Chapter 5

EFFECT OF ENVIRONMENT ON ETHYLENE SYNTHESIS AND COTTON

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INTRODUCTION

Utah is not the first state that comes to mind for cotton research, and, in fact, the total 2009 cotton acreage for Utah came in at 0.02 acres (Fig. 1). On a per acres basis, this is some of the most expensive cotton ever gown in North America. All of it was in either our research greenhouse, or under electric lights in a growth chamber. In this article I describe the past 2 years of research with the plant hormone ethylene. My students and I have found the responses of cotton to be far more interesting than corn or soybeans. Here we explain why.

Figure 1. A photograph of the Utah State University Research Greenhouse complex. The inset shows cotton plants in electronic balances for studies on transpiration rate.

Controlled environments provide a critical intermediate step in scaling from petri dishes to the field (Fig. 2). They allow the separation of individual environmental effects and provide an environment that can be reproduced at any time and in any location around the world. When plants are grown under electric lights the environmental conditions are nearly identical from day to day and from week to week.

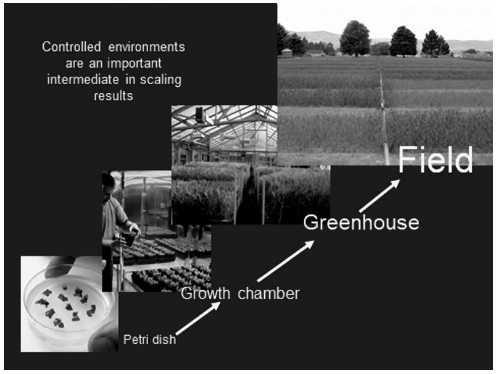

Figure 2. A diagram showing typical locations for scaling research results from a petri dish to the field.

Cotton: A Physiological Paradox

All students in introductory Plant Physiology learn that plants with C3 metabolism are favored by cool temperatures, and plants with C4 metabolism are favored by warm temperatures. Except cotton. The C3 pathway of photosynthesis is universal in cotton and yet it's temperature optimum exceeds many crop plants with C4 photosynthesis.

Part of the explanation for this comes from the high transpiration rates of cotton and the associated evaporative cooling. Figure 3 shows that the canopy temperature of cotton can be 11°C cooler than the air temperature. This astonishing difference only occurs in hot dry climates, but it indicates that the stomates of cotton stay open even in the middle of the day and keep the plant well below the air temperature. Most crop plants would be doing well to achieve a canopy temperature that was 5ºC below air temperature. Cotton has a unique ability to cool itself on hot days.

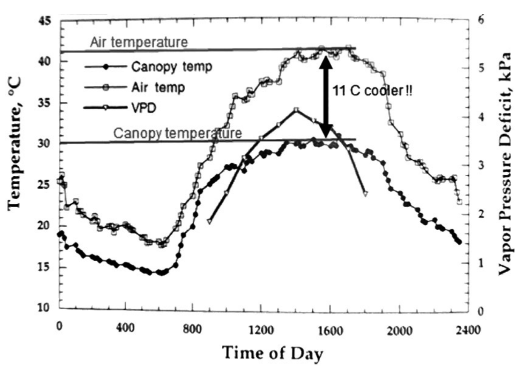

Figure 3. A 24-hour graph of the canopy temperature of cotton leaves (solid circles, left hand axis) and air temperature (open circles, right hand axis), and the driving gradient for transpiration vapor pressure deficit (open circles, right hand axis).

Hormones and Developmental Signaling

Hormones have the same definition in human, animal, and plant systems. A hormone is a molecule that signals responses at extremely low concentrations. In many ways a hormone is like a drum major leading a thousand piece marching band through a complicated parade route.

In animals hormones must be produced in one location and transported to another location, but this definition does not fully apply in plant systems where some hormones can be synthesized on one group of cells and signal an effect a few cells away.

There are five classic plant hormones. Among the most powerful is ethylene, which is widely used to alter plant responses, primarily through the commercial product Ethephon. Ethylene is widely regarded as a growth inhibitor and has long been thought to provide a signal leading to senescence and early aging in plants (Abeles *et al*., 1992). Ethylene is typically called the ripening hormone because of its role in ripening climateric fruit like avacados and banannas. Ethylene, however, is produced by all cells of a plant at all stages of the life cycle (Abeles *et al*., 1992).

Effects of Water Stress on Ethylene Synthesis

Ethylene production is commonly thought to increase during water stress, but there is considerable controversy on this topic. Part of the problem is the result of differences among experimental methods in ethylene research. Several studies have examined desiccation of detached leaves. These studies indicate that water stress increases ethylene production. Studies using intact plants indicate decreased ethylene synthesis (Morgan *et al*., 1990; Narayana *et al*., 1991). Ethylene synthesis was unaffected in maize mutants with variable internal concentrations of abscisic acid (Voisin *et al*., 2006). However, in this study the tissue was detached and placed in a sealed vial for capture of the ethylene. Thus, ethylene synthesis was not captured 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). 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 observations made with different techniques and the molecular data, it seems likely that ethylene synthesis would decrease as a result of water-deficit stress.

Wheeler *et al*. (2004) quantified the ethylene synthesis rate of four crop plants in a large sealed chamber at the NASA-Kennedy Space Center (Fig. 4). They were surprised to find that ethylene synthesis peaked at anthesis in wheat and soybeans. This is likely due to its role in signaling pollination and anther dehiscence. The ethylene synthesis rate of lettuce closely followed its growth rate, indicating that ethylene synthesis in this vegetative plant is a constant fraction of the photosynthetic rate. Although the potatoes in this study grew to be large, high yielding plants, they produced only minimal amounts of ethylene. These results are contrary to the widely held belief that ethylene synthesis is highest just prior to physiological maturity.

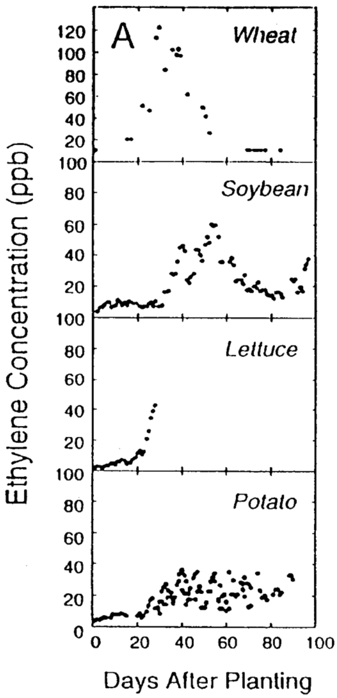

Figure 4. The relative production of ethylene over the life cycle of four crop plant species; wheat, soybean, lettuce and potato. The data are the increase in ethylene in a closed plant growth chamber at the NASA-Kennedy Space Center. Contrary to conventional wisdom, many crops have a peak ethylene synthesis at anthesis, and none of these crops had a peak ethylene synthesis rate during senescence. (From Wheeler *et al.,* 2004).

Effects of Atmospheric Ethylene on Growth and Yield

Ethylene is often called a self-extracting hormone because it is a gas at room temperature. This feature, however, can cause significant problems for the growth of plants in sealed environments where the ethylene cannot disperse with the wind. We recently completed a series of studies to determine the threshold levels at which ethylene alters plant growth. We were surprised to find that ethylene is 10,000 times more toxic to plants than carbon monoxide is to people.

In people, 50 ppm carbon monoxide toxicity starts to cause headaches. In plants, pollination is impaired by only 5 ppb (0.005 ppm; Klassen and Bugbee, 2002, 2004).

Hypothesis: Ethylene Decreases Cell Expansion and Internode Elongation

Ethylene is listed in textbooks as a growth inhibitor based on the classic effects of atmospheric ethylene on cell expansion and internode elongation. In both corn and cotton, ethylene acts like a dwarfing hormone and dwarfs the plants without any visible symptoms of stress (Fig. 5a and 5b*)*. It is likely that the plants have the same number of cells, but cell expansion is inhibited. In soybeans, however, the effect of continuous ethylene exposure was radically different: ethylene inhibited leaf expansion, but increased internode elongation (Fig. 6). We clearly have much to learn about the effects of ethylene on plant development.

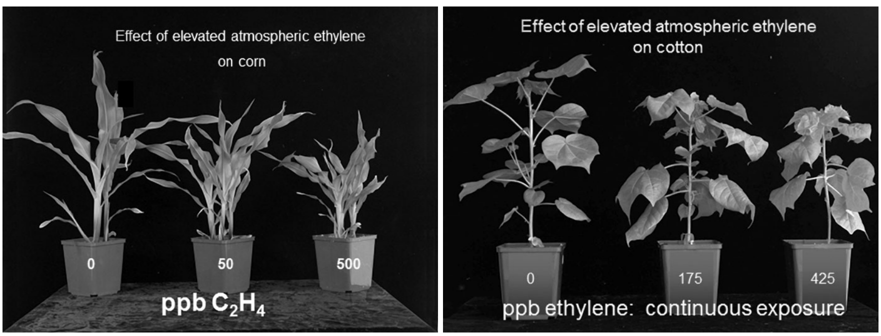

Figure 5 a and b. The effect of elevated ethylene levels on corn and cotton plants continuously exposed to ethylene in the air. In both species the internode elongation and the leaf expansion rates were decreased.

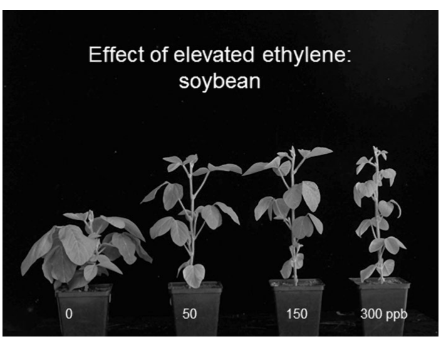

Figure 6. The effect of continuous exposure to ethylene on soybeans. Contrary to other crops, note that ethylene caused an increase in internode elongation. Similar to other crops, however, leaf expansion decreased with increasing ethylene.

The Effects of Stress in Plants

We know that some stress in our lives can lead to our most creative moments. A life without any stress rarely inspires people to greatness. Similarly, one can hypothesize that some stress in plants is also beneficial to trigger reproductive growth and optimal yields. However, this view is controversial and generally not accepted in cotton production. Notwithstanding, the effects of stress are hard to predict: in both plants and people.

Some of our reactions to stress are detrimental to the rapid healing. Our bodies over-react to stress. Swelling at the site of an injury is a good example of this because it reduces blood flow and slows healing. We reduce the swelling of a sprained ankle by using ice packs to cool the tissue, wrapping to compress the tissue, and elevation to reduce the blood pressure. We also use anti-inflammatory drugs to minimize inflammation.

Plants might also overreact to stress – and if they do, oversensitivity to ethylene is a good candidate for signaling the overreaction. In the past few years several groups have been studying a relatively new compound called 1-MCP (1-methylcyclopropene) (Kawakami *et al*., 2010), which blocks the perception of ethylene in plants (Sisler and Serek, 1997). The development of this product has provided physiologists a tool to study the effects of ethylene on growth and development of a wide range of plants. This is a major breakthrough because a biological over-reaction to stress in crop plants has the potential to cause billions of dollars in yield losses. 1-MCP has the potential to act like an anti-inflammatory agent in plants.

Ethylene, Leaf Elongation and Water Stress

We studied the effects of mild drought stress, with and without 1-MCP on leaf elongation in corn (Fig. 7). Our hypothesis was that application of 1-MCP would restore at least some of the normal leaf elongation in the drought-stressed plants. The 1-MCP was applied at 0.5 grams per liter of active ingredient (AFxRD). There was no beneficial effect of the 1-MCP in these studies.

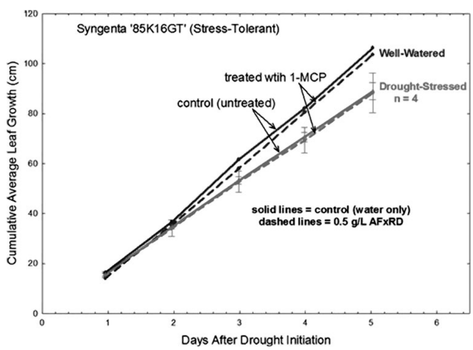

Figure 7. The effect of mild drought stress on cumulative leaf growth in corn. The data is the average of the most recently expanded four leaves. Plants with the dashed lines were treated with a technical grade of 1-MCP called AFxRD.

Similarly, Loka and Oosterhuis (2010) reported that application of 1-MCP to water-stressed cotton plants had no significant effect on leaf gas exchange functions, although carbohydrate metabolism of the pistil was significantly affected. Kawakami *et al*. (2010) reported that there was no significant effect on water-use efficiency and dry matter production water-stressed cotton plants treated with 1-MCP, but individual leaves had higher stomatal resistance and better maintenance of membrane integrity. An antagonistic relationship between ethylene and ABA on stomatal closure of water-stressed plants has also been reported (Wilkinson and Davies, 2010).

We subsequently studied the effect of more severe stress, but with a gradual onset, and with intermittent stress. Again, there was no beneficial effect of the 1-MCP application. The line labeled UTC in Figure 8 is the untreated control plant that was also the well-watered control treatment.

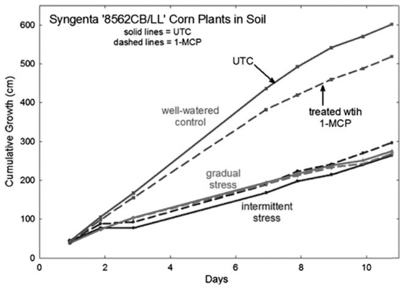

Figure 8. The effect of severe drought stress on cumulative leaf elongation in corn. Blocking the perception of ethylene with sprays of 1-MCP did not restore leaf elongation regardless whether or not the drought stress was gradually imposed or intermittent.

Ethylene, Leaf Elongation and Heat Stress

We subsequently studied the potentially beneficial effect of 1-MCP applications in heat-stressed corn plants. Plants were grown in three matching plant growth chambers (Fig. 9) that had four, 1000 W high pressure sodium lamps in each chamber to provide the equivalent of close to full sunlight at the top of the plant canopy (a photosynthetic photon flux (PPF) of 1600 $\mu mol/m^2/s$).

Figure 9. Three matching growth chambers at Utah State University that provide the equivalent of 80% of full sunlight at solar noon in the summer. Each chamber has been modified to include 4, 1000W high pressure sodium lamps and a recirculating, chilled water filter below the lamps. Cotton growth and development in this high light environment was excellent and representative of the field.

After some preliminary studies, we found that the air temperature needed to be above 33°C to be hot enough to reduce leaf expansion. The three chambers were thus set to 33, 37, and 40°C. There were 12 replicate plants per treatment and the CO_2 was elevated to 900 ppm to partially close stomates and reduce evaporative cooling of the leaves. The elevated temperatures effectively reduced leaf expansion (measured as daily leaf elongation; Fig. 10), but there was no significant effect of blocking ethylene on the restoration of leaf elongation.

Collectively, these studies do not indicate a significant role for ethylene in mediating the effects of either drought or heat stress, at least in corn plants. This is contrary to the conventional wisdom in most textbooks, which suggest that ethylene plays a key role in mediating plants responses to a wide range of environmental stresses.

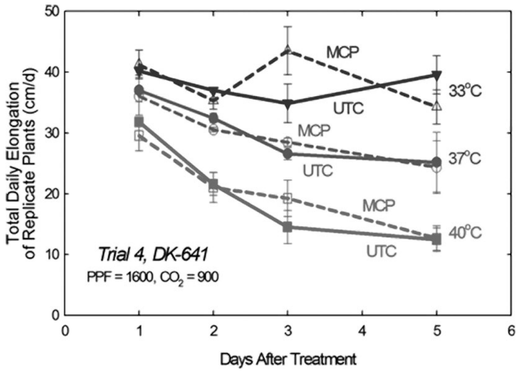

Figure 10. The effect of high temperature stress on leaf elongation of corn, with and without treatment of 1-MCP to block ethylene perception. 1-MCP did not result in a significant increase in leaf elongation at any of the three temperatures.

Development of Techniques for the Real-time Measurement of Whole-plant Transpiration

We have long sought improved techniques for the measurement of whole plant transpiration rates. We recently coupled digital balances to a data acquisition system (Campbell Scientific, model CR1000). This merger of balances and datalogger has allowed us to measure changes in mass of 1 gram and transpiration rates over 10 minute intervals. Figure 11 shows five cotton plants on five balances in a growth chamber. This is what we call a mini-lysimeter system.

We used this mini-lysimeter system to determine the effect of blocking ethylene perception on stomatal aperture. Figure 12 shows the diurnal transpiration rate of cotton plants over a 6 day period. There were two control plants that were sprayed with water and two plants sprayed with 1-MCP at field rates. Plants were initially sprayed with 1x of the field rate (10 g a.i./ha), and then sprayed with 3x the field rate. There was no significant effect on transpiration with either of the two spray treatments.

Figure 11. Five cotton plants on five balances in a growth chamber. This is what we call a mini-lysimeter system.

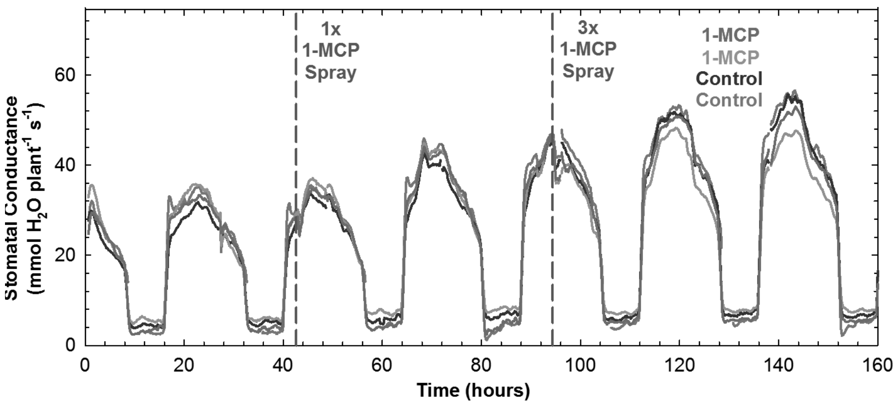

Figure 12. The diurnal transpiration rate of cotton plants over 6 days. Two of the plants were sprayed with 1-MCP to examine the effect of blocking ethylene on transpiration rate.

Jet Lag in Cotton

We used this mini-lysimeter system to determine possible circadian rhythms in several plants. Figure 13 shows the effect of changing the photoperiod on transpiration rate of cotton. Plants were grown with an 8 hour light period and a 16 hour dark period (days 1, 2, and 3 in Fig. 13). The photoperiod was then abruptly changed to a 16 hour light period. The stomates closed by about 2/3 after 8 hours of light, even though the environmental conditions remained exactly the same in this controlled growth chamber.

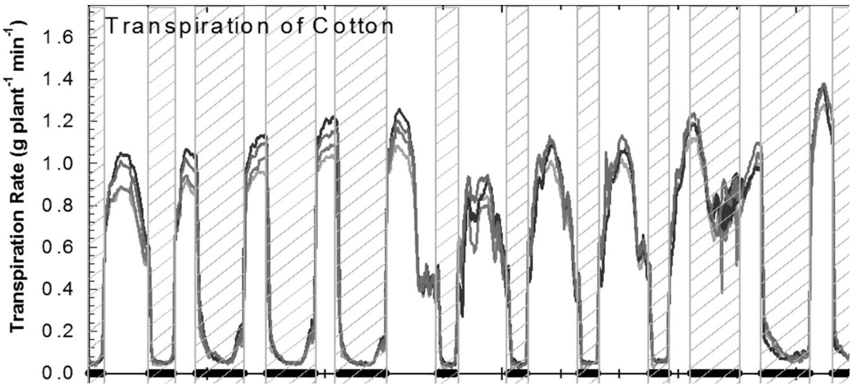

Figure 13. The effect of changing the photoperiod on transpiration rate in cotton.

The plants began to adapt to this longer photoperiod after only one day, and after three days of 16 hour light periods, the photoperiod was changed back to an 8 hour light period. The stomates opened in the dark for the first night, but then quickly adapted so that they closed almost normally in the second consecutive long night. Among the 3 primary crop plants we have studied (corn, soybeans and cotton), cotton has the most profound circadian rhythm. We have called this phenomenon: Jet Lag.

SUMMARY

Cotton has been a fascinating crop to work with. It is highly responsive to environmental signals and has significantly higher transpiration rates per unit leaf area than any other plant we have studied. These high transpiration rates likely help cotton leaves stay cool in environments with high air temperature.

We have not been able to find a role for ethylene in signaling a reduction in leaf elongation caused by either drought stress or heat stress. These studies do not prove that ethylene never has a role in signaling stress in these conditions, but they do indicate that it does not have the universal role that is suggested by textbooks.

Finally, ethylene does not appear to play a role in mediating stomatal aperture in well-watered plants. Cotton does have a profound circadian rhythm, however, which may help it stabilize transpiration rates in variable environmental conditions.

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