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Ethylene production as an indicator of stress conditions in hydroponically-grown strawberries

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ABSTRACT

Ethylene production as an indicator of stress conditions in hydroponically-grown strawberries

By Justin Donald Hogan

With the worldwide phaseout of methyl bromide, the use of hydroponic systems has increased as an economic alternative for the growth of many horticulturally-important crops, including strawberries. In this study, the effect of hydroponics on strawberry plant physiology was examined by first measuring ethylene levels, a plant hormone known to increase due to stressful conditions, and plant growth and yield. Using a gas chromatograph, ethylene was measured from plants which showed that light and temperature have minimal effects, but placement of plants could have an effect on plant growth and yield. Next, the mechanism of ethylene production was examined by measuring levels of the *ACS* gene. Several techniques to obtain RNA from strawberries were tested, but inconclusive results were obtained. In conclusion, the use of ethylene measurements and elucidation of the ethylene pathway could be used as indicators for plant stress to help minimize stress and increase growth and yield.

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CHAPTER I

Introduction

Historical Overview

Plants, like all living organisms, have to adapt to their environment to survive. When at optimal growth, all the different systems within a plant are working to sustain an optimal homeostasis. But, when the environment surrounding a plant changes so that the plant has to respond to that stimulus, different mechanisms are employed to counteract the change in environment. One of the products that can be measured due to environmental changes affecting a plant's homeostasis is ethylene, which is one of the major hormones produced when a plant is not at optimal living conditions. Ethylene was first discovered as a biologically active compound in St. Petersburg by Dimitry Nikolayevich Neljubov in 1886 (Abeles *et al*., 1992). Historical overviews from Buchanan *et al*. (2000) and Abeles *et al*. (1992) have described how Neljubov noticed that when pea seedlings were germinated and grown in the dark, they grew in a horizontal position when exposed to laboratory air, but grew vertically when exposed to outside air. After ruling out cultural practices such as light and temperature, Neljubov discovered that the composition of the air is what caused the seedlings to grow differently, or more specifically, the gas used for illumination caused the abnormal effect. Neljubov noted that the addition of the illuminating gas to outside air resulted in the same growth phenomenon observed in the laboratory air. Subsequent studies that confirmed and expanded on Neljubov's results found that ethylene was the biologically active component of the illuminating gas and smoke, and caused the abnormal growth.

CHAPTER II

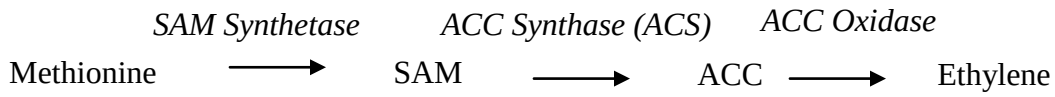
Review of Literature

Overview of Stress-Induced Ethylene Biosynthesis

The Ethylene Biosynthetic Pathway

The first elucidation of ethylene biosynthesis was first described by Lieberman and Mapson who demonstrated that methionine was an ethylene precursor (reviewed in Abeles *et al*., 1992). In 1977, Adams and Yang showed that ethylene was derived from S-adenosylmethionine (SAM or AdoMet). In 1979, in separate experiments by Adams and Yang and also by Lurssen, Naumann, and Schroder, 1-aminocyclopropane-1-carboxylic acid (ACC) was identified as the immediate precursor to the final product of ethylene (reviewed in Abeles *et al*., 1992).

Ethylene is synthesized from the amino acid methionine. Methionine is first converted to S-adenosyl-L-methionine (SAM) by the SAM synthetase, SAM is then converted to either 5'-methylthioadenosine, which can be recycled, or to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS). ACC is then converted to ethylene by ACC oxidase (Buchanan *et al*., 2000). Methionine quantities within plants are too low to sustain normal rates of ethylene production, so it is therefore recycled by the plant. This recycling of methionine is called the methionine cycle or Yang cycle after S. F. Yang who performed much of the early work in the elucidation of the pathway (Abeles *et al*., 1992) which is summarized below:



Stress Conditions Known to Increase Ethylene Biosynthesis

Many physiological effects of ethylene on plants such as seed germination, root and shoot growth, flower development, etc. were reported in early studies, but not until the 1960's did ethylene emerge as an important plant hormone when many other aspects of ethylene production and biosynthesis were found to be of interest due to advances in technology. When this hormone began to be studied extensively, both abiotic and biotic stress conditions were found to increase the ethylene production of plants and this increase in ethylene biosynthesis due to these stress conditions was termed "stress ethylene".

Abiotic stress conditions which stimulate ethylene production include chilling and freezing, heat, flooding, drought, chemical, radiation, mechanical, and bending (Abeles *et al*., 1992). McMichael *et al*. (1972) reported an increase in ethylene production during drought conditions in cotton plants. They noted that sharp increases in ethylene production were found during severe water deficit, but when the plants were watered, ethylene production rates fell rapidly. Similarly, Wright (1977) looked at the relationship between leaf water potential, a function of cell turgidity in the leaf, and ethylene production in wheat leaves. In this study, leaves were allowed to wilt until they had lost 2 to 8% of their fresh weight and ethylene measurements were taken every 135 min for the first 675 min and a final sampling at 24 hrs. Ethylene biosynthesis was found to be greatest in the most severely stressed leaves.

More recently in *Arabidopsis*, Larkindale and Knight (2002) compared oxidative damage and survival rates after heat-induced damage was applied to both an ethylene-insensitive mutant *etr-1* (ethylene response), which has a defective ethylene receptor

subunit and consequently insensitive to ethylene, and wild-type plants. After the heat-induced oxidative damage was performed, ethylene was added exogenously and a thiobarbituric acid-reactive substance (TBARS) assay was performed, which is a common assay for oxidative damage to membranes that measures lipid peroxidation in plants after heating. Thus, higher levels of TBARS are found in plants that are subject to higher levels of oxidative stress (Larkindale and Knight, 2002). The authors found that the *etr-1* mutants in these experiments showed an increased TBARS (>3 fold increase) value and a reduced survival rate (approximately 50%) as compared to the wild type. These values support the hypothesis that ethylene is used by *Arabidopsis* to mediate protection against, or repair of, heat-induced stress (Larkindale and Knight, 2002) and that abiotic stress conditions stimulate ethylene production for protection.

Biotic stress conditions which stimulate ethylene production include infection by viruses, bacteria, fungi, insects, and nematodes (Abeles *et al*., 1992). Lund *et al*. (1998) used tomato plants to determine when and if ethylene has an effect on a plant's defense, specifically leaf necrosis caused by bacterial infection. In this study, an ethylene-insensitive mutant of tomatoes, *Never ripe* (*Nr*), in which the fruit of the plants exhibit only a yellow color and marginal softening after several months of growth, was inoculated with the bacterial pathogens *Xanthomonas campestris vesicatoria* and *Pseudomonas syringae tomatoi*, both of which first cause lesions and later necroses and abscission, and compared to wild type inoculated plants (Lund *et al*., 1998). The authors found that at first there was no visible difference between the number and size of lesions of both the *Nr* and wild type plants. However, 16 days after inoculation, foliar disease symptoms were greatly reduced in the *Nr* leaves when compared to the wild type plants.

The authors then measured electrolyte leakage, which is a quantitative measure of cell injury or death resulting from pathogen infection, to determine the level of cell injury or death resulting from pathogen infection. The authors compared *Nr* and wild type leaves that were inoculated with either *X. c. vesicatoria* or *P. s. tomato* and found that, for both experiments, the *Nr* leaves inoculated with the pathogens had a fourfold decrease in mean electrolyte leakage as compared to the wild type. These findings supported that a reducing effect for ethylene insensitivity on foliar disease development hampers the affects that pathogens cause on the *Nr* leaves. From their study, Lund *et al*. (1998) concluded that foliar disease development can be separated into two stages, the first includes lesion formation that is not affected by endogenous ethylene and the second stage where leaf necrosis develops and requires endogenous ethylene production. These results demonstrate that ethylene plays a significant role in leaf necrosis and that biotic stress conditions stimulate ethylene production for protection.

Regulation of Ethylene Biosynthesis (Transcriptional and Post-transcriptional)

Ethylene production can be regulated at both the transcriptional (synthesis of ribonucleic acid, RNA) and post-transcriptional levels. Since the primary rate-limiting step in the ethylene biosynthetic pathway has been found to be the conversion of SAM to ACC by the protein ACS (reviewed in Rieu *et al*., 2005) and because the level of ACS activity closely parallels the level of ethylene production in most plants (Chae *et al*., 2003), the understanding of the regulation by the ACS protein, both transcriptional and post-transcriptional, is important in determining the biosynthesis of ethylene production by plants.

Typically, ACS is part of a multigene family that encodes different ACS protein isoforms. In *Arabidopsis*, there are nine different ACS forms which produce functional and nonfunctional homodimers (reviewed in Tsuchisaka and Theologis, 2004). In tomato, seven different ACS forms have been identified and found to be differentially expressed (reviewed in Shiu *et al*., 1998). The biological significance for such multigene families, ACS in particular, is unknown (Tsuchisaka and Theologis, 2004). The primary sequence encoded by these genes shows a conservation ranging from 50 to 96% in amino acid sequence identity, with the highest variability at the caroboxy end of the protein (reviewed in Tsuchisaka and Theologis, 2004). In some cases, the expression of specific ACS protein forms is differentially regulated in response to internal or external factors, or alternatively, one ACS member could respond to several different developmental signals (reviewed in Ge *et al*., 2000). For example, in tomato, the *Lycopersicon esculentum* (Le) ACS2 gene is expressed in root, ripe fruit, stamens, and floral senescence. In Geraniums, the *Pelargonium hortorum* (Ph) ACS2 gene is induced in young leaf, leaf bud, stem, and

roots (reviewed in Ge *et al*., 2000). It is also speculated that regulation of ACS could be controlled by a single developmental cue which can induce a coordinated expression of several ACS genes. For example, *Le-ACS2* and *Le-ACS4* genes are simultaneously induced in the pericarp tissue (the wall) of ripening fruit (reviewed in Ge *et al*., 2000). The search to understand all the multiple facets of how ACS effects the biosynthesis of ethylene has lead to an abundance of articles on this topic.

In a study to determine the effect of multiple environmental stress conditions (wounding, light, and chilling) on the regulation of a single ACS gene, Ge *et al*. (2000) identified four new ACS cDNA fragments of tobacco, *Nicotiana tabacum* (*Nt-ACS2*, *Nt-ACS3*, *Nt-ACS4*, and *Nt-ACS5*), along with a previously isolated ACS tobacco gene (*Nt-ACS1*). The authors used a Northern blot analysis to determine when and which of the different ACS isoforms were expressed when subjected to the multiple conditions. For wounding, Ge *et al*. (2000) found that the transcript levels of three genes, *Nt-ACS2*, *Nt-ACS3*, and *Nt-ACS5*, peaked at 6 hrs and declined by 10 hrs after wound induction, while *Nt-ACS4* expression increased to a significant level at 1 hr, reached peak accumulation by 2 hrs, and disappeared by the sixth hour. For light, Ge *et al*. (2000) found that the transcript level of *Nt-ACS2* increased transiently and peaked 0.5 hrs after light induction, and dropped to basal level by 1 hr after light induction, while no other *ACS* transcripts were detectable after light induction. For chilling, Ge *et al*. (2000) found that the level of only the *Nt-ACS2* transcript increased and after 24 hrs of chilling induction increased to a higher level. From their results, Ge *et al*. (2000) demonstrated that multiple stimuli can induce several ACS isoforms simultaneously, such as wounding up-regulating three ACS isoforms, or only a single ACS isoform can be up-regulated, such as chilling only up

regulating ACS2. These findings and others lead to much research needing to be done to elucidate how each specific ACS isoform is regulated.

In *Arabidopsis*, post-transcriptional regulation of ACS has been documented. Woeste *et al*. (2003) described the physiological characterization of etiolated *Arabidopsis* seedlings and found that ACS is regulated by other means than transcriptional regulation. Woeste *et al*. (2003) used *eto1* and *eto3* (ethylene overproducer) mutant etiolated seedlings which cause a triple response in adult *Arabidopsis* plants. This triple response in *Arabidopsis* consists of shortening and radial swelling of the hypocotyl, inhibition of root growth, and exaggerated curvature of the apical hook (Woeste *et al*., 2003). The authors first determined that *eto1* and *eto3* mutants displayed the triple response phenotype as etiolated seedlings, where as wild-type did not, and determined this was caused by an overproduction of ethylene (Woeste *et al*., 2003). The authors then looked at the level of ACS activity within the *eto1* and *eto3* mutants compared to wild-type. The level of ACS from crude extracts of wild-type, *eto1*, and *eto3* were assayed. Woeste *et al*. (2003) found that both mutants showed high elevated levels of ACS activity compared to the wild-type, which indicates the increase in ACS activity may be responsible for the elevated ethylene biosynthesis observed in the mutant seedlings. Finally, Woeste *et al*. (2003) compared several *ACS* mRNA (*ACS4*, *ACS5*, *ACS6*, and *ACS7*) levels from both wild-type and the mutant etiolated seedlings by northern blotting. The authors found that the steady-state levels of expression from the mutants was close to the steady-state level of the wild-type, and for *ACS2* and *ACS4* comparison, the mutant steady-state level was actually lower than the wild-type steady-state level of *ACS* (Woeste *et al*., 2003). From these findings, when the *ACS* level increases, ethylene production increases, but the

regulation is not from translational regulation, but most likely from some type of post-translational regulation (Woeste *et al*., 2003).

A further study to determine the mechanism of post-transcriptional regulation of ACS was conducted by Chae *et al*. (2003) using the *eto3, eto2,* and *eto1* mutations which are located within different ACS genes. The authors found that ethylene biosynthesis is controlled by regulation of the stability of ACS, mediated in part through the C-terminal domain (Chae *et al*., 2003). To confirm this finding, Chae *et al*. (2003) sequenced *Arabidopsis* genomic DNA, and the *eto3* mutant was found to have a T-to-A transversion within the C-terminus of the ACS protein (Chae *et al*., 2003). To determine if this C-terminus change was responsible for the overproducing phenotype of *eto3,* 3.2-kb genomic DNA fragments containing either wild-type *ACS9* ($ACS9^{WT}$) or the *eto3 ACS9* ($ACS9^{eto3}$) coding region and flanking sequences were cloned into a plant transformation vector and introduced into wild-type *Arabidopsis*. All of the $ACS9^{eto3}$ transformants displayed the triple response as etiolated seedlings, and the ethylene production was highly increased over the nontransgenic, wild-type seedlings. The $ACS9^{WT}$ seedlings displayed a wild-type phenotype with ethylene production levels similar to those of the wild-type, nontransgenic plants. Chae *et al*. (2003) then used real-time reverse transcriptase-mediated PCR to show that *ACS9* mRNA levels from *eto3* were comparable to that of wild-type, indicating that the steady state level of *ACS9* transcription in the *eto3* mutant seedlings were the same as wild-type. Thus, the authors concluded the *eto3* mutation affects ethylene biosynthesis through post-transcriptional regulation of ACS (Chae *et al*., 2003).

To further evaluate the mechanism of ethylene overproduction by *eto2*, Chae *et al*. (2003) looked at the stability of the ACS5 protein in *eto2* mutants. They first compared levels of ethylene produced by $ACS5^{WT}$ and $ACS5^{eto2}$ transgenic plants which were quantified by immunoblot analysis. The authors found that the transgenic plants produced approximately equal levels of ethylene at comparable levels of expression of the fusion proteins indicating that the specific activity of the $ACS5^{eto2}$ is not significantly different from the $ACS5^{WT}$. Therefore, they speculated that the stability of ACS5 could be affected by the *eto2* mutation (Chae *et al*., 2003). To test this, Chae *et al*. (2003) determined the half-life of $ACS5^{WT}$ and $ACS5^{eto2}$ by measuring the level of proteins after the inhibition of protein synthesis by cycloheximide-containing MS medium. The level of ACS^{WT} was found to decline rapidly with a half life of 15 min, and reached a minimal level at 45 min after cycloheximide application and from there remained stable (Chae *et al*., 2003). There was a minimal decrease in $ACS5^{eto2}$ protein levels, even 2 hrs after cycloheximide treatment, indicating a much longer half life (Chae *et al*., 2003). Chae *et al*. (2003) concluded that the change in *eto2* increased the ACS5 function by increasing the protein stability. The authors also found that the *eto1* mutation also increases the ACS5 function by increasing the protein stability, but to a lesser extent compared to the *eto2* mutation (Chae *et al*., 2003). These results, along with Woeste *et al*. (2003), demonstrate that an important mechanism by which ethylene biosynthesis is controlled is through post-transcriptional regulation, mainly the stability of ACS.

Hypoxia Effects on Plant Growth and Induction of Elevated Ethylene

In many plants, flooding of the roots results in oxygen deprivation and greatly increases production of ethylene in the leaves (Jackson, 2002). Anaerobic conditions in plant roots inhibit the oxygen-requiring enzyme, ACC oxidase, which catalyzes ethylene production from its immediate precursor, ACC. As a result, ACC accumulates in the roots and is then transported by the vascular system to the stems and leaves where it is rapidly converted to ethylene. Anaerobic conditions also stimulate the synthesis of ACC in the roots, contributing to more ACC to be transported to the leaves. Consequently, higher levels of ethylene in leaves appear to be able to stimulate ACC oxidase synthesis and activity, further increasing ethylene production.

Hydroponic Cultivation of Strawberries

With the worldwide phaseout of methyl bromide as a soil fumigate, the use of hydroponic systems has rapidly increased as an economic alternative for the growth of many horticulturally-important crops (Environmental Protection Agency, 1997; Carpenter *et al*., 2000; VanSickle *et al*., 2000; Federal Register, 2004). As a soilless system, hydroponics eliminates competing weeds and soil-born pests, thus reducing the need for pesticides and avoiding toxic residues that may accumulate in plants. In addition, hydroponic cultivation conserves water and provides conditions that can be quickly altered to suit specific crops. Hydroponic systems provide an economical and viable alternative for the cultivation of strawberry, a crop that has been particularly dependent upon methyl bromide fumigation (Stanley, 1998).

Stress Factors Associated with Hydroponic Cultivation (Strawberries)

In hydroponically-grown plants, stress-induced physiological conditions may arise within the system if nutrient flow is inconsistent, resulting in some plants receiving unequal water supplies. For example, flooding of root systems causes oxygen intake deficiency and interferes with nutrient uptake (Urrestarazu and Mazuela, 2005). Flooding also causes accumulation of high levels of ethylene which may inhibit growth, cause premature ripening, and induce the onset of senescence, potentially reducing plant productivity (Abeles *et al*., 1992; Druege, 2006). In addition to the effects of flooding on ethylene production, other environmental factors such as wounding, light, and temperature may increase ethylene levels in plants. Therefore, careful management of hydroponic systems has become an important consideration for reducing stress conditions that negatively impact yield, in order to increase market profitability by decreasing cultivation costs.

Experimental Objectives

Because stress conditions on plants produce an increase in ethylene levels, ethylene measurements may be a useful tool for identifying conditions that impact plant growth. Therefore, this study was to demonstrate that measurements of ethylene production from leaves of hydroponically-grown strawberry plants could be used as an early indicator of stress conditions within a hydroponic system. This method could be used to identify inconsistencies within a hydroponic system that may cause plant stress and affect subsequent plant growth and fruit production. In addition, changes in *ACS* gene expression levels may also increase resulting from hypoxic conditions within hydroponic systems. The experimental objectives of this study were to:

1. Evaluate ethylene biosynthesis as a stress-indicator of hydroponically-grown strawberries.
2. Determine the transcriptional regulation of ethylene biosynthesis during stress (hypoxic) conditions in the vegetative parts (leaves) of strawberry plants.

CHAPTER III

Materials and Methods

Hydroponics System and Plant Growth Parameters

The hydroponic systems and strawberry growth conditions were designed in collaboration with Dr. Fumiomi Takeda (USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV, USA), and adapted from his procedures (Takeda, 1999). The strawberry cultivar Chandler (*Fragaria X ananassa*), a short day (SD) cultivar (flowers under short days), was used in all experiments except experiment 1, system 1. This cultivar was chosen because it is a day-length neutral cultivar selected for yield and flavor and allows experimentation throughout the year. Chandler strawberries were either purchased as plants (Davon Crest Farm, MD, USA) or grown from runners (Strawberry Tyme Farms, Ontario, Canada) with experimental results not influenced by experimentation on either plants or runners. For experiment 1, system 1, 5 different cultivars were used which included Fern, Honeyoye, Fort Laramie, Tribute, and Quinault.

All plants, prior to planting within the hydroponic systems, were cold treated in a refrigerator for 6 weeks at 4°C to stimulate flowering and condition the plants for growth in the hydroponic systems. Runners were rooted under a misting bench. Each hydroponic system consisted of 10 trays containing 3 plants each (30 plants total), connected to a central nutrient delivery pipe. Plants or rooted runners were planted in 15.24 cm circular net pots with commercial peat-based soilless planting mixture (Premier Horticulture Inc., Red Hill, PA, USA). Pots were placed in Hydroware™ trays (106 x 20.32 x 10.16 cm deep, Sea of Green, Tempe, AZ, USA) lined with plastic screening.

Sifted perlite was placed around the pots and white-on-black plastic mulch (Garden Indoors, Columbus, OH, USA) was lowered over the trays and around the plants to control evaporation and algal growth.

The nutrient solution of commercial fertilizer (Scotts HydroSol Water Soluble Fertilizer 5N-11P-26K), Epson salts ($MgSO_4$), 0.64 $g \cdot l^{-1}$, calcium nitrate ($CaNO_3$), and 0.015 $g \cdot l^{-1}$ ferric chloride ($FeCl_3$) with pH adjusted to 6.2 if necessary, was circulated through the systems by a submersible fountain pump located in a 55 l container. The solution ran through the central nutrient delivery pipe into 1.3 m of irrigation row drip tape per tray with 10 cm emitter spacing (RO-DRIP, Roberts Irrigation Products, San Marcos, CA, USA). The drip tape lay over the perlite and pots and under the plastic mulch of each tray. Pressure in the central pipe and the attached drip tape was controlled by a valve adjacent to the tray furthest from the pump, so that the drip tape was completely expanded over all trays to deliver 5 $l \cdot h^{-1} \cdot m^{-1}$. Troughs were inclined to approximately a 15° angle to aid drainage, with the higher end located at the delivery pipe. Excess solution from the central delivery pipe and each tray was collected by gravity back to the main 55 l container (Fig. 1).

Plants were allowed to acclimate to the hydroponic systems for a minimum of two weeks prior to experimentation. The nutrient solution was changed every 7-10 days to maintain nutrient concentrations, regulate pH, and minimize salt accumulation. Experiments were conducted from October to March under natural short day photoperiod, and supplemented by high pressure sodium lights (1000-W) up to 14 hrs per day. No pesticides were used on the plants during experimentation.

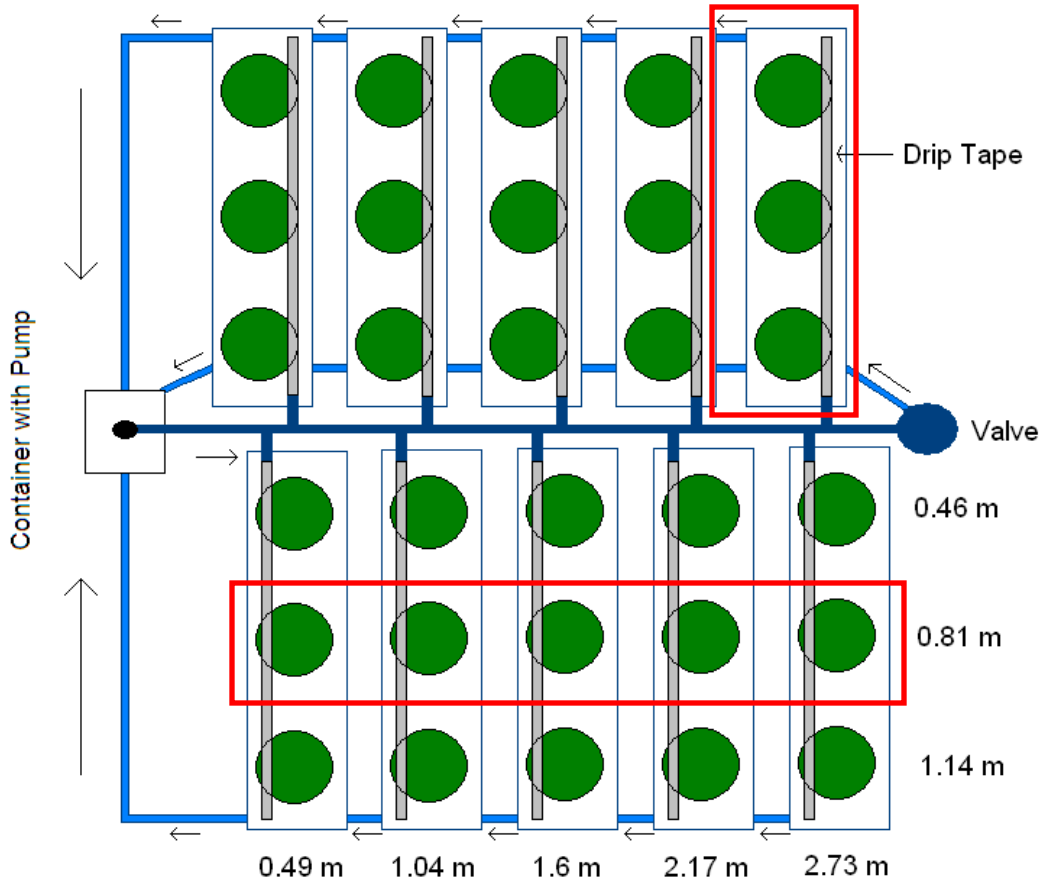


Figure 1. Schematic representation of hydroponic system design with 10 trays containing 3 plants each (30 total). The 55 l container with pump is located at one end of the system and the valve which controls flow rate at the opposite end. Arrows indicate the nutrient flow direction. The relative position of each tray from the pump (0.49, 1.04, 1.6, 2.17, and 2.73 m), position of individual plants from the central nutrient delivery pipe (0.46, 0.81, and 1.14 m), and position of drip tape over each pot is depicted (white-on-black plastic mulch covers tray and drip tape, and is placed around plants). The plants studied for distance from delivery pipe represented 10 plants (5 plants each side of system) and the plants studied for distance from pump represented 6 plants (3 plants each side of system) for the average ethylene production for the respective distances which are depicted by the red boxes.

Figure 2. Hydroponic system within Marshall University's greenhouse.

Ethylene Measurements

Ethylene measurements were obtained from a single excised leaflet of each plant that was folded down the midrib, and rolled to fit into a 2 ml shell vial. Vials were capped with a rubber septum and kept at room temperature for 30 min to allow accumulation of ethylene prior to the onset of wound-induced ethylene. Wound-induced ethylene due to excision was found to begin 40-60 min after leaflet excision, thus, the 30 min incubation time was adequate to measure the stress-induced ethylene (data not shown). To measure the amount of ethylene released by the strawberry leaf within the vial, a 1.0 ml headspace sample was extracted from the vial with a syringe and injected onto an alumina F1 column (0.635 cm X 0.91 m) in a gas chromatograph (Varian 3700, Varian Instrument Division, Walnut Creek, CA, USA) equipped with a flame ionization detector according to the procedure described by Harrison (1997). The nitrogen carrier gas flow rate was 40 $ml \cdot min^{-1}$ and the oven temperature was maintained at 100ºC. Hydrogen and air flow rates to the detector were 40 $ml \cdot min^{-1}$ and 300-400 $ml \cdot min^{-1}$ respectively, and the detector temperature was set at 150°C. Known amounts of an ethylene standard gas (Scott Specialty Gases, Plumsteadville, PA, USA) were analyzed to produce a standard curve for ethylene quantification.

System Analyses

Experiment 1 (Analysis of ethylene production): In 2002, system-wide analyses of three hydroponic systems were conducted to evaluate the effect of daily fluctuations in light intensity and temperature associated with greenhouse growth conditions on ethylene production. Also, ethylene production relative to the central delivery pipe and pump was determined from excised leaflets, along with identification of potential stress-induced ethylene production. For all systems, light was measured using a Basic Quantum Meter (Apogee Instruments Inc., Logan, UT, USA), which measured photosynthetic active radiation (PAR) in µmol (photons)$\cdot m^{-2} \cdot s^{-1}$. Temperature was measured using a thermometer and readings from both instruments were placed beside the plant at the time of excision.

Plant Set 1: A set of system-wide ethylene measurements containing different cultivars was recorded on February 5, 7, 14, and 15, 2002. This system consisted of 5 cultivars, with each cultivar contained in two trays (6 plants/cultivar). The trays of each cultivar were located across from each other corresponding with the distances from the pump. The cultivars were Fern, Honeyoye, Fort Laramie, Tribute, and Quinault located at 0.49 m, 1.04 m, 1.6 m, 2.17 m, and 2.73 m.

Plant Set 2: A set of system-wide ethylene measurements of Chandler leaflets was recorded on February 22, 28, and March 1, 2002. A second set of system-wide measurements of Chandler leaflets was recorded on March 28, 2002.

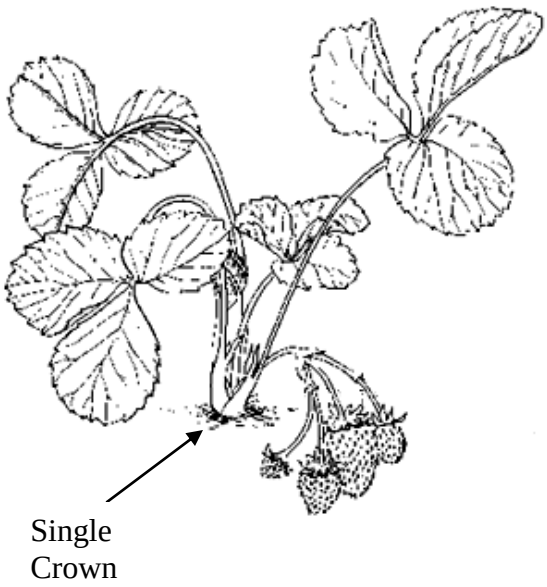
Plant Set 3: A set of system-wide ethylene measurements of Chandler leaflets was recorded on March 5, 12, and 14, 2002. A second set of system-wide measurements of Chandler leaflets was recorded on March 27 and 28, 2002.

Experiment 2 (Analysis of growth and yield): In 2003, two system-wide evaluations were conducted to determine the ethylene production and the growth and yield of strawberry plants grown under ambient greenhouse conditions. Ethylene production relative to the location of the central delivery pipe and pump was determined from excised leaflets. The system consisted of the cultivar Chandler, with trays located across from each other corresponding with the distances from the pump (0.49 m, 1.04 m, 1.6 m, 2.17 m, and 2.73 m). The ethylene measurements were accompanied by measurements of: flower bud number, inflorescence number, crown number, and plant radius (Fig. 3).

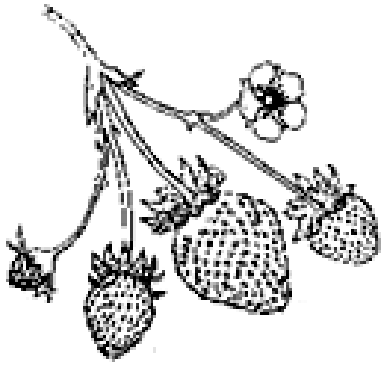
Plant Set 4: A set of system-wide ethylene measurements of Chandler leaflets for plant distance was recorded on February 10 and 13, 2003.

Plant Set 5: Growth and yield measurements of Chandler strawberries were recorded on February 3, March 10, and April 7, 2003.

A)



B)



Prentice-Hall, 1990

Figure 3. (A) Diagram of strawberry plant showing a single crown along with an inflorescence with fruit. (B) Inflorescence of a strawberry plant with a flower bud and 4 fruit.

Experiment 3 (Effect of flooding on ethylene production and growth and yield): In 2003, plant set 6 was used to evaluate the effect of flooding on ethylene production. Two trays were flooded from April 7-April 10 (72 hours). To establish the effect of flooding on ethylene production, measurements were taken before flooding, 24, 48, and 72 hrs while flooded, and 24 hrs after the water was allowed to drain. The trays were flooded by blocking the drainage hole and filling the trays to the top with tap water.

A separate system, plant set 7, was used to evaluate the effect of flooding on flower bud number, inflorescence number, crown number, and plant radius. For this experiment, five trays, on the same side of the system, were allowed to drain at a normal rate, while five trays on the other side of the system were flooded (February 3) with tap water. The trays were filled to the top by blocking the drainage holes. Water was allowed to drain after the 24 hr flooding treatment (February 4).

Plant Set 6: Ethylene measurements from two trays of Chandler plants that were flooded for 72 hrs were recorded on April 7-11, 2003.

Plant Set 7: Growth and yield measurements of Chandler strawberries comparing the flooded to the normal drainage control trays (15 plants/treatment) were recorded on February 3, March 10, and April 7, 2003.

Gene Expression Analysis

Total RNA Isolation (Determination of *ACS* levels): Once the effect of flooding on ethylene production and growth and yield data was determined, potential gene expression changes of the *ACS* gene in strawberry leaves was examined. Total RNA was isolated using three different protocols to determine which would produce adequate yield and purity. For each protocol, excised strawberry leaflets (100 mg) were first frozen in liquid nitrogen and ground to a powder. The Purescript RNA Isolation Kit™ (Gentra Systems, Minneapolis, MN, USA) protocol was followed according to the manufacturer's recommendation except that the Cell Lysis Solution was first added to the mortar for continued grinding of the powdered tissue instead of being placed directly into a tube pestle for grinding. Also, *Arabidopsis* was used with this protocol for a control. The RNEasy Plant Mini Kit (Qiagen, Valencia, CA, USA) protocol was followed according to the manufacturer's recommendation. The TRI REAGENT (Sigma, St. Louis, MO, USA) protocol was followed according to the manufacturer's recommendation except that liquid nitrogen was used to homogenize the leaflets prior to adding the tissue to the TRI REAGENT as opposed to just directly homogenizing the leaflets in TRI REAGENT.

RNA was quantified by O.D. 260 and purity was evaluated by O.D. 260/280 ratio using a ND-1000 spectrophotmeter (NanoDrop Technologies, Wilmington, DE, USA). RNA quality from the Purescript and RNEasy protocols was also evaluated using RT-PCR with the various primers from Table 1. Reaction mixtures of 10 µl using the AccessQuick RT-PCR System (Promega Corp., Madison, WI, USA) protocol was used according to the manufacturer's recommendation with the following mixture: 5 µl 2X master mix, 0.2 µl AMV RT (Avian Myeloblastosis Virus Reverse Transciptase), 0.5 µl

left primer, 0.5 µl right primer, and 3.8 µl RNA. Amplifications were carried out using an iCycler (Bio-Rad, Hercules, CA, USA) with the following temperature parameters: 45 min at 48°C and 2 min at 94°C (to terminate the reverse transcription reaction) followed by 40 cycles (for PCR): 30 sec at 94°C, 1 min at 60°C, 2 min at 68°C. After the 40 cycles, an optional final 7 min extension at 68°C was performed and a final soak at 4°C for 1 hr to overnight. RNA isolation from the RNEasy Plant Mini Kit and TRI REAGENT procedures were separated by electrophoresis on a 2.5% (w/v) NuSieve 3:1 agarose gel (Cambrex BioScience, Rockland, ME, USA). DNA obtained from RT-PCR by the Purescipt procedure was separated on a 4% agarose gel. Both types of gels were run in 1X TAE (Tris-Acetate-EDTA) from a 50X stock solution of TAE (Bio-Rad, Hercules, CA, USA) and stained with ethidium bromide. Gels were imaged on a GelDoc (Bio-Rad, Hercules, CA, USA).

Table 1. Primer pairs for RT-PCR analysis of genes in *Arabidopsis* (At-) and Strawberry (Fa-). Accession numbers are to denote the reference within GenBank.

ACS genes	Acc. #	Primer pairs (location)	Product size (bp)	Anneal. Temp (°C)
At-S18 rRNA	X16077	5'GTGCATGGCCGTTCTTAGTT 3'(1359-1378) 5'ACCGGATCATTCAATCGGTA 3'(1739-1758)	400	60.14 60.16
Fa-RP	U19940	5' GCCATTTGCTGGATCTTCTC 3' (143-162) 5' AACCCAGCAATCAACACCTC 3' (321-340)	198	59.78 59.97
Fa-S18 rRNA (At primers)	X15590	5'GTGCATGGCCGTTCTTAGTT 3' (1272-1291) 5'ACCGGACCATTCAATCGGTA 3' (1648-1667)	396	60.14 62.41

Northern Analysis of *ACS* Expression (Determine presence of *ACS*): Because no sequence exists for any strawberry *ACS* genes, BLAST was used with the *At-ACS9* (GenBank accession no. AF332391) sequence, a known hypoxia regulated *ACS* form, to identify similar sequences of plant species within the same order (Rosales) as strawberry. From the BLAST analysis, ClustalW software was used to develop a phylogentic tree (Fig. 4). These sequences were used to locate a highly conserved region from the various *ACS* sequences to determine a possible probe sequence that could be used with northern blotting. A conserved sequence was identified at position 859 to 919 of *At-ACS5* (Fig. 5).

Although a strawberry probe was not ordered, RNA was extracted using the CelLytic™ PN protocol (Sigma, St. Louis, MO, USA) followed by the North2South Direct HRP Labeling and Detection Kit (Pierce, Rockford, IL, USA) protocol which were both used according to the manufacturer's recommendations with a probe sequence for 18S *Arabidopsis* rRNA of 5'-AATGAGTACAATCTAAATCCCTTAACGAGGATCC ATTGGAGGGCAAGTCTGGTGC-3'. This probe was used on both strawberry and *Arabidopsis* (control) to determine if strawberry would show results since this region is highly conserved.

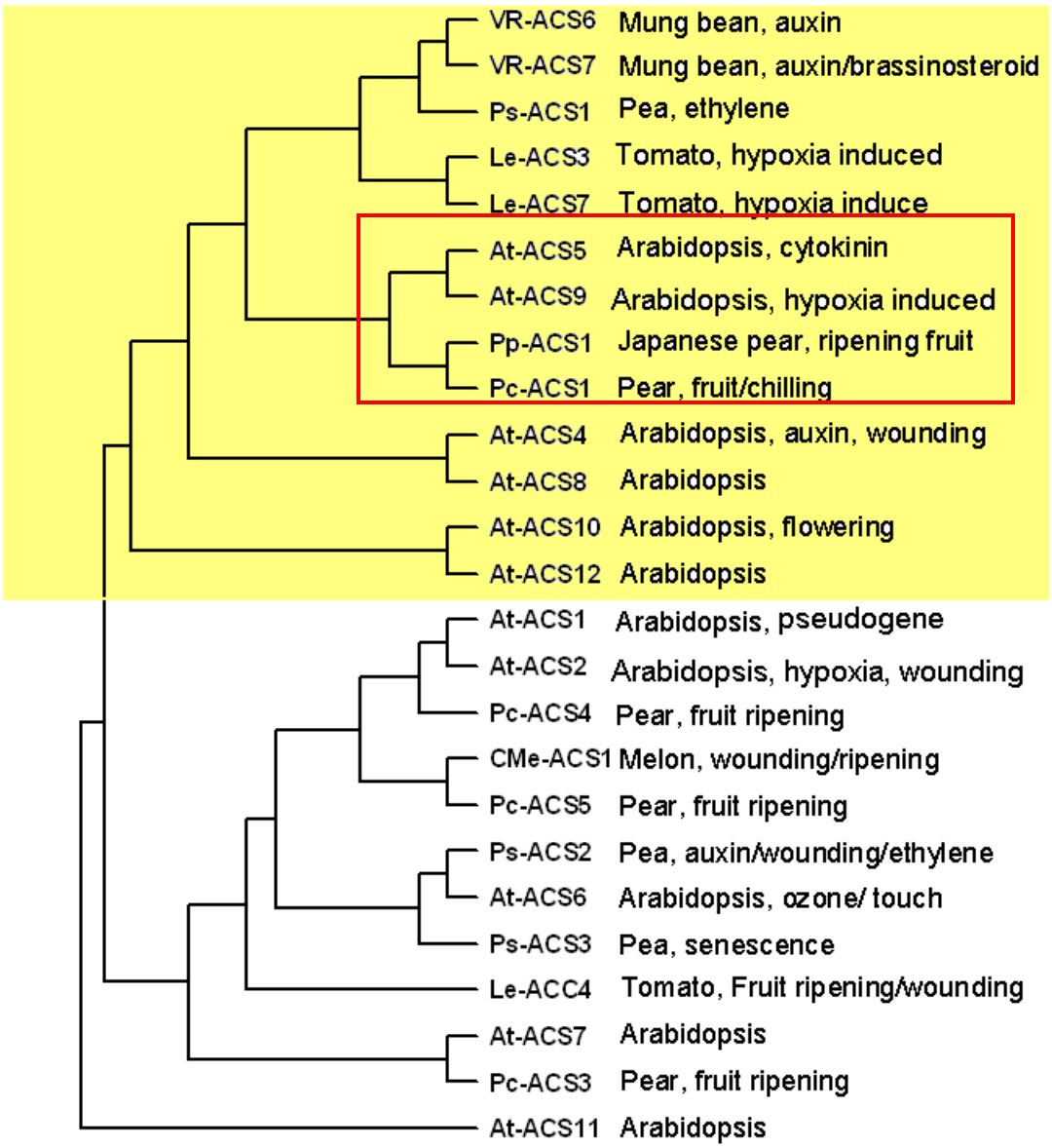


Figure 4. Sequence alignment of *Arabidopsis* and selected cDNA sequences of ACC synthase was performed using ClustalW. *Arabidopsis thaliana***:** *At-ACS1*, U26543; *At-ACS2*, AF334719; *At-ACS4*, AF332404; *At-ACS5*, L29261; *At-ACS6*, AF361097; *At-ACS7*, AF332390; *At-ACS8*, AF334712; *At-ACS9*, AF332391; *At-ACS10*, AF348575; *At-ACS11*, AF332405; *At-ACS12*, AF336920. *Cucumis melo*: *CMe-ACS1*, AB025906. *Lycopersicon esculentum*: *Le-ACS3*, L34171; *Le-ACS4*, M88487; *Le-ACS7*, AF179248. *Pisum sativum: Ps-ACS1*, AF016460; *Ps-ACS2*, AF016459; *Ps-ACS3*, AB049725. *Pyrus pyrifolia: Pp-ACS1*, AB015624. *Pyrus communis: Pc-ACS1*, X87112; *Pc-ACS3*, AF386519; *Pc-ACS4*, AF386518; *Pc-ACS5*, AF386523. *Vigna radiata*: *Vr-ACS6*, AB018355; *Vr-ACS7*, AF151961. Yellow box indicates the subgroup composed of primarily hormone and chilling-inducible forms. Red box indicates target genes for sequence comparison.

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PpACS1    ACGACGACATGGTTGTGGCCGCCGCTACAAAAATGTCAAGCTTTGGTCTTGTTTCTTCTC 1008
PcACS1    ACGACGACATGGTTGTGGCCGCCGCTACAAAAATGTCAAGCTTTGGTCTTGTTTCTTCTC 1020
AtACS5    ACGACGAAATGATCGTTTCAGCAGCTACAAAAATGTCAAGTTTTGGTCTTGTTTCTTCTC 919
AtACS9    ACGACGAAATGGTTGTTTCCGCTGCAACAAAAATGTCAAGTTTCGGTCTCGTGTCTTCTC 919
          ******* *** * **  * ** ** ************** ** ***** ** *******
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Figure 5. Nucleotide sequence comparison using ClustalW of four *ACS* genes from Fig. 4: *Arabidopsis* (*At-ACS5* and *At-ACS9*), Pear (*Pc-ACS1*), and Japanese Pear (*Pp-ACS1*). A possible probe could be used with strawberry from this segment since a long conserved strand is present (indicated by * which represents a match for all four of the nucleotides at their respective positions).

Statistical Analyses

Significance between the means was determined by one-way ANOVA (Microsoft Excel) to evaluate differences in groups of plants. Levels of significance are represented by $P \leq 0.05$. Regression analysis was used to determine significant correlation between light intensity or temperature and ethylene production and different positions in regards to distance from the delivery pipe or pump (Microsoft Excel).

CHAPTER IV

Results

System Analysis

Experiment 1: Evaluation of Strawberry Ethylene Production within the Hydroponic Systems

System-wide analyses were conducted to determine whether temperature or light variations would have an impact on the ethylene production in strawberry leaves under ambient greenhouse conditions. Along with the temperature and light impact, potentially stress-induced plants and positions were identified. We defined "potentially stress-induced" plants as plants producing more than twice the system average of ethylene. To determine potentially stress-induced positions within the system, the average ethylene production from plants located at the same distance from the central delivery pipe (10 plants/position) or pump (6 plants/position) was determined for all plant sets.

Compiled Light and Temperature Measurements:

Plant set 1: Temperatures ranged from 15-37°C and a maximum light intensity of 740 µmol photons $\cdot m^{-2} \cdot s^{-1}$ (PAR). The average ethylene production for the entire system was 27.16 $pl \cdot g^{-1} \cdot min^{-1}$ with ethylene production ranging from 5.84 to 180.90 $pl \cdot g^{-1} \cdot min^{-1}$ (Table 2 and 3).

Plant set 2: For analysis 1, temperatures ranged from 15-35°C and a maximum light intensity of 1508 µmol photons$\cdot m^{-2} \cdot s^{-1}$ (PAR). The average ethylene production for the entire system was 28.54 $pl \cdot g^{-1} \cdot min^{-1}$ with ethylene production ranging from 9.52 to 72.19 $pl \cdot g^{-1} \cdot min^{-1}$ (Table 2 and 3). For analysis 2, temperatures ranged from 20-26°C and

a maximum light intensity of 582 µmol photons·m^{-2}·s^{-1} (PAR). The average ethylene production for the entire system was 28.67 pl·g^{-1}·min^{-1} with ethylene production ranging from 8.38 to 165.32 pl·g^{-1}·min^{-1} (Table 2 and 3).

Plant set 3: For analysis 1, temperatures ranged from 18-30°C and a maximum light intensity of 1075 µmol photon·m^{-2}·s^{-1} (PAR). The average ethylene production for the entire system was 20.25 pl·g^{-1}·min^{-1} with ethylene production ranging from 10.36 to 89.46 pl·g^{-1}·min^{-1} (Table 2 and 3). For analysis 2, temperatures ranged from 20-29°C and a maximum light intensity of 1467 µmol photon·m^{-2}·s^{-1} (PAR). The average ethylene production for the entire system was 42.06 pl·g^{-1}·min^{-1} with ethylene production ranging from 9.23 to 155.16 pl·g^{-1}·min^{-1} (Table 2 and 3).

After the five plant sets' data was compiled, one measurement was found to have a statistical significance, Plant Set 2, analysis 2 between ethylene production and light (P=0.01, Table 3). The whole analysis included potentially stress induced measurements which could have contributed to the measurement being significant because after the potentially stress-induced values were removed, most *P*-values increased, indicating less correlation (Table 3). One *P*-value did decrease to a statistical significance of P=0.05 (Plant Set 3, analysis 2) between ethylene production and light. However, for this measurement the light intensity range was 165-1467 µmol photon·m^{-2}·s^{-1} (PAR), which indicates an extremely high consistent light intensity (Table 3). From the compiled temperature and light intensity data we concluded neither temperature nor light had an effect on the plants that would lead to potentially stress-induced ethylene production from the strawberry plants (Table 2 and 3). Since temperature and light did not have a significant effect on the plants, the ambient greenhouse conditions could be ignored, and

the effect of the systems from different locations could be measured to determine the effect on the plant's ethylene production.

Table 2. Compiled results for Experiment 1 depicting the correlation between temperature (°C) and ethylene production for individual plants in systems built in 2002. For both the ethylene and temperature ranges, "With Stress" indicates measurements with potentially stress-induced plants included and "Without Stress" indicates measurements without potentially stress-induced plants.

* $P \leq 0.05$.

Plant Set Number (dates measured in 2002)	Ethylene Range ($pl \cdot g^{-1} \cdot min^{-1}$)		Temperature (°C) Range	*P*-value	*P*-value
	(With Stress)	(Without Stress)		(With Stress)	(Without Stress)
1	5.84 – 180.90	5.84 – 50.54	15 – 37	0.64	0.97
2, analysis 1	9.52 – 72.19	9.52 – 45.58	15 – 35	0.12	0.21
2, analysis 2	11.54 – 165.32	8.38 – 50.70	20 – 26	0.18	0.32
3, analysis 1	10.36 – 89.46	10.36 – 29.91	18 – 30	0.40	0.31
3, analysis 2	9.23 – 155.16	9.23 – 75.16	20 – 29	0.50	0.78

Table 3. Compiled results for Experiment 1 depicting the correlation between light (µmol photon·m^{-2}·s^{-1} (PAR)) and ethylene production for individual plants in systems built in 2002. For both the ethylene and light ranges, "With Stress" indicates measurements with potentially stress-induced plants included and "Without Stress" indicates measurements without potentially stress-induced plants. * $P \leq 0.05$.

Plant Set Number (dates measured in 2002)	Ethylene Range ($pl \cdot g^{-1} \cdot min^{-1}$)		Light (µmol photons·m^{-2}·s^{-1} (PAR))		
	(With Stress)	(Without Stress)	Range	*P*-value (With Stress)	*P*-value (Without Stress)
1	5.84 - 92.46	5.84 – 50.54	0.2 – 740	0.17	0.55
2, analysis 1	9.52 – 72.19	9.52 – 36.11	420 – 1508	0.57	0.36
2, analysis 2	11.54 – 165.32	11.54 – 50.70	40 – 582	0.01 *	0.32
3, analysis 1	10.36 – 89.46	10.36 – 29.91	63 – 1075	0.19	0.31
3, analysis 2	9.23 – 155.16	9.23 – 75.16	165 – 1467	0.12	0.05 *

Position from the Pipe or Pump Measurements:

Plant Set 1: Three plants within the system were found to have produced potentially stress-induced ethylene (twice the system average of 27.16 $pl \cdot g^{-1} \cdot min^{-1}$). The average ethylene production for plant positions relative to the central delivery pipe (0.46, 0.81, and 1.14 m) showed no statistical significance between the three positions (when compared to each other) with *P*-values ranging from 0.14 to 0.44 (Fig. 6C). However, there was a small increase in average ethylene production for the plants at the 0.81 m position (with average of 41.84 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other two positions (0.46 m with an average of 23.33 $pl \cdot g^{-1} \cdot min^{-1}$ or 1.14 m with an average of 16.30 $pl \cdot g^{-1} \cdot min^{-1}$).

The average ethylene production for plant positions relative to the pump (0.49, 1.04, 1.6, 2.17, and 2.73 m) showed no statistical significance between the five positions (when compared to each other) with *P*-values ranging from 0.07 to 0.85 (Fig. 6D). However, there was an increase in average ethylene production for the plants at the 1.04, 1.6, and 2.17 m positions (with averages of 30.89, 34.00, and 40.80 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other positions of 0.49 and 2.73 m (with averages of 18.10 and 11.98 $pl \cdot g^{-1} \cdot min^{-1}$).

Additionally, Plant Set 1 contained five different cultivars with the following plant positions relative to the pump: Fern 0.49 m, Honeyoye 1.04 m, Fort Laramie 1.60 m, Tribute 2.17 m, and Quinault 2.73 m. Since no statistical significance was found for these position averages with regard to their distance from the pump (Fig. 6D), we concluded that cultivar type had no effect on average ethylene production.

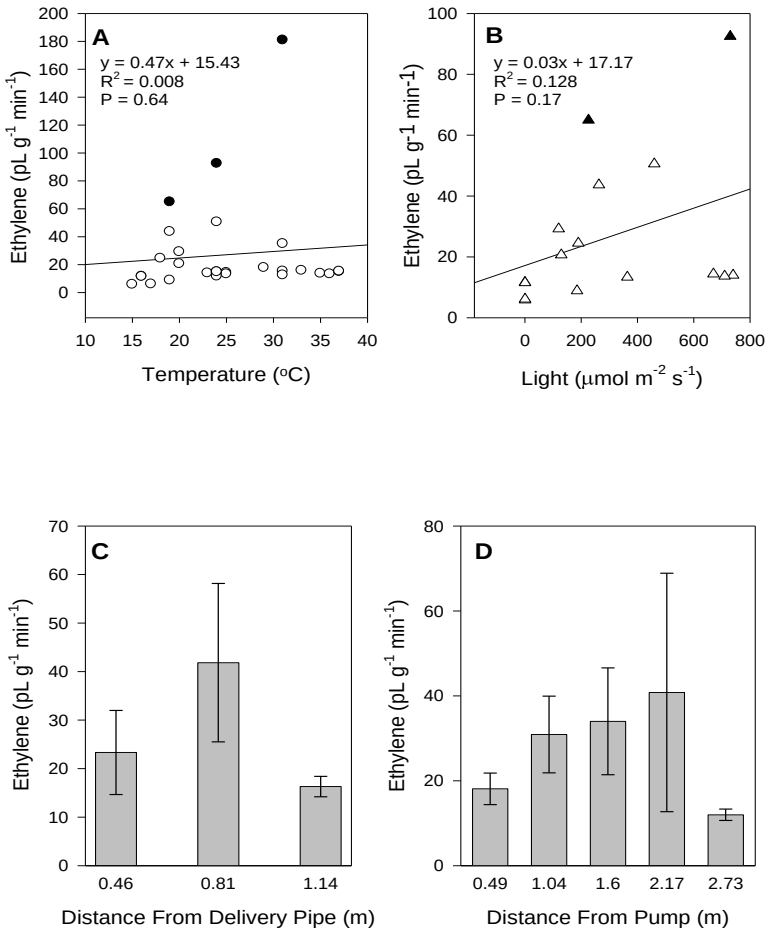

Figure 6. Ethylene measurements from Plant Set 1 that consisted of various cultivar leaflets. (A) Evaluation of temperature effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene production is indicated by filled symbols. (B) Evaluation of light intensity effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene is indicated by filled symbols. (C) Evaluation of average ethylene production produced by plants at different positions from the central delivery pipe. (D) Evaluation of average ethylene production produced by plants at different distances from the pump (Fern 0.49 m, Honeyoye 1.04 m, Fort Laramie 1.6 m, Tribute 2.17 m, and Quinault 2.73 m). n=30. Means ±SE. No statistical significance was found for any of the evaluations.

Plant Set 2: Two plants for the first and four plants for the second system analyses were found to have produced potentially stress-induced ethylene (twice the system average of 28.54 and 28.67 $pl \cdot g^{-1} \cdot min^{-1}$). The average ethylene production for plant positions relative to the central delivery pipe (0.46, 0.81, and 1.14 m) for the first analysis showed no statistical significance between the three positions (when compared to each other) with *P*-values ranging from 0.07 to 0.93 (Fig. 7C). However, there was a small increase in average ethylene production for the plants at the 0.81 m position (with average of 34.91 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other two positions (0.46 m with an average of 25.10 $pl \cdot g^{-1} \cdot min^{-1}$ or 1.14 m with an average of 25.61 $pl \cdot g^{-1} \cdot min^{-1}$). For the second analysis, no statistical significance between the three positions (when compared to each other) was shown with *P*-values ranging from 0.44 to 0.85 (Fig. 8C). However, there was a small increase in average ethylene production for the plants at the 1.14 m position (with average of 33.05 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other two positions (0.46 m with an average of 28.10 $pl \cdot g^{-1} \cdot min^{-1}$ or 0.81 m with an average of 24.86 $pl \cdot g^{-1} \cdot min^{-1}$).

The average ethylene production for plant positions relative to the pump (0.49, 1.04, 1.6, 2.17, and 2.73 m) for the first analysis found a statistical significance between the 2.17 and 2.73 m positions (P=0.05). No other position comparison had a statistical significance, with *P*-values ranging from 0.10 to 0.98 (Fig. 7D). However, there was an increase in average ethylene production for the plants at the 0.49, 1.6, and 2.17 m positions (with averages of 35.21, 34.97, and 28.83 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other positions of 1.04 and 2.73 m (with averages of 23.10 and 20.60 $pl \cdot g^{-1} \cdot min^{-1}$). For the second analysis, no statistical significance between the five positions (when compared to each other) was shown with *P*-values ranging from 0.13 to 0.74 (Fig. 8D). However,

there was an increase in average ethylene production for the plants at the 0.49, 1.6, and 2.73 m positions (with averages of 43.58, 34.24, and 28.44 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other positions of 1.04 and 2.17 m (with averages of 22.38 and 14.71 $pl \cdot g^{-1} \cdot min^{-1}$).

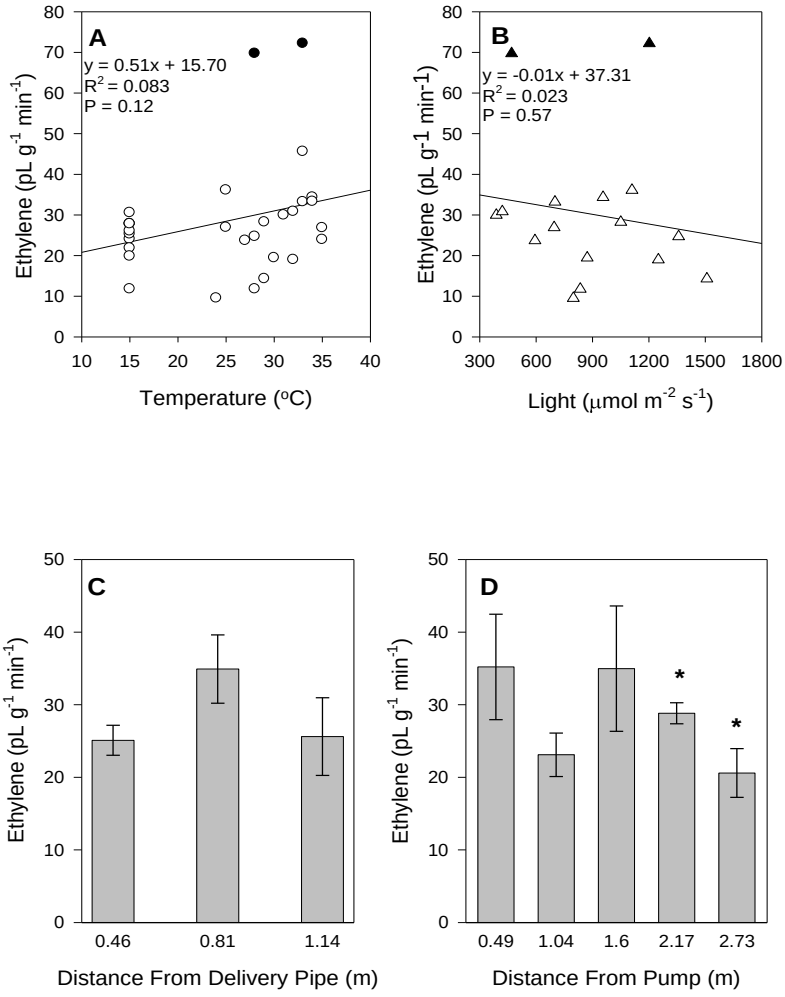

Figure 7. Ethylene measurements from Plant Set 2, analysis 1. (A) Evaluation of temperature effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene production is indicated by filled symbols. (B) Evaluation of light intensity effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene is indicated by filled symbols. (C) Evaluation of average ethylene production produced by plants at different positions from the central delivery pipe. (D) Evaluation of average ethylene production produced by plants at different positions from the pump. n=30. Means ±SE. * $P \leq 0.05$ between 2.17 and 2.73 m positions.

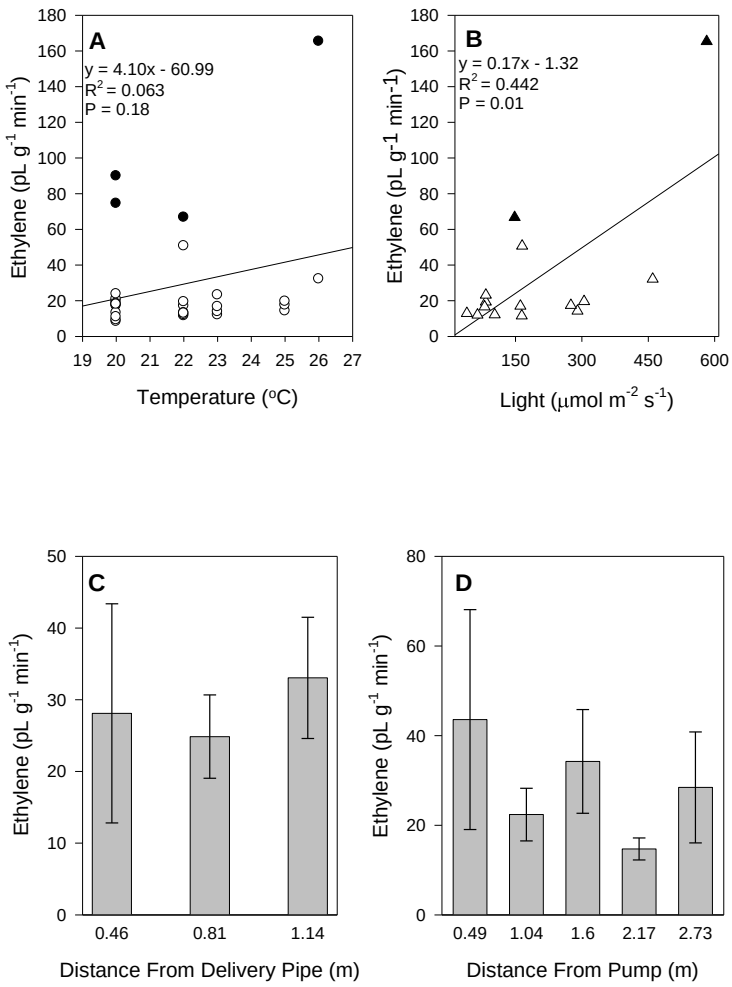

Figure 8. Ethylene measurements from Plant Set 2, analysis 2. (A) Evaluation of temperature effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene production is indicated by filled symbols. (B) Evaluation of light intensity effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene is indicated by filled symbols. (C) Evaluation of average ethylene production produced by plants at different positions from the central delivery pipe. (D) Evaluation of average ethylene production produced by plants at different positions from the pump. n=30. Means ±SE. No statistical significance was found for any of the evaluations.

Plant Set 3: Two plants for both the first and second system analyses were found to have produced potentially stress-induced ethylene (twice the system average of 20.25 and 42.06 $pl \cdot g^{-1} \cdot min^{-1}$). The average ethylene production for plants relative to the central delivery pipe (0.46, 0.81, and 1.14 m) for the first analysis showed no statistical significance between the three positions (when compared to each other) with *P*-values ranging from 0.11 to 0.41 (Fig. 9C). However, there was a small increase in average ethylene production for the plants at the 1.14 m position (with average of 28.13 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other two positions (0.46 m with an average of 15.54 $pl \cdot g^{-1} \cdot min^{-1}$ or 0.81 m with an average of 17.08 $pl \cdot g^{-1} \cdot min^{-1}$). For the second analysis, no statistical significance between the three positions (when compared to each other) was shown with *P*-values ranging from 0.18 to 0.57 (Fig. 10C). However, there was a small increase in average ethylene production for the plants at the 1.14 m plants (with average of 53.72 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other two positions (0.46 m with an average of 39.91 $pl \cdot g^{-1} \cdot min^{-1}$ or 0.81 m with an average of 32.79 $pl \cdot g^{-1} \cdot min^{-1}$).

The average ethylene production for plant positions relative to the pump (0.49, 1.04, 1.6, 2.17, and 2.73 m) for the first analysis showed no statistical significance between the five positions (when compared to each other) with *P*-values ranging from 0.26 to 0.80 (Fig. 9D). However, there was an increase in average ethylene production for plants at the 0.49 m position (with average of 28.80 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other positions of 1.04, 1.6, 2.17, and 2.73 m (with averages of 16.51, 21.54, 18.78, and 15.61 $pl \cdot g^{-1} \cdot min^{-1}$). For the second analysis, a statistical significance was shown between the 1.04 and 2.73 m positions (P=0.04, Fig. 10D) and the 1.6 and 2.73 m positions (P=0.01, Fig. 10D). No other position comparison had a statistical significance, with *P*-

values ranging from 0.10 to 0.87 (Fig. 10D). However, there was an increase in average ethylene production for the plants at the 0.49, 1.04, 1.6, and 2.17 m positions (with averages of 49.71, 45.52, 37.43, and 58.55 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the 2.73 m position (with average of 20.93 $pl \cdot g^{-1} \cdot min^{-1}$).

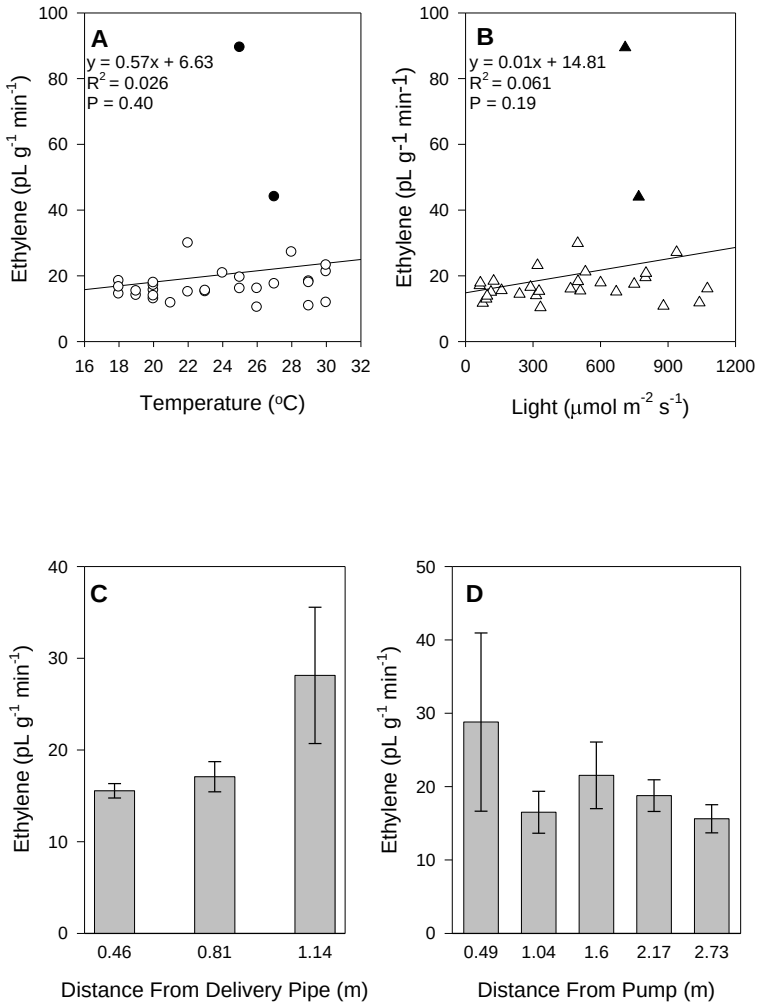

Figure 9. Ethylene measurements from Plant Set 3, analysis 1. (A) Evaluation of temperature effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene production is indicated by filled symbols. (B) Evaluation of light intensity effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene is indicated by filled symbols. (C) Evaluation of average ethylene production produced by plants at different positions from the central delivery pipe. (D) Evaluation of average ethylene production produced by plants at different positions from the pump. n=30. Means ±SE. No statistical significance was found for any of the evaluations.

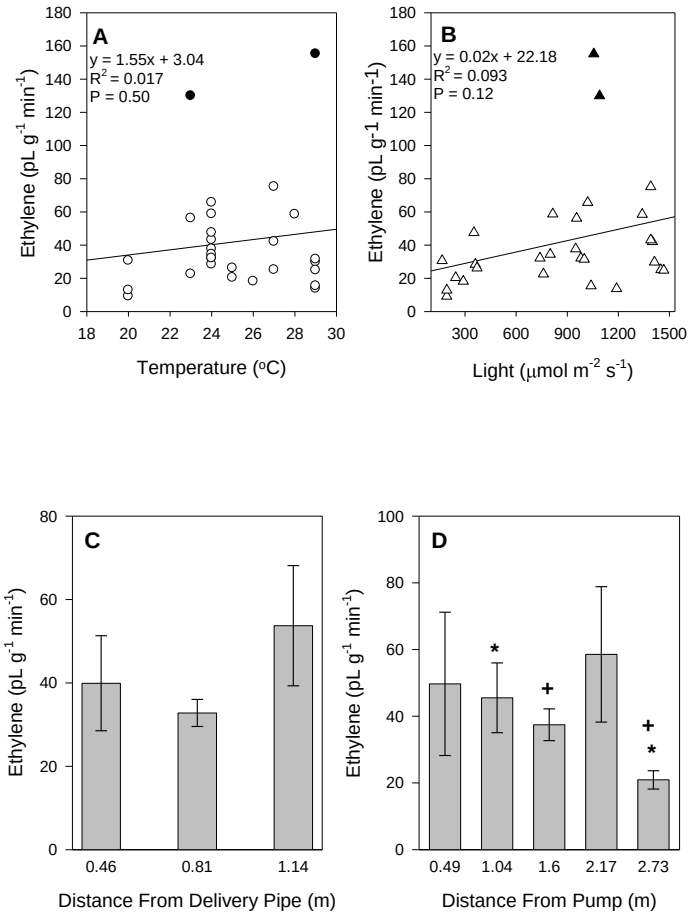

Figure 10. Ethylene measurements from Plant Set 3, analysis 2. (A) Evaluation of temperature effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene production is indicated by filled symbols. (B) Evaluation of light intensity effect on plants exhibiting foliar ethylene production. Potentially stress-induced ethylene is indicated by filled symbols. (C) Evaluation of average ethylene production produced by plants at different positions from the central delivery pipe. (D) Evaluation of average ethylene production produced by plants at different positions from the pump. n=30. Means ±SE. * $P \leq 0.05$ between 1.04 and 2.73 m positions, and + $P \leq 0.05$ between 1.6 and 2.73 m positions.

Once the average ethylene production measurements were determined for the systems, we concluded that the position from the pipe or pump could be used to indicate potentially stress-induced ethylene plants. Our results showed that the position from the pipe or pump was about the same for all systems in their average ethylene production. From the average ethylene production at different positions, the change in average ethylene production at different positions could be explained by the plants that produced twice the average production (potentially stress-induced). Without these potential stress-induced results, all the systems would have been about the same. This leads to the conclusion that plants with elevated ethylene can be identified and can be measured within a system.

For the statistical significance found in Plant Set 2, analysis 1 (P=0.05), the 2.73 m position produced the least amount of average ethylene with a large standard deviation. The 2.17 m position did not have a large standard deviation, but the average ethylene production was more consistent than the 2.73 m position when compared to all other positions. The statistical significance can be attributed to the 2.73 m position having a lower average ethylene production than all the other positions. For Plant Set 3, analysis 2, there were 2 statistical significances found, both involving the 2.73 m position with positions 1.04 and 1.60 m. Potentially stress-induced plants were not involved with any of the 3 positions for this analysis leading to the conclusion that something is affecting the 2.73 m position. This result can also be correlated back Plant Set 2, analysis 1 where the 2.73 m position had a statistical significance to the light measurement for this particular analysis (Table 3) in which once the potentially stress-induced ethylene measurements were taken out, a significance was found. The overall results from

Experiment 1 show that measurements of ethylene production can help pin-point plants that are being affected by the hydroponic systems.

Experiment 2: Ethylene and Growth and Yield Production of Strawberry Plants Grown in a Hydroponic System with Normal Drainage

Once the system-wide analyses were done to determine the ethylene production of strawberry plants within the hydroponic systems, we turned our attention to the growth and yield of the plants. Since these measurements were done a year later, a system-wide analysis was done to determine potentially stress-induced positions within the system and to make sure that the system was acting the same way as the previous year. The average ethylene production from plants located at the same distance from the central delivery pipe (10 plants/position) or pump (6 plants/position) was determined.

To determine whether the hydroponic system had an effect on growth and yield production of the strawberry plants under normal drainage, average base-line measurements were taken of flower bud number, inflorescence number, crown number, and radius of the strawberry plants (cm).

Plant Set 4: The average ethylene production for the entire system was 17.98 $pl \cdot g^{-1} \cdot min^{-1}$ with ethylene production ranging from 4.86 to 64.84 $pl \cdot g^{-1} \cdot min^{-1}$. Three plants within the system were found to have produced potentially stress-induced ethylene (twice the system average). The average ethylene production for plant positions relative to the central delivery pipe (0.46, 0.81, and 2.73 m) showed no statistical significance between the three positions (when compared to each other) with *P*-values ranging from 0.43 to 0.67 (Fig. 11A). However, there was a small increase in average ethylene production for the plants at the 0.46 m position (with average of 20.96 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other two positions (0.81 m with an average of 17.29 $pl \cdot g^{-1} \cdot min^{-1}$ or 1.14 m with an average of 15.70 $pl \cdot g^{-1} \cdot min^{-1}$).

The average ethylene production for plant positions relative to the pump (0.49, 1.04, 1.6, 2.17, and 2.73 m) was found to have a statistical significance between the 1.04 and 2.73 m positions (P=0.04). No other position comparison had a statistical significance, with P-values ranging from 0.06 to 0.63 (Fig. 11B). However, there was an increase in average ethylene production for the plants at the 2.73 m position (average of 31.20 $pl \cdot g^{-1} \cdot min^{-1}$) compared to the other positions of 0.49, 1.04, 1.6, and 2.17 m (with averages of 13.27, 10.26, 15.97, and 18.14 $pl \cdot g^{-1} \cdot min^{-1}$).

Plant Set 5: The average flower bud number was 2.0 for the first month, increased to 4.0 for the second month, and decreased to 1.6 for the third month. The average inflorescence number increased throughout the months going from 1.7 for the first month, 3.8 for the second month, and 10.3 for the third month. The average crown number increased throughout the months going from 1.1 for the first month, 1.3 for the second month, and 1.5 for the third month. The average plant radius decreased from 10.8 for the first month to 10.4 for both the second and third month.

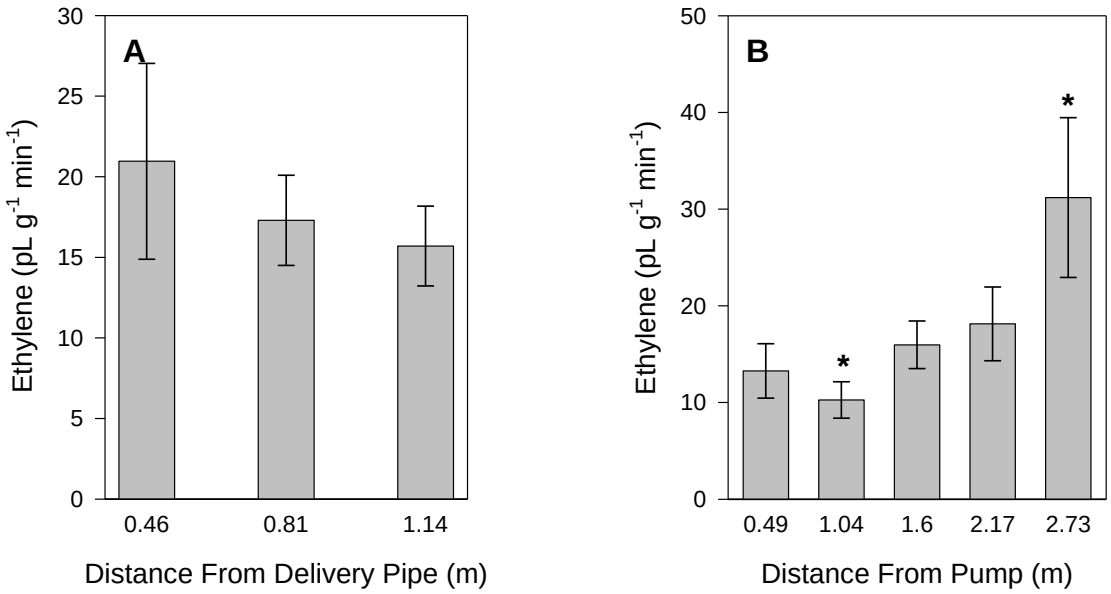

Figure 11. Ethylene measurements from from Plant Set 4. (A) Evaluation of average ethylene production produced by plants at different positions from the central delivery pipe. (B) Evaluation of average ethylene production produced by plants at different positions from the pump. n=33. Means ±SE. * $P \leq 0.05$ between 1.04 and 2.73 m positions

Table 4. Average measurements of different growth variables from strawberry plants grown in a hydroponic system during the Spring of 2003 (Flower bud, inflorescence, crown, and plant radius). n=30. Means ±SE.

	2/3	3/10	4/7
Flower bud #	2 ± 0.40	4.0 ± 0.33	1.6 ± 0.31
Inflorescence #	1.7 ± 0.28	3.8 ± 0.25	10.3 ± 0.56
Crown #	1.1 ± 0.06	1.3 ± 0.09	1.5 ± 0.10
Plant Radius (cm)	10.8 ± 0.26	10.4 ± 0.22	10.4 ± 0.33

Once the system-wide analysis was completed for Plant Set 4, the results were consistent with our previous findings from Experiment 1. The distance from the pipe had comparable average ethylene production for the three positions, but the 0.46 and 0.81 m positions were higher due to each having a potentially stress-induced plant. The distance from the pipe measurements did have a statistical significance between the 1.04 (which produced the least amount of average ethylene) and 2.73 m position. The significance was probably due to to the 2.73 m position having both of the potentially stress-induced plants at this position. Without these potentially stress-induced plants, a statistical significance would not have occurred. We concluded the system was affecting the plants' average ethylene production as the previous results (Experiment 1) indicated.

Since the system was affecting the plants as before, the base-line levels of bud number, inflorescence number, crown number, and plant radius was determined. From our results measured over two months, the results were what we would have expected. The flower bud number increased and then decreased, due to the flower bud developing into fruit. This can be seen by the inflorescence number where it continually rose and had a much higher number from the last measurement. The plants went from blooming with flower bud to producing fruit which would be indicative of the yield increasing. The crown number remained fairly constant because the time range for the measurements would not be enough for the plants to grow and produce extra crowns. Finally, the plant radius stayed constant. This result is somewhat surprising because the plants would be growing with time, but it could be that the plants had already grown to their maximum width when we first began to measure the plants and would not increase further. From these base-line results we could then evaluate the effect of flooded plants.

Experiment 3: Ethylene and Growth and Yield Production of Strawberry Plants Grown in a Hydroponic System with Flooding

Once the system-wide analyses and base-line levels of ethylene and growth and yield production were determined, we next looked at the effect of purposefully flooding the strawberry plants and how that would change their average ethylene and growth and yield production. To evaluate the effect of flooding on the plants' average ethylene production, two trays were flooded for 72 hrs and ethylene was measured at 24 hr intervals with a final measurement 24 hrs after the plants were allowed to have normal drainage. To determine if a flooding event would have an effect on the growth and yield of the strawberry plants, half of a system was flooded and measurements for both the control (normal drainage) and flooded plants were taken on three separate dates.

Plant Set 6: The average ethylene production at the 0 time point was 30.84 $pl \cdot g^{-1} \cdot min^{-1}$. The average ethylene production decreased to 10.82 $pl \cdot g^{-1} \cdot min^{-1}$ after 24 hrs of flooding and then increased to 26.70 $pl \cdot g^{-1} \cdot min^{-1}$ and 57.56 $pl \cdot g^{-1} \cdot min^{-1}$ after 48 and 72 hrs of flooding. The final measurement, 24 hrs after the plants returned to normal drainage, produced a decrease in ethylene production from the 72 hr time point to 44.33 $pl \cdot g^{-1} \cdot min^{-1}$ (Fig. 12).

Plant Set 7: For the growth and yield measurements, the flower bud number, crown number, and radius produced a statistical significance when the control was compared to the flooded for the February 3rd measurements (P=0.01, P=0.03, and P=0.008, respectively) with no other statistical significance found. For the March 10th measurements, the flower bud number and radius produced a statistical significance when the control was compared to the flooded (P=0.01 and P=0.03, respectively) with no other

statistical significance found. For the April 7th measurements, none of the measurements had a statistical significance between the control and flooded plants (Table 5).

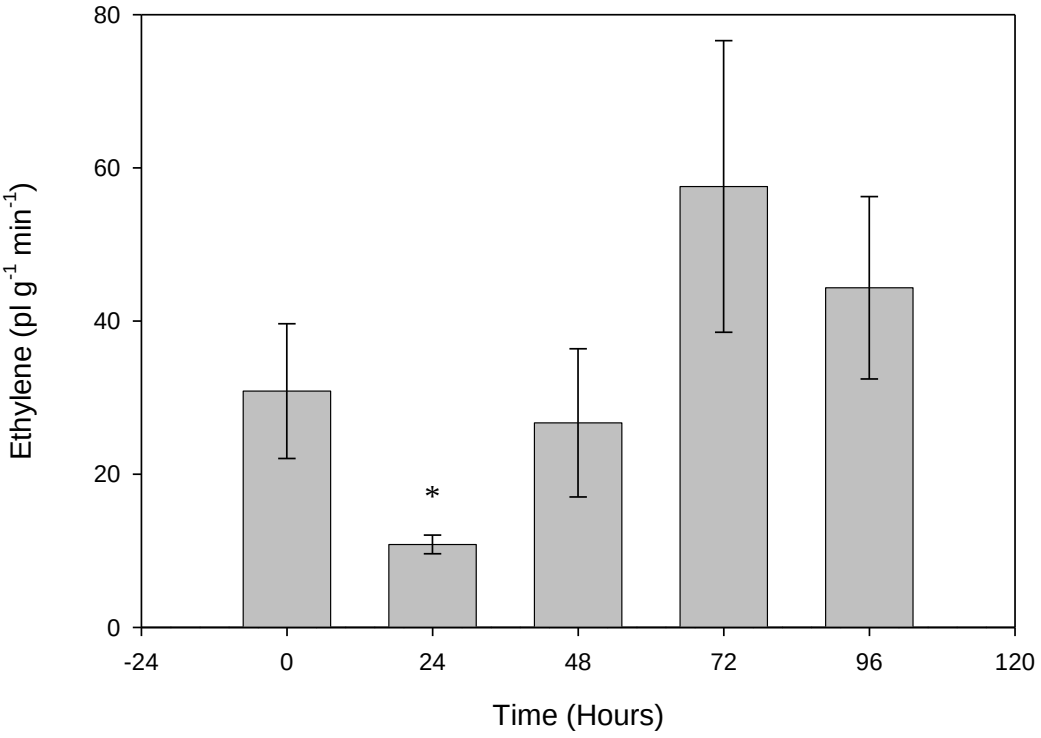

Figure 12. Average ethylene measurements of flooded leaflets from Plant Set 6. Plants had an initial reading before flooding (0 time point), then 24, 48, and 72 hrs while flooded, and a final reading 24 hrs after water was allowed to drain (96 hr time point). All measurements were compared to the 0 time point. n=6. Means ±SE.
* $P \leq 0.05$

Table 5. Average measurements of different growth variables from control and flooded strawberry plants grown in a hydroponic system during the Spring of 2003 (Flower bud, inflorescence, crown, and plant radius). Half the system was flooded on February 3rd (5 trays on the same side of the system) for 24 hrs. All measurements were compared flooded to control. n=15. Means ±SE. * $P \leq 0.05$

	Control			Flooded		
Date	2/3	3/10	4/7	2/3	3/10	4/7
Flower bud #	3.7 ± 0.48	6.5 ± 0.76	3.0 ± 0.51	5.9 ± 0.66*	4.2 ± 0.45*	2.4 ± 0.41
Inflorescence #	3.9 ± 0.35	3.0 ± 0.26	6.3 ± 0.73	2.9 ± 0.42	2.7 ± 0.19	5.9 ± 0.52
Crown #	1.4 ± 0.13	1.4 ± 0.16	1.9 ± 0.17	1.1 ± 0.07*	1.3 ± 0.12	1.7 ± 0.16
Radius (cm)	8.7 ± 0.42	13.7 ± 0.51	10.8 ± 0.56	10.2 ± 0.33*	11.3 ± 0.51*	10.9 ± 0.71

Once the base-line levels were determined for the hydroponic system (Experiment 2), we looked at the effect of flooding the plants on ethylene production and growth and yield production. The results for the average ethylene production showed that the initial reading (0 time point) had a similar average ethylene production as compared to all other experiments previously performed (approximately 30 $pl \cdot g^{-1} \cdot min^{-1}$). After 24 hrs of flooding, the ethylene production decreased significantly (P=0.05). The reason for the decrease is unknown. At the 48 hr time point, the ethylene production increased to about the base-line level and at 72 hrs increased to almost twice the average ethylene base-line level. The expected results would have been for the ethylene production to increase at the 24 hr time point and continue to increase up to the 72 hr time point. The 96 hr time point decreased from the 72 hr time as would be expected because the water was allowed to drain and the plants could return to ambient conditions.

Taking the flooding results and comparing back to previous findings (Experiment 2), the only statistical significances that were found involved the 2.73 m position, and this position had lower average ethylene production compared to the other positions. By combining the results from this experiment and previous experiments, the reason the 2.73 m position produced significant differences when having a lower average ethylene production was because those plants were being flooded and our recording of the measurements were done at the beginning of the flooding.

The growth and yield data showed that flooding had an adverse effect on the plants. From our results, the flower bud number for the control group acted as our base-line results (Table 4), but the flooded plants had less flower buds over time with a statistical significance when control to flooded was compared (Table 5). The

inflorescence number acted the same when the control and flooded were compared with no statistical significance between them. Also, the inflorescence number was consistent with the base-line levels (Table 4). The crown number produced a statistical significance on the first date measured, but this was due to a large range for the control group as compared to the flooded because the control and flooded plants acted the same as our base-line levels (Table 4). However, the radius of the plants produced a statistical significance. Even though the plants from both the control and flooded acted like our base-line plants (Table 4), the control group grew larger than the flooded group. From these results, we concluded that the flooding especially affected the flower bud number and radius of the plants. This can be seen from the flooded plants being on average larger in flower bud number and radius during the first measurement, and being smaller after the last measurement. Because the plants were flooded for only a short time, long term affects on the plants won't be seen in this experiment, but even with short term flooding, the state of the plant can be affected, especially for the yield of the plant which was shown in flower bud number decreasing, which turns into yield, and the robustness of the plant decreasing, which is shown through the radius of the plant or the smaller the plant, the less yield that can be produced.

Gene Expression Analysis

RNA Isolation: After determining ethylene levels physiologically, attempts to acquire good quality RNA for *ACS* isolation failed in this investigation. Total RNA isolation was attempted by three different protocols: Purescript RNA Isolation Kit by Gentra Systems, RNEasy Plant Mini Kit by Qiagen, and TRI REAGENT by Sigma. After each kit was used, the quantity and purity was determined using a ND-1000 spectrophotmeter. Each kit produced little to no yield from the strawberry leaflets (confirmed by low O.D. 260 and O.D. 260/280 measurements). After the Purescript protocol was performed and RNA products obtained, RT-PCR was performed using the Fa-RP primers (Table 1) for the strawberry sample and no bands were present (Fig. 13A). However, bands were present with the *Arabidopsis* sample (Fig. 13A) using the Fa-S18 rRNA primers (Table 1). The RNEasy protocol showed faint bands of RNA (Fig. 13B), but when RT-PCR was performed with the products using the Fa-RP primers (Table 1), no bands were present (data not shown). The TRI REAGENT protocol did not show any bands after the kit was used (Fig. 13C) and RT-PCR was not performed.

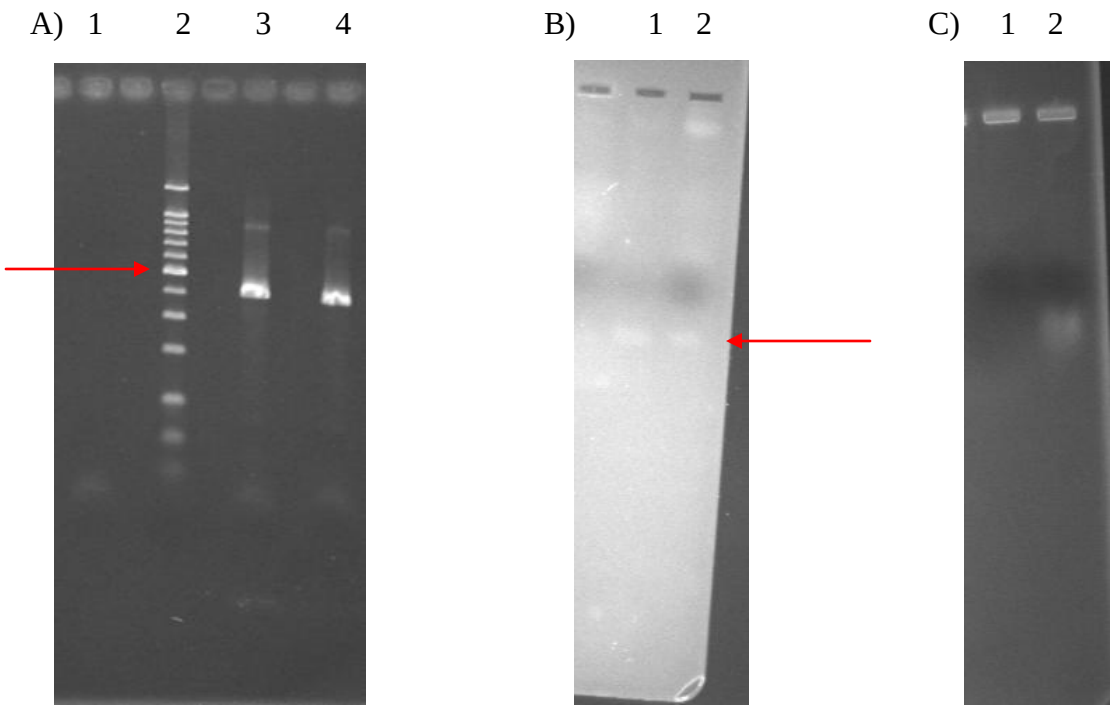

Figure 13. Analysis of RNA isolation using: (A) DNA from first using the Purescript RNA Isolation Kit and then RT-PCR. Lane 1-Strawberry, Lane 2-Ladder (100 bp DNA Step Ladder, Promega Corp., Madison, WI, USA), Lanes 3 and 4-*Arabidopsis*. Red arrow indicates the 500 kb band of the ladder, with the *Arabidopsis* bands approximately at the 396 bp position. (B) RNA from the RNEasy Plant Mini Kit. Lane 1 and 2-Genomic RNA. Bands are shown by red arrow. (C) RNA from the TRI REAGENT protocol. Lanes 1 and 2-Genomic RNA. For both (B) and (C), a ladder was not used because our goal was to determine if any bands would be present with further extractions to be done if bands were present that looked correct.

Northern Dot Blot: A Northern Dot Blot procedure was conducted using RNA from the RNEasy sample with the 18S ribosomal RNA protein probe. Both *Arabidopsis* and strawberry had two blots each that were probed on the same blot paper. Since no sequence exists for a strawberry *ACS* sequence, this was a preliminary experimental procedure to determine if northern blotting would work to evaluate gene expression in strawberry using a probe based on the conserved region of the *ACS* gene.

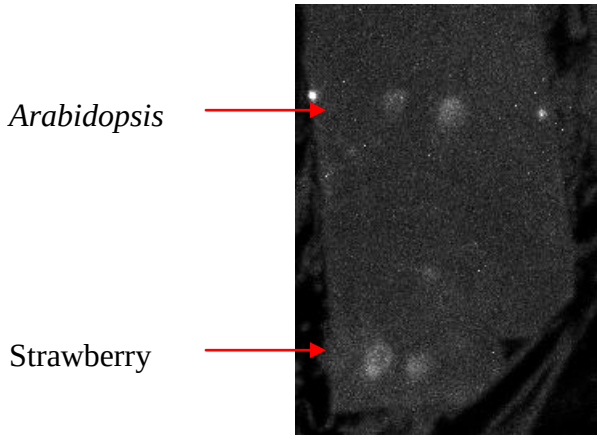

Figure 14. Northern dot blot using the North2South procedure. Total RNA was extracted using the CelLytic™ PN protocol (Sigma, St. Louis, MO, USA) followed by the North2South Direct HRP Labeling and Detection Kit (Pierce, Rockford, IL, USA) protocol and probed with a probe sequence for 18S *Arabidopsis* rRNA of 5'-AATGAG TACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGC-3'. The two blots at the top of the paper were of *Arabidopsis* and the two blots at the bottom were of strawberry.

From the results, attempts to isolate RNA using various protocols were unsuccessful. Even though the spectrophotometric measurements showed RNA was present within our samples, actual visualization confirmation was not able to be achieved. We performed RT-PCR for the *ACS* gene with the Purescript protocol and the positive controls showed that the protocol worked, but our sample for the strawberry *ACS* gene showed no bands (Fig. 13A). The RNEasy Plant Mini Kit protocol produced faint bands for genomic RNA (Fig. 13B), but RT-PCR was unsuccessful (data not shown). The TRI REAGENT protocol did not isolate any genomic RNA and RT-PCR was not performed (Fig. 13C).

We also evaluated a northern dot blot procedure to see if the 18S probe would be able to visualize strawberry RNA. Our probe showed that 18S-RNA was present, as the intensity of the blot was about the same as our control with *Arabidopsis*. However, given the quality of the strawberry RNA, this experiment did not progress further and the protocol to isolate a better quantity of RNA needs to be refined (Fig. 14).

CHAPTER V

Discussion

With the worldwide phaseout of methyl bromide as a soil fumigate, the use of hydroponic systems has rapidly increased as an economic alternative for the growth of many horticulturally-important crops (Environmental Protection Agency, 1997; Carpenter *et al*., 2000; VanSickle *et al*., 2000; Federal Register, 2004), especially strawberry, a crop that has been particularly dependent upon methyl bromide fumigation (Stanley, 1998). With the phase out of methyl bromide and an increase in the use of hydroponic systems, along with little information on the affect of hydroponics on plants, this study was undertaken to demonstrate that measurements of ethylene production from leaves of hydroponically-grown strawberry plants could be used as an early indicator of plant stress.

Since ethylene production is known to increase due to various factors within plants, we wanted to narrow down the scope of the exact cause of stress on plants within our hydroponic systems and to demonstrate that specific areas of the system can be measured to give an early warning for potentially stressed plants so that the problem can be rectified before yield is affected. The reported data suggest that sampling time could be a significant factor when evaluating ethylene production of strawberry plants under our experimental conditions. Circadian rhythms for ethylene production have been reported for sorghum (Finlayson *et al*., 1998), cotton (Jasoni *et al*., 2000), and *Arabidopsis thaliana* (Thain *et al*., 2004). For these species, ethylene production peaks during midday and is lowest during the dark cycle, a rhythm that may reflect a midday temperature optimum for ethylene production. A temperature optimum of 30°C has been

reported for ethylene production by apple fruit and mung bean hypocotyls (Yu *et al*., 1980). Temperatures above 35°C often represent heat-stress levels that inhibit ethylene production (Yu *et al*., 1980), however the temperature optimum and sensitivity to higher temperature is cultivar-dependent. For example, Balota *et al*. (2004) found increased ethylene production at temperatures as high as 38°C for wheat seedlings. In addition, Finlayson *et al*. (1998) found that while both light and temperature cycles were required to maintain circadian rhythm in sorghum, a SD plant, and temperature cycles can override the light signal in controlling circadian ethylene production. Therefore, their results suggest a circadian rhythm and that fluctuation in temperature was likely the critical factor in regulating ethylene production. Since the average ethylene production values attributed to sampling time were lower than the values used to indicate stress conditions, we conclude that the high values observed for some plants were caused by an inherent stress to the plant rather than by a circadian rhythm. Also, the temperature range during the experimental time course did not reach heat-shock conditions, and no decline in ethylene production was observed at higher temperatures. These data indicate that system-wide analysis should be conducted within a consistent time period to minimize differences due to a circadian effect even though with our results no correlation was found for light or temperature and ethylene production over an inconsistent time period.

For our system design, higher ethylene levels occurred in plants within areas that may have had inconsistencies in nutrient delivery or drainage pattern (manifested as pump pressure or drainage control by the valve on the delivery pipe). Higher flow rate may lead to excessive watering, which contributes to accumulation around the roots, especially for plants located at the lower end of the trays. However, the observed pattern

of stress-induced ethylene production may represent an edge effect where plants along the perimeter receive more mechanical stimulation and show a wound-induced ethylene response. In plant set 7, plants that averaged an increase in ethylene also showed a decrease in flower number and plant radius, demonstrating ethylene analysis as a method for predicting negative impacts on yield. When plants were flooded over a period of time, ethylene production increased in a manner consistent with increased ethylene levels observed in flooded tomato plants (English *et al*. 1995). Hypoxic conditions negatively affect the yield in horticulturally-important crops grown hydroponically (Urrestarazu and Mazuela, 2005). Urrestarazu and Mazuela (2005) report than even small changes in oxygen can be limiting for crops such as sweet pepper and melon, and note that increasing oxygen content by supplying an oxygen generator (potassium peroxide) through fertigation increased yield in these plants. They conclude that daily changes in oxygen content and watering level may be subtle, but could reflect a significant change in yield over the life of the plants. Our results suggest that measurements that indicate increased ethylene production may reflect inconsistencies within the system, such as reduced oxygen to the roots that could then be evaluated and adjusted to increase overall crop yield.

Besides trying to gain an understanding of how the hydroponic systems affected the strawberry plants physiologically by measuring ethylene, we also tried to determine how ethylene was regulated by the plant itself, specifically through the control of the *ACS* gene. The pathway for ethylene has been studied extensively (Yang and Hoffman, 1984) and the level of ACS activity has been shown to closely parallel the level of ethylene, meaning if ACS increases, then ethylene increases (Chae *et al*., 2003). But, these factors

have been studied mostly with *Arabidopsis* and not much work has been attempted with strawberry plants. According to Manning (1994), little information is available to characterize the ripening (in which ethylene is a major contributor) of non-climacteric (lack of increased respiration and ethylene production as the fruit changes color) fruits, such as strawberry, at the molecular level because of difficulties encountered in obtaining suitably pure RNA (Manning, 1994). This is consistent with our results in obtaining suitably pure strawberry RNA, even though our experiments dealt with the leaves and not with the fruit itself. Using three different RNA isolation protocols with strawberry, we were unable to obtain any decent amount of RNA, if at all, and why this occurred is unknown. According to Manning (1994), some possible explanations for the difficulties could be that strawberry fruits have been found to have temporary disappearance of mRNA in immature fruit and developing strawberry has more mRNA changes than any other fruit (Manning, 1994). For our studies, we did not use the fruit, but, if the fruit has many RNA changes, then the other parts of the plant may also have an increased change in mRNA levels as compared to other fruit plants.

Another possibility leading to difficulties in obtaining RNA could be RNase. Plants are known to alter the levels of RNase activities in response to a variety of endogenous and exogenous stimuli and so the induction of RNases specific for mRNA degradation can play a part in plant responses (Yen and Green, 1991). Perhaps in our studies RNases contributed significantly to the results of not being able to obtain suitable RNA. The exact reason for our difficulties in obtaining suitably pure RNA from strawberries needs to be investigated further so that the ACS role within strawberries can be determined more throughly.

Since a good quantity of total RNA was not able to be obtained through conventional kits, we then performed a northern dot blot. Here we used a probe for 18S ribosomal protein (which should be highly conserved) from *Arabidopsis* and did produce a positive result for the strawberry blot. This at least demonstrated that RNA was present within the leaves and further work is possible to determine the exact reason why RNA was not able to be obtained.

CHAPTER VI

Summary and Conclusion

With the use of hydroponic systems as a rapidly increasing economic alternative for the growth of many horticulturally-important crops (Environmental Protection Agency, 1997; Carpenter *et al*., 2000; VanSickle *et al*., 2000; Federal Register, 2004), the need to understand the best way to utilize this technique has become more important. Strawberry has not been studied extensively and in this investigation several hydroponic systems that contained various cultivars of strawberries were constructed over several years to determine the effect of the hydroponic systems on the strawberry plants by measuring the average ethylene production from various positions within the systems. Light, temperature, position from the central delivery pipe, and position from the pump were the first variables that were considered for an overall assessment of the plants' response while grown in the hydroponic systems. Next, the mechanism of ethylene production by plants was attempted in strawberry plants because not much work has been conducted with this plant species.

The conclusion from this investigation was that measurements of ethylene production could be used as an early indicator to determine potentially stress-induced plants within a hydroponic systems This was shown through the ambient conditions from light and temperature not producing an effect that would hamper the results of trying to determine where potentially stress-induced ethylene production occurred within the hydroponic systems. On the other hand, the position from the pipe or pump was shown to be able to be used to indicate potentially stress-induced plants due to the variations within the systems. How flooding affected the plants was then determined through actual

flooding of the plants and the plant's internal mechanism of ethylene production was investigated. The pathway of ethylene production is well understood, but the regulation of this pathway needs to be studied further. Our results to understand this regulation better were inconclusive because RNA was not able to be obtained and why this occurred is not understood.

Overall, the findings from all the experiments showed that ethylene production measurements can be a technique used to pin-point unhealthy plants. If these plants can be found early, then the cause of the potentially stress-induced ethylene can be determined and steps can be taken to fix the reason for the plants stress-induced ethylene. The techniques used in this investigation hopefully help to shed some light on the complex mechanisms that plants employ when not at optimal homeostasis and in turn allow hydroponics to become an even greater economic alternative to the existing techniques already in use.

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