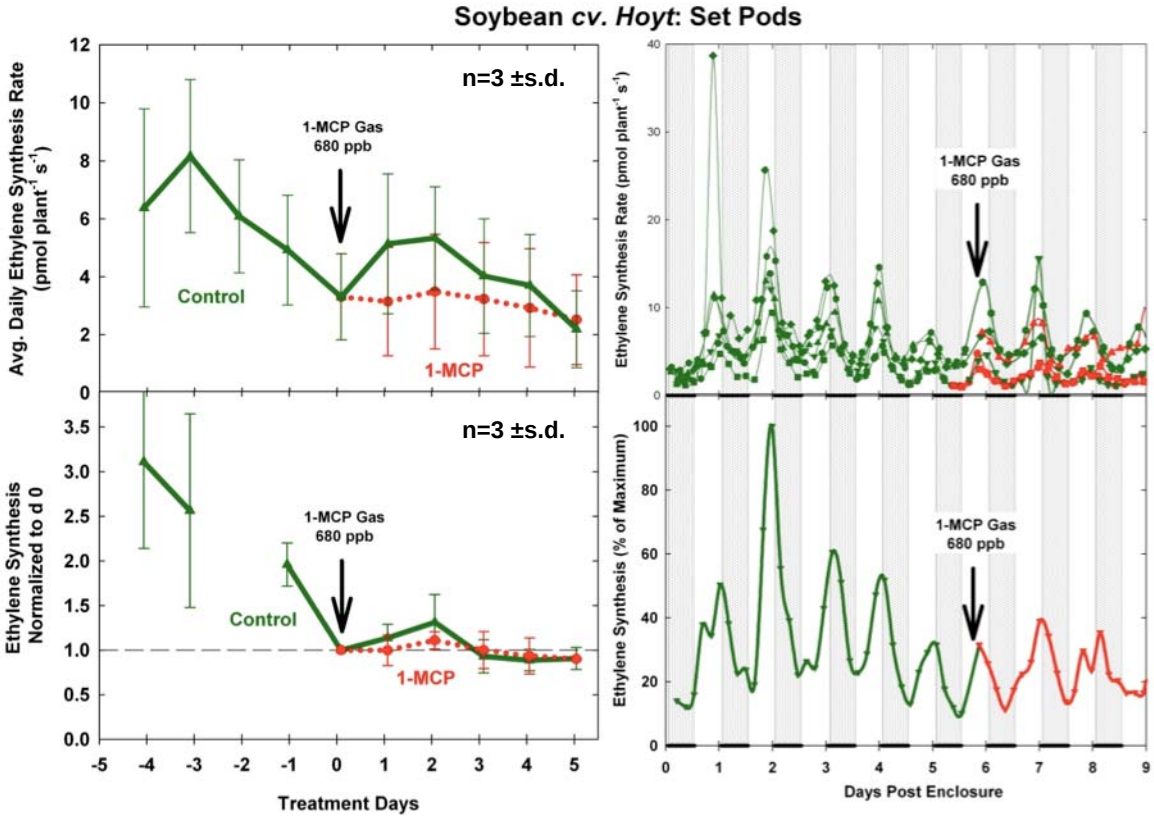

Figure 4-4. Average daily ethylene synthesis rate, ethylene synthesis rate, normalized rate of synthesis and ethylene synthesis expressed as a percent of maximum rate for soybean plants. When treated and control plants are analyzed during individual days, application of 1-MCP to healthy soybean plants did not significantly affect ethylene synthesis or diurnal cycling. When treatment days are aggregated and analyzed, the trend towards decreased ethylene synthesis is slightly siginificant. The data presented for the diurnal fluctuation is a representative replicate chamber.

Florida 47 tomato plant ethylene synthesis was increased for four days post treatment (Fig. 4-5). However, this result is likely due to an interaction with the intumesence stress present in the plants. Although in-chamber ethylene concentrations did not go higher than 9 ppb, the plants exhibited signs of ethylene stress including upwardly curled leave and flower abortion. Elevated humidity in the chambers (~80% R.H.) and a lack of ultraviolet light likely

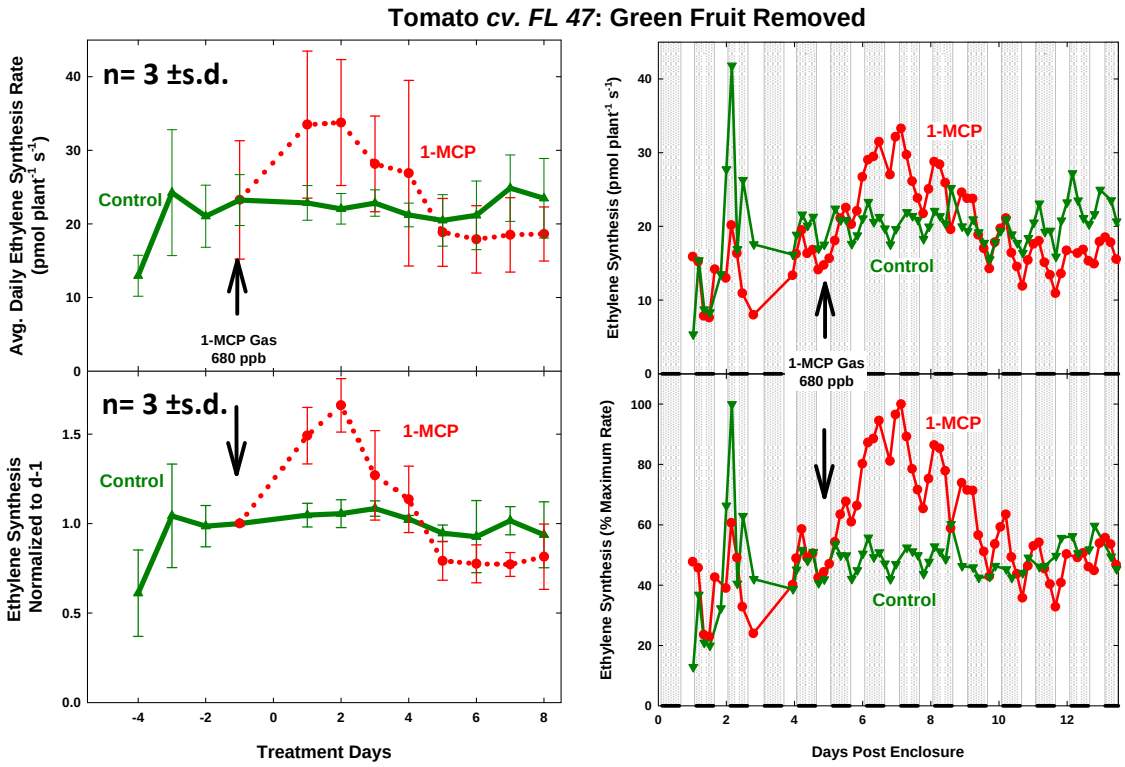

Figure 4-5. Ethylene synthesis for *Florida 47* tomatoes with green fruit removed. Application of 1-MCP increased ethylene synthesis almost twofold compared to control plants. Diurnal cycling was altered as synthesis rates rose and fell. It is almost certain that the increase in synthesis is the result of an interaction with intumesence in the plant triggered by high chamber humidity and a lack of ultraviolet light. It is also possible that the plants were already ethylene stressed when treatment began. Thus, these results are not representative of healthy unstressed tomato plants.

contributed to intumesence injury. Thus, this data represents an interaction of 1-MCP gas application with stressed plant growth.

Beefsteak tomato plants did not have the horticultural problems seen in *Florida 47.* Due to a higher airflow rate through the chambers, ethylene concentrations did not exceed 5ppb. UV lights, which do not photodegrade ethylene, were installed in the chambers, and the relative humidity was decreased to ~40%. The application of 1-MCP resulted in an almost 2x increase

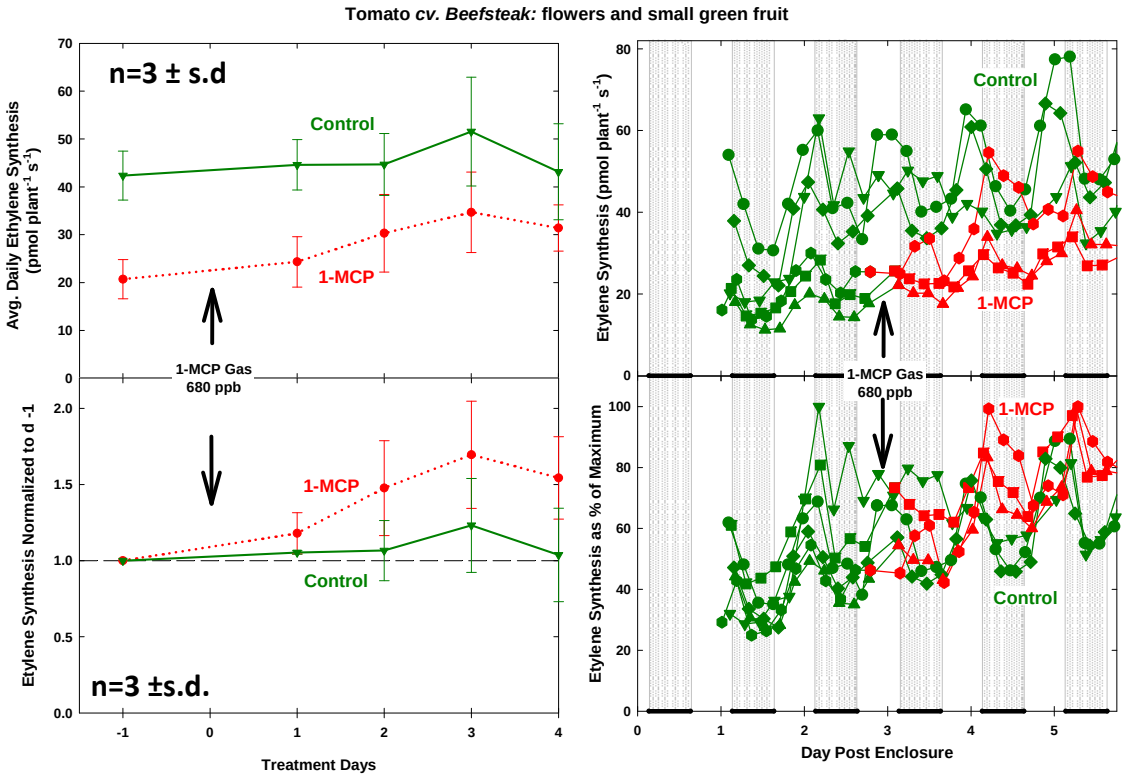

Figure 4-6. Ethylene synthesis in beefsteak tomato plants. Data points represent the average and standard deviation of three chambers. Although 1-MCP (680 ppb) treated plants had the lowest rate of ethylene synthesis, when synthesis was normalized to the day prior to treatment a rise in ethylene synthesis is evident. ANOVA analysis for individual days is not statistically significant. When multiple days are pooled and analyzed, the trend is significant. Diurnal fluctuations in ethylene synthesis, although present, were not as apparent as in other species and cultivars. 1-MCP application at 680 ppb does not appear to affect the pattern of diurnal cycling that is present.

in ethylene when compared to the synthesis rate of the day prior to application, an increase similar to that seen with *FL 47* plants. Although the results are not statistically significant due to experimental error between chambers, tomato plants appear to be the only species tested that had the potential for a significant increase in ethylene response due to 1-MCP application.

Diurnal cycling was present to a greater or lesser extent in all species tested (Fig. 4-6). In all cases tested, 1-MCP application did not affect this cycle

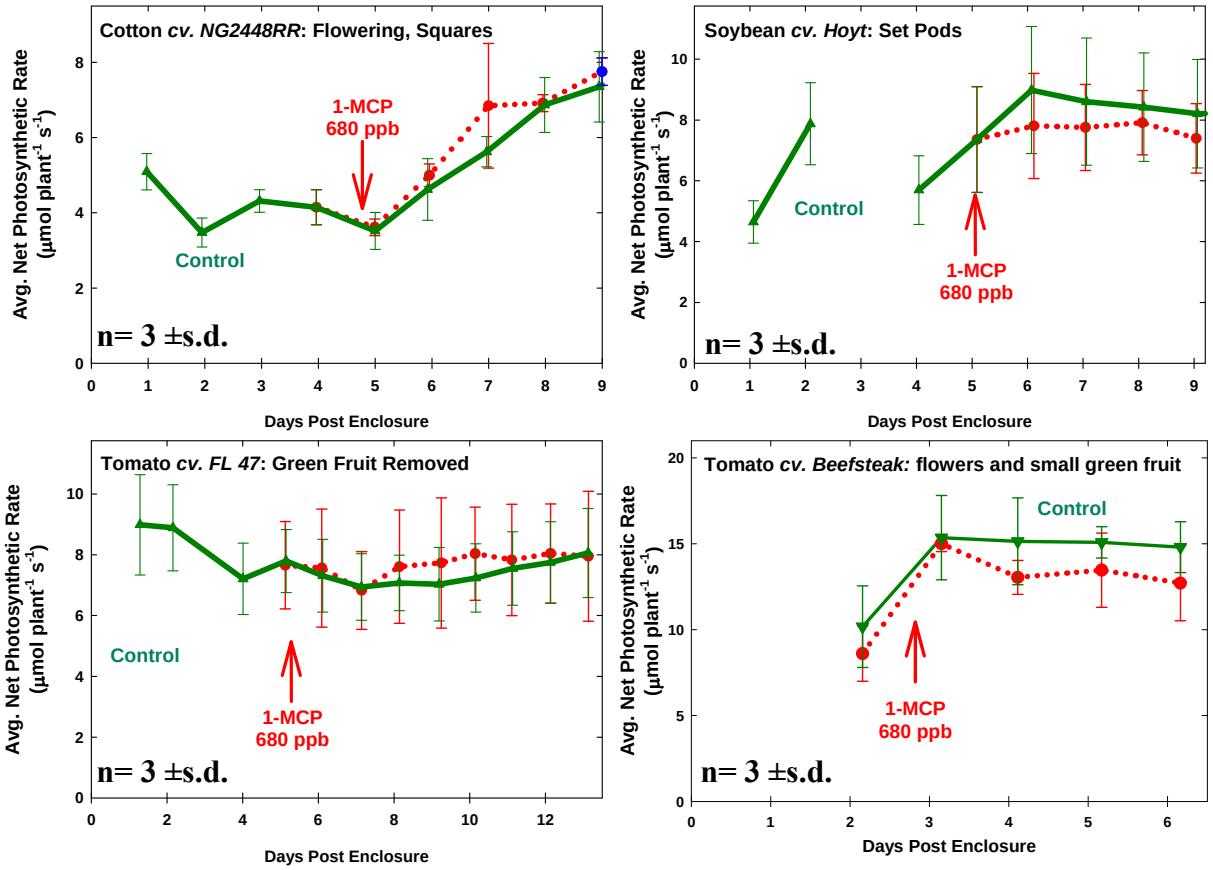

Figure 4-7. The application of 1-MCP to cotton, soybean, and tomato plants did not alter the daily net photosynthetic rate. For all plants, uptake rate was either constant or increased throughout the duration of the experiment.

although it was somewhat attenuated during the increase in ethylene synthesis experienced by both *Florida 47* and *Beefsteak* tomato plants. In all species tested, the maximum rate of ethylene synthesis occurred just prior to turning the electric lights in the chamber off. Also, the minimum rate of synthesis corresponds to the time just prior to turning on the electric lights in the system.

Net photosynthetic rate was not significantly affected by 1-MCP application (Fig. 4-7). P_{net} remained constant or increased over the duration of the experiment at a range of 5-15 µmol plant^{-1} s^{-1} for all control plants. Although

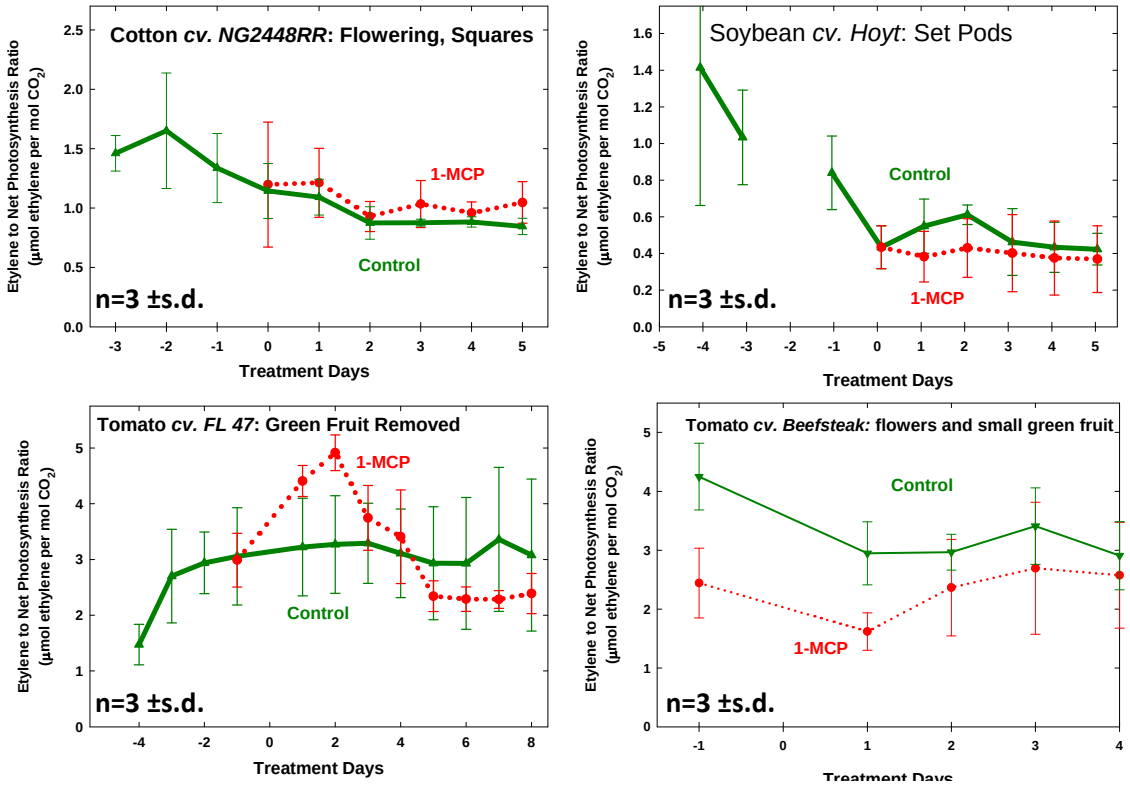

Figure 4-8. Ratios of average daily ethylene synthesis to net photosynthetic rate for cotton, soybean, and two tomato cultivars. Treatment with MCP did not significantly affect this ratio except for a temporary increase in intumesence stressed *FL 47* tomato plants. Cotton and soybean plants decreased over time whereas both tomato plants remained relatively constant.

not significant when compared to control plants, cotton and *FL 47* tomato both tend to have a slight increase in P_{net}, whereas both soybean and *Beefsteak* tomatoes tend to have a slight decrease. Neither alteration was capable of significantly affecting ethylene synthesis rates expressed as a ratio to net carbon uptake rate.

The ratio of ethylene synthesis to net photosynthetic rate ranged from 0.4 in mature soybean plants to a high of 6 for stressed tomatoes (Fig. 4-8). This is a much more narrow range than per-plant ethylene synthesis rates. There was no

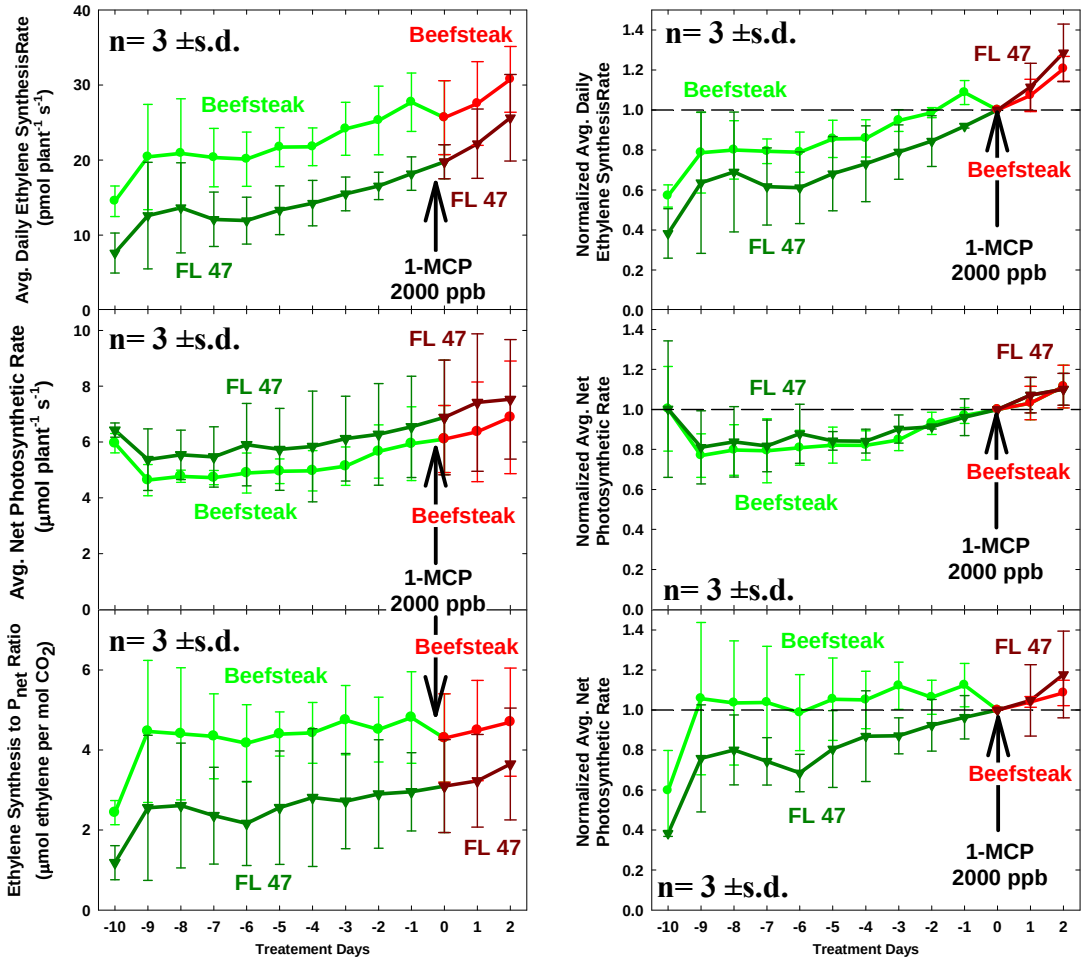

Figure 4-9. A comparison of tomato cultivars *Beefsteak* and *Florida 47*. Ethylene synthesis and net photosynthetic rate increased throughout the study. The ethylene synthesis to net photosynthetic rate ratio remained unchanged. Application of 1-MCP at a concentration of 2000 ppb did not alter either the rate of ethylene synthesis or net photosynthesis.

consistent trend for a change in ethylene synthesis rate between all the crops studied. Although stressed *FL 47* tomato plants did have a significant increase in synthesis, in the absence of stress this may not always be so.

A direct comparison of the tomato cultivars *Beefsteak* and *Florida 47* revealed that the *Beefsteak* cultivar had a greater rate of ethylene synthesis both on a per-plant and per-metabolic-unit basis (Fig. 4-9). Both cultivars had

increasing rates of ethylene synthesis and net photosynthesis. The ratio of ethylene synthesis to net photosynthesis, however, was constant. The application of 1-MCP at 2000 ppb did not alter either ethylene synthesis or net photosynthetic rate (Fig. 4-9).

Discussion

Tomato fruit is classified as climacteric. Contrary to what would be expected from work in climacteric fruit (Lurie, 2005), 1-MCP increased ethylene synthesis in whole *Florida 47* tomato plants. A similar, but not significant, trend was also seen in *Beefsteak* tomato plants. In the case of *FL 47* tomato plants, the most likely cause for the increase in synthesis was due to a possible interaction with intumesence stress caused by a lack of UV light and high humidity as described in Lang and Tibbitts (1983) and Morrow and Tibbitts (1987, 1988). Also, although ethylene concentrations never were higher than 9 ppb, *FL 47* tomatoes exhibited signs of ethylene stress including upwardly curled leaves (Abeles et al., 1992) and aborted flower buds. These conditions were addressed when *Beefsteak* tomato plants were tested. A hypothesis that the green fruit on the plant could be contributing to the ethylene increase is unlikely since this is counter to the decreased rates of ethylene synthesis and respiration documented for a wide variety of climacteric fruits, and the response was seen in plants with and without fruit. Also, neither soybeans that had setting pods nor corn (when gassed and not sprayed) and cotton exhibit the same response to 1-MCP application. As a reproductive crop, tomatoes are the most sensitive to

exogenous ethylene (see Figure 2-12). It is possible that this sensitivity to ethylene plays a role in the response to 1-MCP application.

A follow-up study that directly compared both *Beefsteak* and *Florida 47* cultivars under nonstressed conditions (Fig. 4-9) supports the data from the first *Beefsteak* trial (Figs. 4-7 and 4-8). At a high dosage of 2000 ppb, 1-MCP had no significant effect on ethylene synthesis or net photosynthetic rate. Altogether, the tomato data highlight that ethylene synthesis and the impact of 1-MCP is likely dependent on how stressed the plants are. This is bolstered by the stress effects seen by Hayes et al. (2007) and Faust and Lewis (2004).

Although for the all of the unstressed crops tested there was no ethylene response shown, the differences between 1-MCP response seen in two wheat cultivars (Hays et al., 2007) and the increase in synthesis from poinsettia cuttings seen by Faust and Lewis (2004) highlight that a uniform response for all applications of 1-MCP is not to be expected. Nor do data from post-harvest fruit provide a predictive indicator of plant response. Indeed, the non-effect on net photosynthetic rate shown in Fig. 4-7 is contrary to any expectation one would have using climacteric fruit, which consistently shows a decrease in respiration (Lurie, 2005), as a guide.

An effect of 1-MCP on diurnal cycling appears to be non-existent for all of the crops tested. Both tomato cultivars exhibited diurnal cycles even during the increase in ethylene synthesis rate. The timing of the minimum and maximum rates of synthesis agrees with our own prior work (see Ch. 3) and with the observations of Rikin et al. (1984) and Jasoni et al. (2000). This, however, is to

be expected since 1-MCP affects only the binding site of the ethylene receptor proteins which do not appear to be regulated by the circadian clock (Thain et al. 2004). This bolsters the contention that ethylene signaling does not play a role in circadian rhythms in plants.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

As a plant hormone, ethylene is responsible for stress signaling and the moderation of developmental change. As such, mastery of ethylene synthesis and sensitivity has the potential to impact numerous areas of agricultural importance. These include flood and drought stress, postharvest storage and transport, controlled environment plant growth, and control of developmental change.

Although ethylene effects have been used throughout history, only within the last 50 years have techniques developed to the point at which quantification of both sensitivity and synthesis have been possible. Initial experiments often used detached plant parts and long accumulation times in static atmospheres in order to quantify ethylene levels. These techniques can lead to artifacts in the data and an incorrect picture of ethylene function and effects as researchers scale from a tissue to a whole-plant level. By using automated thermal desorption techniques coupled with sensitive gas chromatography equipment, we measured the ethylene synthesis and sensitivity of intact plants in controlled environments under steady-state conditions.

A similar revolution has occurred in the tools available for manipulating ethylene perception. Prior to 1996 or so, the only tools available for ethylene perception blocking were the compounds silver nitrate and silver thiosulfate. The dissociated silver ion from both of these compounds is the active agent that displaces the copper cofactor of the ethylene receptor protein. Although effective,

these compounds suffer from toxicity issues. They must be applied as a liquid which does not ensure consistent uptake from one application to the next, and they are expensive and thus not suited for commercial application. Also, the specificity of the silver ion to just the ethylene pathway cannot be guaranteed.

The development and application of 1-methylcyclopropene as an ethylene perception blocker has provided a potent new tool for the investigation of ethylene perception. Unlike STS or silver nitrate, 1-MCP is a gas. Thus, uniformity of application in a laboratory environment is not problematic. Also, since physical occlusion of the ethylene receptor binding site is the action mechanism of 1-MCP, the odds for alternate-pathway nonspecificity are decreased. Since 1-MCP is a small molecule with structural characteristics similar to ethylene, it has the potential to diffuse through plant tissue in a manner similar to ethylene gas. Finally, the potential for field-application of 1-MCP to growing plants as an analgesic for plant stress, coupled with the novelty and potential benefits gained from application, merited the examination of 1-MCP effects on ethylene synthesis.

In order to provide more insight and quantification of ethylene effects, we conducted basic research into the following three areas of inquiry: ethylene sensitivity of vegetative (radish) and reproductive (pea) dependent crops; the effect of acute water deficit stress and flood induced hypoxia on ethylene synthesis; and, finally, the effect of ethylene perception blocking using 1-MCP. These studies represent a diverse array of plants and conditions and lay the

foundations both for further studies that examine combined effects and expectations for field-trials.

Ethylene Sensitivity

Ethylene Dose-Response Curves

Sensitivity of plants to atmospheric ethylene is an important factor not only in areas where there is poor gas exchange, such as storage and shipping containers, but also in areas where there is increased air pollution. Examples of areas with increased air pollution include industrial greenhouses that use forklifts and other combustion-based equipment, farmland near cities, the International Space Station, and areas near polyethylene manufacturing plants. Our study of the sensitivity of plants to atmospheric ethylene allowed us to identify target concentrations of ethylene gas that do not appear to have a significant impact on the growth of the plant.

Prior work performed in our laboratory recorded in Klassen and Bugbee (2004) provided data on the sensitivity of wheat (*cvs. Apogee* and *Perigee*), rice (*cv. Super Dwarf*), lettuce (*cv. Grand Rapids*), and tomato (*cv. Reimann Philippe*). Our work added two crops to this collection of data: peas (*cv. Earligreen*) and radish (*cv. Cherry Belle*). We also drew a distinction between those crops whose yields were dependent upon vegetative growth (i.e., radish, mustard) and those that required reproductive growth and development (i.e., peas, tomato). These were expressed as yields normalized as percentages of control (0 ppb ethylene) plants. Two-parameter exponential decay lines were

then used to fit lines representing dose-response curves to the data. From this analysis, three different conclusions can be reached.

First, the calculated potential yield loss for a crop depends upon whether or not the yield is from reproductive or vegetative organs. Peas and radish plants are at both ends of this spectrum. A corollary effect to this is that not all vegetative or reproductive plants have shown the same sensitivity. For example, tomato and pea plants were more sensitive than both cultivars of wheat tested. These inter-species variations can be due to a number of possible factors.

An example of two possible factors can be seen in a comparison of pea and tomato sensitivity. As documented by former master's student Tim Hudelson (2006), the flower abortion due to elevated ethylene levels was the primary factor behind loss of potential yield in tomato plants. In his work, significant loss of flower buds occurred at ethylene levels as low as 10 ppb. Vegetative growth, however, remained unaffected. Pea plants, however, exhibited a combination of both vegetative loss and flower bud abortion. Thus, the loss of radiation capture potential due to decreased leaf size lowers the amount of photosynthate available to the pea plant with which to construct new reproductive organs. If, however, reproductive organs are constructed, the detrimental effects of elevated ethylene are also able to interfere with the proper development of that tissue.

Other potential avenues for exploration would include the use of molecular techniques to predict sensitivity of a given cultivar to ethylene. Examples would include ethylene receptor protein levels at key times, localization of ethylene synthesis apparatus and quantity over the life cycle of a plant, alterations to

mRNA levels for receptors and synthesis proteins in response to stress events, etc. An ideal goal would be to tie the parameters of the exponential-decay curve equation, or some other mechanistic equation, to fundamental components of the ethylene synthesis and perception pathways. This could then lead to the development of testing kits and other services that could be used by commercial customers and agricultural consumers to evaluate the sensitivity of their crop to ethylene at a given time. Should an increase in sensitivity be determined, especially if a stress event were about to occur, proactive treatment could then be applied to the crop (such as 1-MCP application to prevent ethylene perception) to prevent a loss of yield.

Using ethylene sensitivity data across a wide variety of plant species, Pierik et al. (2006) proposed a biphasic model of ethylene action on plant growth. As part of this model, they proposed four different dose-response curve types categorized as: “Type I,” wherein ethylene applied at any level decreased plant growth, “Types II and III,” wherein ethylene up to a certain level increased plant growth and leaf expansion, and “Type IV,” wherein growth remained unaffected over a broad range of ethylene concentrations and then increased. Although these curve types were created using data from tissue elongation, it is relevant to determine if they have any application or predictive power for plant yield. According to these definitions, all of our tested crops fit into the “Type I” category of growth. This classification, however, is not necessarily to be expected if one is classifying plants based on area of species adaptation (wetland vs. dry land).

The example used for “Type I” growth by Pierik et al. (2006) is data from cucumber plants which represent our data for ethylene sensitivity as well. The other plants used as examples for curve “Types II and III,” *Arabidopsis* and wheat would also be considered representative of typical crop plants. Indeed, of the four plants given as examples, only *Rumex palustris* plants could be considered of wetland origin. It is of particular note that the *Hong Mang Mai* wheat tested has an ethylene sensitivity curve that is significantly different from either *Apogee or Perigee* (Klassen and Bugbee, 2004; Pierik et al., 2006).

All of these differences serve to highlight the immense variation in ethylene sensitivity, even within a single species. This demonstrates the need for the development for a more fundamental means of predicting ethylene sensitivity. Additional work with *Oryza, Zea,* and *Ananas* genera would serve to highlight the differing sensitivities of a wetland-adapted C_3 plants, C_4 plants, and CAM plants. It may yet be possible that the curve types posited by Pierik et al. (2006) will be observed in plant yields.

Ethylene Sensitivity – PPF Interaction

The investigation into an ethylene sensitivity-PPF interaction focused on a relatively insensitive vegetative crop (radish) and a reproductive crop with high sensitivity (peas). Prior studies indicated that ethylene synthesis responded to changes in both light quantity (shade avoidance) and light quality (also part of the shade-avoidance mechanism). Also, there were observations that ethylene synthesis exhibited a diurnal fluctuation that could be tied into light-entrained circadian rhythms. These observations, coupled with the idea that plants grown

in higher light were stronger than their etiolated counterparts led to the hypothesis that an increase in PPF would lead to a decrease in sensitivity.

For each, crop plants were grown under a range of PPF intensities and a control (0 pp) or a “high” (50 ppb for pea and 200 ppb for radish) ethylene concentration was imposed. For both crops tested, ethylene sensitivity was unaffected by increased PPF intensity. This suggests that ethylene sensitivity is not linked to or controlled by any light sensing mechanism within the plant. If there were any variance in sensitivity similar to the diurnal fluctuations in synthesis, this test would not be indicative of presence or absence of such a cycle. For the time frame involved in such studies, only molecular work would be able to ascertain the presence of such a cycle in sensitivity.

Ethylene Synthesis

Plants are the primary source of ethylene production in controlled environments. Although there are means to block ethylene perception for a temporary time using chemicals, a different approach is to obtain direct control over ethylene synthesis. This has been accomplished both by using chemicals such as AVG, AOA, and Co^{2+}, which interfere with enzymes in the ethylene synthetic pathway, and by using genetic techniques that either reduce the amount of substrate available to synthesis proteins (i.e., ACC deaminase production) or regulate the level of the proteins themselves. Although some of these techniques have been developed for a number of years, they have not been combined with steady-state measurements of ethylene synthesis or with stress conditions. Indeed, ethylene synthesis rates reported in the literature have

a 100-fold range, sometimes for the same crop. This large range of synthesis rates reflects the plethora of techniques, tissues, times, and stresses used to address this question. Our work focused on gathering data for three key effects on ethylene synthesis: water deficit stress, flood stress, and the effect of blocking perception on synthesis. An unexpected windfall from examining these effects was the observation of diurnal cycles in ethylene synthesis.

Diurnal Ethylene Cycling

Diurnal cycles in ethylene synthesis were first observed in the 1970's in tomato leaves (El-Beltagy et al., 1996) and subsequently in cotton (Rikin et al., 1984; Jasoni et al., 2000), *Stellaria longipes* (Kathiresan et al., 1996), *Tillandsia usneoides L.* (Beβler et al., 1998), sorghum (Finlayson et al., 1998, 1999), and *Arabidopsis* (Thain et al., 2004) plants. The bulk of this research was concerned mainly with the verification of the fluctuation and identifying the components that regulate the cycle. Our studies extended this research by looking at the effects of water deficit, flood, and perception blocking on the cycle in addition to providing data on reproductive cotton, soybean, tomato, and vegetative corn plants.

The diurnal cycling of ethylene synthesis from such a diverse range of plants has broad implications for the design and interpretation of data from experiments aimed at quantifying synthesis rates and factors that impact them. First, it is clear that headspace-accumulation methods that rely on time periods of several hours or more can no longer be considered reliable measurements of ethylene synthesis. This is of particular concern if the time of day is not accounted for. For example, measurements taken early in the morning would

indicate a rapid increase in ethylene concentration and thus would over-estimate the rate of synthesis. Following the peak of ethylene synthesis, which generally occurs late in the evening about 2-3 h before the onset of dark, the decline in ethylene synthesis would lead to an underestimate of ethylene synthesis. The error bars of the average daily rates of ethylene synthesis presented in Chapters 3 and 4 highlight the uncertainty in such a number due to the effects of diurnal cycling.

The methods used in our work revealed another possible shortcoming. Although we used an automated chromatography system to acquire six data points from each chamber over the course of a day, these six points would not always occur at the same time each day. Thus, it is possible that the maximum or minimum rate of synthesis each day could be missed and the data points would be slightly out of phase with the actual cycle. A prime example of this is given by the %Maximum rate data for corn presented in Chapter 4. It is quite possible that the rapid nature with which corn synthesis varies precluded the capture of the maximal rate of synthesis, thus leading to broader less-defined peaks in synthesis. An ideal system would have a sampling density great enough to capture the cycle with a high degree of accuracy and timing. Thus, it would be virtually assured that the maximum and minimum rates would not be missed or significantly altered. Such experiments will become possible as advances in measurement technology cut sampling times. Indeed, the potential for our own measurement times to be halved exists if the two instruments involved were converted to work with packed-column chromatography instead of capillary

columns. This would allow for either double the chambers to be tested or double the data from existing test chambers.

Acute Water Deficit Stress

Acute water deficit stress differs from classical drought stress in two ways: time and location. These differences were classified when the nomenclature for differentiating water stress types was proposed in the classic review of ethylene and plant responses to stress by Morgan and Drew (1997). Morgan and Drew (1997) proposed that the term “drought stress” should apply to plants growing in large volumes of soil such that the supply of water declines over the course of days to weeks. The term “water deficit stress” or “acute water deficit stress” would then apply to plants grown in small soil volumes where the water availability would decline over the course of hours to days. By using this proposed nomenclature, Morgan and Drew sought to bring clarity to the well-documented confusion that has marred the field. This is important since the primary source of the confusion seems to have arisen from effects due to duration of imposition and subsequent stress. By the definitions outlined above, our studies inquired into the effect of acute water deficit stress on ethylene synthesis.

The crop tested, cotton, had a significant decrease in ethylene synthesis as a result of water deficit stress. Although this was expected from the literature, what was not expected was that ethylene synthesis returned to a normal rate following rewatering. Although the literature is clouded on the subject, one point that was clear was that upon re-watering, a “burst” of ethylene synthesis was to

be expected. This “burst” of synthesis was seen both in detached leaves and in young mandarin seedlings. In fact, in the mandarin seedling study (Tudela and Primo-Millo, 1992) ACC was found to accumulate in roots during the period of water deficit; upon rewatering, ACC was then transported to the shoots and converted to ethylene, thus resulting in the measured burst of synthesis. Although our result is unusual, if our experience with ethylene sensitivity is a guide, it is quite possible there exist an array of responses to this type of stress similar to the curves for ethylene sensitivity proposed by Pierik et al. (2006).

Flood Stress Induced Hypoxia

The literature for flood stress and its effect on ethylene synthesis has been more consistent than the effects reported for drought stress. What is expected from the literature is that ethylene response to flood conditions is determined by how adapted the species is to a wetland environment. This relates to the ability of the plant to maintain aerenchyma tissue in response to flood conditions. Rice and corn, as flood adapted plants for example (Justin and Armstrong, 1987), are expected to have a greater increase in ethylene synthesis in response to flooding as opposed to nontolerant plants, such as peas and soybeans. Indeed, one could hypothesize that the magnitude of the ethylene synthesis response to flooding would correlate to the difference in root tissue porosity following a flood event. Since most of the techniques used to quantify ethylene synthesis were based on measures ethylene concentrations, usually following a hold-and-headspace sample procedure, what has been difficult to grasp from the literature is a clear picture whether or not the elevated concentration in ethylene was a result of

actual increased synthesis or an increase in concentration due to the diffusion barrier that water represents.

Both crops tested were representative of a flood sensitive (soybean) and an intermediate tolerant (corn) plants. Based on the hypothesis that the flood adapted plant would have a greater response to ethylene than the non-adapted plant, it was surprising to see that both plants had an increase in ethylene synthesis in response to flood stress. Although both crops showed an increase in ethylene synthesis, corn synthesis significantly increased more than 10x from 0.1-1.0 pmol plant^{-1} s^{-1}. Soybeans, however, only had a nonsignificant 2x increase from 2-4 pmol plant^{-1} s^{-1}. This trend remains the same when the rates are normalized for rate of metabolism, which corrected for differences in plant size and metabolic rate. Therefore, for the two crops tested, the hypothesis that flood sensitivity is a predictor of ethylene synthesis response is upheld. Also, since the changes in ethylene concentration were measured from a free-flowing atmosphere moving through a controlled environment, it is clear that the measured change in ethylene synthesis is the result of a true increase in ethylene synthesis rather than an accumulation effect due to water acting as a diffusion barrier or as a result of wound-induced ethylene synthesis. Future refinements to the system could allow for the separate measurement of gases diffusing from the root zone vs. the shoot zone, further characterizing the nature of the ethylene synthesis.

Perception Blocked by MCP

The gaseous compound 1-methylcyclopropene has great potential to alter the control of ethylene sensitivity in crop plants. Prior work with climacteric fruits has demonstrated a decrease in both ethylene synthesis and respiration as a result of treatment. Observations of nonclimacteric fruit, however, have yielded a variety of results including the occasional reevaluation of a fruit from nonclimacteric to climacteric status. Unrooted poinsettia cuttings, however, increased in both ethylene synthesis and respiration rate. However, this change was measured in an enclosed environment that was not temperature controlled. Treated kernels and embryos from heat-sensitive wheat plants exhibited a 6-7x increase in ethylene synthesis when compared to similarly heat-stressed controls Hays et al. (2007). These observations, coupled with the negative-feedback aspect of ethylene percept led us to hypothesize that ethylene synthesis would increase when MCP was applied.

Contrary to our hypothesis, MCP application did not affect ethylene synthesis rate in corn, cotton, or soybean plants. Both cultivars of tomato, however, showed almost a 2x increase in ethylene synthesis in response to treatment. However, this increase was only significant when the effects of intumesence stress were present. In no case did MCP treatment affect net photosynthetic rate. These results agree with those of Hays et al. (2007) and Faust and Lewis (2004) in that blocked ethylene perception under stress conditions perhaps leads to an increase in ethylene synthesis. When

unstressed, however, a biologically significant increase in synthesis does not occur.

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APPENDICES

Appendix A: Waterlogged Soils: Consequences for Ethylene Diffusion and Plant Health

Waterlogged Soils: Consequences for Ethylene Diffusion and Plant Health

Abstract

In this paper we briefly review the literature on soil ethylene synthesis and soil gas transport models. Saturated conditions in root zone soils trigger roots of flood-tolerant adapted plants to form aerenchyma. Aerenchyma formation initiates when a localized build-up of ethylene gas in root tissue triggers the release of cellulase and pectinase enzymes. Additionally, the onset of the fermentative metabolic pathway is controlled by ethylene concentrations. Although ethylene synthesis in roots may increase under a variety of stress conditions, soil water content is the main factor governing the diffusion of ethylene away from plant roots into the surrounding soil. Ethylene production in soils is primarily through microorganisms. Under normally aerated conditions a balance between production and consumption is maintained. Under hypoxic and anoxic conditions production drastically increases while consumption is virtually eliminated. The bulk of this occurs in the upper 20 cm of the soil where there is abundant C and N sources.

Effects of Ethylene on Plant Roots

Ethylene is a potent, gaseous, plant hormone responsible for fruit ripening, leaf senescence and abscission, fruit ripening, and floral development (Abeles, 1992). Once in the root, ethylene has the potential to not only affect root development, but to also be transported, primarily through the aerenchyma to the

shoot (Colmer, 2003). In the root itself, elevated concentrations of ethylene have been shown to initiate the formation of lysigenous aerenchyma tissue by triggering the release of cellulase and pectinase enzymes (Jackson and Armstrong, 1999; Drew, He and Morgan, 2000). Elevated ethylene concentrations act as a signal of hypoxic conditions ultimately leading to the use of the fermentative respiration pathway. Under anoxic conditions, however, ethylene synthesis is completely blocked (Drew, 1997). Soil ethylene concentrations have been observed as high as 10 µl l^{-1} (10 ppm) when conditions favor production over degradation (Smith and Dowdell, 1974).

Ethylene Production and Consumption

Ethylene exchanges through roots either via diffusion from soil sources or due to internal production from the ethylene synthesis pathway.

Internal Root Production

The ethylene synthesis pathway in plants involves three enzymes to convert methionine into ethylene. Two of these enzymes are involved in the formation and oxidation of the immediate precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC). ACC-synthase converts S-Adenosylmethionine (AdoMet) into ACC and is the rate-limiting step in the pathway. ACC-oxidase catalyzes the conversion of ACC to ethylene. Ethylene synthesis inhibitors disrupt the pathway by targeting either ACC-synthase (eg. AVG, AOA) or ACC-oxidase (eg. Co^{2+}, AIBA; Abeles, 1992). Since this pathway

depends on oxygen's presence to catalyze the formation of ethylene it does not function under anoxic conditions.

Soil Ethylene Under Normal Conditions

In soils ethylene is primarily produced by microorganisms and, under aerobic conditions, is simultaneously consumed. Thus production and consumption are balanced under normal conditions (Zechmeister-Boltenstern & Nikodim, 1999; Fukuda, *et al.*, 1984; De Bont, 1976). In soil samples taken from montane and lowland regions in Austria it was found that under aerobic conditions ethylene degradation rates exceeded production rates in the presence of acetylene (an ethylene consumption inhibitor) by a factor 10-100 (Zechmeister-Boltenstern & Nikodim, 1999). In waterlogged conditions, however, the balance tips and ethylene accumulates to concentrations high enough to affect plant growth (Smith & Russell, 1969).

Soil Ethylene Under Varying Water Tensions

Zechmeister-Boltenstern & Nikodim (1999) used samples from Austrian montane and lowland soils at differing water tensions to determine which conditions are most favorable for the production and consumption of ethylene. Soil samples were from elevations of 150, 1400 and 1500 m above sea level. Soil types included Phaeozem, Umbric Gleysol, Umbric Podzol, Gleyic Cambisol, and Eutric Cambisol, which encompassed a variety of soil textures (Table 1).

Soil samples were adjusted in air-tight flasks to water tensions of 3, 30 and 300 kPa. By injecting either ethylene gas or acetylene and measuring the subsequent steady-state ethylene content rates of ethylene production and consumption were determined. Ethylene production rates were greatest under anaerobic conditions (Fig. 1 A, B). This suggests that the oxygen-dependant methionine based pathway is not prevalent under normal soil conditions. This hypothesis is consistent with other observations reported in Frankenberger & Arshad (1995).

Under anaerobic soil conditions ethylene formation and degradation rates in the montane soils exceeded those of the lowland soils (Fig. 1. A, B). For lowland soil samples, fine-textured loamy soils had 3-30 times the rate of ethylene production than coarser-textured sandy soils (Fig. 1. A). Under anaerobic conditions ethylene formation was strongly positively correlated with clay content, humus concentration and total nitrogen (Fig. 1., A). At a water tension of 3 kPa, ethylene degradation rates were also correlated to humus concentrations and total nitrogen.

These results led the authors to suggest several possible mechanisms for the significant increase of ethylene in waterlogged soils. First, in the transition from aerobic to anaerobic conditions aerobic microorganisms, which are the main consumers of ethylene production, are killed. Their remains subsequently become substrate for anaerobic producers. Second, the correlation of increased production with high clay particle and organic matter might indicate a desorption of ethylene and other hydrocarbons from the particles. Therefore, more ethylene,

or ethylene substrates, would be released as water tension decreases and the retention potential of the soil is reduced. Finally, soils rich clay and organic matter content may support a more active microfloral community capable of acting as a sink for ethylene under normal aerobic conditions.

Ethylene Production in a Vertical Profile

Jäckel, Schnell and Conrad (2004) examined ethylene production rates at different depths and water treatments of a deciduous forest soil. Soil samples were taken from a slope in a deciduous forest near Marburg, Germany and was classified as a cambisol with a loamy sand texture (Henckel, *et al.*, 2000). Samples were incubated in glass stoppered glass flasks at 25˚C in the dark. Headspace gas samples were taken using gas-tight syringes and analyzed on a gas chromatograph.

Ethylene accumulation after 28 h of anoxic incubation was highest in the upper soil layer (0-2 cm depth) and gradually decreased with soil depth (Fig. 2). The high rates of production corresponded with increased C and N levels in the upper layers of the soil surface. Increasing soil water content weakly stimulated ethylene production but only in the upper 4 cm of the soil. Adding methionine, with a final concentration of 1.6 µmol g^{-1} soil, to the soil samples did not affect ethylene production during 25 h of anoxic incubation. This agrees with the hypothesis stated above that the methionine based ethylene synthesis pathway is not the predominant ethylene production pathway operating in the soil. Furthermore, autoclaving the soil samples and then testing for ethylene evolution

resulted in a 98% drop in synthesis activity. This provides further evidence that the bulk of ethylene production in soils is of biological, not mineralogical, origin.

A major drawback of the water tension and vertical profile studies is the use of incubated samples under disturbed conditions. In both studies, sample storage conditions prior analysis could have affected the microbial population profile. Also, headspace analysis has the ability to artificially inflate rates of production or degradation by altering the gas composition when samples are taken. Also, with the soil samples taken out of their natural environment, allowance must be made for microbial community nutrient supply and maintenance. Finally, variability due to diurnal and seasonal temperature and/or lighting fluctuations was not accounted for when calculating final average production rates for the soils.

Gas Diffusion Models in Undisturbed Soil

Moldrup *et al.*, 2004 provides a review of gas diffusion coefficient (D_P) models dependent on air-filled porosity (ε) and proposed a new model for D_P, as a function of ε, the total porosity Φ, and the macroporosity. Termed the three-porosity model, prediction of $D_P(\varepsilon)$ requires measuring only one point of the soil water curve (SWC) at $^{-}100$ cm of water potential. This model and its predecessors are used to understand the control of gas transport and fate in natural undisturbed soil systems where diffusive, rather than convective, gas transport is the norm. The importance of water content in the root zone is demonstrated by the fact that all of the models used to determine D_P have provisions to specify the water content of the soil in question. In fact, the authors

conclude that the choice of the model used and the subsequent accuracy of the prediction is heavily dependent on knowledge of a given soil's SWC. Although such models are primarily used to determine oxygen availability to plant roots, any factors that will alter the diffusivity of the soil will impact ethylene's accumulation and distribution in the soil system and, ultimately, a plant's response and subsequent growth.

Conclusions

From this literature review several main points governing ethylene in soil systems become clear. First, biological agents as opposed to physical or chemical means primarily carry out the bulk of ethylene synthesis in soil systems. Second, under well-aerated conditions ethylene production by plant roots and soil microbes is balanced by consumption. Next, under anoxic or partially waterlogged conditions ethylene production increases and its ability to be consumed or diffuse out of the soil or plant root is increasingly impaired, leading to a buildup in ethylene concentrations. Also, the increased production of ethylene under anoxic conditions suggests that the oxygen-dependent methionine based pathway for ethylene synthesis is not widely used by anaerobically producing microflora. Finally, ethylene production in soils is limited chiefly to the upper layers where there are abundant C and N sources.

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Table A1.1. Description of soil samples. Taken from Zechmeister-Boltenstern & Nikodim,

Soil no	Region	Area	Soil type	Texture or bedrock
1–4	Lowland	Siemdorf	Phaeozem	Sand
5–8	Lowland	Hohenau	Umbric Gleysol	Loam
9	Montane	Teufelsstein	Umbric Podzol	Phyllite
10–12	Montane	Teufelsstein	Gleyic Cambisol	Phyllite
13–16	Montane	Scheuchegg	Eutric Cambisol (stagnic properties)	Agrillite

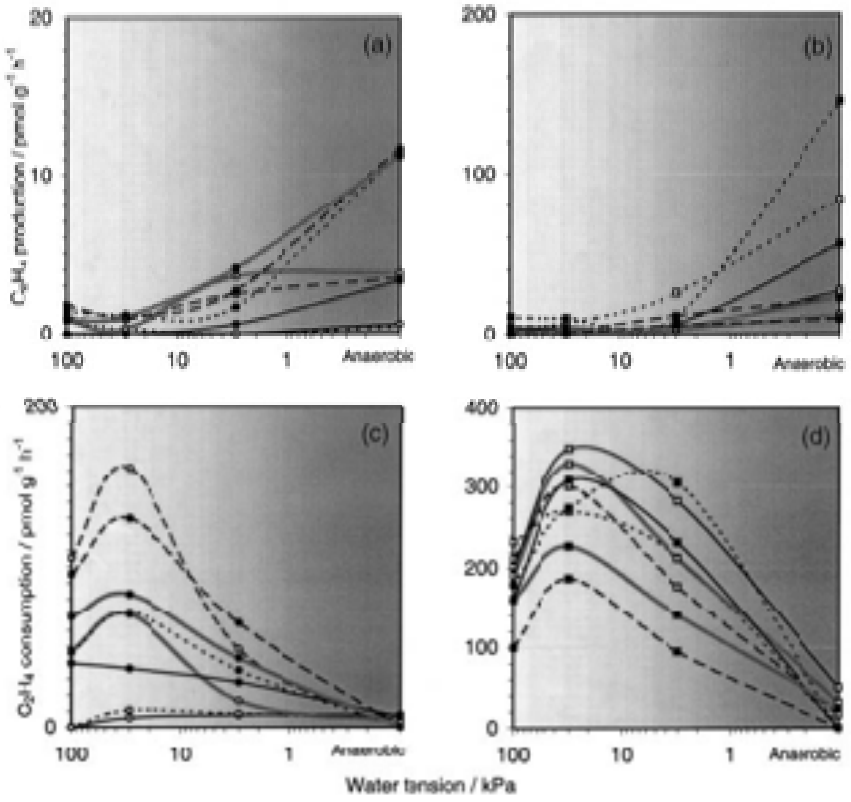

Figure 1 Effect of water tension on ethylene production and consumption measured after 24 h of incubation. (a) and (b) ethylene production; (c) and (d) ethylene consumption. (a) and (c) lowland soils: · · · · · ·, coniferous forest; ——, grassland; - - - - - -, deciduous woodland; ——, arable field; ○, Siemdorf (sand); ●, Hohenau (loam). (b) and (d) montane soils: · · · · · ·, spruce forest; ——, pasture; - - - - - -, pasture with clover; ——, spruce forest clear-cut; □, Teufelsstein: ■, Scheuchegg. Shading indicates increasing soil moisture

Taken from Zechmeister-Boltenstern & Nikodim, 1999.

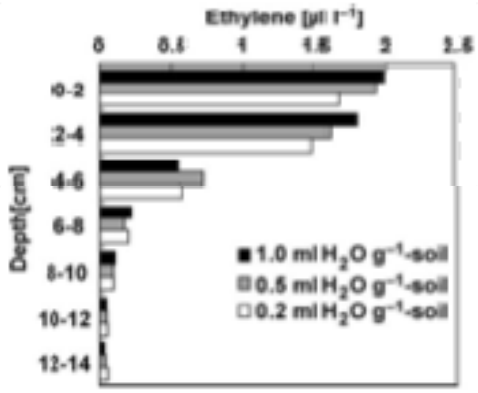

Figure 2. Ethylene accumulated after 28 h incubation under a N_2 atmosphere using samples from different soil layers to which different amounts of water were added. Taken from Ilzkel, *et al.*, 2004.

Appendix B: Selection of Earligreen Pea Plants

'Earligreen' a Super-Dwarf Pea Cultivar for use in Controlled Environment Research

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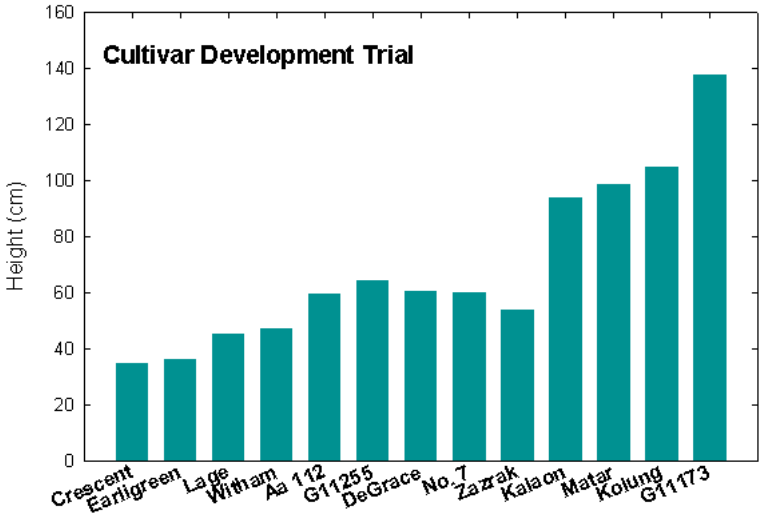
Earligreen is ideal for controlled environment studies due to its fast life cycle, short height, and excellent growth in low light. *Earligreen* peas typically grow 18 to 35 cm tall and flower 20 to 25 days after emergence with the first fresh seed ready at 40 days. Optimal temperature is 20 to 25˚C. *Earligreen* grows well under a wide range of light levels (photosynthetic photon flux (PPF), 100 to 1000 µmol m^{-2} s^{-1}) and a photoperiod of 16 to 24 hours. Leaves display a characteristic silver speckling pattern.

Earligreen was developed in 1950 at the Morden research station in Manitoba, Canada. *Earligreen* (PI 365417) is a hybrid of *Engress* and an unknown early maturing field pea. C. Walkof from the Canada Department of Agriculture donated *Earligreen* seed to the ARS-GRIN network in June of 1971. Germplasm has not been commercially available for at least 20 years.

Study 1: Cultivar Development Trial

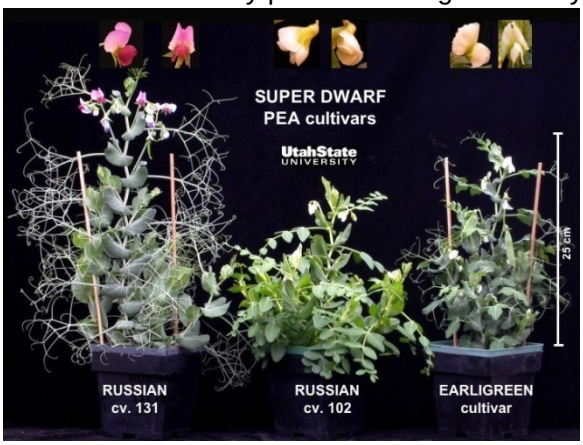
Earligreen growth and development were compared to twelve other cultivars listed as less than 25 cm tall in the ARS-GRIN database. Plants were greenhouse grown with supplemental high pressure sodium light to provide a sixteen hour photoperiod and were watered twice daily with a dilute nutrient solution. After 43 days plant height and developmental progress were recorded. *Earligreen* plants were first to flower and were shorter than 11 of the selected cultivars.

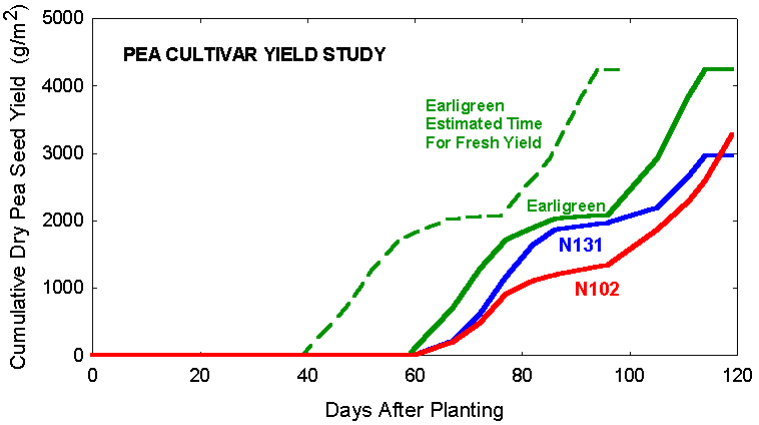
Cultivar	Days to First Flower
Earligreen	20
Zazrak	21
No. 7	21
Matar	24
DeGrace	25
Kalaon	25
Kolung	27
G11255	31
Witham	32
G11173	32
Crescent	>43
Lage	>43
Aa 112	>43



Study 2: Cultivar Yield Study

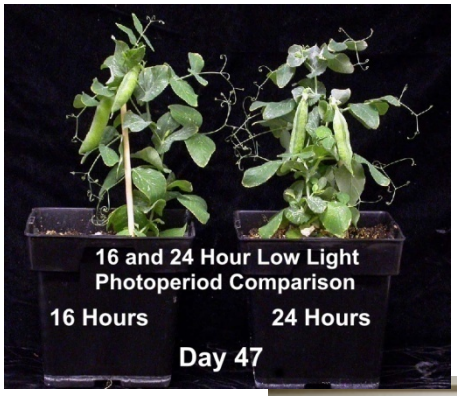
Earligreen was compared to two Russian cultivars (cv. 131 and cv. 102), which have been grown on the International Space Station. Plants were greenhouse grown with supplemental high pressure sodium light to provide a sixteen hour photoperiod and were watered twice daily with a dilute nutrient solution. Fully matured dry pods were harvested. Yield was cumulatively calculated and averaged for each cultivar. *Earligreen* flowered earlier and continuously produced a higher seed yield per unit area.





Study 3: Low Light: 16 and 24 hour Photoperiod Comparison

Earligreen plants were grown under cool white fluorescent lights at a PPF of 90 µmol m^{-2} s^{-1} and a photoperiod of either 16 or 24 hours. Osmocote Plus was mixed into the media with approximately 7 g per 2 L pot. Plants were watered with tap water twice daily. Plants were grown in ambient laboratory conditions. Lab temperature was maintained between 20 and 25˚C. The three replicate plants in each treatment were harvested 65 days after emergence. No evidence of chlorosis was seen in plants grown under either photoperiod. Although time until first flower was unaffected, plants grown under continuous low light had a slightly higher yield and harvest index than those grown using a 16 hour photoperiod.



Parameter	16 hr	24 hr
1st Flower	26	26
Plant Fresh Weight (g)	9.3	9.8
Plant Dry Weight (g)	1.5	1.8
No. Pods per Plant	2.0	1.7
No. Peas per Pod	2.5	3.4
Dry Mass per Seed (g)	0.25	0.27
Yield (g/plant)	1.3	1.6
Harvest Index (%)	46	47

Appendix C: Helium Quality Affects Thermal Desorber Calibration

Helium Quality Affects Thermal Desorber Calibration

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Thermal desorption technology increases the sensitivity of gas chromatography, but it also can concentrate contaminants from any gas stream that passes over a trap.

If contaminants interfere with the elution of the compound of interest, it is impossible to get a clean blank run (no sample applied yet there is still a peak) and the calibration curve will not pass through zero (Fig. AB1, top line). This may be the result of contamination in either the gases used to blend the standards (trap tubes) or gases used internally by desorber (cold trap). However, when combined with an inability to get a clean zero, the evidence suggests that the problem is with gases internal to the instrument. The carrier gas, which passes through the cold trap at several stages of operation, is the most likely source. We compared contamination from two He standards (Fig. AB 2).

Conclusion

The total hydrocarbon contamination specification in helium cylinders is more important than using UHP Grade helium.

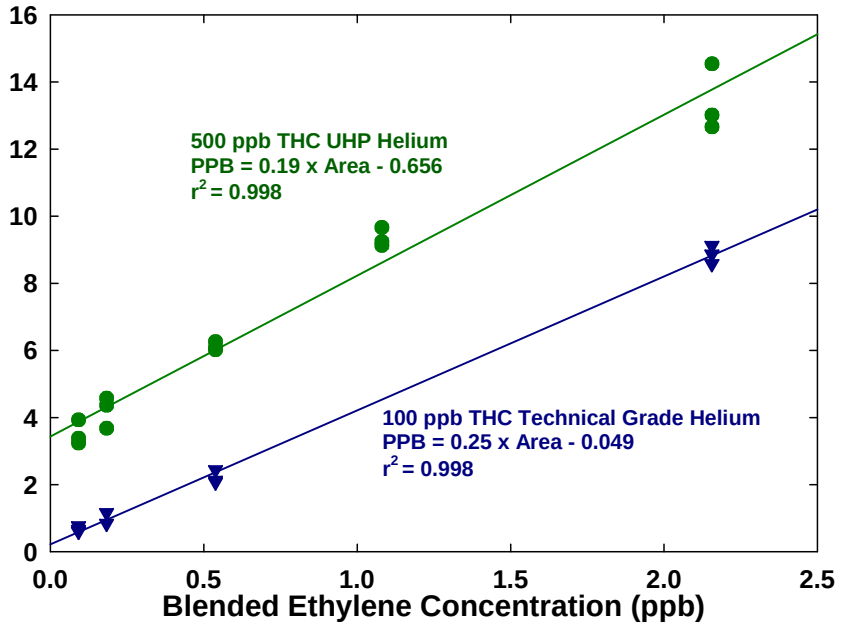

Figure AB.1. The effect of carrier gas hydrocarbon contamination on zero offset. Ultra-high purity helium was specified at 500 ppb THC. Technical grade helium was specified at 100 ppb THC.

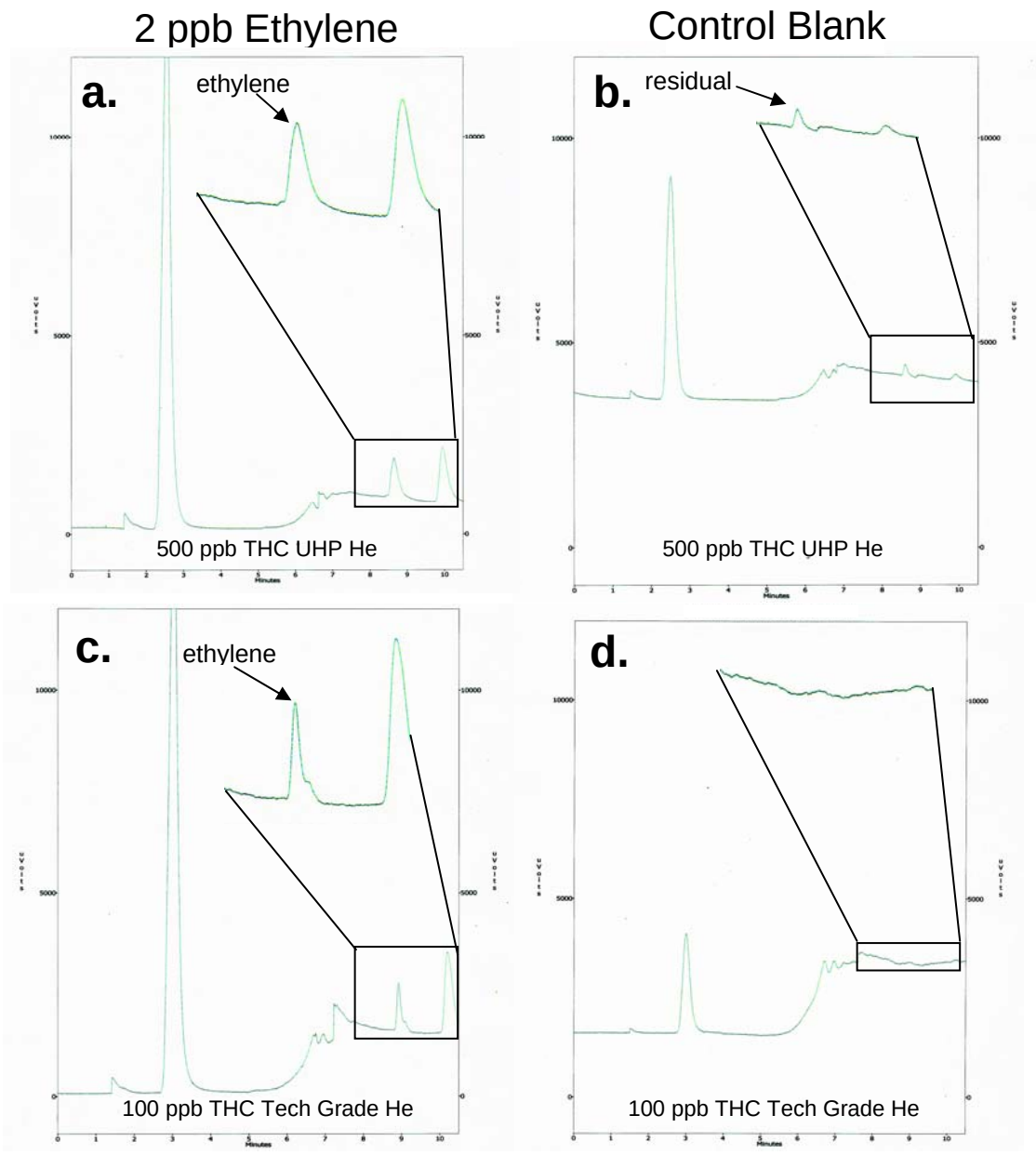

Figure AB.2. Chromatograms generated with and without contamination demonstrate residual peak interference. Although peak shape for a 2 nmol mol^{-1} (parts per billion, ppb) standard appeared adequate (a), a control blank still had a residual peak at the same retention time (b). Adjusting column temperature and pressure programs did not separate the contaminant peak from the ethylene peak. Although ultra-high purity (UHP) grade helium (99.9995% purity) was used, the gas contained 500 ppb total hydrocarbon contamination (THC) per cylinder. Technical grade helium (99.995% purity) with 100 ppb THC, coupled with an inexpensive hydrocarbon filter (Scottgas #5344H, ~$50) removed the residual peak (d).

Appendix D: Validation of Controlled Environment Chambers and Gas Chromatography used in Ethylene Synthesis Measurements

Validation of Controlled Environment Chambers and Gas Chromatography used in Ethylene Synthesis Measurements

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A unique array of challenges and obstacles must be overcome for the successful measurement of ethylene synthesis from intact plants in controlled environments. This is made doubly-difficult since errors arise not only from the environment design and construction, but also from the instrumentation used to make the measurements. The two largest questions that arise from the construction of a system designed to accomplish this goal are: Is the system stable? And, is the data obtained the result of the plants or an artifact of the system? Here, we discuss three techniques used to validate the experimental chambers and gas chromatography systems used in our research:

1. Measurements of volume fraction remaining (VFR) curves compared to modeled values.

2. Measurement of incoming filtered air compared to source air.

3. Measurement of a continuous steady-state source of ethylene.

The system components tested with these techniques included: experimental chambers, filtered air supply, external air, and the gas chromatography system.

Volume Fraction Remaining

The calculation of the volume fraction remaining of a gas in an otherwise closed environment with gas-flow is completed using the equation:

$$Modeled\ VFR = e^{-\left(\frac{Flow\ Rate}{Chamber\ Volume} \times Elapsed\ Time\right)}$$

Thus, turnover time in a chamber can be modeled and measurements can be compared to the model to determine the accuracy of the overall system. The values from the equation can be multiplied by 100 in order to obtain percent fraction remaining. If the measured and modeled data agree, then several variables can be eliminated as sources of error: stability of chromatography system, accuracy of flow meters into the chamber, and isolation of the system from contamination. This test is also a proxy for testing the quantitative accuracy

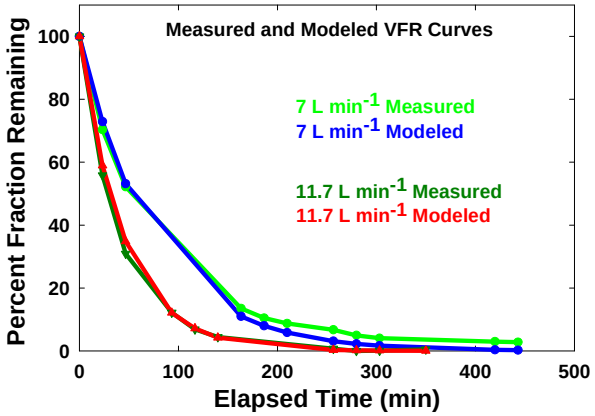

Figure 1. Measured and modeled VFR curves for 7 and 11.7 L min^{-1} flow rates. In our 524 L chambers, the higher flow rate had a greater degree of overlap with the model than the lower flow rate.

of the chromatography system but it is not a substitution for the creation of a rigorous standard curve. Measured and modeled data for 7 and 11.7 L min^{-1} flow rates is presented in Figure 1. In our 524 L chambers, the lower flow rate was likely unable to maintain enough chamber pressure, thus resulting in deviations from the modeled value due to contaminant influx from outside the chamber.

The technique is performed by first establishing a constant flow rate into the chamber and then spiking the chamber with your gas of interest, ethylene in this case, to a concentration that is at the high end of the calibration curve for the chromatograph. Regular samples are removed from the chamber and the gas concentration analyzed. It is important that the gas flow through the chamber is greater than flow rate removed by the sampling system so that a positive chamber pressure is maintained. The measured values can be converted into a percent volume fraction remaining by using the following equation:

$$Measured\ VFR = \left({}^{Next\ Measured\ Concentration}/_{Initial\ Concentration}\right)$$

The value from this equation can also be multiplied by 100 to obtain the percent fraction remaining. Thus, measured and modeled values can be compared side by side with each other independent of actual concentration values.

Filtered vs. Source Air Measurements

This technique determines: the reliability of the air filter, if leaks are entering into the chamber or sampling lines from outside sources, and the variance of the system for a low value repeatedly measured. Also, the technique establishes the lowest level which can reliably be determined as

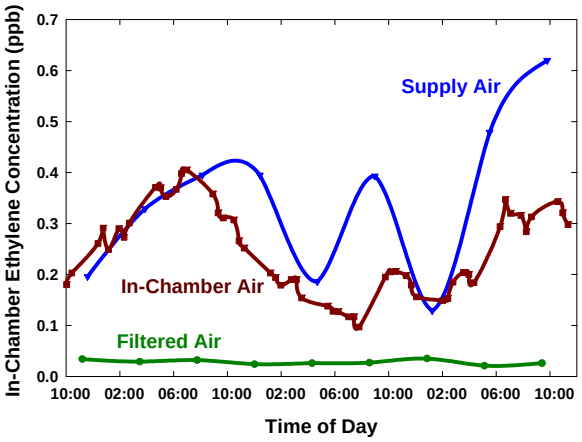

Figure 2. Supply, filter and in-chamber ethylene concentrations over a 36 h period. In this example, the in-chamber air closely follows that of the supply, suggesting a leak into the chamber from the outside air. Note that the filtered air is never at zero concentration.

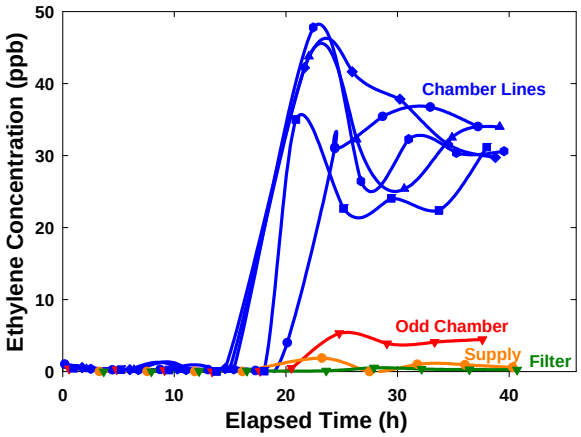

Figure 3. Example of the application of a steady-state ethylene source to chambers. One odd chamber is distinctly lower than the others, possibly as a result of a leak in the sample line. High concentrations are above the standard curve for the instrument, leading to a larger variance between lines than normal.

"signal" from the plants compared to background "noise" passed through the filter. Background levels from the filter should be subtracted from the chamber concentration value before synthesis rates are calculated. Thus, the monitoring of external source and filtered air supply is a routine part of the experiment without which the experiment cannot proceed. Figure 2 gives an example of a chamber that was leaky over the course of the 36 h monitoring period. The filtered air supplied to the chamber was at a low, stable, ethylene concentration. In contrast, the ethylene concentration in the chamber mirrors the concentration of the outside air surrounding the chamber. This situation is rectified through either tighter chamber sealing, increased airflow into the chamber, or both.

Steady-Source Measurement

This technique uses the introduction of a steady source of the gas of interest, ethylene in this case, so that system stability can be tested. Additionally, leaks introduced into the system from components under negative pressure will also show up. In figure 3, for example, the odd chamber is lower than all of the other lines from the chamber, possibly as a result of a leak in the sample lines, which are under negative pressure. The large variance in the sample lines is likely due to an over-range of the standard curve leading to unreliable peak measurement and integration by the gas chromatography system.

Conclusion

The above techniques are not limited in application to ethylene gas, or to chromatography systems. These techniques will work with almost any combination of an input-sensor environment where samples must be taken and analyzed. Also, another source of validation, not discussed here, is the benefit of a proper calibration curve for the instrument used to measure the samples. That, alone, will reveal many problems with the instrument without the interference of the rest of the system. When all these factors have been accounted for, one can then be confident that the data obtained are indeed "signal" instead of "noise."

Appendix E: NASA GSRP Fellowship Proposal and Yearly Reports

Ethylene Synthesis and Control in Dwarf Crop Species

Joseph Romagnano, Ph.D. Candidate, Utah State University Crop Physiology Lab

Introduction

The International Space Station attempts to maintain ethylene levels at 50 ppb but achieving this set point is not always possible (Perry & Peterson, 2003). Elevated atmospheric ethylene levels cause a variety of abnormal responses including inhibited root and hypocotyl elongation, leaf epinasty, reduced growth, premature leaf senescence, and sterility (Abeles *et al.*, 1992; Klassen and Bugbee, 2002, 2004; Mattoo and Suttle, 1991; Morison and Gifford, 1984; Smalle and Van Der Straeten, 1997).

Previous studies in our lab clearly show that levels as low as 20 ppb significantly reduce plant growth and yield, particularly in flowering plants (Klassen and Bugbee, 2002; 2004). Plants are the primary source of ethylene on the space station and ethylene production can increase tenfold during stress. Thus, it is extremely difficult to maintain atmospheric levels below 20 ppb only using physical/chemical means of ethylene control. However, it may be possible to reduce the crop contribution to the ethylene burden by chemically and genetically controlling their ability to synthesize ethylene.

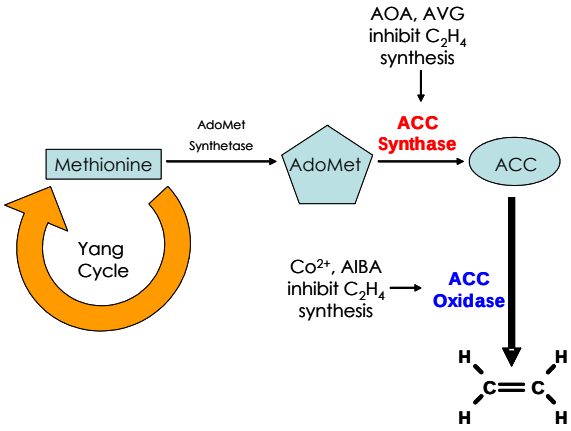

Figure 1. The ethylene synthesis pathway. Aminoethoxyvinylglycine (AVG) and Aminoethoxycetic acid (AOA) disrupt ACC Synthase and Cobalt (Co^{2+}) and α-aminois-butyric acid (AIBA) disrupt ACC Oxidase.

Ethylene Synthesis

The ethylene synthesis pathway involves three enzymes to convert methionine into ethylene (Fig. 1). Two of these enzymes are involved in the formation and oxidation of the immediate precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC). ACC-synthase converts S-Adenosylmethionine (AdoMet) into ACC and is the rate-limiting step in the pathway. ACC-oxidase catalyzes the conversion of ACC to ethylene. Ethylene synthesis inhibitors disrupt the pathway by targeting either ACC-synthase or ACC-oxidase.

Despite the extensive literature on biological ethylene production, rates of whole plant synthesis are not well characterized. Rates of synthesis range 200 fold from 0.01 to 2.0 nmol kg_{DW}^{-1} s^{-1} in roots and shoots of healthy plants and production rates are 2 to 10

times higher in stressed plants. Klassen and Bugbee (2004) summarized the literature on ethylene production by crop plants. The majority of these studies measured ethylene synthesis from excised tissues in closed containers. It is well known that mechanical perturbations and excision promote "wound ethylene" production. Many studies may predict artifically high estimates of production rates in intact plants (Abeles *et al.*, 1992; Morgan and Drew 1997). Rates of ethylene production also vary with environmental conditions, which are often sub-optimal in microgravity.
Ethylene synthesis rates over the lifespans of tomato, wheat, soybean, lettuce and potato were measured as part of a whole-stand photosynthesis experiment conducted at Kennedy Space Center (Wheeler, *et. al.*, 1996; 2004). Calculations based on Wheeler's reported data show ethylene synthesis rates of 0.17 nmol kg^{-1} s^{-1} for lettuce and 5.35 nmol kg^{-1} s^{-1} for tomatoes (assuming 200 g dry weight per m^2) were measured. These values are for unstressed plants and could be much higher if the plants were stressed. Also, this study used a sealed chamber and a parthenocarpic tomato cultivar (*cv.* 'Reimann Philipp') that may have autocatalyitically produced ethylene, both factors that may have contributed to an overestimated synthesis rate.

Chemical Control of Ethylene Synthesis

The commercially available chemicals aminovinyl glycine (AVG) and aminooxyacetic acid (AOA) inhibit ethylene synthesis by interfering with the activity of ACC-oxidase (Abeles, *et al.*, 1992). Two other compounds, aminoisobutyric acid (AIBA) and Co^{2+} interfere with ACC-oxidase activity (Abeles, *et al.*, 1992). Varying concentration and inhibitor types may be used to manipulate ethylene synthesis rates in plants.

Decreasing ethylene synthesis rates may provide the additional benefit of limiting ethylene perception. Klee (2004) suggested that increased ethylene synthesis might be associated with increased receptor synthesis. Once a receptor binds ethylene, it may be permanently disabled. Plants that are less able to synthesize ethylene may be less likely to synthesize receptors and thus less sensitive to external ethylene. To reduce sensitivity, research efforts need to identify the relative importance of the ethylene synthesis and the response pathways. Chemical inhibitors of ethylene synthesis can facilitate this research effort, allowing us to begin immediate assessment of ethylene synthesis effects.

Genetic Control of Ethylene Synthesis

Genetic manipulation techniques have been effective in reducing ethylene production in tomato (Klee and Clark, 2002) and broccoli (Henzi, 1999). Antisense gene insertions of ACC synthase or ACC oxidase to suppress the ethylene synthesis enzymes can reduce up to 99% of the ethylene production in tomato plants. One of the advantages to the antisense approach is to produce plants with varying rates of ethylene synthesis (Klee and Clark, 2002). Additional control methods also exist, over-

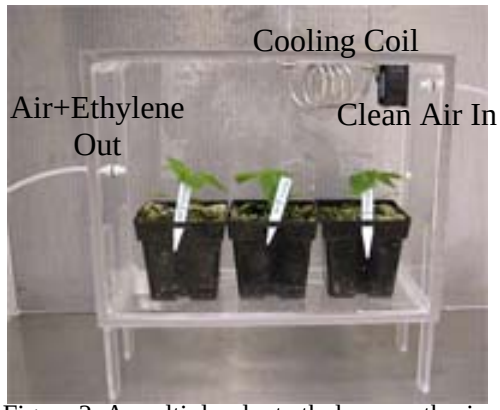

Figure 2. A multiple-plant ethylene synthesis chamber.

expression of a bacterial ACC deaminase effectively blocked ethylene production by removing the ethylene precursor ACC (Klee and Clark, 2002). Also, reduction in ethylene production significantly delayed tomato fruit ripening (Klee and Clark, 2002) and decreased apple fruit-drop (Sato et al., 2004).

Objective

The proposed research seeks to precisely quantify the effects of drought and hypoxic stress on ethylene synthesis rates throughout the life cycle of dwarf crop plants. Chemical and genetic controls will then be used to decrease ethylene synthesis in dwarf tomato plants.

Proposed Research

Normal and Stressed Rates of Ethylene Synthesis

Rationale: Since literature values of ethylene synthesis in crop plants vary widely in technique, cultivars, and obtained synthesis rates (Klassen and Bugbee, 2004), it is necessary to determine unstressed rates of ethylene synthesis. Drought and hypoxia, which are known to increase ethylene synthesis rates, (Abeles, *et al.*, 1992) will be applied to simulate imperfect watering of the root zone.

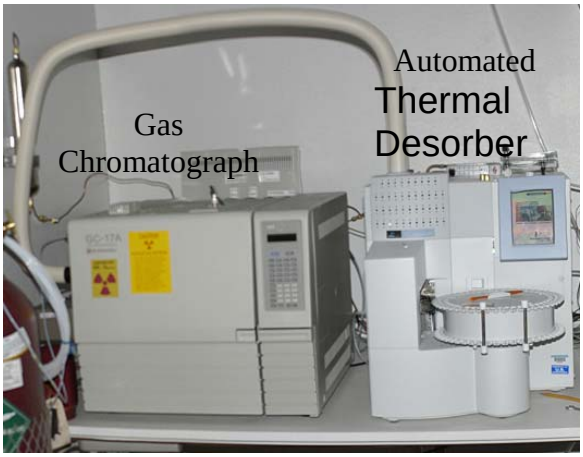

Figure 3. In-house automated thermal desorber mated to a computer controlled gas chromatograph. The system is capable of automatically obtaining samples from 31 chambers.

Procedures: Initial studies will characterize ethylene synthesis in unstressed, healthy plants throughout their life cycle. Studies will be conducted in flow-through chambers (Fig. 2) at a near-optimal CO_2 level (1200 ppm), a baseline PPF of 400 micromoles per m^2 per second, 16 hour photoperiod, 25˚C day/20˚C night temperature; and optimal root-zone water and oxygen. Drought and hypoxia in the root zone will be applied by manipulating water applied through a porous tube nutrient delivery system. Soil water content will be monitored using time domain reflectometry.

The lab is equipped with an automated gas chromatography (GC) system for continuous ethylene monitoring of 31 chambers for our ethylene sensitivity studies (Klassen and Bugbee, 1999). A modified version of that system which integrates an automated thermal desorbtion system, already in the lab (Fig. 3), will be used to measure ethylene production in our small chambers. Combining the thermal desorbtion system with the gas chromatography system decreases the ethylene detection limit from parts per billion to

parts per trillion. This enables the use high airflows in the growth chambers, thus avoiding the ethylene build-up and autocatalysis problems associated with prior techniques.

Expected Results: It is expected that ethylene synthesis rates will vary not only between species but also over the life cycle of the plant. Therefore techniques for controlling ethylene levels in an advanced life support system may only need to be used during certain stages of plant growth, or with only certain types of plants. Thus the usage of physico-chemical control is decreased. Also, certain stress conditions may adversely affect ethylene synthesis rates more than others necessitating a stronger or weaker response dependant upon the stressor.

Chemical inhibition of ethylene synthesis

Rationale: Application of chemical inhibitors will allow immediate modification of ethylene synthesis rates without the time required to form transgenic plants. Both AVG and AOA greatly reduce ethylene synthesis and have been applied to both whole plants and detached organs. The physiological significance of the remaining ethylene production is not known (Abeles, *et. al.*, 1992).

Procedures: Studies will be conducted on dwarf tomatoes using an ACC-synthase inhibitor (AVG) and an ACC-oxidase inhibitor ($CoCl_2$) to determine if inhibition of C_2H_4-synthesis will improve final yield in a high plant density environment. A range of inhibitor concentrations will be applied to identify the concentrations that will confer C_2H_4 insensitivity without disrupting final yield. Evaluations will include measurements of ethylene evolution with different inhibitor concentrations and within different stages of plant development to determine inhibitor efficiency. Physiological analyses and digital imagery (Klassen, *et al.*, 2003). will be collected at regular intervals.

Expected Results: Values for ethylene synthesis rates throughout the life cycle of dwarf tomato plants will be identified. A dose-response curve of final yield to synthesis inhibitor concentrations will be generated. Tomato plant reproductive development is expected to improve in high ethylene environments without significant impact to ethylene-dependent plant development. A guideline will be developed around which to format genetic approaches.

Genetic Insertion into Micro-Tina and Micro-Tom Dwarf Tomato Cultivars

Rationale: Ethylene production of Micro-Tom tomato will be genetically modified. A lowered rate of ethylene synthesis will decrease the ethylene burden an ALS system would experience, thus reducing the need for ethylene controls. The success of creating such a plant will serve as a model for space plant production in the future.

Procedures: Leaf disc co-cultivation with agrobacteria, followed by tissue culture and plant regeneration on selective media will be used to transfer various constructs into the plant. For reducing ethylene production, antisense constructs of ACC synthase or ACC oxidase will be used. To reduce ethylene sensitivity, mutated ETR-1 from Arabidopsis will be over-expressed in the plants. Dr. Klee at the University of Florida per agreement will provide the constructs. These constructs have been effective in full size tomato plants (Klee and Clark, 2002; Wilkinson et al., 1997). PCR and RT-qPCR methods will be used to confirm transgene presence and expression in the transgenic plants. The transgenic plants will be evaluated for ethylene evolution, plant size and fruit production under various growth conditions, especially at high-level ethylene conditions.

Expected results: A transgenic dwarf tomato will be created with reduced ethylene production. The fruit production will be improved in high-ethylene compared with non-transformed plants by carefully selecting plants with right the combination of ethylene production and sensitivity. However, some difficulty in generating ethylene-insensitive plants from tissue culture will be experienced due to low efficiency in root regeneration (Klee and Clark, 2002). Elevated ethylene in the tissue culture vessels should encourage root regeneration.

Potential Spin-Off Applications

Since ethylene-induced deterioration decreases produce shelf life this research may increase the shelf life of produce on Earth.

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Ethylene Synthesis and Control in Dwarf Crop Species

End of Year Progress Report: Year 1

Joseph Romagnano, Ph.D. Candidate, Utah State University Crop Physiology Lab

Introduction

Efficient food production in all NASA environments requires a complete understanding of ethylene physiology. Plants are the main source of ethylene in controlled environment chambers and levels as low as 20 nmol mol^{-1} (ppb) can reduce yield. However, since ethylene is required to regulate developmental change it is important to understand how much ethylene synthesis or sensitivity can be reduced without affecting development. This requirement leads to three broad objectives for this research:

1. Quantify rates of ethylene synthesis and sensitivity in healthy and stressed plants.
2. Determine the potential of chemical inhibitors to reduce ethylene synthesis and sensitivity.
3. Create a genetically modified dwarf tomato plant with a reduced rate of ethylene synthesis

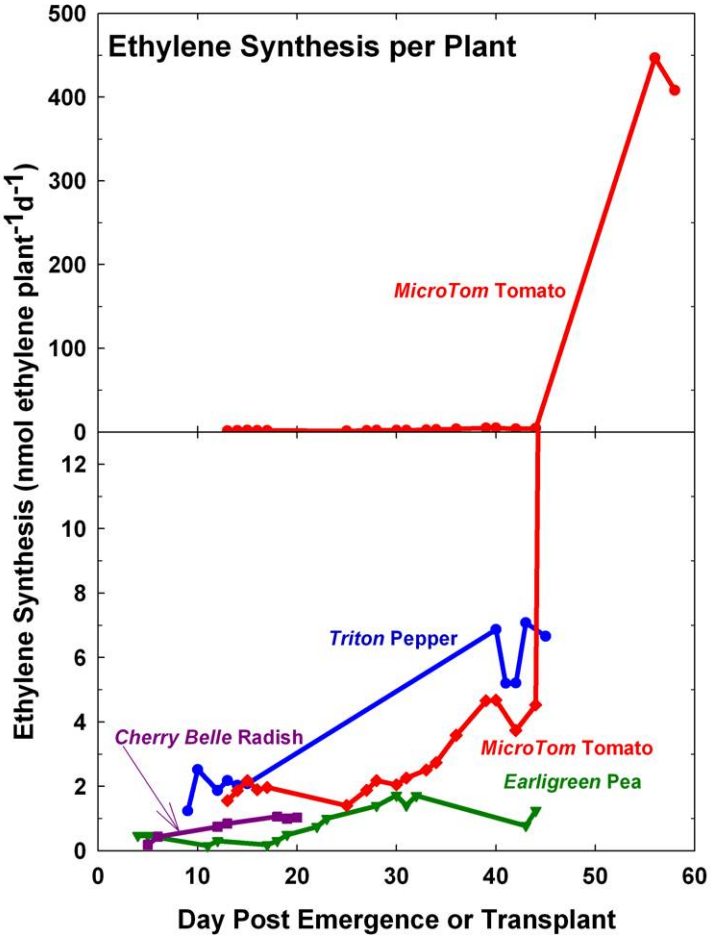

Figure 1. Ethylene synthesis per plant for: *Cherry Belle* radish, *Earligreen* pea, *Triton* pepper, and *MicroTom* tomato. Ethylene synthesis in tomato markedly increased at the onset of fruit ripening.

This past year efforts focused on the first objective. Four areas were studied:

1. Rates of ethylene synthesis in unstressed plants.
2. The effect of light intensity on ethylene sensitivity
3. Ethylene autocatalysis.
4. Impact of ethylene on root system architecture.

Ethylene Synthesis

Preliminary synthesis studies using a low-flow-through chamber were conducted with the following salad crops: *Cherry belle* radishes, *Triton* peppers, *Earligreen* peas, and *MicroTom* tomatoes. *MicroTom* tomatoes had per plant ethylene synthesis profiles similar to chamber ethylene concentration data for *Reimann Philipp* tomato presented in Wheeler, *et al.* (2004). Specifically, there was steady ethylene production (or accumulation in the chamber with Wheeler's work) with a marked increase in synthesis at the onset of fruit ripening (Fig. 1). *MicroTom* ethylene synthesis rates increased from 1 to 4 nmol plant^{-1} d^{-1} during the first 44 days post emergence (DPE). When the fruit started to ripen (> 50 DPE) ethylene synthesis per plant rose above 450 nmol plant^{-1} d^{-1}. *Triton* peppers, however, *did not* show a similar increase in per plant ethylene synthesis with the onset of fruit ripening (Fig. 1), but they did increase six fold (from 1 to 6 nmol plant^{-1} d^{-1}) over the life cycle of the plant. *Cherry belle* radishes and *Earligreen* peas also had similar per plant ethylene synthesis profiles (Fig. 1). These data show trends similar to findings presented in Wheeler *et al.* (2004). It appears that ethylene synthesis is tied to plant growth rate. Future work will focus on quantifying ethylene synthesis using non-destructive digital imaging to measure plant size (Klassen, *et al.*, 2003, for techniques). The results from this work will be presented at the Habitation 2006 conference.

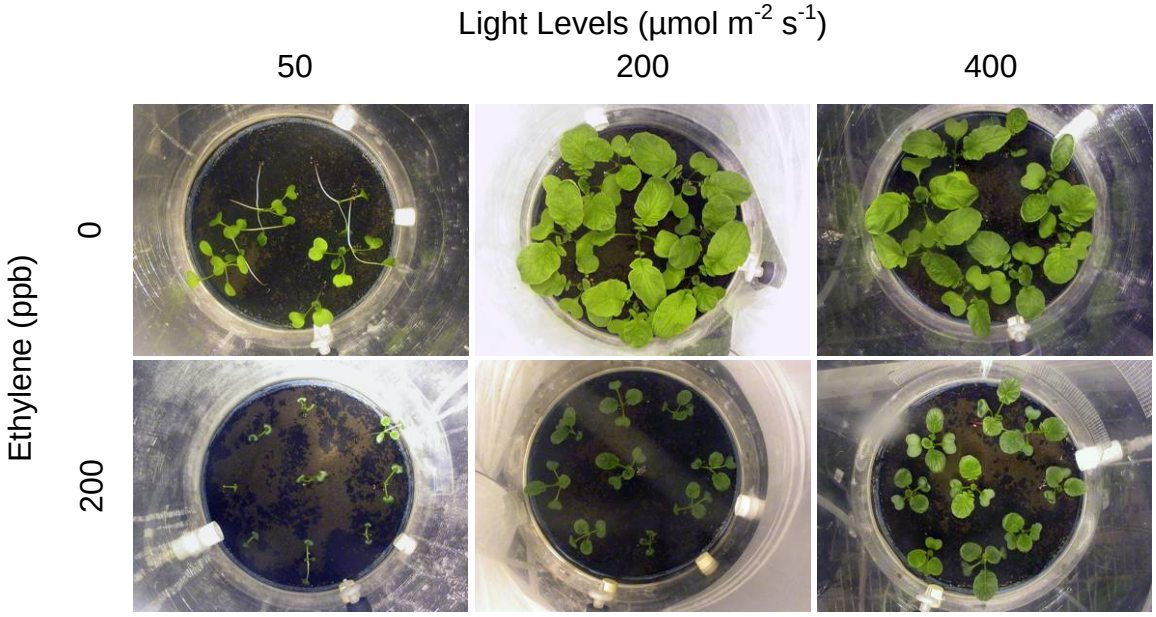

Figure 2. Effect of PPF level on ethylene sensitivity. Increased light levels did not decrease sensitivity to ethylene.

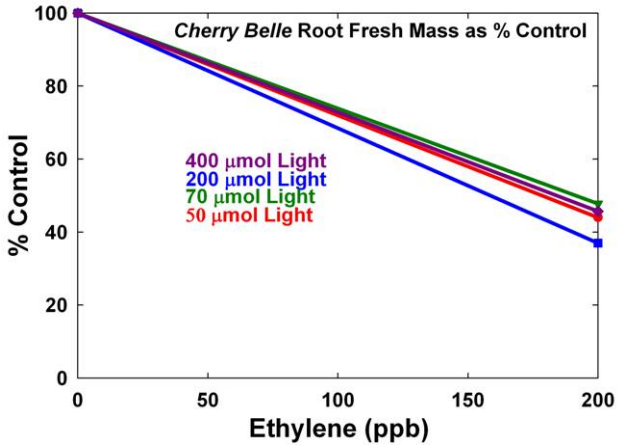

Figure 3. Effect of ethylene on *Cherry belle* radish plants grown under different PPF levels. Root fresh mass significantly decreased as a result of ethylene treatment.

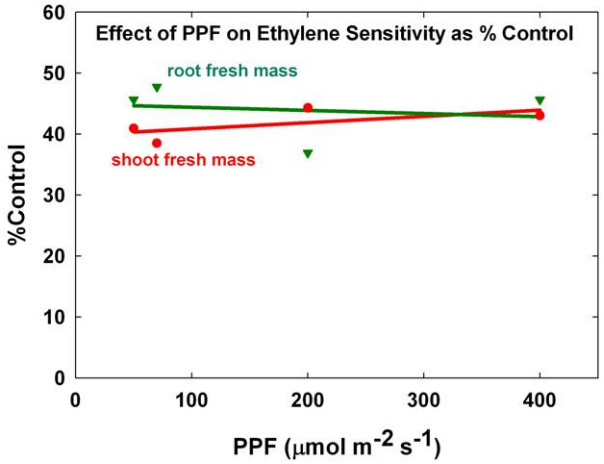

Figure 4. Effect of PPF on *Cherry belle* radish plants grown at 200 ppb ethylene. Increased light levels did not decrease sensitivity. All plants were approximately 45% of controls.

Ethylene-Light Interactions

Light intensity is a key controller of plant growth rate. An experiment to determine the effect of light intensity on ethylene sensitivity was done using *Cherry belle* radish plants (Fig. 2). Light levels at 50, 200 and 400 µmol m^{-2} s^{-1} and ethylene concentrations of 0 and 200 ppb were used. Root fresh mass decreased as a result of the ethylene treatment (Fig. 3). However, plants grown at different light levels and 200 ppb ethylene were all approximately 45% of control and not significantly different from each other (Fig. 4). This suggests that

increasing light intensity will not decrease the ethylene response of the plant. Since ethylene synthesis may be linked to metabolic rate, which is primarily controlled by light intensity, future work will investigate if light intensity alteration will control the rate of ethylene synthesis.

Ethylene Autocatalysis

In order to determine if there is a relationship between ethylene synthesis rates and ethylene sensitivity, ethylene dose-response studies were conducted using *Kristen* and *Sharon* mum plants. Remarkably, *Kristen* mums were able to tolerate exceptionally high (640 ppb) concentrations of ethylene without significant decreases in flower or plant growth. Furthermore, *Kristen* plants exposed to this high level of ethylene did not have a significantly greater rate of ethylene synthesis than control plants grown at 0 ppb (0.83 pmol m^{-2} s^{-1} for control vs. 0.34 pmol m^{-2} s^{-1} for treated). Surprisingly, *Kristen* plants treated with ethephon seventeen days prior to measurement had synthesis rates of 96 pmol

m^{-2} s^{-1}, a rate 115 times higher than the gassed or untreated plants! Stoichiometric calculations of ethephon to ethylene conversion rates showed that this high level of synthesis could not be sustained by the initial application of ethephon. Indeed, calculations showed that 5 mL of applied ethephon at spray concentration would have converted to ethylene and dissipated within 14 h of the application if the high rate of ethylene synthesis seen was maintained. This strongly suggests that ethylene autocatalysis does occur in these plants. However, it appears that the autocatalysis is triggered by a high acute dose of ethylene (ethephon) as opposed to a chronically elevated level. This is especially important to the growth of plants in controlled environments. If ethylene is not produced through autocatalysis during vegetative growth and floral development then plants with reduced rates of ethylene synthesis should be able to develop normally with a minimum of ethylene removal equipment required. Furthermore, prior ethylene synthesis studies conducted using sealed chambers and non-steady-state techniques may not be fatally flawed, as suggested in Klassen and Bugbee (2004), provided the levels of ethylene accumulation in the chambers were not high enough to trigger autocatalysis.

Ethylene and Root System Architecture

Root architecture describes root growth over time and space. Prior studies have examined the effects of ethylene and nutrient deficiency using ethylene precursors or inhibitors in combination with nutrient deficiency (review: López-Bucio *et al.*, 2003). To test if ethylene gas alone could alter root architecture in young pea plants, a 30 ppb ethylene concentration was maintained through a column root zone in a preliminary study. Although not statistically significant, the data trend shows that roots grown without ethylene were longer, had more lateral branches, and supported larger shoots (Table1). This is contrary to literature that shows ethylene induces root growth under nutrient deficiency. This suggests that under nutrient sufficient conditions ethylene may act as a root growth inhibitor. This would prevent the plant from investing carbon in unneeded root growth.

Table 1. Effect of 30 ppb ethylene on root growth of 10 DPE Earligreen pea plants. Significantly different measurements are bolded.

Parameter	**0 ppb mean**	**30 ppb mean**	**p-value** (ANOVA, α=0.05)
Root Fresh Mass (g)	3.8	3.3	0.588
Radicle Length (cm)	31.5	29.7	0.334
Number of Lateral Roots	85.0	80.0	0.705
Shoot Length	7.5	**7.0**	**0.272**

Future work should include ethylene at multiple higher levels in order to validate and further quantify these observations. The introduction of ACC positive controls and combinations with selected nutrient deficiency would further define the role ethylene plays in root growth regulation.

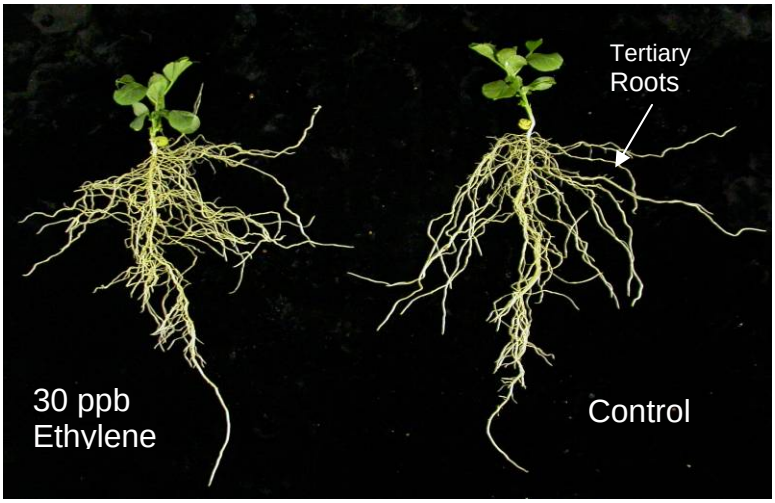

Figure 5. Roots and shoots from ethylene treated (left) and control (right) columns. Although the control treated roots had higher average root mass, root length and lateral root number the difference was not significant. Control roots had tertiary root tissue whereas treated roots did not. This effect was not quantified.

Year Two Plans

In addition to the future work highlighted above, I plan to conduct ethylene synthesis studies using high-volume flow-through chambers. This will allow baseline and stress synthesis studies in non-ethylene-accumulating conditions to be performed. Studies evaluating the use of chemicals to control ethylene synthesis will also be initiated. This work will be performed as outlined in the original project proposal.

Educational Outreach Activities

One of the goals of any NASA researcher is to educate others about the research underway and its use not only in space but also on the ground. In the past year I led two outreach activities. First was an annual gathering of second grade students from River Heights Elementary School for a “space plants” day. Over fifty students attended this year’s event. Students were given a tour of the Crop Physiology Laboratory and planted dwarf pea (*Earligreen*) and tomato (*MicroTina*) plants for further study in the classroom (Fig.6). The students were also led through an interactive presentation highlighting NASA’s efforts to create an advanced life support system and the role plants would play in such a system. In addition to the “space plants” day I was invited to represent the Crop Physiology Lab at the Adams Elementary School science fair. The fair

Figure 6. Second grade students from River Heights Elementary school, assisted by their teacher Mrs. Keren Lundhal, plant dwarf peas and tomatoes during the “space plants” day.

included students in the 4th and 5th grades, many of whom were participating in their first science fair.

Summary of Travel

In the past year I traveled to Salt Lake City, Utah to participate in the American Society of Agronomy's 2005 Annual Meeting. I was co-author of a poster entitled "*1-methylcyclopropene (1-MCP) Blocks Ethylene Perception in Peas in High-Ethylene Environments"* which summarized and presented the research efforts of summer intern Joel Wilkinson. The poster was awarded first place in the student competition. February 5th through the 8th of 2006 I will be presenting a summary of my work with radish plants at the NASA sponsored Habitation 2006 conference. A visit to present and share data with Kennedy Space Center researchers is in the early planning stages. These three activities represent the trips itemized and approved in the initial budget proposal.

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Klassen, S., and B. Bugbee. 2004. Ethylene synthesis and sensitivity in crop plants. *HortScience* 39: 1546-1552.

Klassen, S.P., G. Ritchie, J.M. Frantz, D. Pinnock, and B. Bugbee. 2003. Real-time imaging of ground cover: relationships with radiation capture, canopy photosynthesis, and daily growth rate, p. 3-13 Digital Imaging and Spectral Techniques: Applications to Precision Agriculture and Crop Physiology, Vol. 66. ASA, Madison, WI.

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Ethylene Synthesis and Control in Dwarf Crop Species

End of Year Progress Report: Year 2

Joseph Romagnano, Ph.D. Candidate, Utah State University Crop Physiology Lab

Introduction

Efficient food production in NASA environments requires a complete understanding of ethylene physiology. Plants are the main source of ethylene in controlled environment chambers and levels as low as 10 nmol mol^{-1} (ppb) can reduce yield (Klassen and Bugbee, 2004). However, since ethylene is required to regulate developmental change it is important to understand how much ethylene synthesis or sensitivity can be reduced without affecting development. This requirement leads to two broad objectives that were examined this past year:

4. Quantify rates of ethylene synthesis & sensitivity in healthy & stressed plants.

5. Determine the potential to chemically alter ethylene synthesis & sensitivity.

An Inexpensive Gas Exchange Box

For the following studies, plants were placed in 81-L polycarbonate boxes sealed with closed-cell foam tape and an acrylic top (Fig. 1). A battery powered fan was used to circulate internal air. Each box had a 5-10% d^{-1} leak rate and cost under $200 per unit. Polypropylene boxes were found to be unsuitable for use since polyethylene decomposes into appreciable ethylene quantities.

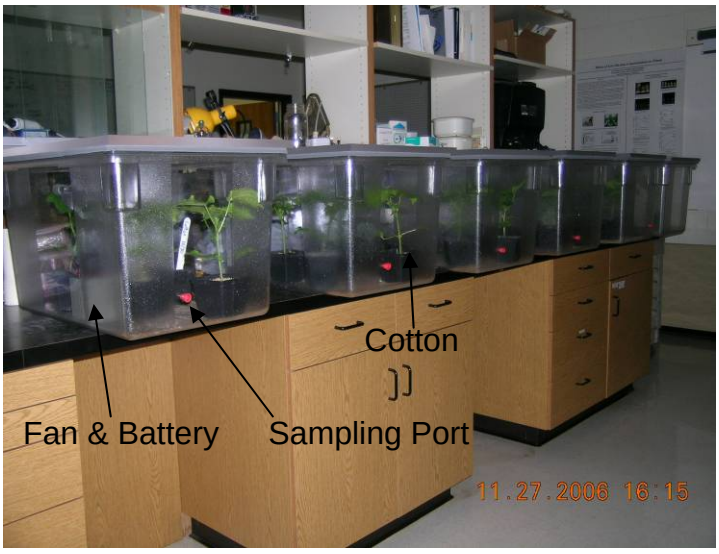

Figure 1. Inexpensive boxes for gas exchange. Plants were placed inside for 8-20 h while ethylene and CO_2 accumulation were measured. The boxes are constructed out of injection-molded polycarbonate and the lid out of cast acrylic. Closed cell foam weatherstripping seals the two together.

Quantifying Wound Ethylene Production

It is common knowledge that plants, when wounded or stressed exhibit a "wound ethylene" response (Abeles, *et al.*, 1992, León, *et al.*, 2001). Although much is

known about the Genet. Mol. Biol. behind wound ethylene (Guo and Ecker, 2004) there is little data quantifying the amount produced and the impact it has on ethylene synthesis. As noted in Klassen and Bugbee (2004), the majority of ethylene synthesis studies have used detached organs in enclosed chambers or flasks.

Figure 2. Detached pea flower in sealed flask.

By counting and weighing the organ of interest, we converted data from detached organs in flasks (Fig. 2) and combined it with whole plant data from the gas exchange boxes (Fig. 1). The detachment of organs from the plant resulted in ethylene synthesis increases from 44-1250x (Fig. 3). Such a result cannot be predicted from molecular techniques. Molecular biology can quantify the amount of ACC present, the amount of synthesis proteins and the amount of transcripts, but not the actual amount of ethylene evolved. Detached organs may, therefore, result in misleading predictions for whole-plant behavior. Future work will focus on quantifying this in other salad crops of interest such as radish, lettuce, and pepper.

Blocking Ethylene Perception

Chemical control of ethylene synthesis has been achieved with silver thiosulfate, aminovinyl glycine (AVG), aminooxyacetic acid (AOA) aminoisobutyric acid (AIBA), and Co^{2+}. Although these compounds have been used with success they must be dissolved and sprayed onto the plant, which means that uptake is variable. Also, several of these compounds are toxic to humans.

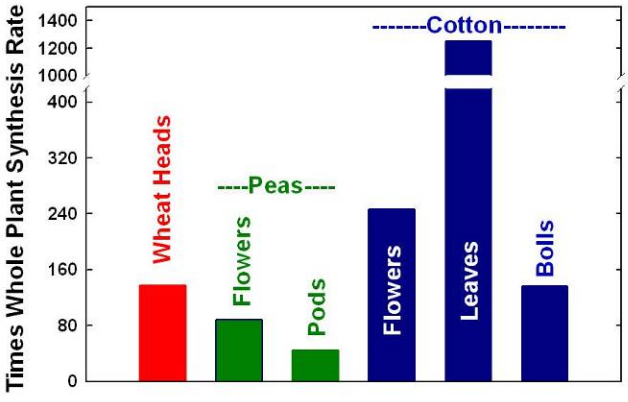

Figure 3. Ethylene synthesis for detached organs expressed as a multiple of whole plant ethylene synthesis. For all cases tested ethylene synthesis for detached organs was significantly greater than in the whole plant.

MCP is a non-toxic alternative that can be homogeneously applied as a gas. Most studies of MCP have focused on its effects in post-harvest physiology (Blankenship and Dole, 2003). MCP appears to decrease both ethylene synthesis and respiration of climacteric fruit. Limited information on non-climacteric fruits indicates that the effect of MCP is inconsistent and needs to be evaluated on a case-by-case basis (Lurie, 2005). For example, ethylene synthesis increased in citrus fruits, was unaffected in strawberries (Lurie, 2005), and decreased in grapes (Chervin, *et al.*, 2005). Although the effects of MCP on harvested organs are of importance for increasing shelf life and storage, there is sparse information for the effect of MCP in whole plant physiology. MCP could potentially mitigate the effects of drought and hypoxia, which are especially common in microgravity.

1-MCP Increases Respiration and Ethylene Synthesis

The effect of MCP on ethylene synthesis and respiration for whole plants and detached organs was studied in five common crop plants. We hypothesized that, similar to harvested fruit, MCP would decrease the respiration and ethylene synthesis of whole plants in an enclosed chamber.

Plants were placed in gas exchange boxes and kept in the dark for 8-20 h. Ethylene and CO_2 (respiration) accumulation were quantified. Length of time in box was determined by the minimum amount of time needed for a measurable amount of ethylene to accumulate (5 ppb minimum). The ratio of the ethylene to CO_2 synthesis rates was calculated to determine ethylene synthesis as a function of respiration, which eliminates metabolic rate and plant size as variables. Calculating this ratio allows us to test the hypothesis that ethylene signaling is tied more to metabolic rate than to plant size. Small rapidly growing plants can produce more ethylene than large, slow growing ones; however, per unit metabolism, they may be identical.

MCP increased the respiration and ethylene synthesis for all intact plants tested (Fig. 4, a). This was

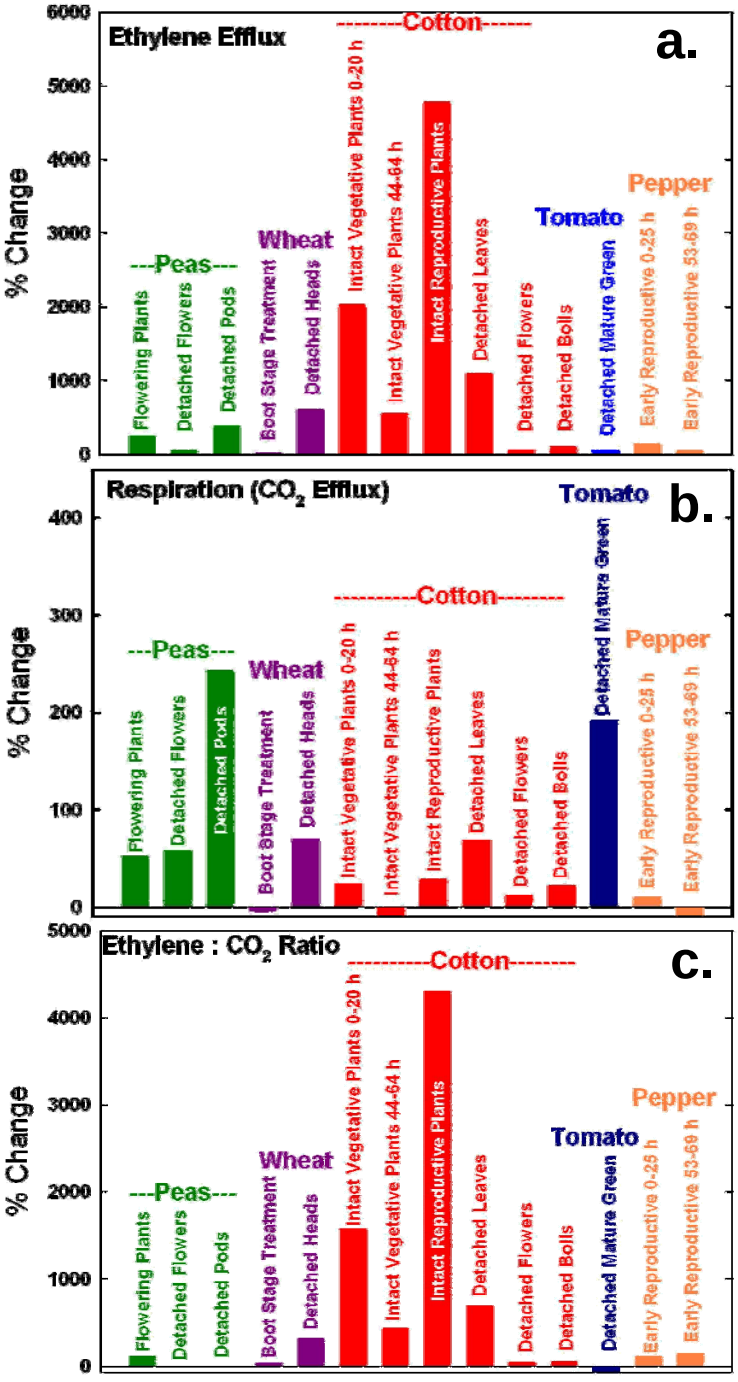

Figure 4. The effect of MCP on ethylene synthesis (a), respiration (b) and the synthesis to respiration ratio (c) expressed as percent change. MCP increased ethylene synthesis and respiration for all plants and organs studied. The synthesis to respiration ratio also increased, indicating that ethylene synthesis was increased greater than respiration.

unexpected given the effects observed in climacteric post-harvest physiology. Respiration increased for all plants during the first 24 h of treatment (Fig. 4, b). Plants that were treated earlier (boot stage wheat) or returned to the gas exchange box after 24 h (cotton, pepper) showed a decrease in respiration (Fig. 4, b). This may be due to a wearing off of the MCP effect. MCP increased the rate of ethylene synthesis more than respiration except in pea flowers and pods and detached tomato fruit (Fig. 4, c). For the detached organs, the increase in respiration was significantly larger than the increase in ethylene synthesis. This further highlights the value of tying synthesis to respiration.

Since alterations in ethylene synthesis serve as an indicator of stress conditions, this differential increase in synthesis would, under normal circumstances, lead to the conclusion the plants are stressed. However, since ethylene is regulated via a negative-feedback mechanism this data may indicate the beginning of autocatalytic ethylene production.

The long-term consequences of whole plant exposure to MCP have yet to be studied. Although MCP has the potential to mitigate ethylene contamination, the possibility remains that, upon the generation of new receptors, the plant may become more sensitive to the ethylene already present. Future work will study this hypothesis.

Flood Stress Increases Ethylene Synthesis

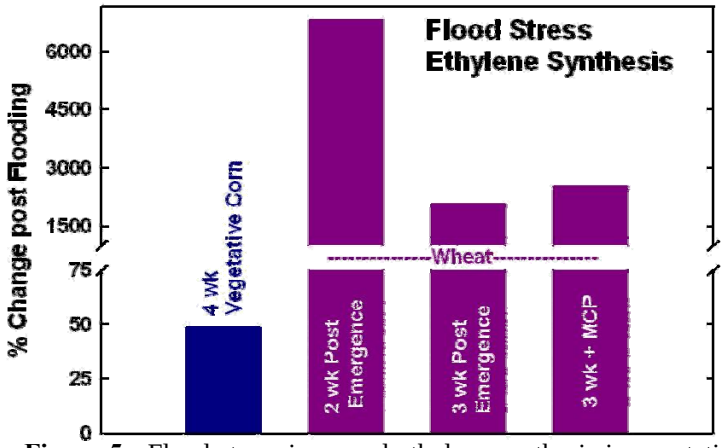

Figure 5. Flood stress increased ethylene synthesis in vegetative corn and wheat plants. MCP treated wheat plants had a higher synthesis increase than control plants.

Plants were kept in gas-exchange boxes as described above. Flood stress was applied by soaking non-draining pots with water until standing pools formed. Flood stress increased ethylene synthesis for all plants studied except vegetative tomato (Fig. 5). Flood stress had the greatest impact on 2-week post emergence wheat plants. Wheat plants tested one week later had significantly less change due to flood stress (Fig.5). This may be due to acclimatization from the prior-week's test. MCP treated wheat plants were more sensitive to flood stress than control plants (Fig. 5). This may mean that MCP is amplifying the ethylene stress signal. This is consistent with the effect seen in unstressed plants (Fig. 4).

When MCP blocks an ethylene receptor, the signal is not transduced to the synthesis pathway (Blankenship and Dole, 2003). Thus, a signal for autocatalytic synthesis should not be present. However, in all whole-plant cases examined, synthesis increased as a result of MCP application. This suggests that the plant is either compensating for a lack of perceived ethylene or that autocatalytic synthesis has been triggered.

Manuscript Development

To date, we have 3 manuscripts in development and one in review:

Romagnano, J.; Bugbee, B.. 2007. Low PPF does not increase ethylene sensitivity in radish or pea. *Plant Growth Regulation.* (in review)

Romagnano, J.; Bugbee, B.. 2007. 1-MCP increases ethylene synthesis more than respiration in whole plants. *Journal of Experimental Botany*. (in preparation)

Romagnano, J; Bugbee, B.. 2007. Quantifying wound ethylene synthesis. *Plant Physiology*. (in preparation)

Romagnano, J.; Bugbee, B.. 2007. Dwarf Crop Responses to Continuous (24 h) Photoperiod. *Hort Science.* (in preparation)

Final Year Plans

In addition to the work highlighted above, I plan to conduct ethylene synthesis studies using high-volume flow-through chambers. This will allow baseline and stress synthesis studies in non-ethylene-accumulating conditions to be performed. Drought, hypoxia and MCP effects will be of particular interest. This work will be performed as outlined in the original project proposal. This year is also the final year of my doctoral studies. As such, much time will also be devoted to manuscript and dissertation preparation.

Educational Outreach Activities

One of the goals of any NASA researcher is to educate others about the research underway and its use not only in space but also on the ground. In the past year I led two outreach activities. First, I traveled to meet with second grade students at River Heights Elementary School for a "space plants" day. Again, over fifty students attended this year's event. Students planted dwarf pea (*Earligreen*) and tomato (*MicroTina*) plants for study in their classrooms. The students were also led through an interactive presentation highlighting NASA's efforts to create an advanced life support system and the role plants would play in such a system. Also, we continue to receive communications from students asking for help on science fair projects and other classrooms that have used our seed for their own projects. In addition to the elementary school visit, our lab has hosted many visitors, including a group of senior citizens from the Brigham City Senior Center (Fig. 6).

Figure 6. The author (far right) with members of the Brigham City Senior Center. The seniors toured the facilities and learned about the space plant effort.

Summary of Travel

February 5th through the 8th of 2006 I traveled to the NASA sponsored Habitation 2006 conference. A visit to present and share data with Kennedy Space Center researchers is in the early planning stages. These activities represent the trips itemized and approved in the initial budget proposal.

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Appendix F: Dwarf Crop Responses to Multiple Photoperiod Regimes

Dwarf Crop Responses to Multiple Photoperiod Regimes

Joseph Romagnano, Bruce Bugbee

Introduction

Electric lighting in greenhouses and growth chambers is a well-established technique to increase photoperiod and daily light integral (Werner, 1942; Stevenson and Clark, 1933; Langhans and Tibbitts, 1997). Day length is responsible for triggering developmental changes in short and long day plants. Day length and light intensity combined give a plant's daily light integral, a key factor in determining plant growth (Chabot *et al.*, 1979). The primary goal of supplemental light systems is usually to maximize the daily light integral without extending the photoperiod beyond 16 h (Hurd and Thornley, 1974; Langhans and Tibbitts, 1997; Withrow and Benedict, 1936; Bonner, 1940). Standard greenhouse practice for vegetable crop production, exemplified by Hannon (1998) and Nelson (2003), generally recommends supplemental light intensity in the 100-200 µmol m^{-2} s^{-1} (6.5-10.8 klux) range. Neither author recommends a photoperiod longer than 18 hours. The primary obstacle behind the use of a continuous photoperiod is the perception that plants need a dark period to transport accumulated photosynthate to sink tissues and that development will be negatively affected. This perception arose from early research into photoperiod requirements.

In addition to coining the term "photoperiodism," Garner and Allard (1923) reviewed and extended inquiries into plant growth and development in response to photoperiod. They identified the defining characteristics of short and long-day

plants. Most important, was the identification of short day length requirements to initiate flowering and reproductive development in some crop species. They also showed that extremely low levels of supplemental light were enough to prevent many plants from entering winter dormancy. Due to the technology at the time, however, the extension of the photoperiod to a full 24 hours at light intensities high enough to increase growth was not possible.

Arthur, Guthrie and Newell (1930) were later able to use multiple high-intensity incandescent lights in climate-controlled rooms to grow plants under photoperiods up to 24 hours in length. In addition to morphological characteristics they reported nitrogen and carbohydrate content data for buckwheat, lettuce, radish, tomato and salvia plants grown under different CO_2 concentrations, photoperiods, and irradiance levels. In nearly all cases the use of a 24 hour photoperiod decreased the mass per plant and increased the percent total carbohydrates when compared to plants grown under short or intermediate photoperiods. Tomato plants were the most sensitive of the plants tested. Foliar injury occurred under the 24 hour photoperiod regardless of intensity tested (typical intensity: 280 µmol m^{-2} s^{-1}).

Hillman in 1956, Kristofferson in 1963, Dorais *et al.* 1996, and Dorais *et al.*, 2003 have also reported chlorosis in the leaves of tomato plants under high light intensities. The early research led to the hypothesis that a high level of starch accumulation in the leaves of tomatoes leads to leaf chlorosis and a resultant loss in photosynthetic capacity (Dorais *et al.*, 2003). Thorne and Koller, 1974, demonstrated a decrease in CO_2 influx as the result of increased starch

content in the leaf. Subsequent work in single-rooted soybean leaves showed that leaves with high starch content were unable to increase their photosynthetic rates as rapidly as low starch leaves when CO_2 partial pressures were increased (Sawada *et al.*, 2001). Furthermore, photosynthetic rates were negatively correlated with both sugar and starch content in the leaves (Sawada *et al.*, 2001). This evidence lends support to the theory that photosynthesis is limited by photosynthate transport out of source tissues. If chlorosis occurs when the capacity to transport photosynthate is less than the photosynthesis rate at high light intensity and increased photoperiod, it should be possible to grow tomato, or any other crop, at 24 hour photoperiods so long as the light intensity results in a photosynthetic rate that does not exceed the photosynthate transport capacity.

Hurd and Thornley (1974) appear to be the first to successfully grow tomatoes using a continuous photoperiod and several different light integrals. Their plants were grown using NFT hydroponics and light integrals ranging from 1 – 47 mol m^{-2} d^{-1}. Tomatoes grown in continuous light treatments had high growth and net assimilation rates (NAR). Plants grown in the highest light treatments had mottling on their leaves. Both high and low light plants had substantial drops in energy conversion efficiency after 30 d. Plants grown under intermediate light levels, however, showed no drop in efficiency or chlorosis when harvested 24 d after planting. Hurd and Thornley (1974) also noted that there were cultivar differences in the ability to handle extended photoperiods. This further suggested that 24 h photoperiods could successfully grow crops under the right conditions.

For multiple crop species we compared the yield production efficiency of 16 and 24 h photoperiods at an extremely low light level. We hypothesized that plants grown in continuous low light would have 1.5x more growth than those grown using a 16 h photoperiod at the same intensity since the light level would be low enough to prevent photosynthate accumulation in the leaves. We then used higher light intensities and a constant light integral to examine the effects of 16, 20, and 24 h photoperiods.

Materials and Methods

Low-Light Plants and Growing Conditions

Tomato (cv., *Micro-Tina*), radish (cv., *Cherry Belle*), pea (cv. *Earligreen*), and pepper (cv., *Triton*) plants were grown in ambient lab conditions under cool white fluorescent lights at a PPF of 90 µmol m^{-2} s^{-1} and a photoperiod of either 16 (5.2 mol m^{-2} d^{-1}) or 24 (7.8 mol m^{-2} d^{-1}) hours. Plants were watered with tap water once each day. Nutrients were supplied by Osmocote Plus slow-release fertilizer mixed into the 50/50 peat / perilite media at approximately 7 g per 2 L pot.

Constant Light Integral Plants and Growing Conditions

Tomato (cv., *Micro-Tina*), radish (cv., *Cherry Belle*), pea (cv. *Earligreen*), mustard (cv., *Mizuna*) and pepper (cv., *Triton*) plants were grown three controlled environment chambers (EGC, inc., Chagrin Falls, OH). Each chamber had 1.25 m^{-2} of surface area. Pots (2 L) were filled with 1:1 peat / perilite media. Nutrients were provided by watering twice daily with Peters 5-11-26 HYDRO-SOL supplemented with 10 µM Fe EDDHA, 1.4 mM $CaNO_3$, and 10 µM

$Na_2SiO_3*9H_2O$. Plants were grown under HPS lamps at either 500 μmol m^{-2} s^{-1} for 16 hours, 400 μmol m^{-2} s^{-1} for 20 hours, or 333 μmol m^{-2} s^{-1} continuous for a final light integral of 28.8 mol m^{-2} d^{-1} for all chambers. Carbon dioxide was elevated to 1200 μmol mol^{-1}. Relative humidity was maintained at 70-80% day-night.

Statistics

All experiments used single pot spaced plants except Mizuna, which had 4 plants per pot. For low-light experiments, replicate pots for each photoperiod were randomly placed under the lights. Measurements for each experimental unit were analyzed using one-way or multi-variate ANOVA using type 1 sums of squares and α=0.05 (SPSS software Macintosh v. 11.0.4).

Results

The results from this experiment are still in need of refinement. Tables that follow 1.x are from the low-light experiments. Tables that follow the 2.x convention are from the high-light / constant light integral experiment. Bolded values indicate where significant differences were observed.

Table 1.1. Effect of 16 vs 24 h photoperiod on growth and development of Micro-Tina tomato. Significantly different measurements are bolded.

Parameter	**16 h mean**	**24 h mean**	**p-value** (ANOVA, α=0.05)
Shoot Fresh Mass (g)	80.6	**116.7**	**0.013**
Shoot Dry Mass (g)	9.7	**17.7**	**0.007**
Shoot Dry Mass Photosynthetic Efficiency (mg/mol Photons)	14.2	17.1	0.205
Shoot Percent Dry Mass (%)	12.0	**15.1**	**0.025**
Days to Flower (d)	34.5	33.0	0.589
Days to Fruit (d)	47.0	47.0	1.000
# Red Fruit	20.0	15.8	0.427
Red Fruit Fresh Mass (g)	58.3	43.2	0.291
Red Fruit Photosynthetic efficiency (g/ mol Photons)	**84.9**	42.0	**0.047**
# Green Fruit	5.8	1.5	0.191
Green Fruit Fresh Mass (g)	10.4	1.90	0.135

Table 1.2. Effect of 16 vs 24 h photoperiod on growth and development of Earligreen pea plants. Significantly different measurements are bolded.

Parameter	**16 h mean**	**24 h mean**	**p-value** (ANOVA, α=0.05)
Plant Fresh Mass (g)	9.3	9.8	0.764
Plant Dry Mass (g)	1.5	1.8	0.409
Plant Dry Mass Photosynthetic Efficiency (mg/mol photons)	4.6	3.5	0.217
Plant Percent Dry Mass (%)	16.7	**18.3**	**0.030**
Days to Flower (d)	26.8	26.3	0.769
Days to Fruit (d)	28.3	27.7	0.727
# Pods / Plant	2.0	1.7	0.286
# Seeds / Plant	5.8	5.0	0.615
Mass Seed / Plant	1.5	1.3	0.783
Seed Mass Photosynthetic Efficiency (mg / mol photons)	4.4	2.7	0.175

Table 1.3. Effect of 16 vs 24 h photoperiod on growth and development of Cherry Belle radish plants. Significantly different measurements are bolded.

Parameter	**16 h mean**	**24 h mean**	**p-value** (ANOVA, α=0.05)
Shoot Fresh Mass (g)	5.8	6.8	0.609
Shoot Dry Mass (g)	0.44	0.59	0.215
Shoot Dry Mass Photosynthetic Efficiency (mg/mol Photons)	4.0	3.6	0.668
Shoot Percent Dry Mass (%)	7.7	9.0	0.062
Root Fresh Mass (g)	5.8	**15.7**	**0.005**
Root Dry Mass (g)	0.31	**0.85**	**0.011**
Root Dry Mass Photosynthetic Efficiency (mg / mol photons)	2.8	5.2	0.052
Root Percent Dry Mass	5.3	5.3	0.995

Table 1.4. Effect of 16 vs 24 h photoperiod on growth and development of Triton pepper plants. Significantly different measurements are bolded.

Parameter	16 h mean	24 h mean	p-value (ANOVA, α=0.05)
Shoot Fresh Mass (g)	50.7	54.8	0.250
Shoot Dry Mass (g)	8.1	7.9	0.768
Shoot Dry Mass Photosynthetic Efficiency (mg/mol Photons)	**12.3**	7.9	**0.005**
Shoot Percent Dry Mass (%)	16.0	14.3	0.119
Days to Flower (d)	39.0	37.0	0.071
Days to Fruit (d)	42.0	45.3	0.064
# Fruit	2.0	2.0	1.000
Fruit Fresh Mass (g)	104.2	70.2	0.163
Fruit Photosynthetic efficiency (mg/ mol Photons)	**157.8**	70.8	**0.017**

Table 2.1. Effect of three photoperiods with a constant light integral of 28.8 mol d^{-1} on the growth and development of Mizuna mustard plants. Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	**16 h mean**	**20 h mean**	**24 h mean**	**p-value** (ANOVA, α=0.05)
Leaf Area (cm^2)	268.6^{a}	286.1^{a}	279.9^{a}	0.520
Shoot Fresh Mass (g)	13.0^{a}	14.6^{a}	13.7^{a}	0.173
Shoot Dry Mass Photosynthetic Efficiency (mg / mol photons)	3.7^{a}	**4.4^{b}**	**4.6^{b}**	**0.016**
Shoot Dry Mass (g)	1.8^{a}	**2.2^{b}**	**2.3^{b}**	**0.016**
Shoot Percent Dry Mass (%)	14.1^{a}	$15.0^{a,b}$	16.6^{b}	0.092

Table 2.3. Effect of three photoperiods with a constant light integral of 28.8 mol

Table 2.2. Effect of three photoperiods with a constant light integral of 28.8 mol d^{-1} on the growth and development of Cherry Belle radish plants. Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	**16 h mean**	**20 h mean**	**24 h mean**	**p-value** (ANOVA, α=0.05)
Leaf Area (cm^2)	254.6[a]	359.8[a]	210.2[a]	0.232
Shoot Fresh Mass (g)	10.0[a]	15.3[a]	8.6[a]	0.258
Shoot Dry Mass (g)	1.1[a]	1.6[a]	1.0[a]	0.418
Shoot Dry Mass Photosynthetic Efficiency (mg / mol photons)	1.9[a]	2.80[a]	1.8[a]	0.418
Shoot Percent Dry Mass (%)	11.8[a]	10.3[a]	12.4[a]	0.366
Root Fresh Mass (g)	41.5[a,b]	62.5[b]	23.5[a]	0.068
Root Dry Mass (g)	2.2[a,b]	3.1[b]	1.4[a]	0.109
Root Dry Mass Photosynthetic Efficiency (mg/ mol photons)	3.8[a,b]	5.4[b]	2.4[a]	0.109
Root Percent Dry Mass (%)	5.5[a]	5.0[a]	6.2[a]	0.241

d^{-1} on the growth and development of Earligreen Pea plants. Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	16 h mean	20 h mean	24 h mean	p-value (ANOVA, α=0.05)
Primary Shoot Length (cm)	31.6^{a,b}	**35.8^{b}**	28.4^{a}	**0.011**
Number of Nodes in Primary Shoot	17.4^{a}	18.3^{a}	16.8^{a}	0.386
Internodal Length (cm/ node)	1.8^{a,b}	**2.0^{b}**	1.67^{a}	**0.044**
Number of Secondary Shoots	8.8^{a,b}	**9.3^{b}**	6.8^{a}	0.051
Shoot Fresh Mass (g)	43.9^{a}	**85.9^{b}**	27.6^{a}	**<0.001**
Shoot Dry Mass (g)	7.6^{a}	**21.4^{b}**	7.7^{a}	**<0.001**
Shoot Dry Mass Photosynthetic Efficiency (mg / mol photons)	6.1^{a}	**17.2^{b}**	6.2^{a}	**<0.001**
Shoot Percent Dry Mass (%)	19.1^{a}	25.2^{a}	32.3^{a}	0.146
Number of Pods	29.6^{a}	**53.0^{b}**	25.2^{a}	**<0.001**
Pod Fresh Mass (g)	29.3^{b}	**76.7^{c}**	12.9^{a}	**<0.001**
Pod Dry Mass (g)	5.8^{a}	**14.2^{b}**	4.9^{a}	**<0.001**
Pod Dry Mass Photosynthetic Efficiency (mg/ mol photons)	4.7^{a}	**11.5^{b}**	4.0^{a}	**<0.001**
Pod Percent Dry Mass (%)	22.3^{a}	18.7^{a}	**46.1^{b}**	**0.024**
Number of Seeds	126.0^{a}	**180.5^{b}**	113.2^{a}	**<0.001**
Number of Seeds per Pod	4.3^{a,b}	3.5^{a}	4.5^{b}	0.071
Seed Fresh Mass (g)	55.8^{a}	**29.3^{b}**	37.1^{a}	**0.003**
Seed Dry Mass (g)	**17.5^{c}**	5.8^{a}	15.3^{b}	**<0.001**
Seed Dry Mass Photosynthetic Efficiency (mg/ mol photons)	**14.1^{c}**	4.7^{a}	12.4^{b}	**<0.001**
Seed Percent Dry Mass (%)	32.5^{b}	19.7^{a}	**42.6^{c}**	**<0.001**
Fresh Mass Per Seed (mg / seed)	**409.8^{c}**	161.6^{a}	327.6^{b}	**<0.001**
Dry Mass Per Seed (mg / seed)	**139.3^{b}**	32.0^{a}	**136.1^{b}**	**<0.001**

Figure 1. Cherry Belle radish and MicroTina tomato plants grown under 16 or 24 hours of low light. Radish plants had a significantly larger root mass when grown under 24 hours light. Tomato plants had no differences in fruit size, fruit number, fruit mass, or days to fruit. Shoot mass and shoot percent dry mass, however, were significantly greater in tomato plants grown using continuous light. No evidence of leaf chlorosis was seen in tomato plants grown using continuous light.

Table 2.4. Effect of three photoperiods with a constant light integral of 28.8 mol d^{-1} on the growth and development of *Triton* pepper plants. Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	16 h mean	20 h mean	24 h mean	p-value (ANOVA, α=0.05)
Primary Stem Length (cm)	24.2^{a}	22.4^{a}	21.6^{a}	0.501
Stem Fresh Mass (g)	41.3^{a}	**56.5^{b}**	34.4^{a}	**0.009**
Stem Dry Mass (g)	**5.8^{b}**	**6.2^{b}**	4.1^{a}	**0.014**
Stem Dry Mass Photosynthetic Efficiency (mg / mol photons)	**2.9^{b}**	**3.2^{b}**	2.0^{a}	**0.014**
Stem Percent Dry Mass (%)	**14.2^{b}**	11.3^{a}	11.8^{a}	**<0.001**
Number of Fruit	**19.8^{c}**	9.4^{a}	13.6^{b}	**<0.001**
Fruit Fresh Mass (g)	**195.6^{b}**	97.5^{a}	**183.5^{b}**	**0.028**
Fruit Dry Mass (g)	**10.6^{b}**	5.2^{a}	**9.1^{b}**	**0.018**
Fruit Dry Mass Photosynthetic Efficiency (mg/ mol photons)	**5.4^{b}**	2.6^{a}	**4.6^{b}**	**0.017**
Fruit Percent Dry Mass (%)	**5.7^{b}**	5.4^{a,b}	4.9^{a}	0.066
Number of Leaves	63.2^{a}	69.6^{a}	61.0^{a}	0.814
Leaf Fresh Mass (g)	63.7^{a}	44.4^{a}	58.3^{a}	0.112
Leaf Dry Mass (g)	**10.6^{b}**	6.2^{a}	7.4^{a,b}	0.078
Leaf Dry Mass Photosynthetic Efficiency (mg/ mol photons)	**5.3^{b}**	3.1^{a}	3.7^{a,b}	0.078
Leaf Percent Dry Mass (%)	**16.2^{b}**	14.0^{a}	12.7^{a}	0.004
Leaf : Fruit (g leaf / g fruit)	2.2^{a}	2.8^{a}	1.4^{a}	0.293

Table 2.5. Effect of three photoperiods with a constant light integral of 28.8 mol d^{-1} on the growth and development of *MicroTina* tomato plants. Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	16 h mean	20 h mean	24 h mean	p-value (ANOVA, α=0.05)
Plant height (cm)	21.6[a]	28.2[a,b]	**39.0[b]**	**0.017**
Shoot Fresh Mass (g)	287.1[a]	252.4[a]	274.4[a]	0.598
Shoot Dry Mass (g)	43.1[a]	37.2[a]	42.4[a]	0.561
Shoot Dry Mass Photosynthetic Efficiency (mg / mol photons)	24.1[a]	20.8[a]	23.7[a]	0.561
Shoot Percent Dry Mass (%)	14.9[a]	14.7[a]	15.5[a]	0.331
Number of Red Fruit	11.2[a]	4.6[a]	6.6[a]	0.199
Red Fruit Fresh Mass (g)	44.5[a]	25.7[a]	24.3[a]	0.362
Red Fruit Dry Mass (g)	3.3[a]	1.9[a]	1.9[a]	0.358
Red Fruit Dry Mass Photosynthetic Efficiency (mg/ mol photons)	1.8[a]	1.1[a]	1.1[a]	0.357
Red Fruit Percent Dry Mass (%)	7.9[a]	5.7[a]	7.4[a]	0.299
Number of Green Fruit	24.2[a]	**69.6[b]**	19.4[a]	**<0.001**
Green Fruit Fresh Mass (g)	40.6[a]	**143.9[b]**	32.2[a]	**<0.001**
Green Fruit Dry Mass (g)	4.9[a]	**15.4[b]**	3.4[a]	**<0.001**
Green Fruit Dry Mass Photosynthetic Efficiency (mg/ mol photons)	2.7[a]	**8.6[b]**	1.9[a]	**<0.001**
Green Fruit Percent Dry Mass (%)	12.1[a]	**10.8[b]**	10.4[a]	**0.006**

Table 2.?. Effect of photoperiod with a constant light integral of 28.8 mol d^{-1} on photosynthetic efficiency (mg dry mass / mol photons). Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	16 h mean	20 h mean	24 h mean	p-value (ANOVA, α=0.05)
Micro Tina Shoot	24.1^{a}	20.8^{a}	23.7^{a}	0.561
Micro Tina Red Fruit	1.8^{a}	1.1^{a}	1.1^{a}	0.357
Micro Tina Green Fruit	2.7^{a}	**8.6^{b}**	1.9^{a}	**<0.001**
Triton Stem	**2.9^{b}**	**3.2^{b}**	2.0^{a}	**0.014**
Triton Fruit	**5.4^{b}**	2.6^{a}	**4.6^{b}**	**0.017**
Triton Leaf	**5.3^{b}**	3.1^{a}	3.7^{a,b}	0.078
Earligreen Shoot	6.1^{a}	**17.2^{b}**	6.2^{a}	**<0.001**
Earligreen Pod	4.7^{a}	**11.5^{b}**	4.0^{a}	**<0.001**
Earligreen Seed	**14.1^{c}**	4.7^{a}	12.4^{b}	**<0.001**
Cherry Belle Shoot	1.9^{a}	2.8^{a}	1.8^{a}	0.418
Cherry Belle Root	3.8^{a,b}	5.4^{b}	2.4^{a}	0.109
Mizuna Shoot	3.7^{a}	**4.4^{b}**	**4.6^{b}**	**0.016**
Grand Rapids Leaves	14.7^{a}	**17.3^{c}**	11.9^{b}	**<0.001**

Table 1.?. Effect of photoperiods with light integrals of 5.2 or 7.8 mol d^{-1} (16 or 24 h respectively) on photosynthetic efficiency (mg dry mass / mol photons). Significantly different measurements are bolded.

Parameter	16 h mean	24 h mean	p-value (ANOVA, α=0.05)
Micro Tina Shoot	14.2	17.1	0.205
Micro Tina Red Fruit	**84.9**	42.0	**0.047**
Triton Shoot	**12.3**	7.9	**0.005**
Triton Fruit	**157.8**	70.8	**0.017**
Earligreen Shoot	4.6	3.5	0.217
Earligreen Seed	4.4	2.7	0.175
Cherry Belle Shoot	4.0	3.6	0.668
Cherry Belle Root	2.8	5.2	0.052
Mizuna Shoot	In Progress		

Table 2.6. Effect of three photoperiods with a constant light integral of 28.8 mol d^{-1} on the growth and development of *Grand rapids* lettuce plants. Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	**16 h mean**	**20 h mean**	**24 h mean**	**p-value** (ANOVA, α=0.05)
Leaf Area (cm^2)	2418.6^b	2494.0^b	1858.0^a	**0.007**
Shoot Fresh Mass (g)	153.0^b	168.7^b	118.3^a	**<0.001**
Shoot Dry Mass Photosynthetic Efficiency (mg / mol photons)	14.7^a	17.3^c	11.9^b	**<0.001**
Shoot Dry Mass (g)	10.6^b	12.5^c	8.5^a	**<0.001**
Shoot Percent Dry Mass (%)	6.9^a	7.3^a	7.2^a	0.321

Table 2.3A. Effect of three photoperiods with a constant light integral of 28.8 mol d^{-1} on the growth and development of Earligreen Pea plants. Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	16 h mean	20 h mean	24 h mean	p-value (ANOVA, α=0.05)
Primary Shoot Length (cm)	31.6^{a,b}	**35.8^{b}**	28.4^{a}	**0.011**
T2	37.0	35.4	37.6	0.478
Number of Nodes in Primary Shoot	17.4^{a}	18.3^{a}	16.8^{a}	0.386
T2	19.2	21.0	20.4	0.144
Internodal Length (cm/ node)	1.8^{a,b}	**2.0^{b}**	1.67^{a}	**0.044**
T2	1.9	1.7	1.8	**0.023**
Number of Secondary Shoots	8.8^{a,b}	**9.3^{b}**	6.8^{a}	0.051
T2	9.4	10.4	9.8	0.349
Shoot Fresh Mass (g)	43.9^{a}	**85.9^{b}**	27.6^{a}	**<0.001**
T2	63.2	84.6	67.3	**0.054**
Shoot Dry Mass (g)	7.6^{a}	**21.4^{b}**	7.7^{a}	**<0.001**
T2	10.8	14.8	11.2	**0.024**
Shoot Dry Mass Photosynthetic Efficiency (mg / mol photons)	6.1^{a}	**17.2^{b}**	6.2^{a}	**<0.001**
T2	8.7	11.9	9.1	**0.024**
Shoot Percent Dry Mass (%)	19.1^{a}	25.2^{a}	32.3^{a}	0.146
T2	16.9	17.4	16.9	0.625
Number of Pods	29.6^{a}	**53.0^{b}**	25.2^{a}	**<0.001**
T2	37.0	49.8	38.4	0.075
Pod Fresh Mass (g)	29.3^{b}	**76.7^{c}**	12.9^{a}	**<0.001**
T2	44.5	66.1	50.7	**0.016**
Pod Dry Mass (g)	5.8^{a}	**14.2^{b}**	4.9^{a}	**<0.001**
T2	6.3	9.7	6.9	**0.010**
Pod Dry Mass Photosynthetic Efficiency (mg/ mol photons)	4.7^{a}	**11.5^{b}**	4.0^{a}	**<0.001**
T2	5.1	7.9	5.5	**0.010**
Pod Percent Dry Mass (%)	22.3^{a}	18.7^{a}	**46.1^{b}**	**0.024**
T2	12.2	14.7	13.6	**0.009**
Number of Seeds	126.0^{a}	**180.5^{b}**	113.2^{a}	**<0.001**
T2	155.6	168.8	154.6	0.791
Number of Seeds per Pod	4.3^{a,b}	3.5^{a}	4.5^{b}	0.071
T2	4.3	3.4	4.1	**0.023**
Seed Fresh Mass (g)	55.8^{a}	**29.3^{b}**	37.1^{a}	**0.003**
T2	61.5	65.8	51.4	0.302
Seed Dry Mass (g)	**17.5^{c}**	5.8^{a}	15.3^{b}	**<0.001**
T2	14.7	15.4	11.2	0.159
Seed Dry Mass Photosynthetic Efficiency (mg/ mol photons)	**14.1^{c}**	4.7^{a}	12.4^{b}	**<0.001**
T2	11.9	12.5	9.0	0.159
Seed Percent Dry Mass (%)	32.5^{b}	19.7^{a}	**42.6^{c}**	**<0.001**
T2	24.0	23.5	21.6	0.057
Fresh Mass Per Seed (mg / seed)	**409.8^{c}**	161.6^{a}	327.6^{b}	**<0.001**
T2	396.6	395.3	330.2	0.068
Dry Mass Per Seed (mg / seed)	**139.3^{b}**	32.0^{a}	**136.1^{b}**	**<0.001**
T2	95.3	93.2	72.0	0.047

Table 2.3. Effect of three photoperiods with a constant light integral of 28.8 mol d^{-1} on the growth and development of Earligreen Pea plants. Significantly different measurements are bolded. Superscript letters indicate Duncan grouping.

Parameter	16 h mean	20 h mean	24 h mean	p-value (ANOVA, α=0.05)
Primary Shoot Length (cm)	31.6^{a,b}	**35.8^{b}**	28.4^{a}	**0.011**
Number of Nodes in Primary Shoot	17.4^{a}	18.3^{a}	16.8^{a}	0.386
Internodal Length (cm/ node)	1.8^{a,b}	**2.0^{b}**	1.67^{a}	**0.044**
Number of Secondary Shoots	8.8^{a,b}	**9.3^{b}**	6.8^{a}	0.051
Shoot Fresh Mass (g)	43.9^{a}	**85.9^{b}**	27.6^{a}	**<0.001**
Shoot Dry Mass (g)	7.6^{a}	**21.4^{b}**	7.7^{a}	**<0.001**
Shoot Dry Mass Photosynthetic Efficiency (mg / mol photons)	6.1^{a}	**17.2^{b}**	6.2^{a}	**<0.001**
Shoot Percent Dry Mass (%)	19.1^{a}	25.2^{a}	32.3^{a}	0.146
Number of Pods	29.6^{a}	**53.0^{b}**	25.2^{a}	**<0.001**
Pod Fresh Mass (g)	29.3^{b}	**76.7^{c}**	12.9^{a}	**<0.001**
Pod Dry Mass (g)	5.8^{a}	**14.2^{b}**	4.9^{a}	**<0.001**
Pod Dry Mass Photosynthetic Efficiency (mg/ mol photons)	4.7^{a}	**11.5^{b}**	4.0^{a}	**<0.001**
Pod Percent Dry Mass (%)	22.3^{a}	18.7^{a}	**46.1^{b}**	**0.024**
Number of Seeds	126.0^{a}	**180.5^{b}**	113.2^{a}	**<0.001**
Number of Seeds per Pod	4.3^{a,b}	3.5^{a}	4.5^{b}	0.071
Seed Fresh Mass (g)	55.8^{a}	**29.3^{b}**	37.1^{a}	**0.003**
Seed Dry Mass (g)	**17.5^{c}**	5.8^{a}	15.3^{b}	**<0.001**
Seed Dry Mass Photosynthetic Efficiency (mg/ mol photons)	**14.1^{c}**	4.7^{a}	12.4^{b}	**<0.001**
Seed Percent Dry Mass (%)	32.5^{b}	19.7^{a}	**42.6^{c}**	**<0.001**
Fresh Mass Per Seed (mg / seed)	**409.8^{c}**	161.6^{a}	327.6^{b}	**<0.001**
Dry Mass Per Seed (mg / seed)	**139.3^{b}**	32.0^{a}	**136.1^{b}**	**<0.001**

CURRICULUM VITAE

Joseph F. Romagnano

Education:

Ph.D. 2008 Utah State University
Crop Physiology

M.S. 2003 Worcester Polytechnic Institute
Biotechnology

B.S. 2001 Worcester Polytechnic Institute
Biology & Humanities and Arts

Experience:

Jan. 2004 – Present **Graduate Research Assistant / Ph. D. Candidate**
Utah State University Crop Physiology Laboratory
Logan, UT (Dr. Bruce Bugbee supervising)

- Developed controlled environment chambers to measure ethylene synthesis from intact plants under steady-state conditions
- Developed gas chromatography system incorporating automated thermal desorption for continuous monitoring of ethylene synthesis
- Effects on ethylene synthesis by abiotic stressors (***flood***, ***drought***, *etc*) studied
- Publication pending review process

Nov. 1999 – Dec. 2003 **Undergraduate/Graduate Research Assistant**
Worcester Polytechnic Institute
Worcester, MA (Dr. Pamela Weathers supervising)

- Designed and constructed experimental systems for delivering nutrients to plants in microgravity
- One paper submitted for publication currently undergoing revision

Jun. 2001 – Dec. 2003 **Biology/Biotechnology Dept. Teaching Assistant**
Worcester Polytechnic Institute
Worcester, MA

- Maintained an active presence in class lectures and was also responsible for the setup and management of laboratory classes
- Classes TA'd include: Plant Physiology, Fermentation, Animal Cell Culture, Photomicroscopy, Electron Microscopy, and Bioprocessing

Jun. 2002 – Aug. 2002 **Field Diver**
Lycott Environmental
Southbridge, MA (summer contract)

- Responsible for identification and removal of Eurasian milfoil and other aquatic nuisance species from Lake George, NY and other lakes
- Surveyed milfoil beds, harvested Eurasian milfoil through hand and suction methods.

Feb. 2001 – Aug. 2001 **Electron Microscopy Technician**
University of Massachusetts Medical School
Worcester, MA (position terminated - lack of funds)

- Prepared samples for viewing with TEM (i.e., fixed, embedded, performed microtomy and imaged)
- Responsible for developing & printing negatives, maintaining stock solutions

Aug. 1999 – Dec. 1999 **Student Researcher**
MBL Semester in Environmental Science Program
Woods Hole, MA

- Offered each fall by The Ecosystems Center, Marine Biological Laboratory (MBL), SES is a 15-week program in environmental science offered to advanced students enrolled in colleges participating in MBL Consortium in Environmental Science.
- The final five weeks of the Semester in Environmental Science are dedicated to independent group research projects where students present their findings in public symposium and write up their project results in a scientific paper format and a journalistic format suitable for presentation to a lay readership.
- Project completed entitled “Reconstructing Lake History Through the Use of Sediment Cores.”

Skills:

- Gas Chromatography coupled with Automated Thermal Desorption
- Campbell scientific dataloggers and automated data acquisition
- Digital analysis of plant growth
- Enzyme extraction and activity analysis
- Electron Microscopy (Transmission and Scanning)
- Photomicroscopy
- Sterile Tissue Culture
- Atomic Adsorption Spectroscopy
- LaChat Ion Chromatography
- CHN isotope analysis
- LECO sulfur analysis

Awards:

- NASA Graduate Student Research Program (3 years)
- NASA Travel Award to 2007 International Astronautical Congress
- Sigma Xi Scientific Honor Society Associate Member
- Eagle Scout, Boy Scouts of America

References available on request