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DO CORN PLANTS SELF-PRIME AT DUSK?

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ABSTRACT

Green leaf volatile (GLV) emission as a result of abrupt light to dark transitions, termed darkshock, has been previously observed, but its mechanism and physiological importance has yet to be determined. Here we show that three cultivars of *Zea mays*, Delprim, B73, and W438, respond to darkshock by synthesizing and emitting GLVs within minutes of darkshock. Within the first 15 minutes after darkshock, Z-3-Hexenal is the predominant GLV. During the second 15-minute interval, Z-3-Hexen-1-ol and Z-3-Hexenyl Acetate begin to replace Z-3-Hexenal. During the third 15-minute interval, Z-3-Hexenyl Acetate is the predominant GLV with only trace amounts of the alcohol emitted. Gene expression of two maize lipoxygenases (LOXs), ZmLOX10, a key enzyme in the GLV biosynthesis pathway, and ZmLOX8, a key enzyme in the jasmonic acid (JA) biosynthesis pathway, was examined. ZmLOX10 was present in leaf tissue, but was not induced in response to darkshock. This suggests that LOX10 activity, rather than presence or absence, limits GLV emission. ZmLOX8 transcript levels, on the other hand, are induced after darkshock, but induction trails GLV emission by peaking between 30 and 60 minutes after darkness. The pattern of LOX8 induction suggests GLVs induce LOX8 as they percolate through the leaf before being emitted; yet, LOX8 transcript levels do not translate into measurable JA levels. The question whether the GLV burst after darkshock primes the plant remains undecided as JA levels, the typical telltale of priming, remain unchanged.

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Chapter 1

Introduction

Plants face stress during growth. Stressors can be grouped into two categories, biotic and abiotic. Biotic stressors include herbivores, pathogens, and fungi. Abiotic stressors include among others, mechanical damage, drought, and nutrient deficient soil. To cope with stress, plants have evolved numerous defense mechanisms. While some defense mechanisms are constitutive (i. e. they are always present), others are induced after the plant perceives a threat (Frost et al, 2008). One of these induced defense mechanisms is the production and release of volatile organic compounds (VOCs).

Green Leaf Volatiles

Green leaf volatiles (GLVs) are C_6-aldehydes and C_6-alcohols synthesized in plant leaves in response to wounding and stress (Matsui, 2006). There are nine GLVs commonly produced in plants (Matsui, 2006). Prior to threat perception, GLV levels in leaves are low (Matsui, 2006). Upon onset of stress, GLVs are rapidly synthesized from freed linolenic and linoleic acids (Jardine, 2012). Synthesis begins with a 13-lipoxygenase (LOX) catalyzed oxygenation of the 13-carbon of free linolenic acid, producing 13-hydroperoxylinolenic acid (HPLA) (Jardine, 2012). HPLA is then converted to Z-3-hexenal by hydroperoxide lyase (HPL) (Jardine, 2012). Z-3-hexenal, which is toxic to the plant, is rapidly converted into the alcohol Z-3-hexenol and the acetate Z-3-hexenyl acetate (Matsui, 2006, Matsui et al, 2012).

After wounding, GLVs are synthesized rapidly within the disrupted tissue. Although most concentrated at wound sites, GLVs are not limited to the wounded tissue. GLVs diffuse through the plant

from wounded leaves to unwounded leaves, turning on defense genes that will lead to a systemic response (Matsui, 2006). When a full systemic response is occurring, all leaf tissue emits GLVs (Rose et al, 1996).

GLVs have been linked to many physiological functions. GLVs produced at wound sites combat bacteria and fungi that gain access through disrupted tissue. Eight GLVs tested in a study by Nakamura and Hatanaka (2002) showed antimicrobial properties (Nakamura and Hatanaka, 2002). The growth of five bacteria strains tested (*E. coli, MRSA, S. aureus, O-157:H7, and Sal. enteritidis*) was inhibited when GLVs were present at 12.5μg/mL or higher.

GLVs play multiple roles in the interactions between herbivores and their host plants. Oligophages, such as the Colorado potato beetle, use GLVs released from their hosts, E-2-hexenol, Z-3-hexenol, and E-2-hexenal, as a means to determine which plants are suitable for oviposition and feeding (Visser and Ave, 1978). *Manduca sexta* uses GLVs as a feeding stimulant. *Manduca sexta* feeding on tobacco with HPL inactivated, and therefore no GLV production, fed less often and grew less than those feeding on wild type plants (Halitschke et al, 2004). The moth *Heliothis virescens* uses GLVs to determine suitable plants for oviposition. Nocturnal volatile profiles of compounds released by tobacco plants being fed on by herbivores are recognized by this moth (De Moraes et al, 2001). Moths respond to these signals by pursuing a different host plant to avoid depositing eggs on a host that has competing herbivores already feeding.

GLVs also play a critical role as signals in indirect plant defenses (Matsui, 2006). Indirect plant defenses provide protection by attracting parasitoids from higher trophic levels (Arimura, 2005). Species attracted by GLVs parasitize or consume herbivores attacking the plant. Some parasitoids, such as *Cardiochiles nigriceps*, a parasitic wasp, distinguish between herbivore specific volatile blends. The wasp can distinguish between plants attacked by *Heliothis virescens* and plants attacked by *Helicoverpa zea*, allowing it to find and parasitize its host, *Heliothis virescens*, more efficiently (De Moraes et al, 1998).

Finally, GLVs also play a role in inter- and intra-plant communication. GLVs produced in a damaged leaf can diffuse through the plant to undamaged leaves, turning on defense genes that will mount a systemic defense against the threat (Matsui, 2006). Volatilized GLVs can be sensed by neighboring plants as an alarm signal, allowing these plants to turn on defense genes in preparation for future attack. This is known as priming. When attacked by an herbivore, primed plants respond more rapidly with potent inducible defense responses, including JA production and volatile emission, than unprimed plants (Engelberth et al, 2004).

Darkshock

Darkshock is the term given to rapid light to dark transitions. Plants respond to darkshock with emission of GLVs. Previous works have studied the GLV burst after darkshock, but few have attempted to determine the mechanism of GLV synthesis and the physiological reason for the GLV burst. This study investigates darkshock in *Zea mays* and begins to shed light on the mechanism and purpose of GLV bursts after darkshock.

Graus et al (2004) first showed that GLVs were emitted by plants after darkshock using poplar leaves. Proton transfer reaction-mass spectrometry, a technique that identifies volatile compounds and analyzes emissions in real-time, indicated that large amounts of hexenal, followed by hexenol and hexenyl acetate, were emitted within 30 seconds of darkshock (Graus et al, 2004). Along with identification, the study focused primarily on how aerobic vs anoxic conditions changed the observed pattern of volatile organic compounds after darkshock (Graus et al, 2004). No GLV emission was observed under anoxic conditions, showing that the lipoxygenase pathway, which requires oxygen as a substrate, is involved in the formation and release of GLVs (Graus et al, 2004, Jardine et al, 2012).

Additionally, work using African grasses showed a significantly increased amount of volatile emissions in the first hour of darkness (Chamberlain et al, 2006). Of the total increase in emission, 95%

was due to GLVs. The majority of these GLVs, 74%, were emitted during the first 10 minutes of darkness (Chamberlain et al, 2006). Chamberlain et al (2006) used experimental conditions that mimicked nightfall at the equator, providing evidence that the GLV emission observed in laboratory conditions also occurs in the field.

Recent publications have studied GLV emission following darkshock in more detail. The identities of GLVs emitted were determined by Brilli et al (2011) using a proton transfer reaction-time of flight mass spectrometer. The study determined that Z-3-hexenal and E-3-hexenal were emitted rapidly after darkness, dissipating quickly after peaking due to their volatility. Alcohols (Z-3-hexenol, E-3-hexenol, and E-2-hexenol) and Z-3-hexenyl acetate were emitted following the aldehyde, as they were synthesized using aldehyde as a substrate (Brilli et al, 2011).

Volatile emission following darkshock has also been reported in mesquite branches. GLV emission patterns corroborated the findings of Graus et al (2004) and Brilli et al (2011). It was suggested that the GLV burst occurred due to the activation of LOXs in chloroplasts of darkshocked plants, causing the rapid oxidation of free linolenic acids (Jardine et al, 2012).

Priming

Priming is the physiological process by which a plant prepares to more quickly or aggressively respond to stress (Frost et al, 2008). To reduce the length of the lag period between threat detection and defense induction, plants have evolved the ability to sense signals, such as GLVs, which indicate an attack may be imminent. This ability allows for defense mechanisms to be prepared before an attack occurs. Priming is observed in plants as the up-regulation of defense-related genes in response to challenge (Frost et al, 2008). One of the telltales of priming is an increase in JA levels within 30 minutes of exposure to GLVs, which disappears within two hours (Engelberth et al, 2004).

Lipoxygenases

Lipoxygenases (LOX) are involved in both GLV and JA biosynthetic pathways. LOXs are nonheme iron-containing dioxygenases that catalyze the addition of oxygen to unsaturated fatty acids. Oxygen can be added to either end of the pentadiene system, resulting in either 9- or 13- hydroperoxy linolenic acid (Siedow, 1991). LOXs adding oxygen to the 9-carbon are 9-LOXs and LOXs adding oxygen to the 13-carbon are 13-LOXs. The unsaturated fatty acid hydroperoxide products of LOX-mediated reactions are highly toxic to plant cells, causing them to be quickly used as substrates for one of the seven downstream branches of the LOX pathway (Feussner and Wasternack, 2002, Porta and Rocha-Sosa, 2002, Gao et al, 2007).

In corn TS1/LOX8, a 13-LOX localized in plastids, catalyzes the first step in the biosynthesis of JA responsible for sex determination (Acosta et al, 2009). Wound-induced JA is also synthesized via TS1/LOX8 (Christensen et al, 2013). In fact LOX8 transcripts are induced within 30 minutes of wounding and disappear within 2 hours after wounding (Christensen et al, 2013). 13-Hydroxy linolenic acid produced by LOX8 is used as a substrate by allene oxide synthase (AOS) leading to production of JA (Christensen et al, 2013). Experiments with LOX8 mutants showed that wounded wild-type plants produced 66% higher JA levels than wounded mutants. This indicates that while LOX8 plays a primary role in producing substrate for JA biosynthesis, other LOXs are also providing substrate to the pathway (Christensen et al, 2013).

In corn, ZmLOX10 is a 13-LOX that acts in the GLV biosynthesis pathway. LOX10 catalyzes the oxygenation of the 13-carbon of linolenic acid, yielding 13-hydroperoxy linolenic acid that is further modified by HPL which converts 13-hydroperoxy linolenic acid into GLVs (Christensen et al, 2013). This activity has been confirmed using LOX10 mutants. Mutants showed no GLV emission after wounding, indicating LOX10 is required for GLV synthesis and no other LOX provides substrate to the GLV biosynthesis pathway (Christensen et al, 2013). LOX10 is expressed in non-chloroplast organelles of the leaf tissue (Nemchenko et al, 2006, Mohanty et al, 2009, Christensen et al, 2013). Exogenous

application of JA and wounding induces ZmLOX10 transcript levels, indicating the gene plays a part in wound response (Nemchenko et al, 2006).

LOX10 transcripts contain light-responsive elements, making them light-dark dependent (Nemchenko et al, 2006). LOX10 levels increase rapidly following daybreak, reaching maximum expression six hours following light exposure. Transcript levels remain high until nightfall, at which time they decrease, reaching lowest levels six hours after darkness (Nemchenko et al, 2006).

ZmLOX11 is a homolog to ZmLOX10 that likely arose due to a gene duplication event. The amino acid sequences of the two genes are 90% homologous (Nemchenko et al, 2006). LOX11 is expressed at greatest levels in silks and does not follow a circadian rhythm pattern (Christensen et al, 2013). Wounding and exogenous JA application does not induce LOX11 (Christensen et al, 2013).

While LOX10 appears to be the only LOX that controls GLV production, crosstalk occurs in the JA biosynthesis pathway. LOX10-mutants showed decreased induction of LOX8, indicating that LOX10 is required for proper JA biosynthesis and that LOX10 may provide substrate for production of both JA and GLVs (Christensen et al, 2013). When GLVs were applied to LOX10 mutants, JA levels were partially restored (Christensen et al, 2013). Therefore, GLVs may play a role in wound-induced JA biosynthesis. The model of LOX8/LOX10 interaction suggested by Christensen et al. 2013 shows LOX10 derivatives produced in damaged tissue inducing the transcription of ZmLOX8, leading to increased JA biosynthesis (Christensen et al, 2013).

In this thesis, I investigated the following questions:

- Under which conditions do corn plants emit GLVs after darkshock and do corn cultivars respond to darkshock equally with respect to emitted GLVs?
- Does the timing of darkshock affect corn GLV emission?
- Does an induction of the biosynthetic enzymes of GLVs (LOX10, LOX11, or HPL1 transcripts) explain the emission of darkshock-induced GLVs; i. e. what is the mechanism of darkshock?

- Do darkshock-induced GLVs prime the plant; i. e. are LOX8 transcripts induced? If so, is JA biosynthesis induced in darkshocked plants?

Chapter 2

Do maize cultivars respond to darkshock equally with respect to emitted GLVs?

The pattern of GLV emission after darkshock has been documented in a number of studies (Graus et al, 2004, Brilli et al, 2011, Jardine et al, 2012). None of these studies, however, have analyzed GLV emission after darkshock in *Zea mays*. We tested three cultivars of *Zea mays*, Delprim, W438, and B73, to determine whether different cultivars respond to darkshock similarly and to identify the most responsive cultivar to use in further experiments. Twelve-day-old seedlings were cut at 9am and placed in water or Linolenoyl-L glutamine. At 1:30pm a subset of each treatment group was subjected to dark shock.

All three cultivars showed GLV emission following darkshock. Hexenyl acetate was the only GLV detected during 90-minute collection intervals. The pattern of emission was similar in all three cultivars, with hexenyl acetate emissions peaking within 1.5 hours of darkshock. However, hexenyl acetate peak emission amounts differed between cultivars.

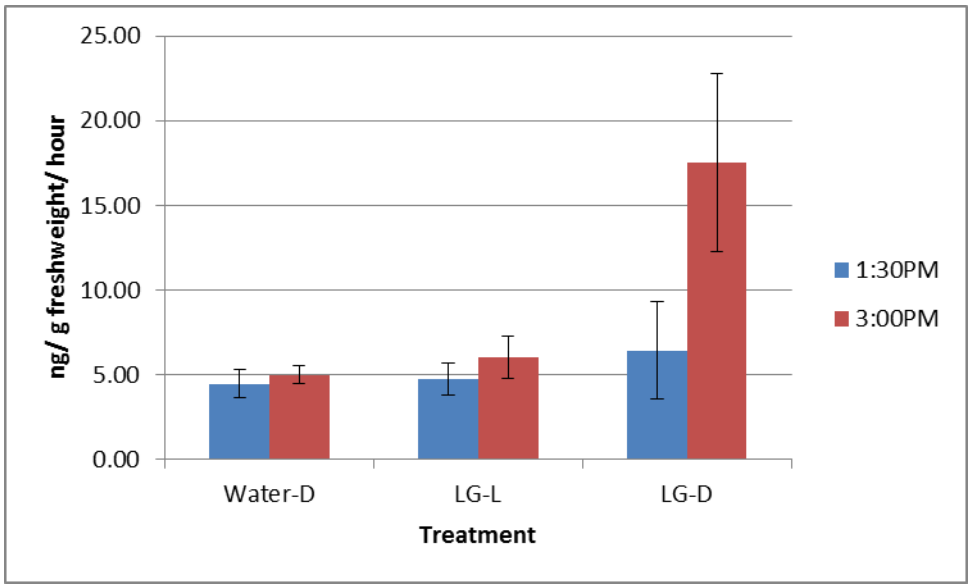

Figure 1: Hexenyl acetate emission in cut Delprim seedlings. Delprim seedlings induced with Ln L-Glutamine and darkshocked at 1:30pm showed a 2.7-fold increase in hexenyl acetate in the first 1.5 hours of darkness compared to

hexenyl acetate emissions of the 1.5 hour period before darkshock. Uninduced plants darkshocked at 1:30pm did not show an increase in hexenyl acetate emission. Induced seedlings left in the light showed no difference in hexenyl acetate emission in either collection interval. The graph is the average of two independent experiments. Each bar represents the average of eight seedlings (±SE). Seedlings were cut at 9:30am. Axis Labels- Water-D: uninduced seedlings darkshocked at 1:30pm, LG-L: induced seedlings remaining in the light, LG-D: induced seedlings darkshocked at 1:30pm.

As seen in Figure 1, Delprim cut stem seedlings only showed response to darkshock after induction with elicitor solution. Uninduced seedlings placed directly into water did not respond to darkshock with a measurable burst of hexenyl acetate. Induced seedlings showed hexenyl acetate emission in the range of 5 to 50ng/g fresh weight/ hour in the first 1.5 hour interval after darkshock.

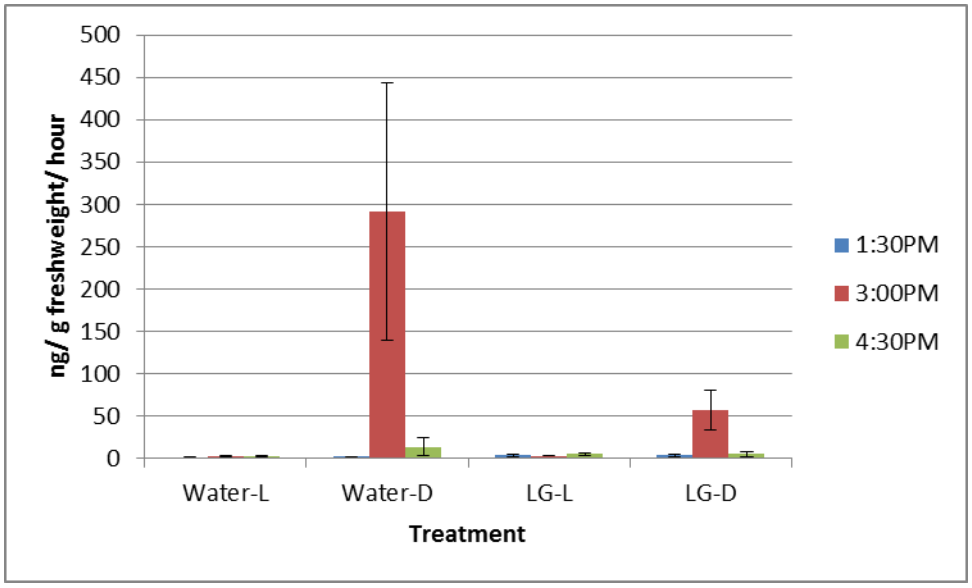

Figure 2: Hexenyl acetate emission in cut B73 seedlings. GLV emission after darkshock was observed in both seedlings induced with Ln L-Glutamine and uninduced seedlings. Average hexenyl acetate emission was 5-fold greater in the first 1.5 hours of darkness in uninduced seedlings than in induced seedlings. High variation in hexenyl acetate emission was observed in darkshocked seedlings. Induced and uninduced seedlings kept in the light showed baseline levels of hexenyl acetate emission during all collection periods. Each bar represents the average of three seedlings (± SE). Seedlings were cut at 9:30am. Axis Labels- Water-L: uninduced seedlings remaining in the light, Water-D: uninduced seedlings darkshocked at 1:30pm, LG-L: induced seedlings remaining in the light, LG-D: induced seedlings darkshocked at 1:30pm.

Both B73 induced and uninduced seedlings responded to darkshock as seen in Figure 2. Induced seedlings showed hexenyl acetate emission averaging 57ng/g fresh weight/ hour in the first 1.5 hour interval after darkshock. Uninduced seedlings showed hexenyl acetate emission averaging 292ng/ g fresh weight/ hour in the first 1.5 hour interval after darkshock. Large variation between seedlings was observed in B73.

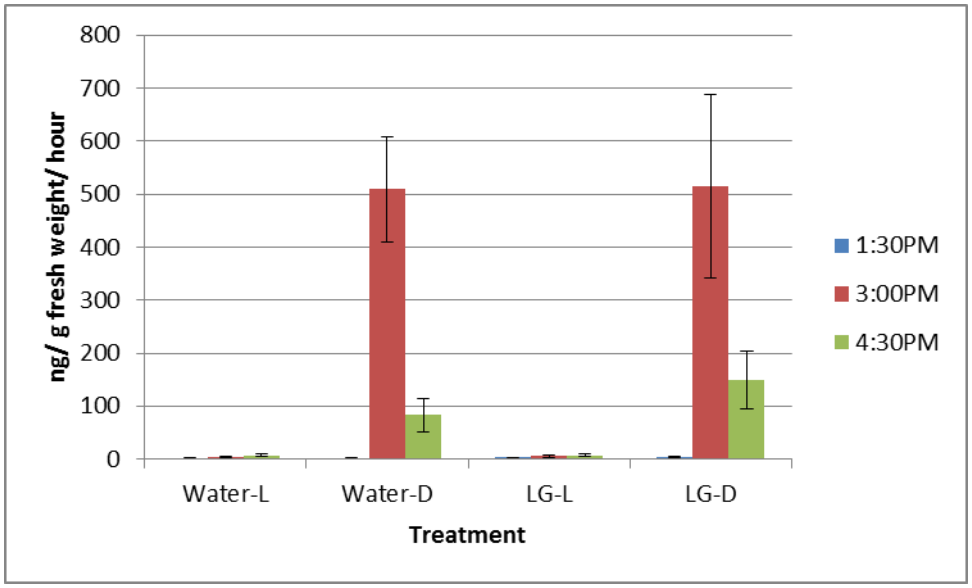

Figure 3: Hexenyl acetate emission in cut W438 seedlings. Darkshocked induced and uninduced W438 seedlings emitted similar amounts of hexenyl acetate in the 1.5 hours after darkshock. Variation between samples was less in uninduced seedlings. Seedlings of both treatments that remained in the light showed baseline hexenyl acetate emissions at all time-points. Each bar represents the average of 3 seedlings (± SE). Seedlings were cut at 9:30am. Axis Labels- Water-L: uninduced seedlings remaining in the light, Water-D: uninduced seedlings darkshocked at 1:30pm, LG-L: induced seedlings remaining in the light, LG-D: induced seedlings darkshocked at 1:30pm.

As seen in Figure 3, both induced and uninduced W438 cut stem seedlings showed similar responses to darkshock. Induced seedlings emitted 514ng/ g fresh weight/ hour hexenyl acetate during the first 1.5 hours after darkshock. Uninduced seedlings emitted 509 ng/ g fresh weight/ hour in the first 1.5 hours after darkshock. W438 seedlings showed the largest burst of GLVs of any cultivar tested following darkshock.

In summary, each cultivar showed a large increase in hexenyl acetate emission in the first 1.5 hours following darkshock. However, variation was observed between the cultivars. Delprim seedlings required induction to produce a GLV burst and emitted a small amount of hexenyl acetate compared to B73 and W438 seedlings. Induced Delprim seedlings emitted just 3% of the hexenyl acetate of W438 seedlings and 6% of the hexenyl acetate of B73 seedlings. B73 seedlings showed a large GLV burst in uninduced seedlings with high seedling to seedling variation. Induced and uninduced W438 seedlings emitted the largest amounts of hexenyl acetate following darkshock with less variation than B73. Therefore all subsequent experiments were conducted using uninduced cut W438 seedlings.

It was not determined why each cultivar varied in its response to darkshock. Further experiments could be conducted that examine the GLV bursts following darkshock in greater detail. Transcript and hormone levels could also be tested to determine if gene expression differences between cultivars account for the differing responses to darkshock.

How do intact seedlings respond to darkshock with respect to emitted GLVs?

Cut seedlings are mechanically wounded. To determine if undamaged seedlings respond differently, intact, uncut W438 seedlings were subjected to darkshock.

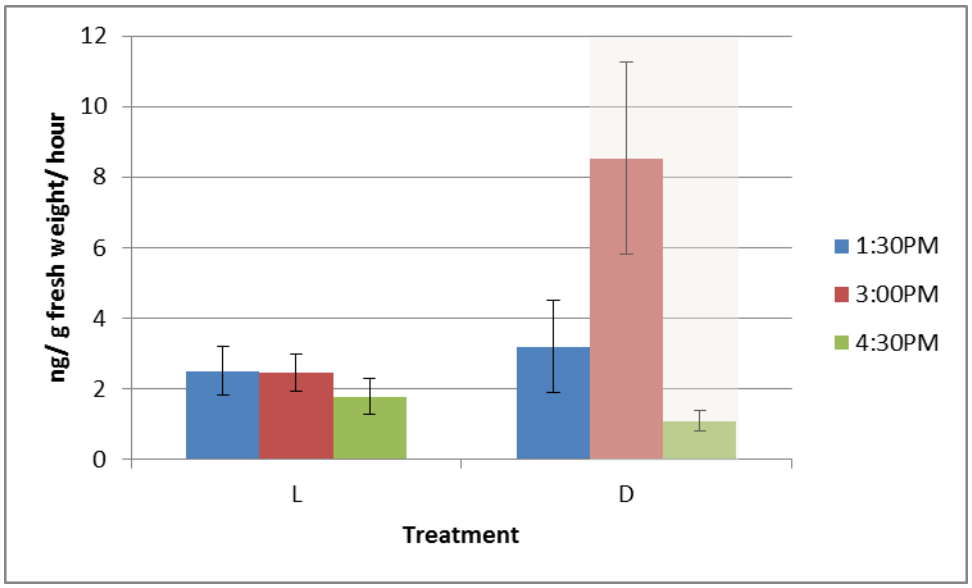

Figure 4: Hexenyl acetate emission in W438 intact seedlings. Intact seedlings darkshocked at 1:30pm showed the same pattern of hexenyl acetate emission as cut stem seedlings. But, emission for darkshocked plants in the first 1.5 hours after darkness was only 8.5 ng/ g fresh weight / hour. Each bar represents the average of three seedlings (± SE). The graph is representative of two independent experiments. Axis Labels- L: seedlings remaining in the light, D: seedlings darkshocked at 1:30pm.

Hexenyl acetate emission following darkshock of intact seedlings followed a similar pattern of emission to cut plants. During the 1.5 hour period following darkshock, plants emitted the largest amount of hexenyl acetate. Emission rates, however, were significantly lower compared to cut plants. Emission totaled just 8.5 ng/g fresh weight/ hour during the first collection period following darkshock, less than two percent of the emissions seen in cut stem W438.

Low emission in intact plants indicates that wounding is required for significant hexenyl acetate emission. Wounding may liberate linolenic acid that is used as substrate, allowing for a substantial GLV burst. Additionally, LOX levels may be induced by wounding, increasing enzyme activity and emission levels.

Chapter 3

How does timing of darkshock affect GLV emission?

LOX10 follows a diurnal rhythm and transcript levels are low at night (Nemchenko et al, 2006). After daybreak, LOX10 transcripts increase for the first six hours of daylight, reaching maximum expression at 2pm (Nemchenko et al, 2006). If the quantity of GLVs emitted after darkshock is limited by LOX10 activity, it would be expected that GLV emission rates would mirror LOX10 transcript levels.

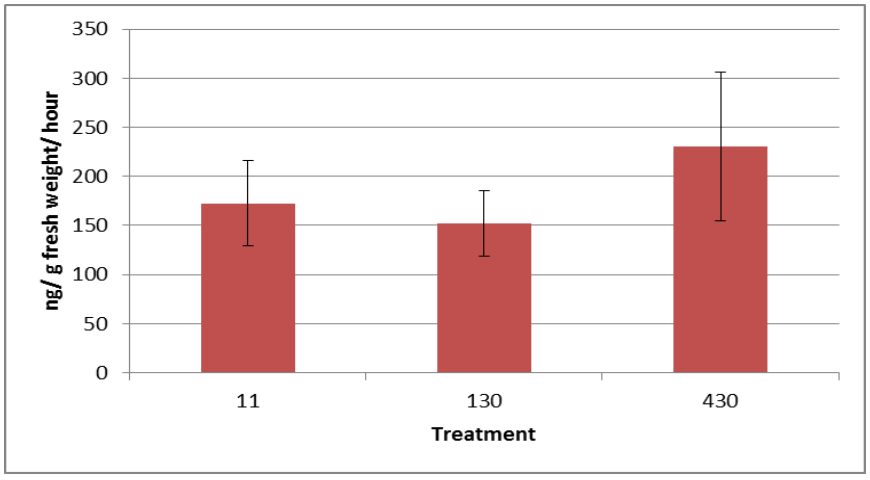

Figure 5: Hexenyl acetate emission following darkshock of cut W438 seedlings at three different times. Each bar represents the 1.5 hour time period following darkshock. Emission totals were similar regardless of time of day. Each bar represents the average of four seedlings (± SE). Seedlings were cut at 9:30am. Axis Labels- 11: seedlings darkshocked at 11:00 am, 130: seedlings darkshocked at 1:30 pm, 430: seedlings darkshocked at 4:30 pm.

W438 seedlings cut at 9:30am were darkshocked at 11:00 am, 1:30 pm, and 4:30 pm. GLV emission following darkshock was observed at all three times. As seen in Figure 5, significant differences were not observed between times of darkshock. The 11:00 time point is within the six hour period when LOX10 transcript levels increase following daybreak according to Nemchenko et al (2006). If LOX10 transcripts were wholly responsible for GLV emission rates, we would have expected that GLV emission rates at the 11:00 time point were slightly decreased compared to emission levels at 1:30 and 4:30 when LOX10 transcript levels were near their peak according to Nemchenko et al (2006).

We also conducted a more detailed time course for seedlings exposed to the normal onset of darkness with a 30 minute simulated dusk period while emitted GLVs were collected at 30 minute intervals. Seedlings were cut at 9:30am or 5:30pm and subjected to a 30 minute dusk period between 9:00 pm and 9:30 pm. As seen in Figure 6 and Figure 7, seedlings showed baseline GLV emissions during the 30 minute dusk period. GLVs were only emitted following full darkness. The time interval elapsed between cutting and dusk did not change the pattern of GLV emission or the rate at which GLVs were emitted following full darkness.

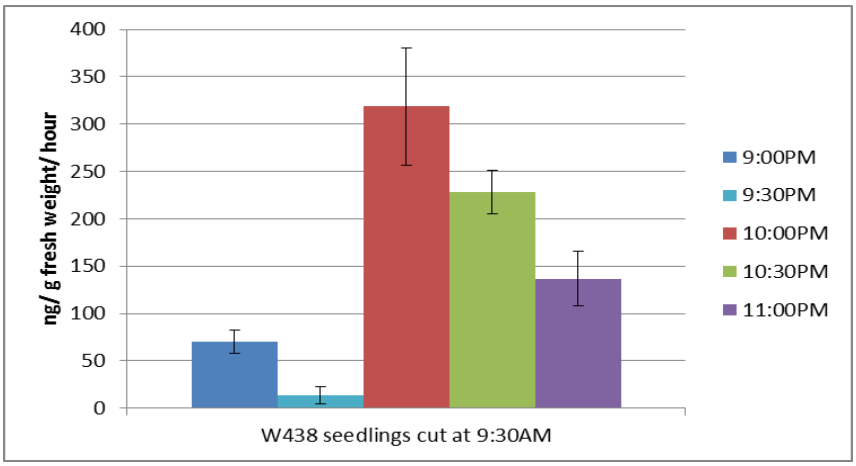

Figure 6: Hexenyl acetate emissions in seedlings cut at 9:30 am and exposed to artificial dusk. Hexenyl acetate emission was not observed during the 30 minute artificial dusk. Each bar represents the average of four seedlings (± SE). 9:30PM bar: volatiles collected during the 30 minute dusk period, 10:00PM bar: volatiles collected during the first 30 minutes of full darkness.

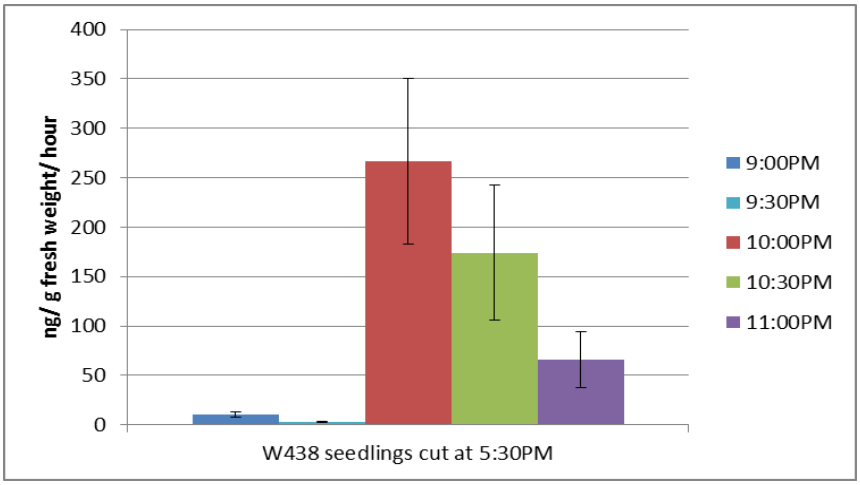

Figure 7: Hexenyl acetate emissions in seedlings cut at 5:30 pm and exposed to artificial dusk. Hexenyl acetate emission was not observed during the 30 minute artificial dusk. Each bar represents the average of four seedlings (± SE). 9:30PM

bar- volatiles collected during the30 minute dusk period, 10:00PM bar- volatiles collected during the first 30 minutes of full darkness.

In summary, darkshock induced GLV emission was independent of the time elapsed between cutting the seedlings and darkshock treatment. Inducing darkshock at different times of the day did not result in different GLV emission amounts, indicating that GLV emission amounts do not mirror LOX10 transcript levels. Adding an artificial dusk did not change the pattern of GLV emission upon the onset of full darkness.

Chapter 4

What is the mechanism of darkshock?

Many studies have shown that plants respond to rapid light-dark transitions with a burst of GLVs, but little research has focused on the mechanism that leads to GLV emission. In order to gain a better understanding of the GLV biosynthetic pathway, a volatile collection time course with darkshock at 1:30pm and collection periods of 15 minutes was matched to seedling gene expression. Genes in the GLV biosynthesis pathway were tested to determine if up-regulation of a specific gene was causing the GLV burst that was observed following darkshock.

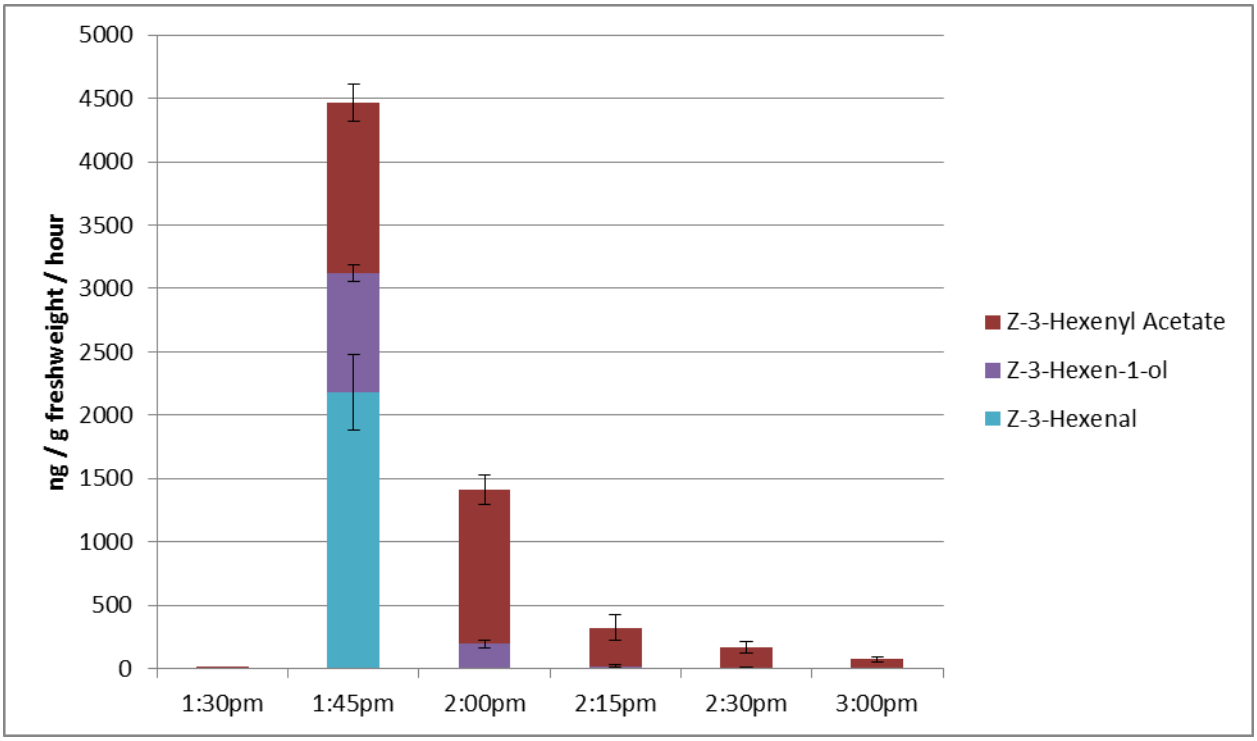

Figure 8: GLV emission of cut W438 seedlings cut at 9:30 am and darkshocked at 1:30 pm. Three GLVs made up the total GLV emission spectrum. Z-3-hexenal was emitted in the first 15 minute collection interval only. Z-3-hexen-1-ol was emitted during the first 30 minutes following darkshock. Z-3-hexenyl acetate was emitted at all collection time points following darkshock. Each bar represents the average of six seedlings (±SE). The graph summarizes two independent experiments.

When GLVs were collected at 15 minute intervals, two GLVs, Z-3-hexenal and Z-3-hexen-1-ol, were detected that were not observed when GLVs were collected for longer intervals. These GLVs likely broke through the SuperQ filter before the filter was removed when longer collection intervals were used. Z-3-hexenal and Z-3-hexen-1-ol may also have been lost in the cut stem assay of Delprim and B73. As seen in Figure 8, Z-3-hexenal, the first GLV produced in the GLV biosynthesis pathway, is emitted only in the first 15 minutes following darkshock. By the fifteen minute time point, all Z-3-hexenal has been emitted or been converted into its downstream GLVs. Similarly, Z-3-hexen-1-ol was emitted in the first two 15 minute collection intervals before it was emitted or converted into Z-3-hexenyl acetate. Z-3-hexenyl acetate was emitted over a 1.5 hour period as observed in previous experiments.

Seedlings used for gene expression were harvested at the same experimental time points as the filter changes for the volatile collection experiment. Tissue collection began after cutting all seedlings at 9:30am. All seedlings remained in the light until 1:30pm when half of the remaining seedlings were darkshocked. Prior to darkshock, four seedlings were collected at each time point to determine baseline transcript levels and if transcript levels responded to wounding. After 1:30pm when darkshock was induced, four darkshocked seedlings and four control seedlings that remained in the light were collected at each time point. The gene expression experiment was run twice independently. The first was carried out in August 2014 and the second in November 2014.

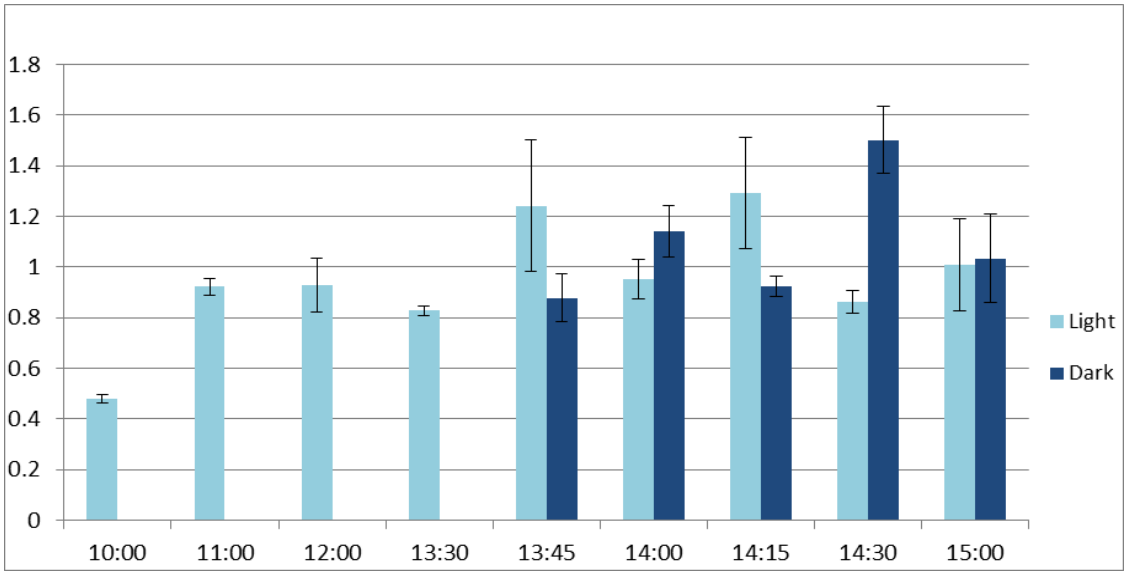

Figure 9: LOX10 transcript levels replicate one. LOX10 levels in light tissue increased between 10:00 and 11:00 before stabilizing. Darkshocked tissue showed no induction. Each bar represents the average of four seedlings (±SE). Seedlings were cut at 9:30am.

As seen in Figure 9 and Figure 10, LOX10 in light samples increased in the morning hours from approximately 10:00 to 12:00. These time points are within the period after daybreak when LOX10 transcript levels increase to daytime levels (Nemchenko et al, 2006). Transcript levels stabilized between 11:00 and 12:00 and remained stable through the remainder of the experiment.

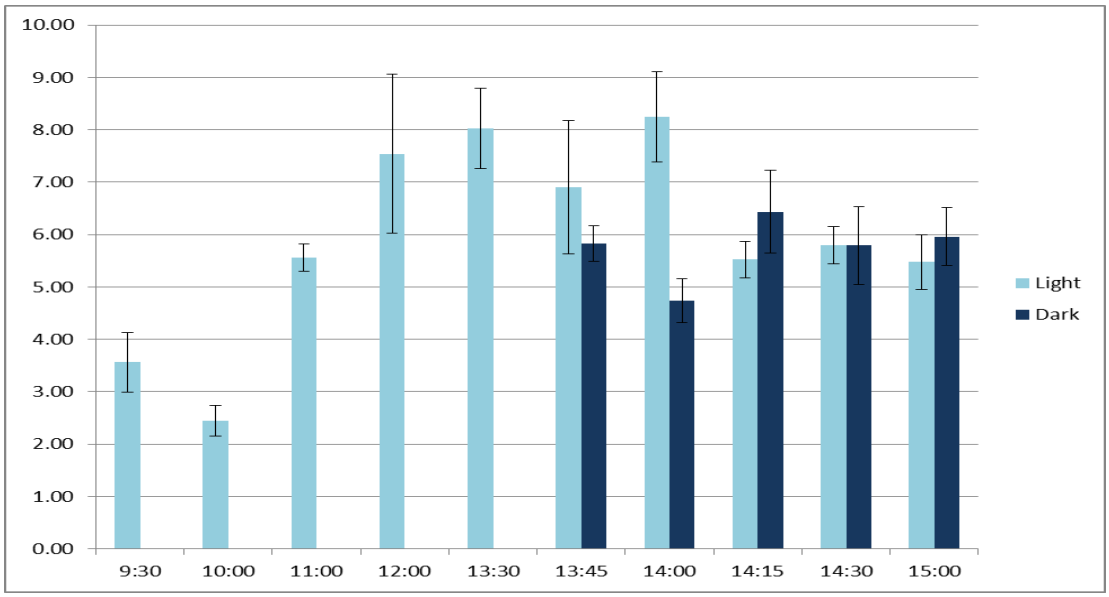

Figure 10: LOX10 transcript levels replicate two. LOX10 levels in light tissue increased between 10:00 and 12:00 before stabilizing. Darkshocked tissue showed no induction. Each bar represents the average of four seedlings (±SE). Seedlings were cut at 9:30am.

LOX10 in darkshocked samples showed no induction following darkness. Transcript levels remained approximately equal in darkshocked and light control seedlings through the duration of the experiment after darkshock was induced. Both darkshocked and light samples contained similarly high quantities of LOX10 transcripts, therefore, it does not appear that LOX10 up-regulation is responsible for the GLV burst following darkshock.

Although not the limiting factor for the GLV burst, LOX10 is still required for GLV synthesis (Christensen et al, 2013). The high total amounts of LOX10 transcripts suggest that the transcript sits in leaf tissue at high levels during the day. If the transcript levels correlate with protein levels, large amounts of LOX10 protein are also likely to be present throughout leaf tissue.

If LOX10 proteins residing in the leaf tissue during daylight hours are constitutively active, the production of GLVs via the GLV biosynthesis pathway may be substrate limited. After darkshock, linolenic acid freed during darkshock would be immediately oxygenated by LOX10 (Brilli et al, 2011). This would lead to rapid synthesis of GLVs by the rest of the GLV biosynthesis pathway. Without darkshock, linolenic acid is membrane bound and would therefore not be available for oxygenation by LOX10 proteins. No GLVs would be synthesized or emitted by the plant.

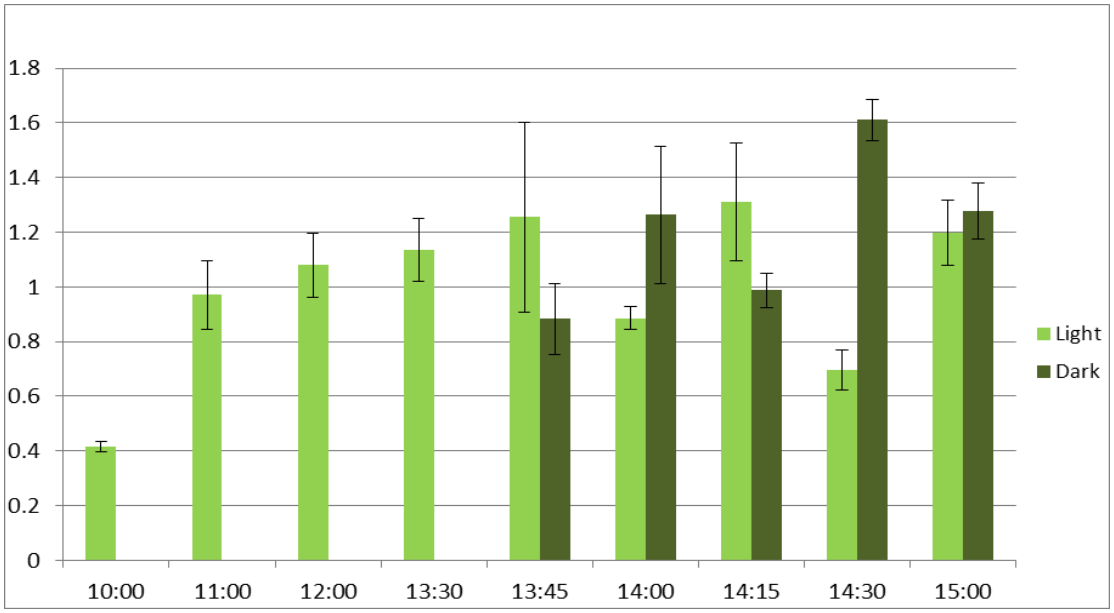

Figure 11: LOX11 transcript levels. LOX11 transcript levels mirrored LOX10 transcript levels. No induction was observed in darkshocked plants. Each bar represents the average of 4 seedlings (±SE). Seedlings were cut at 9:30am.

LOX11, a homolog of LOX10, was also tested to determine if transcript levels respond to darkshock. LOX11 likely arose due to a gene duplication of LOX10. This would reduce the selective pressure on LOX11, possibly allowing LOX11 to gain a new function. Because of the sequence similarity between LOX10 and LOX11, it is possible that LOX11 may have evolved to control the GLV biosynthesis pathway.

As seen in Figure 11, the pattern of LOX11 transcripts mirrored LOX10 transcripts. However, in accordance with Nemchenko et al (2006) we also found that LOX11 transcript levels were significantly lower than LOX10 transcript levels in leaf tissue. As no induction was observed after darkshock, we did not measure LOX11 transcript levels in later experiments.

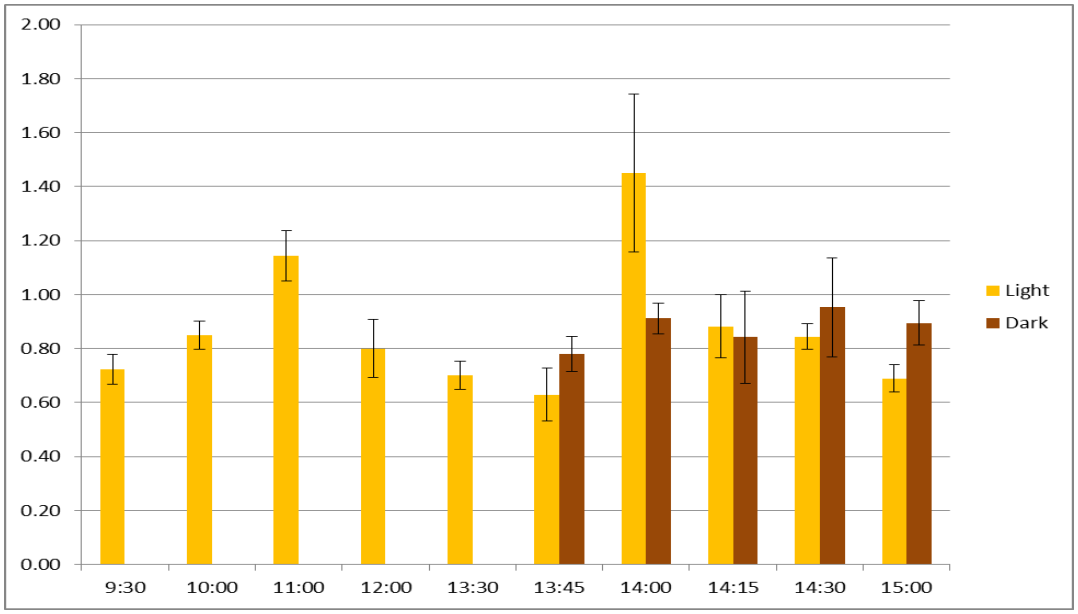

Figure 12: HPL1 transcript levels. HPL1 transcript levels were not induced after darkshock. Each bar represents the average of four seedlings (±SE). Seedlings were cut at 9:30am.

HPL is a downstream enzyme of LOX10 in the GLV biosynthesis pathway. As LOX10 induction does not appear to be responsible for GLV biosynthesis after darkshock, HPL1 transcripts were investigated to determine if this downstream enzyme limited GLV emission. As seen in Figure 12, HPL1 transcripts were not induced by darkshock.

If darkshocked seedlings are getting primed, we would expect that the LOX of the JA pathway would be induced with a subsequent increase of JA levels. LOX8 has been shown to be a key enzyme in the JA biosynthesis pathway and transcript levels of LOX8 are induced after wounding (Christensen et al, 2013). LOX8 transcript levels were measured to determine if transcript levels were induced.

As seen in Figure 13 and Figure 14, LOX8 transcripts were highly induced due to wounding at 10:00, 30 minutes after seedlings were cut. Transcript levels decreased to baseline throughout the course of the morning and remained at baseline levels for the duration of the experiment in control plants. This corroborates the work of Nemchenko et al (2013) in which LOX8 transcript levels followed a similar induction pattern after mechanical wounding. In that study, LOX8 transcripts were highly induced within

30 minutes of mechanical wounding. The induction tapered off over a four hour period (Nemchenko et al, 2012).

Darkshocked seedlings showed a second induction of LOX8 following darkshock that began between 15 and 30 minutes after darkness as seen in Figure 13 and Figure 14. The induction peaked 30 minutes to an hour after darkshock. Samples showed a maximum LOX8 induction of 4-times control levels before tapering off over the final 45 minutes of the experiment. While LOX8 transcript levels were induced following darkshock, induction was not as great as induction due to wounding.

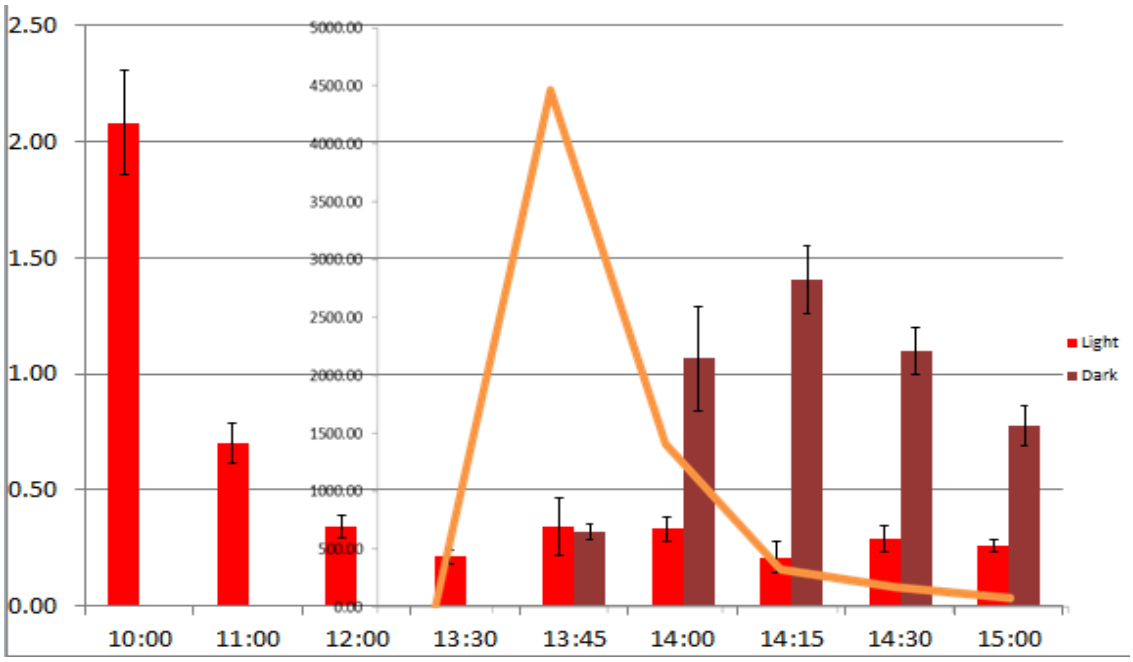

Figure 13: LOX8 transcript levels replicate one. Transcript levels were induced due to wounding 30 minutes after cutting. Transcripts decreased to baseline levels by 12:00pm. Darkshocked seedlings showed a second induction of LOX8 beginning between 15 and 30 minutes post-darkshock. Peak induction occurred 30 to 60 minutes post-darkshock before tapering off towards baseline levels. The orange overlay shows total GLV emission at each time point following darkshock in ng/ g fresh weight/ hour. Seedlings for both volatile collection and tissue collection experiments were cut at 9:30am. Tissue collections began at 10:00am. Seedlings were darkshocked at 1:30pm in both volatile collection and tissue collection experiments. Each bar represents the average of four seedlings (±SE).

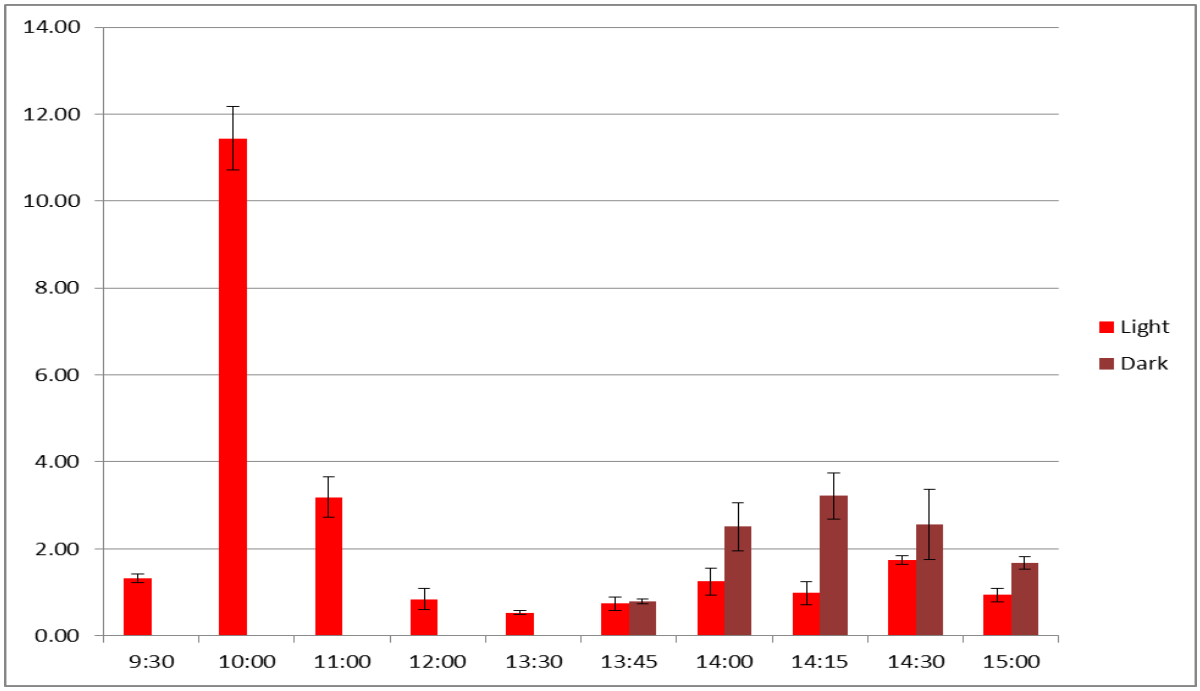

Figure 14: LOX8 transcript levels replicate two. Transcript levels were induced due to wounding 30 minutes after cutting. Transcripts decreased to baseline levels by 12:00pm. Darkshocked seedlings showed a second induction of LOX8 beginning between 15 and 30 minutes post-darkshock. Peak induction occurred 30 to 60 minutes post-darkshock before tapering off towards baseline levels. Each bar represents the average of four seedlings (±SE). Seedlings were cut at 9:30am.

Christensen et al (2013) suggested that there is an interaction between LOX10 and LOX8. In that study, LOX10 mutants had JA levels partially restored by the application of GLVs. This suggests that GLVs may be able to induce LOX8 levels, leading to JA production. As seen in Figure 13, in which GLV emission is overlaid with LOX8 transcript levels, LOX8 induction occurs approximately 15 minutes after peak GLV emission. The GLV burst immediately after darkshock may be responsible for the induction of LOX8 transcripts.

The research by Christensen et al (2013) was conducted on LOX10 mutants, with exogenous GLVs applied. JA levels were partially restored after GLV application indicating that LOX8 was induced by the GLV application. In this experiment, similar results were observed; however, the GLVs inducing LOX8 were not exogenous but produced by the plant. This raised the question of whether induced LOX8 transcript levels following darkshock were resulting in JA production.

To determine if the LOX8 induction following darkshock was leading to JA synthesis, hormone analysis was conducted. In two independent experiments, JA levels did not increase above baseline levels following darkshock, indicating that the increase in LOX8 transcript levels did not translate to JA synthesis. This may have occurred due to a lack of substrate. The JA and GLV biosynthesis pathways both use linolenic acid as a substrate. If the GLV burst used all of the freed linolenic acid substrate that resulted from darkshock, none would remain to be synthesized into JA when LOX8 induction occurred. There is an ongoing debate in the JA community on whether the LOX responsible for GLVs and the LOX responsible for JA synthesis are competing for substrate.

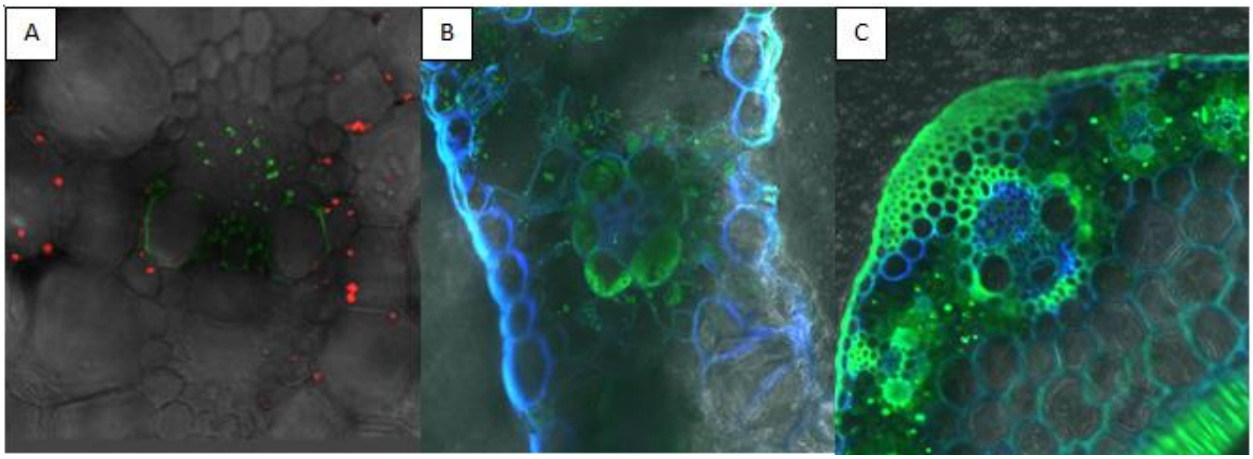

Figure 15: Confocal laser scanning microscope images of LOX10-YFP. (A)- LOX10-YFP in cross section of maize leaf vascular bundle. Green- LOX10, Red-Chlorophyll. Image prepared by Stacy DeBlasio, David Jackson Lab, Cold Spring Harbor Laboratory, One Bungtown Road Cold Spring Harbor, NY. (B)- Cross section of maize leaf containing a vascular bundle. LOX10 is only visible in the interior of the leaf. Green dots- LOX10, Blue-DAPI stain for visualizing cell walls. (C)- Cross section of maize mid-rib. LOX10 is only visible in the interior of the leaf. Green dots- LOX10, Blue-DAPI stain for visualizing cell walls.

LOX8 induction following darkshock GLV emission may provide clues to a possible mechanism of GLV biosynthesis. GLVs were shown to induce LOX8 in this study and by Christensen et al (2013). In order for GLVs produced by the plant to induce LOX8, they must be synthesized in the mesophyll or vasculature of the leaf. As seen in the image provided by Dr. David Jackson of the Cold Spring Harbor Laboratory (Figure 15, A), LOX10 tagged with yellow fluorescent protein is present in the interior of the leaf. In LOX10-YFP seedlings supplied by the Jackson Lab, the protein was also only visible in the interior of the leaf (Figure 15, B, and C). This suggests that LOX10 protein is localized to the mesophyll

or vasculature. Once synthesized, GLVs percolate through the leaf tissue before being emitted from the leaf surface. While moving through the leaf tissue, GLVs induce LOX8. This would explain the induction of LOX8 observed 15 minutes after GLV emission in gene expression experiments.

Chapter 5

Do corn seedlings self-prime?

Herbivore attack is a constant threat to plants. In order to survive, plants must mount defenses to ward off attackers. These defenses can be split into two categories. Constitutive defenses are always present; induced defenses are only mounted when the plant is threatened. By only mounting induced defenses when attack is imminent, plants reduce the energy costs associated with the defense and gain a fitness advantage over plants with constitutive defenses (van Hulten et al, 2006).

Relying on induced defenses might save energy, but leaves the plant vulnerable to attack, resulting in damage during the interval between attack and the start of the induced defense response. In order to reduce this lag period, plants have evolved the ability to prime. Priming is the process by which a plant prepares to more quickly or aggressively respond to future stress (Frost et al, 2008).

Priming occurs when a plant senses and responds to environmental cues that signal an imminent threat. GLVs are one of these cues, acting as inter- and intra-plant signaling molecules. Plants growing in close proximity can sense GLVs emitted by a neighboring plant under attack, allowing intact plants to induce defenses. This can also occur within a single plant, when leaves being attacked by herbivores emit volatiles which induce defense genes in healthy leaves.

Ton et al (2006) examined the effect of priming in maize to determine if primed plants showed improved defenses compared to unprimed plants when attacked. Plants were primed with volatiles emitted by plants infested with *Spodoptera littoralis*. Both direct and indirect defenses were found to be improved in primed plants. *S. littoralis* growth on plants primed with volatiles from caterpillar-infested plants was reduced by fifty percent compared to *S. littoralis* feeding on unprimed plants (Ton et al, 2006). Reduced caterpillar growth showed that direct plant defenses were stronger in primed plants. An increase in primed plant attractiveness to parasitic wasps showed that priming also improved indirect plant

defenses. Plants exposed to volatiles from infested plants attracted more parasitic *Cotesia marginiventris* wasps than plants exposed to clean air (Ton et al, 2006).

Priming is only evident when plants are challenged by an attacker. Unchallenged primed plants exposed to volatiles from infested plants were not found to have higher volatile emissions than unprimed plants. After challenge, however, primed plants showed enhanced emission of aromatic volatiles and terpenoid compounds (Ton et al, 2006). Ton et al (2006) identified six genes that were induced by both *S. littoralis* feeding and by exposure to volatiles from *S. littoralis*-fed plants. This allowed future studies to use these genes' expression as a marker for priming.

In a separate study also using maize, JA was shown to be induced upon exposure to GLVs (Engelberth et al, 2004). Intact seedlings exposed to GLVs responded with an induction of JA that reached a maximum 30 minutes following exposure (Engelberth et al, 2004). AOS and AOC, key enzymes in the JA biosynthesis pathway, were induced after exposure to GLVs.

After herbivore attack, GLV emission from the wounding site might prime locally during the day. If an herbivore is attacking the plant, GLVs can be efficiently synthesized and emitted from the disrupted tissue due to the abundance of LOX10. If priming occurs in the undamaged leaf tissue close to the herbivore's feeding site, then the plant's defense system is one step ahead of the herbivore.

At night, however, LOX10 levels are significantly decreased (Nemchenko et al, 2006, Christensen et al, 2013). This does not allow for efficient GLV production. Therefore, localized priming is not as effective, leaving the plant vulnerable to continued herbivore feeding during the nighttime hours. Darkshock induced GLVs might therefore prime the plant prophylactically and systemically.

According to this model, as the sun sets, LOX10 transcript levels remain high through full darkness. The oncoming darkness causes the freeing of linolenic acid, which is rapidly converted by LOX10 into GLVs. These GLVs prime the plant systemically as they percolate through the leaf tissue before being emitted. This allows for augmented defense gene expression throughout the plant in the case

of an attack during the night. Therefore, the plant does not need to rely on GLVs produced in disrupted tissue for localized priming during the nighttime hours.

This also may explain why GLV emission following darkshock is only observed in wounded plants. Plants that are not under attack do not need to use the energy to prime defense genes. Therefore, intact plants do not emit the large GLV burst that is observed in plants with wounded tissue.

Our results are in accordance with published data (Brilli et al, 2011, Christensen et al, 2013): GLVs were rapidly synthesized in darkshocked seedlings, and GLVs seem to be necessary for induced LOX8 transcript levels. Christensen et al (2013) suggested that GLVs are necessary for complete wound-induced LOX8 and consequently JA levels. Given that LOX10 transcripts were high throughout the day without any change in darkshocked seedlings, but GLVs were rapidly synthesized in darkshocked seedlings, we have to assume that LOX10 was present and active to allow for the rapid GLV synthesis following darkshock. We observed a delayed peak for LOX8 transcript levels compared to GLV emission, which also supports the notion of Christensen et al (2013) that GLVs are required to induce LOX8 transcripts. Further experiments with LOX8 mutants will allow for this connection to be studied in greater detail. LOX8 mutants may not respond to wounding, and therefore may not emit a burst of volatiles upon darkshock. The LOX8 mutants may act as intact seedlings because defense genes are not activated upon wounding.

Many new questions have arisen from this study. What causes each cultivar to respond to darkshock differently? Are transcript levels responsible for the observed difference in GLV emission following darkshock? How do plant responses to GLVs synthesized by the plant differ from those synthesized by neighboring plants? No JA synthesis was observed after darkshock; could darkshocked plants be using all of the available substrate to produce GLVs, leaving none for JA synthesis? Where does the substrate for the large GLV burst observed come from? Why is GLV synthesis increased immediately following darkshock? Priming efficiency is reduced during the night due to a reduction in

LOX10 transcript levels. Is darkshock used as a rapid systemic priming technique to prepare wounded plants for further attack during the night?

Further experiments that may provide answers to these questions include a detailed study of the differences between cultivars. Examining gene expression in light-control and darkshocked plants of each cultivar would provide gene expression data that could be paired with volatile emission data from this thesis. If LOX10 transcript levels are necessary for the GLV burst after darkshock, it would be expected that Delprim would have reduced LOX10 levels compared to B73 and W438. Studying the transcript levels of a gene that is induced by JA would confirm that JA is not produced as a result of LOX8 induction following darkshock. This would provide further evidence that the GLV and JA biosynthesis pathways use the same substrate. Finally, priming experiments in which one set of seedlings is primed with exogenous GLVs and a second set of seedlings is primed with endogenous GLVs (via darkshock) could determine whether the priming response differs based on the location of GLV synthesis.

This study has shown that different cultivars respond to darkshock with a burst of GLVs, however, emission amounts differ greatly between cultivars. LOX10 transcript levels in seedlings left in the light are not different from those in darkshocked seedlings; therefore, LOX10 transcript levels do not explain the GLV burst after darkshock. Finally, LOX8 transcript levels are induced within 15 minutes of the GLV burst, indicating that the GLVs may be priming the plant as they percolate through the leaf tissue before emission. Interestingly, the LOX8 induction does not result in measurable increases in JA levels. These findings provide a foundation from which the cultivar differences, LOX10/LOX8 interplay, and priming aspects of darkshock can be studied further.

Chapter 6

Methods

Growing Plants

Delprim (Semances et Plantes SA, Delley Switzerland), B73, and W438 seedlings were planted into autoclaved, moistened Sunshine MVP (Sun Grow Horticulture, Agwam, MA, USA) potting soil in 6" round pots. One quarter teaspoon of Osmocote Plus 15-9-12 (Scotts®, Marysville, OH, USA) slow release fertilizer was placed on top of the soil at the time of planting. Plants were placed in a growth chamber with lights of equal number metal halide and high pressure sodium lamps with outputs of 400 watts each for the duration of the 14-day growing period. The light cycle was as seen in Table 1. The temperature cycle was 25 degrees Celsius from 6:00 to 22:00 and 23 degrees Celsius from 22:00 to 6:00. Plants were watered as needed.

Table 1: Growth Chamber Light Cycle

6:00-6:30	1 Metal Halide Lamp	1 High Pressure Sodium Lamp
6:30-21:30	3 Metal Halide Lamps	3 High Pressure Sodium Lamps
21:30-22:00	1 Metal Halide Lamp	1 High Pressure Sodium Lamp
22:00-6:00	Dark	Dark

Volatile Collections

Volatile collections were carried out in the growth chamber in which seedlings were grown. 14-day-old seedlings in the V2 stage with two visible leaf collars were used for volatile collections. Seedlings selected were of uniform size. A razor was used to cut seedlings just above the first leaf.

Individual seedlings were immediately placed into either a 10mL autosampler vial containing double distilled water (ddH_2O) (for uninduced seedlings) or an elicitor solution of 250µL of 25mM phosphate buffer at a pH of 7.8 containing 400ng Ln L-Glutamine per seedling in 250mL phosphate buffer (for induced seedlings). After the elicitor solution was taken up, induced seedlings were placed into individual 10mL autosampler vials containing ddH_2O. Once in autosampler vials, seedlings were slid into individual glass volatile collection tubes.

Positive air pressure was maintained inside the collection tubes throughout the experiment to ensure outside air did not enter the system. Filtered air was forced into the collection tubes at 1L per minute. A volatile collection system (Analytical Research Systems Inc., Gainesville, FL USA) removed air at 0.5L per minute. SuperQ filter traps were used to collect volatiles. Filter changes were done by hand with the cap to the collection chamber being unscrewed for 15 seconds to allow the used filter to be removed and a new filter to be inserted.

Darkshock was not induced until baseline emission levels were determined by collecting volatiles under light conditions. Darkshock was induced by wrapping individual tubes containing one seedling with aluminum foil. Each tube was wrapped in a single piece of aluminum foil so that no light could infiltrate the tube. After wrapping, tubes were placed back into their prior position for the duration of the experiment. Seedlings in tubes that were left unwrapped were used as a control.

At the conclusion of the experiment, seedlings were removed from the collection tube and vial of ddH_2O. The bottom portion of the stalk that was submerged in ddH_2O was removed with a razor blade. Seedlings were massed.

Gene Expression/Hormone Analysis Tissue Collection

Seedlings were selected and cut as stated above. Sets of four seedlings were placed in beakers of ddH_2O immediately after cutting. Beakers were left uncovered in the growth chamber until 1:30pm, at

which time half of the beakers were placed under a dome covered with aluminum foil to induce darkshock and half were placed under a clear dome as controls. Four control plants were harvested at each time point prior to darkshock at 1:30pm. Four darkshocked and four control plants were harvested at each time point after darkshock at 1:30pm. Tissue was collected by removing seedlings from the beaker of ddH_2O, cutting off the submerged portion of the stalk, and placing the seedling in a screw cap vial. Seedlings were immediately flash frozen in liquid nitrogen.

Gas Chromatography – Flame Ionization Detector

"Filters containing collected volatiles were eluted using two aliquots of 50µL (100µL total) hexane:dichloromethane (1:1, vol:vol) containing 400µg nonyl acetate as an internal standard. Filters were blown dry using a stream of nitrogen. Gas chromatogram retention time allowed for volatiles emitted from plants to be identified. Samples were analyzed in an Agilent 6890 GC-FID (Agilent, Santa Clara, CA, USA) with an Equity-5 column (30m x 0.2mm x 0.2µm film thickness; Supelco, Bellefonte, PA). Helium was the carrier gas at an average linear velocity of 26 cm s^{-1}. One microliter of each sample was injected in the spitless mode. The injector was changed to split mode after 0.75 minutes. The initial oven temperature was held at 40^{o}C for 1 minute, then programmed to increase at 8^{o}C per minute to 180^{o}C. This was followed by ramping at 30^{o}C per minute to 300^{o}C and holding at 300^{o}C for five minutes. The injector and the detector temperatures were set to 280^{o}C and 300^{o}C respectively (Seidl-Adams et al, 2014)." Compounds were identified using the retention time of the peak. Emission amounts were quantified in ng/ g fresh weight/ hour. This was achieved by dividing the peak area of the compound by the peak area of the standard, multiplying by 400 to correct for the amount of sample injected into the GC, dividing by the plant mass, and multiplying/dividing to adjust the length of the collection interval to a one hour period.

Gene Expression

RNA was extracted using a QIAGEN RNeasy Mini Kit. RNA was extracted from tissue samples according to the RNeasy Mini Handbook. Approximately 100mg of ground plant tissue was added to a 2mL microcentrifuge tube containing 600µL buffer RLT and vortexed vigorously. The lysate was transferred to a QIAshredder spin column and centrifuged in a tabletop centrifuge at full speed for 2 minutes. The supernatant was mixed with 250µL pure ethanol and transferred into an RNeasy spin column and centrifuged at 8,000 x g for 15 seconds. The flow-through was discarded. The column was washed using 700µL of buffer RW1 and 500µL buffer RPE, each followed by centrifugation at 8,000 x g for 2 minutes. The column was dried by centrifuging the column in a dry 2mL collection tube at full speed for 1 minute. RNA was eluted by placing the column in a 1.5mL collection tube, adding 35µL of RNase-free water, and centrifuging at 8,000 x g for 1 minute.

"RNA was quantified using a NanoDrop (Thermo Scientific, Waltham, MA). One microliter of each sample was placed on the nanodrop to obtain the concentration of RNA. Genomic DNA in 3µg total RNA was digested in a 50µL reaction with the Turbo-DNA-free™ kit (Applied Biosystems, Carlsbad, CA USA) according to the manufacturer's protocol (Seidl-Adams et al, 2014)."

"In a 20µL reaction with SMART™ MMLV Reverse Transcriptase (Clontech, Mountain View, CA USA), 550ng of DNase-treated total RNA was reverse transcribed according to the manufacturer's protocol using a mix of anchored 18mer polydT and random 8mer oligo primers (Genomics Core Facility, Penn State University, University Park, PA USA) at a final concentration of 3.75µM (Seidl-Adams et al, 2014)."

"cDNA was diluted 1:10 and used in 5µL aliquots as template in 20µL PCR reactions. The final primer concentration was 0.5µM. All other components necessary for qPCR were contained in the SsoFast™ EvaGreen® Supermix (Bio-Rad, Hercules, CA, USA). All PCRs were run in triplicate. A water control and a standard curve were run on each of the 96-well plates. The standard curve was generated using five threefold serial dilutions of pooled cDNA from highly expressing samples as

template. The highest concentrated template for the standard curves (3x) was obtained by diluting 1.5µL undiluted cDNA into 3.5µL H_2O and scaled up correspondingly so that on all plates standard curves were generated from the same template pool, guaranteeing that the same amount of template was used in all reactions. Standard curves were constructed assigning 81 arbitrary units as the starting amount of the transcript of interest when the 3x pooled cDNA was used as template. To control for the amount of cDNA in each of the samples, adenine phosphate transferase 1 (AmAPT1) was used as a reference gene. Its expression is invariant in experimental conditions used. All transcript levels of the genes of interest were expressed as the ratio of their and ZmAPT1 starting quantities based on the respective standard curves (Seidl-Adams et al, 2014)." Primer sequences used for qPCR reactions are seen in Table 2.

Table 2: Primer Sequences

Gene	Forward Primer	Reverse Primer
APT-1	APT1F380: AGGCGTTCCGTGACACCATC	APT1R541: CTGGCAACTTCTTCGGCTTCC
LOX8	8LOXF2005: CGGTACACGCTCAAGATCAA	8LOXR2186: ACGGCCATTCCTCTTCTGAT
LOX10	10LOXF2665: AGGAGTACATGGGGGAGTTC	10LOXR2916: GCATGCTGAGGATGGATCAG
LOX11	11LOXF2645: AGGAGTACATGGGAGAGTTG	11LOXR2896: GAGAGGACGGACGGGTCAA
HPL-1	HPL1F1360: AGCACCTCTTCTGGTCCAAC	HPL1R1499: CACCTCGAAGTCGTCGTAGC

Jasmonic Acid Extraction

Plant tissue was ground while frozen in a Genogrinder with three metal balls of diameter 6mm for two minutes at 1200 strokes per minute. JA was measured using the protocol outlined by Schmelz et al (2004). 50mg-100mg of ground plant tissue was added to a Fast-Prep tube containing one smidgen scoop of Zirmil beads, 400µL isopropanol:H_2O (2:1, vol:vol) adjusted to pH 3 with HCl, and 20µL of internal standard mix. Plant tissue was homogenized in a FastPrep FP120 tissue homogenizer (BIO101 ThermoSavant, QBiogene, Carlsbad, CA USA) at speed 6 for 20 seconds. The tissue was made acidic by adding 3µL 2N hydrochloric acid. Samples were vortexed at speed 7 and centrifuged at 10,000 rpm for one minute on a tabletop centrifuge. The organic phase was transferred into a glass vial immediately after centrifugation and dried under a stream of nitrogen. The remaining deposit was re-dissolved in 200µL ether:methanol (9:1, vol:vol). 3µL of 2N trimethylsilyldiazomethane in hexane was added to methylate carboxylic acids in the solution. Samples were vortexed at speed 3 and incubated at room temperature for 30 minutes. The methylation reaction was stopped with 3µL hexane:acetic acid (88:12, vol:vol). Samples were vortexed at speed 3 and incubated at room temperature for 25 minutes. The remaining solvent was evaporated and glass vials were placed into a 180^{o}C heating block for two minutes. Volatiles were collected on SuperQ filters inserted into the cut cap septum (Engelberth et al, 2003).

"Compounds adhered to the filters were eluted with three 50µL aliquots of dichloromethane and blown dry with a stream of nitrogen. One microliter of elutant was analyzed in a GC-mass spectrometry (MS) system consisting of an Agilent 6890N GC interfaced with an Agilent 5973N mass selective detector. The column and GC conditions were equivalent to those used in the GC-FID. The MS was used in chemical ionization mode with the default temperature settings (ion source: 230^{o}C and quadrupose: 150^{o}C)" (Seidl-Adams et al, 2014). JA was identified using the retention time of the peak.

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The Pennsylvania State University, University Park, Pennsylvania
Schreyer Honors College — Expected May 2015
B. S. in Biology, Genetics & Developmental Biology Option
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Work Experience:

The Pennsylvania State University, University Park, PA — Fall 2012, Fall 2013-Present
Undergraduate Researcher, Chemical Ecology Lab, Dr. James H. Tumlinson

- Assist researchers with vacuum filtration collection of volatiles emitted by maize
- Analyze Gas Chromatography/Mass Spectrometry data of maize volatiles to allow for comparisons of volatile emission between maize varieties
- Develop research methods to quantitatively compare stomata numbers on maize leaves
- Analyze the effect of salicylic acid on volatile emission of maize
- Determine the effect of dark shock on GLV emission in maize

Tom Laser Woodturning, Fairfield, PA — Summer 2010, 2011, 2012
Wood/Metal Shop Assistant

- Assisted in designing and building a custom saw to be used by American Micro Industries Inc.

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