# EFFECTS OF LIGHT INTENSITY AND NITROGEN SOURCE ON PAC CHOI (BRASSICA RAPA L.), AND INTERACTION WITH THE DIAMONDBACK MOTH (PLUTELLA XYLOSTELLA L.)

by

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## Abstract

Greenhouse studies were conducted to examine direct effects of light intensity and nitrogen source on primary and secondary metabolism of pac choi (*Brassica rapa* L. var. *chinensis* cv. 'Mei Qing Choi') and indirect effects on diamondback moth (*Plutella xylostella* L.) (DBM). In the first study, plants were exposed to high and low light intensities during different times of the year, resulting in a range of light intensities. From four experiments, plants exhibited higher phenolic content, greater shoot biomass, and higher C:N ratios under high light intensity, whereas plants under low light intensity contained higher protein. Ferulic acid increased under high light intensity, and this increase was negatively correlated with male DBM body weights. However, DBM developed faster on plants in the August experiment (high light), compared to the July experiment (lower light). This implies that light intensity may not be affecting DBM through plant-mediated changes unless reduced male weights confer a reduction in larval consumption.

In the nitrogen source study, application of an organic source of nitrogen (fish hydrolysate fertilizer) was compared to a conventional fertilizer to determine whether nitrogen source directly impacts pac choi chemistry and biomass, thus indirectly impacting DBM fitness. In two experiments, there was no significant effect of fertility treatment on pac choi nutrients or biomass, with the exception of percent leaf phosphorus, which was significantly higher in the conventional fertility treatment. For DBM, percent survival and cohort development were significantly reduced on pac choi receiving the organic fertilizer. Calcium and magnesium were significantly higher in pac choi infested with DBM larvae than plants without DBM. In addition, calcium was negatively correlated with female DBM body weights in one experiment for the

organic treatment. Overall, this study demonstrated that pac choi plants that received the organic fertilizer were similar to pac choi plants that received a conventional fertilizer with the exception of phosphorus. Furthermore, female DBM body weights were negatively impacted by calcium in the organic treatment. As multiple fitness traits for DBM were negatively affected in the organic treatment, pac choi crops grown with fish hydrolysate fertilizer may experience less feeding from DBM.

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# Table of Contents

List of Figures ..... ix
List of Tables ..... xi
Acknowledgements ..... xiv
Dedication ..... xv
Chapter 1 - Introduction and Literature Review ..... 1
Introduction ..... 1
Importance of Study ..... 4
Study System ..... 4
Objectives and Hypotheses ..... 8
Literature Review ..... 12
Plant Defenses against Herbivores ..... 12
Insect Herbivore Nutrition ..... 13
Secondary Metabolites ..... 14
Alternative Crop Production Practices ..... 20
Chapter 2 - Effects of light intensity on pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') chemistry and interactions with the diamondback moth (*Plutella xylostella*) ..... 36
Introduction ..... 36
Materials and Methods ..... 40
Results ..... 49
Discussion ..... 53
Tables and Figures ..... 63
Chapter 3 - Effects of nitrogen source on pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') chemistry and interactions with the diamondback moth (*Plutella xylostella*) ..... 75
Introduction ..... 75
Materials and Methods ..... 80
Results ..... 90
Discussion ..... 93

Tables and Figures ..... 99
Chapter 4 - Summary and Conclusions ..... 111
Summary ..... 111
Conclusions ..... 112
Limitations ..... 116
Suggestions for Future Research ..... 120
References ..... 124
Appendix A - Final *Plutella xylostella* age-class distributions associated with light intensity and nitrogen source studies. ..... 179
Appendix B - Tables of light intensity analysis of variance (ANOVA) for each light intensity experiment. ..... 180
Appendix C - Effect of ferulic acid on *Plutella xylostella* in artificial diet study. ..... 184
Appendix D - Effect of light intensity on pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') total phenolic content under tunnel and field conditions. ..... 188
Appendix E - 'Bellows method' for collecting adult *Plutella xylostella* moths. ..... 194

# List of Figures

Figure 2-1. Relationship of light intensity associated with ambient (amb) and shade treatments (N = 40) and carbon:nitrogen (C:N) ratios in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Equations for ambient and shade were significant at $P \leq 0.05$..... 70

Figure 2-2. Relationship of light intensity associated with ambient (amb) and shade treatments (N = 40) and protein content in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Equations for ambient and shade were significant at $P \leq 0.05$. ..... 71

Figure 2-3. Relationship of light intensity associated with ambient (amb) and shade treatments (N = 40) and total phenolic content in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Starred equations are significant at $P \leq 0.05$..... 72

Figure 2-4. Relationship of ambient (N = 17 plants with male data) and shade (N = 13 plants with male data) treatments between male adult *Plutella xylostella* body weight and concentration of ferulic acid in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Starred equations are significant at $P \leq 0.05$..... 73

Figure 2-5. Relationship of ambient (amb) and shade light intensity and concentration of ferulic acid in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Starred equations are significant at $P \leq 0.05$. N = 40 plants used in analysis. ..... 74

Figure 3-1. Relationship between percent (%) survival of *Plutella xylostella* and percent (%) magnesium in *Brassica rapa* leaves for the spring and fall experiments. The best-fit relationships (quadratic) are shown, along with line equations and coefficients of determination. Starred relationships are significant at $P \leq 0.05$. Points represent survival per block (N = 4) used in the analysis..... 109

Figure 3-2. Relationship between adult female body weight (mg) and percent (%) leaf calcium in *Brassica rapa* for the spring experiment associated with the organic (org) fertility treatment (N = 6 plants with female data) and conventional (con) fertility treatment (N = 8 plants with female data). The best-fit relationships (linear) are shown, along with line equations and coefficients of determination. Starred relationships are significant at $P \leq 0.05$. ..... 110
Figure 4-1. A conceptual model summarizing study results, with arrows indicating examined relationships, + indicating a relationship was detected, – indicating a relationship was not detected, and ? indicating an unknown relationship. For the light intensity study, it was determined that 1) increases in light intensity were associated with higher phenolic contents and shoot biomass, and lower protein contents in pac choi (*Brassica rapa*) and 2) higher ferulic acid content was associated with lower male diamondback moth (*Plutella xylostella*) body weights. For the nitrogen source study, it was determined that 3) there were no significant differences between organic and conventional nitrogen sources on *B. rapa* chemistry (except levels of leaf P) or plant growth parameters; however 4) *P. xylostella* larval feeding was associated with high percent leaf calcium and magnesium contents, and survival and development of larva were negatively impacted by the organic fertility treatment. It is still not known if light intensity or nitrogen source directly affects *P. xylostella*. ..... 123
Figure D-1. Low tunnels and adjacent field plots consisted of four rows of ten plants each used to determine the effects of light intensity on *Brassica rapa* phenolic content. ..... 192
Figure D-2. Design specifications for low tunnel construction. ..... 193
Figure E-1. Foot pump device used to collect adult *Plutella xylostella* moths from cages. ..... 194

# List of Tables

Table 2.1 Analysis of variance (ANOVA) values for light intensity, temperature, *Brassica rapa* and *Plutella xylostella* response variables, by experiment, light treatment, light treatment replicate, and the two-way interaction between experiment and light treatment. Values in bold are significant ($P \leq 0.05$ using Fisher's Protected LSD). ..... 64

Table 2.2. Mean (± SE) light intensity and temperature inside light treatment cages (ambient and shade) by light treatment, pooled over the four experiments (July, August, February, and March). ..... 65

Table 2.3. Mean (± SE) light intensity inside light treatment cages (ambient and shade), with data pooled for the four experiments (July, August, February, and March). ..... 65

Table 2.4. Mean (± SE) temperature inside greenhouse cages by experiment (July, August, February, and March), with pooled data for ambient and shade treatments. ..... 66

Table 2.5. Mean (± SE) concentrations for total phenolics and sinapic acid in *Brassica rapa* by ambient and shade treatment, pooled across the four experiments (July, August, February, March). ..... 66

Table 2.6. Mean (± SE) *Brassica rapa* responses to differences in ambient and shade treatment by experiment (July, August, February, and March). ..... 67

Table 2.7. Mean (± SE) *Plutella xylostella* (diamondback moth, DBM) female and male body weights, pooled across ambient and shade treatments, by experiment (July, August, February, and March). ..... 68

Table 2.8. Mean (± SE) percent survival of *Plutella xylostella* in artificial diet study, by experiment. ..... 69

Table 3.1. Final concentration of nutrients applied to *Brassica rapa* containers from three applications of Neptune's Harvest fish hydrolysate (Neptune's Harvest; Gloucester, MA). 99

Table 3.2. Final concentration of nutrients applied to *Brassica rapa* containers from three applications of an inorganic salt solution, formulated to match the fish hydrolysate (Neptune's Harvest; Gloucester, MA). ..... 100

Table 3.3. Mean (± SE) differences in greenhouse light intensity, temperature, and relative humidity for the spring and fall experiments. ..... 101

Table 3.4. Analysis of variance (ANOVA) values for block (1-8), experiment (spring and fall), fertility (conventional and organic), and sample time (pre-sample: prior to DBM introduction, and post-sample: end of experiment), and the experiment by sample time interaction associated with the leachate variables. Values in bold are significant ($P \leq 0.05$). ..... 102
Table 3.5. Mean (± SE) leachate variables in *Brassica rapa* containers at pre-sampling (7 days after first fertilizer application) and post-sampling (7 days after third fertilizer application).[1] ..... 103
Table 3.6. Analysis of variance (ANOVA) values for *Brassica rapa* response variables for block (1-8), experiment (spring and fall), fertility treatment (conventional and organic), and herbivore (with and without *Plutella xylostella*). Values in bold are significant at $P \leq 0.05$ (as determined by Fisher's Protected LSD). ..... 104
Table 3.7. Mean (± SE) total and specific phenolics in *Brassica rapa* for spring and fall experiments. Means followed by common letter within a row are not significantly different between experiments as determined by Fisher's Protected LSD at $P \leq 0.05$. ..... 105
Table 3.8. Mean (± SE) differences in *Brassica rapa* response variables between the spring and fall experiments across plants with and without *Plutella xylostella*. ..... 105
Table 3.9. Mean (± SE) differences in selected *Brassica rapa* response variables between the spring and fall experiments and between plants with and without *Plutella xylostella*. ..... 106
Table 3.10. Analysis of variance (ANOVA) values for *Plutella xylostella* response variables for block (1-8), experiment (spring and fall), fertility treatment (organic and conventional), and the experiment by fertility interaction. Values in bold are significant as determined by Fisher's Protected LSD at $P \leq 0.05$..... 107
Table 3.11. Mean (± SE) *Plutella xylostella* cohort development and survival per plant between organic and conventional fertility treatments across experiments (spring and fall). ..... 107
Table 3.12. Mean (± SE) *Plutella xylostella* body weights and larval feeding equivalents between the spring and fall experiments across fertility (organic and conventional) treatments. .... 108
Table A.1. Differences in *Plutella xylostella* age-class distributions (means ± SE) on *Brassica rapa* plants in July, August, February, and March. ..... 179
Table A.2. Differences in *Plutella xylostella* age-class distributions (means ± SE) on *Brassica rapa* plants for the spring and fall experiments. ..... 179

Table B.1. July Experiment. Results of analysis of variance (ANOVA) for light intensity and temperature, plant (*Brassica rapa*) response variables, and insect (*Plutella xylostella*) response variables, by light (ambient and shade treatments), light replicate (5 replicates each of ambient and shade treatment), herbivore (plants with and without *P. xylostella*). Values in bold are significant ($P \leq 0.05$). ..... 180
Table B.2. August Experiment. Results of analysis of variance (ANOVA) for light intensity and temperature, plant (*Brassica rapa*) response variables, and insect (*Plutella xylostella*) response variables, by light (ambient and shade treatments), light replicate (5 replicates each of ambient and shade treatment), herbivore (plants with and without *P. xylostella*). Values in bold are significant ($P \leq 0.05$). ..... 181
Table B.3. February Experiment. Results of analysis of variance (ANOVA) for light intensity and temperature, plant (*Brassica rapa*) response variables, and insect (*Plutella xylostella*) response variables, by light (ambient and shade treatments), light replicate (5 replicates each of ambient and shade treatment), herbivore (plants with and without *P. xylostella*). Values in bold are significant ($P \leq 0.05$). ..... 182
Table B.4. March Experiment. Results of analysis of variance (ANOVA) for light intensity and temperature, plant (*Brassica rapa*) response variables, and insect (*Plutella xylostella*) response variables, by light (ambient and shade treatments), light replicate (5 replicates each of ambient and shade treatment), herbivore (plants with and without *P. xylostella*). Values in bold are significant ($P \leq 0.05$). ..... 183
Table D.1. Light intensity, temperature, and total phenolic content (mean ± SE) in *Brassica rapa* grown in open field and low tunnel plots for spring and fall experiments, along with means associated with pac choi grown under different light treatments (ambient and shade) in a greenhouse study. ..... 191

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# Dedication

I dedicate this work to my husband, Sam, who has proven to me that nothing is worth doing unless it's done in love.

# Chapter 1 - Introduction and Literature Review

## Introduction

The use of protected environments is becoming increasingly popular for growing vegetable crops (Vandermeer 1995; Knewtson 2008; Willer and Kilcher 2009). Growing crops in protected environments, such as high tunnels and greenhouses, may extend the growing season and potentially increase profits to producers (Wells and Loy 1993). Some studies have reported that protected environments may reduce insect herbivore outbreaks by restricting insect movement into crops (Parrot and Marsden 2002; Jett and Reed 2003). However, it is not known if protected environments lead to changes in plant chemistry that impact susceptibility to insect herbivores. For example, it is unclear if reductions in light intensity associated with protected environments mediate host plant chemistry favorable to herbivores, such as decreasing the production of defensive phenolic compounds (Zhao and Carey 2009). Herbivore interactions with host plants may be influenced by plant quality based on the presence of primary and secondary metabolites (De Pascale et al. 2007). Because vegetable production in protected environments is increasing, it is important to understand if plant protection against herbivores is influenced by changes in light intensity, which is typically lower in protected environments compared to field settings (Ames et al. 1995; Oh and Rajashekar 2009). Ingersoll et al. (2010) found that total phenolic content of spicebush (*Lindera benzoin*) increased with increasing light intensity. Due to their toxicity, higher phenolic content tends to reduce herbivory (Mole and Waterman 1988). In another study, lettuce (*Lactuca sativa*) was reported to have significantly

higher levels of phenolics when grown under field conditions compared to high tunnels (Oh et al. 2011). This may be due to an increase in photosynthesis, which results in an increased accumulation of carbon resources in plant tissues (Cullen et al. 2006), making it possible to re-allocate carbon from primary to secondary metabolites, such as phenolics (Schwachtje and Baldwin 2008). Plants with higher phenolic content are important because they are typically associated with negative effects on herbivores (Dixon and Paiva 1995).

In addition to growing crops in protected environments, many producers utilize organic production systems, primarily due to the perception that organic practices are lower-risk to the environment (Willer and Kilcher 2009). One component of organic production is fertility, which is the use of various types of organic fertilizers such as composts, fish waste, and manures, in place of conventional soluble inorganic fertilizers in organic systems. Due to the available forms of nitrogen (N) in some organic-based fertilizers, their nutrient availability tends to be lower than conventional fertilizers. Similar to light intensity, the influence of nutrient availability may modify host plant chemistry, thus influencing susceptibility to herbivores (De Pascale et al. 2007; Gawlik-Dziki 2008). For example, increasing N concentration tends to reduce carbohydrate assimilation in plant tissues, which then redirects metabolic activities to produce proteins using N (Wilkens et al. 1996). Therefore, plants receiving conventional fertilizers may produce fewer carbon-based secondary metabolites such as phenolics (Bryant et al. 1983). Consequently, it is important to determine if changes in N sources impact plant-insect interactions (Ames et al. 1995; Oh and Rajashekar 2009). Nitrogen sources in organic fertilizers tend to release N more slowly than conventional fertilizers, which supply readily available N (Gaskell and Smith 2007). Lower levels of available N in organic fertilizers may influence defensive chemistry (Roorda van Eysinga 1984). For example, Li et al. (2008) determined that

reduced soil N affected secondary metabolite levels in leaf mustard (*Brassica juncea foliosa*), where phenolic content was considerably lower due to increasing conventional N fertilization.

In addition to being affected by 'bottom-up' environmental factors such as light and soil N, the composition of primary metabolites (e.g., protein) and secondary metabolites (e.g., phenolics) in plants may also be influenced by the 'top-down' presence of herbivores (De Pascale et al. 2007; Gawlik-Dziki 2008). In general, plants respond to herbivory by decreasing the assimilation of primary nutrients via reducing photosynthesis (Welter 1989; Zangerl et al. 2002). Furthermore, transcript levels of photosynthesis-related genes are commonly down-regulated during herbivory (Hui et al. 2003; Ralph et al. 2006; Tang et al. 2006), which may protect plant tissue from oxidative damage during tissue maceration from chewing herbivores (Niyogi 2000). This shows that changes in plant chemistry are also subject to change from herbivores pressures.

The interaction of the bottom-up and top-down factors can be complex. Changes in plant chemistry during herbivory may be affected by alterations in the environment. Decreased N fertilization and lower light intensity have been shown to increase protein content and lower the phenolic content of certain plants (Mooney and Gulmon 1982; Mole and Waterman 1988). Protein and phenolic content are important factors in determining host plant quality for herbivores. In general, higher levels of protein are beneficial for insects, and higher levels of phenolics are detrimental (Behmer 2009). Collectively, changes in light, N, and/or herbivory may alter plant growth and productivity, and may impact susceptibility to insect herbivores (Mole and Waterman 1988; Dudt and Shure 1994; Parr and Bolwell 2000; Gent 2002; Le Bot et al. 2009). Therefore, the goal of this research is to determine, under greenhouse conditions, if

plants receiving different light intensities and fertilizer types vary in plant nutrient and phenolic content, and assess the potential impact of this on insect herbivore fitness.

## *Importance of Study*

A survey determined that 85% of Midwest high tunnels are used for organic production (Knewtson 2008). Due to the implementation of these growing practices, it is important to understand if plant-insect interactions are mediated by changes in both light intensity and N source (including organic versus conventional N sources) (Louda et al. 1989; Karban and Baldwin 1997; Bezemer and Jones 1998; Gatehouse 2002; Schoonhoven et al. 2005; Zheng and Dicke 2008). The research reported in this dissertation focuses on the direct effects of light intensity and soil nutrition on plant chemistry, and corresponding plant effects on insect herbivory. By evaluating these effects on plant-insect interactions, it may be possible to improve pest management strategies. This research utilized pac choi (*Brassica rapa* var. *chinensis*) and the diamondback moth (*Plutella xylostella*) as components of the plant-herbivore study system for all greenhouse experiments.

## *Study System*

Diamondback moth (DBM) was used in this research because of its widespread pest status (Liu et al. 2002; Talekar et al. 2003). DBM is also easily obtained and maintained in colonies. DBM is a specialist feeder on cruciferous crops because it utilizes N- and sulfur-containing glucosinolate compounds present in crucifers (Ahuja et al. 2010). Moderate concentrations of glucosinolate compounds stimulate larval feeding and female ovipostion on crucifers (Gupta and Thorsteinson 1960; Li et al. 2000). Since DBM is a serious pest of

crucifers, any means to enhance management practices would benefit producers. In fact, during outbreaks, DBM can cause up to 90% crop loss (Talekar and Shelton 1993; Verkerk and Wright 1996). Moreover, it is estimated that 1 billion US dollars is spent world-wide annually to control DBM in *Brassica* production systems (Sarfraz et al. 2009). DBM larval feeding can devastate crops if populations are not managed (Parker et al. 1995). Management is difficult because DBM is expanding its geographic distribution and because it can quickly develop resistance to insecticides (Miyata et al. 1986; Wakisaka et al. 1992; Shelton 2001). Current management tactics for DBM include the use of resistant glossy leaf varieties of crucifers, which are only moderately effective against first instar larvae, resulting in less feeding (Eigenbrode and Shelton 1990). In addition, rainfall and high relative humidity (>80%) may reduce numbers of early instars, as water droplets drown the first three larval instars located inside feeding sites (Waterhouse 1987). This has resulted in using overhead irrigation to manage DBM (McHugh and Foster 1995). However, constant moisture from irrigation leads to plant disease problems (McHugh and Foster 1995). Therefore, the primary means of managing DBM is the use of insecticides. Conventional insecticides disrupt natural enemy populations, thus significantly influencing their impact on DBM. This leads to more frequent insecticide applications and increased risk for DBM resistance (Furlong et al. 2004). As such, alternative management practices are needed for DBM.

Management of DBM may be improved by modifying the quality of host plants. Insect development, survival, and reproduction are affected by host plant quality (Tsai and Wang 2001; Morgan et al. 2001; Kim and Lee 2002; Liu et al. 2004; Yasar and Gungor 2005; Kumar et al. 2009). Growth and development of DBM larvae on host plants is highly dependent on various factors of the environment (Ko and Fang 1979). DBM has four larval instars with an average

development time of four days per instar. Larvae are 6-18 mm in length and are very active (Capinera 2009). Plant damage is characterized by holes or 'windowpanes' in leaves. Severe damage to cruciferous crops occurs if high numbers of larvae infest growing tips (Capinera 2009). In the tropics, DBM remains active year-round and is reported to have as many as 28 generations per year (Talekar et al. 1985; Talekar and Shelton 1993). Females can lay up to 300 eggs during their ten-day lifespan, although oviposition is influenced by photoperiod, temperature, and larval food quality (Harcourt, 1957); the latter may be associated with host plant chemistry. If changes in plant chemistry result in negative impacts on DBM, populations and feeding damage may be reduced.

To explore host plant quality, this research used pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') because prior studies have established that primary and secondary metabolism in this plant may be influenced by high tunnel and organic production systems compared to typical field production using conventional fertilizers (Zhao et al. 2007; Altimimi et al. 2009). Also, pac choi is a cool-season crop grown extensively in Asia, and is gaining in popularity in the United States (Harwood and Plucknett 1981; Rochfort et al. 2006). Consumption of vegetable *Brassica* species is increasing due to their nutritional value, which has been associated with reducing incidences of cardiovascular disease and cancer (Cartea et al. 2011). Health benefits may be due to the presence of antioxidants, vitamins, carotenoids, fiber, minerals, glucosinolates and phenolic compounds (Verma et al. 1988; Elongavan et al. 1994; Verhoeven et al. 1997; Duthie et al. 2000; Harbaum et al. 2007; Podsedek 2007; Jahangir et al. 2009). Pac choi is known to contain a high amount of phenolic compounds, including quercetin glucosides, kaempferol, and esters of hydroxycinnamic acids (ferulic and sinapic) (Harbaum et al. 2007; Lin and Harnly 2010). The dietary benefit of some phenolics may be because they act

as antioxidants, which alleviates the effect of damaging free radicals generated by natural cell processes and external stress (Kurilich et al. 1999). The nutrient content of pac choi is important for human consumption, but may also influence insect herbivores.

### *Brassica-Diamondback Moth Interactions*

Previous studies have demonstrated that *Brassica*-DBM interactions are affected by variations in light intensity, mainly associated with varying phenolic levels in *Brassica* plants (Sousa et al. 2005 and 2008). DBM larvae may respond differently to cabbage leaves exposed to varying light intensities due to changes in phenolic content. For example, Caputo et al. 2006 evaluated DBM herbivory in a field study under ambient and shade conditions. In this study *Arabidopsis* plants were fed upon three times more by DBM larvae under shade conditions than plants growing under ambient light conditions. However, this study did not mesaure total or specific phenolic content in plants grown under both light conditions with DBM herbviory (Caputo et al. 2006). Measuring relative concentrations of total and individual phenolics may allow for detecting ratios of phenolics that are important in plant-herbivore interactions (Close and McArthur 2002). In addition, available information on plant responses to changes in environmental factors, such as light intensity and N source, is limited, especially in relation to specific phenolics (Bryant et al. 1983; Close and McArthur 2002). However, research examining the effects from environmental factors has received more attention. In fact, there is interest in enhancing the concentrations of certain phenolics in horticultural crops through genetic improvement or environmental manipulation such as the use of organic fertilizers (Takeda 2001; Milkowski and Strack 2010). As such, it is important to identify the presence of primary and secondary components in plants that are unfavorable for DBM growth and survival (Awmack and Leather 2002).

Previous studies have established that *Brassica*-DBM interactions are affected by variations in soil nitrogen (N). For example, in a field study, Jansson et al. (1991) found that increasing conventional N fertilization to cabbage (*Brassica rapa*) enhanced DBM abundance, which may have been associated with larger plants receiving higher N. These plants may have attracted ovipositing females through increased volatile production of glucosinolate compounds. Moreover, compounds known to be toxic to DBM in *Brassica* plants are affected by soil N. For example, high levels of soil N reduce the availability of zinc (Zn) for plant uptake (Camp and Fudge 1945). Likewise, Seymour (1951) reported that *Brassica* plants provided with high N did not translocate copper (Cu) to the leaves. Moreover, studies have shown that Zn and Cu are toxic to DBM larvae (Pollard and Baker 1997; Jhee et al. 1999; Jhee 2004; Behmer et al. 2005; Coleman et al. 2005). DBM larval feeding may be reduced as levels of leaf Zn and Cu increase (Jhee et al. 2006; Park et al. 2009). It has been suggested that the negative effects of Zn and Cu may be additive on DBM by interacting with other plant defensive compounds, such as certain phenolics or defense proteins like poly-phenoloxidase (Boyd 1998).

## *Objectives and Hypotheses*

Although it has been reported that host plant quality can impact development and reproduction of DBM (Umeya and Yamada 1973; Sarnthoy et al. 1989; Shirai 2000), what is not well-known about *Brassica*-DBM interactions is how factors of the growing environment may impact DBM via changes in primary and secondary metabolites in leaf tissues. Furthermore, although the effects of glucosinolate compounds on DBM are well understood, the impact of specific phenolic compounds is not. Simultaneously evaluating both primary nutrients (C:N ratio and protein) and secondary compounds (phenolic content) may provide additional insight into

*Brassica*-DBM interactions. As such, the goals of this research are to determine the direct effects of light intensity and nitrogen source on pac choi chemistry, and corresponding effects on DBM fitness parameters and herbivory. The specific objectives are to:

1) determine the effect of light intensity on pac choi chemistry and impact on DBM body weights, survival, larval consumption, and cohort development;
2) compare the effect of an organic and a conventional N source on pac choi chemistry and on DBM performance and survival; and
3) evaluate herbivory of DBM larvae on pac choi chemistry under varying light intensities and N sources.

This research will address several knowledge gaps, including DBM responses to specific and total phenolic content (Sarfraz et al. 2010a,b; Staley et al. 2010); light intensity effects on pac choi chemistry, both quantitatively and qualitatively (Harbaum et al. 2007); effects of organic and conventional nitrogen sources on pac choi chemistry (Zhao et al. 2007); and influence of macro- and micronutrients on plant-herbivore interactions (Dale 1988b; Coleman et al. 2005; Schoonhoven et al. 2005). A potential outcome of this research is recognizing that crop risk from DBM may be influenced by the crop production system used. If so, modified pest management programs for DBM may need to be considered on crops grown under either protected environments or organic fertilization. Information related to changes in primary and/or secondary plant chemistry may also contribute to what is known about the effects of protected environments on pac choi quality.

***Objective 1***

Effects of light intensity on pac choi chemistry and DBM larval responses will be tested in a series of greenhouse experiments, repeated four times. Light treatments will involve

exposing pac choi to both ambient light and shade conditions, and inoculating plants with DBM larvae. Selected pac choi chemical responses and DBM fitness responses will be assessed under various light conditions. Based on previous literature and hypotheses (Coley et al. 1985), plants grown under high light intensity should have a higher leaf carbon, phenolic content, and greater shoot biomass than plants exposed to low light intensity. Another prediction for plants grown under high light intensity is that DBM should have lower body weights and larvae should consume less leaf tissue than plants grown under low light intensity. Body weight has been recognized as a measure of DBM fitness (Shirai 1995). As such, body weight will be used as the primary herbivore response variable in this study.

***Objective 2***

Effects of an organic and conventional fertilizer on various aspects of pac choi chemistry and DBM responses will be determined in a greenhouse experiment, conducted twice (spring and fall). Fertility treatments will include applications of an organic-based fish hydrolysate fertilizer containing N content composed of a high $NH_4^+$ - N to $NO_3^-$ - N ratio and a conventional fertilizer formulated comparable to the fish hydrolysate nutrient content, but with a higher total N content composed of a high $NO_3^-$ - N to $NH_4^+$ - N ratio. Growing medium and plant macro- and micro-nutrients will be compared between plants receiving the organic and conventional fertilizer treatments. DBM survival and adult body weights will be assessed in the experiments. Based on previous literature and predictions from the carbon-nitrogen balance hypothesis (Bryant et al. 1983), plants receiving an organic fertilizer should have less available N in the growing medium for uptake, contain higher leaf carbon and a higher phenolic content, and have a lower shoot biomass. For DBM, predictions include lower body weight, lower survival, and longer

development times on plants using the organic fertilizer compared to those receiving the conventional fertilizer.

***Objective 3***

For treatments in each study, plant chemical and growth responses will be compared between plants exposed to DBM larval feeding and plants not exposed to DBM. This will make it possible to determine if herbivory, as a separate factor, affects pac choi chemistry (Karban and Myers 1989; Gatehouse 2002). This assessment is important in the likelihood that herbviory affects plants responses (either via increases or decreases) in relation to direct responses to the primary factors (light intensity and nitrogen source) in the pac choi growing environment. The prediction is that herbivory will increase phenolic content levels compared to plants not exposed to herbivores.

# Literature Review

## *Plant Defenses against Herbivores*

Insect herbivores are abundant in nature and their interactions with host plants are complex, having evolved over millions of years (Bernays and Graham 1988; Bernays 2001; Schoonhoven et al. 2005; Zheng et al. 2007; Wu and Baldwin 2010). The diversity of plant species may be due to selection pressure by herbivores when they feed on and reduce plant fitness (Maron and Gardner 2000; Berenbaum and Feeny 2008). Ehrlich and Raven (1964) initiated the term 'reciprocal adaptive radiation' to describe 1) genetic variation that allows plants to survive insect attack, and 2) the subsequent adaptations of insect herbivores, allowing them to overcome plant defenses. However, plants have developed a variety of defensive strategies against herbivores, including physical and chemical barriers, either of which may be constitutive or induced (Berenbaum and Zangerl 1993; Karban and Baldwin 1997; Baldwin and Preston 1999; Schoonhoven et al. 2005; Price 2008; Ahuja et al. 2010; Snoeren et al. 2010). A constitutive defense, which is always present, may be an external feature that reduces or inhibits feeding such as leaf thickness and toughness, surface waxes, or trichomes (plant hairs) (Karban and Myers 1989; Walling 2000). Leaf thickness and toughness are associated with fiber or lignin content. As such, in order for herbivores to utilize tissue with a high fiber or lignin content, herbivores need specialized mandibles that do not degrade over time (Sanson et al. 2001). Constitutive defenses may also include rapid growth to compensate for consumed plant tissue (Smith 2005). Induced defenses are responses associated with exposure to stress, such as herbivore damage (Walling 2000). These defenses include the synthesis and accumulation of

secondary metabolites and release of volatile compounds (Turlings et al. 1995; Alborn et al. 1997; Gatehouse 2002). Volatiles are used as signals in some plants to attract natural enemies or warn neighboring plants (Turlings et al. 1990; Walling 2000; Kessler and Baldwin 2001). Secondary metabolites are affiliated with repairing damaged tissue, inhibiting herbivory, or reducing the nutritional quality of plant tissue (Edwards and Wratten 1983). Aside from the production of secondary metabolites, anti-digestive proteins such as poly-phenoloxidase may influence growth and behavior of herbivores, or alter the nutritional quality of plant tissue by interacting with secondary metabolites (Felton 1996; Karban and Baldwin 1997; Stout et al. 1998; Anttila et al. 2010). In some cases, insects have developed mechanisms to utilize or sequester defensive compounds in plant tissue to avoid attack from natural enemies (Cates 1980). As the composition of plant components change, so does plant quality for herbivores.

### *Insect Herbivore Nutrition*

Host plant quality, as a function of nutritional value and plant defense, may impact insect herbivore fitness in terms of survival or reproduction (Fraenkel and Blewett 1943; Awmack and Leather 2002). In general, herbivores require relatively large concentrations of dietary N in the form of amino acids for growth and reproduction (Mattson 1980; Slansky 1990). In order to obtain required N, herbivores must sequester N from plant tissues. Plants consist mainly of carbohydrates and, to a lesser extent, protein, either as protein-N or non-protein N (Mattson 1980). The composition of such metabolites are regulated both by genetic and environmental factors, and may be affected by season, plant age, and diel (24 hr) cycling (Mattson 1980).

Protein and carbohydrate (sugar) intake is essential to insect growth and development, and is regulated during feeding (Behmer 2009). A study of caterpillar species, including

*Heliothis* spp., *Heliocoverpa* spp., *Manduca* spp., *Malacasoma* spp., and *Spodoptera* spp., and their nutrient intakes, indicated that caterpillars tend to consume protein at a rate equal to or greater than carbohydrates (Behmer 2009). The need for greater amounts of protein than carbohydrates has been associated with enhanced herbivore survival, growth, and female fecundity (Mattson 1980; Felton 1996). In addition, protein quality may affect caterpillar consumption, where lower protein quality induced a reduction in feeding rates (Karowe and Martin 1989). Herbivore response to inadequate nutritional quality, including protein content, may result in increased consumption rates (compensatory feeding) and/or longer feeding periods to counteract nutrient imbalances or deficiencies (Slansky and Feeny 1977; Felton 1996). Nutrient intake may also be affected by the presence of defensive compounds, which include diverse classes of secondary metabolites (Mattson 1980).

### *Secondary Metabolites*

Secondary metabolites are ubiquitous in plants but do not have clearly-defined roles (Schoonhoven et al. 2005). It has been suggested that the array of secondary metabolites present in plants serve as a defense against herbivores (Fraenkel 1959). Furthermore, specialist herbivores have evolved to feed on plants with specific secondary metabolites (Close and McArthur 2002). For example, pierid caterpillars (*Pieris* spp.) feed exclusively on plants in the Brassicaceae family because feeding is stimulated by the presence of sinigrin, a mustard oil glycoside specific to Brassicaceae (Berenbaum and Zangerl 2008). Generalist herbivores, however, tend to feed on a range of plants in different families without adaptations for consuming secondary metabolites (Bernays et al. 1994; Bernays 2001). Although secondary metabolites have been widely studied (Dixon and Paiva 1995), their specific functions in plants

are still not well-understood. However, studies have indicated potential roles in plant growth regulation, pigmentation of fruits and flowers, and cell wall integrity (reviewed by Parr and Bolwell 2000; Taiz and Zeiger 2002). Accumulation of secondary metabolites is also associated with a wide range of abiotic and biotic stresses including herbivory, temperature extremes, soil nutrient deficiencies, and insufficient or excessive light exposure (Dixon and Paiva 1995; Close and McArthur 2002; Young et al. 2005). Secondary metabolites are divided into three classes based on their biosynthetic pathways: terpenes, phenolics, and N-containing compounds (Bassman 2004). Each class has been shown to have negative effects on herbivores (Taiz and Zeiger 2002). Terpenes, such as monoterpene esters, occur in certain species of chrysanthemum (*Dendranthema* spp.) and are toxic to a wide range of insect herbivores (Gershenzon and Croteau 1992).

Phenolic compounds are associated with resistance to insects due to their toxic effects and role in reducing palatability of plant tissue (Schoonhoven et al. 2005). For example, the hydroxycinnamic acids are phenolic compounds that enhance leaf toughness by forming layers of lignin (Parr and Bolwell 2000). The rigidity of lignin deters herbivores because it is not digestible (Karban and Myers 1989). Lignin components also bind to proteins, reducing the nutritional quality of plant tissue post-ingestion (Kurz 1989). Flavonoids are phenolic compounds that help protect leaf surfaces from ultraviolet light damage by alleviating the formation of free radicals in the epidermis (Li et al. 1993; Dixon and Paiva 1995). Low levels of ultraviolet light stimulate production and accumulation of flavonoids and other phenolics in the leaf epidermis (Mazza et al. 2010).

Nitrogen-containing secondary metabolites include alkaloids and cyanogenic glycosides (Castells et al. 2005), which are extremely toxic to both vertebrate and invertebrate herbivores,

including humans (Kurz 1989). Other N-containing compounds include glucosinolates and non-protein amino acids. Non-protein amino acids, such as L-cavanine found in some plants including alfalfa (*Medicago sativa*), are toxic to herbivores (Rosenthal 1977). However, some insect herbivores have adapted to consuming L-cavanine through modified biochemical pathways that detoxify the compound (Taiz and Zeiger 2002). Similarly, specialists that feed on *Brassica* crops have adjusted to the accumulation of glucosinolate compounds, whereas generalist feeders are typically repelled (Karban and Myers 1989). Diamondback moth, a specialist herbivore of *Brassica* plants, uses sulfatase activity to desulfate glucosinolates, thus avoiding the transformation of toxic glucosinolate breakdown products (Ratzka et al. 2002).

Secondary metabolites may interfere with insect herbivore nutrient uptake and utilization (Felton 1996). Feeny (1970) proposed that the combination of both secondary metabolites and primary plant nutrients may have greater impact on herbivores than secondary metabolites alone. For example, protein composition may reduce quality for herbivores (Karowe and Martin 1989), and intraspecific changes in protein quality, resulting from plant stress, such as herbivory, may affect herbivore performance (Bi et al. 1994). Furthermore, a low protein content induces higher consumption rates, which may lead to increased consumption of toxic secondary metabolites (Slansky and Wheeler 1992). Phenolic compounds, in particular the hydroxycinnamic acids (caffeic, sinapic, and ferulic), can bind to and precipitate out proteins in macerated leaf tissue, which may affect survival, growth, and fecundity of some insects (Dixon and Paiva 1995). The effect of secondary metabolites as defensive compounds against herbivores is well-understood (Dixon and Paiva 1995; Close and McArthur 2002; Berenbaum and Zangerl 2008). However, little is known regarding interactions of primary and secondary metabolites with herbivores

(Slansky 1990; Felton 1996). Changes in the composition of primary and secondary metabolites in response to herbivore feeding are unclear.

### *Induction of Secondary Metabolites*

Secondary metabolites are not exclusively present in plants but can be synthesized after wounding by herbivores (Karban et al. 1999; Bruinsma and Dicke 2008). For example, in cells surrounding herbivore feeding sites, elevated levels of phenolics are commonly detected (Uritani 1978). However, this reaction is dependent on feeding behavior. Chewing insects tend to induce the production of secondary metabolites more so than piercing-sucking insects because most of these compounds are released from cell wall-bound storage sites such as vacuoles during feeding (Walling 2000). Piercing-sucking insects are not exposed to secondary metabolite storage sites unless their mouthparts actively damage cells during feeding in the phloem or xylem (Schoonhoven et al. 2005). Furthermore, components in the saliva of chewing insects may induce defense responses in plant tissues, including the accumulation of secondary metabolites (Musser et al. 2004; Major and Constabel 2007). Overall, defense responses are complex and vary depending on plant-herbivore interactions. For example, signaling of defense responses in plants is regulated by plant hormones such as jasmonic acid, salicylic acid, and ethylene (Winz and Baldwin 2001). These signals may instigate multiple responses, such as induction of phenolics or release of volatiles by stimulating overlapping precursors in biosynthetic pathways (Gatehouse 2002; Major and Constabel 2007). These stimuli may initiate transient fluctuations in secondary metabolites or systemic-acquired-resistance (SAR), a continuous type of defense response (Pieterse and Van Loon 2004). Stimulation of defense responses and production of defensive compounds may involve responses to a plant's growing environment. For example, light intensity and changes in soil nutrition may affect the biosynthetic pathways associated with

production of phenolics by impacting levels of the precursor, phenylalanine (Young et al. 2005). More specifically, changes in light exposure (Rowe and Potter 2000) and soil N (Gershenzon 1984) are associated with changes in carbon assimilation, and therefore, changes in carbon-based defenses (Slanksy and Scriber 1985).

### *Phenolic Compounds*

Phenolic compounds may have both positive and negative effects on herbivores (Rosenthal and Berenbaum 1991; Karban et al.1999). They may act directly as harmful pro-oxidants by exerting oxidative stress on cells, serving as beneficial antioxidants via alleviating oxidative stress on cells, or by reducing the nutritional quality of plant tissues (Felton et al. 1989; Appel 1993; Summers and Felton 1994). However, all of these responses are dependent on plant-herbivore interactions because feeding behavior and midgut pH may mediate the harmful or beneficial effects of phenolics (Bernays et al. 1983; Boyer et al. 1988; Bi and Felton 1995; Johnson and Felton 2001). For example, caterpillars typically have an alkaline midgut (pH > 7), with an oxidizing redox potential that promotes formation of damaging free radicals post-ingestion of phenolic compounds (Summers and Felton 1994). Phenolics may also impact caterpillars through prolonged development, which then increases exposure to natural enemies (Price et al. 1980; Price 1991). However, phenolics may be sequestered by specialist herbivores, thus decreasing their susceptibility to predators and parasitoids (Price 2008).

Several studies have demonstrated the varied effects of phenolics on herbivores (Zangerl and Berenbaum 1993; Bi and Felton 1995; Johnson and Felton 2001; Ferreres et al. 2009). For example, parsnip (*Pastinaca sativa*) varieties comprised of different phenolic contents were exposed to the parsnip webworm (*Depressaria pastinacella*). It was found that when exposed but not fed upon, plants contained twice the phenolic content, including furanocoumarins,

xanthotoxins, and sphondins, than plants that were fed upon (Zangerl and Berenbaum 1993). Furthermore, parsnip webworm larvae feeding on artificial diets containing higher concentrations of furanocoumarins had reduced growth (Berenbaum 1978; Berenbaum et al. 1986). The negative effects of phenolics on herbivores may include covalent and non-covalent binding to proteins, which impair enzymatic functions and reduce protein digestibility, and also decrease availability of amino acids (Berenbaum et al. 1989; Bi and Felton 1995). Ingestion of chlorogenic and caffeic acid by *Helicoverpa zea* larvae resulted in oxidative damage to the midgut epithelium, including lipid perioxidation, protein and nucleci acid oxidation, and a reduction in midgut antioxidants (Summers and Felton 1994). These findings suggest that some phenolics may disrupt metabolism when consumed by herbivores. However, other studies have reported that consuming plants containing phenolics may benefit certain insect herbivores. For example, Johnson and Felton (2001) reported that tobacco (*Nicotiana tabacum*) lines with modified levels of chlorogenic acid did not inhibit feeding of the tobacco budworm (*Heliothis virescens*). Plants fed upon by the tobacco budworm, that over-expressed chlorogenic acid production, resulted in no effect on metabolism, and oxidative stress markers were present in the tobacco budworm midgut whereas this did not occur with larvae feeding on plants under-expressing chlorogenic acid. Based on these results, it appeared that the expected pro-oxidant effects associated with chlorogenic acid were ameliorated by the antioxidant capacity of the caterpillar midgut (Johnson and Felton 2001). This suggests that the effects of phenolic compounds is specific, depending on herbivore and the concentration of the phenolic compound, which may also be influenced by a number of factors, including plant age, prior wounding by herbivores, and environmental stress (Koricheva et al. 1998; Hemming and Lindroth 1999; Parr and Bolwell 2000).

## *Alternative Crop Production Practices*

A trend in vegetable crop production is the adoption of growing plants in protected environments under organic production systems (Parrot and Mardsen 2002; Altieri and Nicholls 2003). This system may directly alter the growing environment, either by reducing light transmission or by supplying organic N sources, thus potentially impacting plant growth as well as susceptibility to herbivores (Jansson et al. 1991; Ingersoll et al. 2010). By experimentally investigating light intensity and N source, there is an opportunity to assess whether they directly impact plant chemistry with corresponding indirect effects via plant-insect interactions. As such, this research has implications for pest management associated with minimizing herbivore outbreaks.

### *High Tunnel Vegetable Production*

Vegetable production in high tunnels is common in the Mediterranean region and Asia (Wein and Pritts 2009). It is also increasingly popular in the USA (Wells and Loy 1993, Gent 2002; Knewtson 2008). High tunnels are covered frames, similar in structure to Quonset-type greenhouses, but use polyethylene film as a covering over metal, plastic, or wood frames. High tunnels utilize solar radiation for heating and passive ventilation for cooling (Blomgren and Frisch 2007). The costs associated with high tunnel construction and maintenance are considerably less compared to typical greenhouse construction and maintenance, which relies on electricity for heating and cooling (Wells and Loy 1993). Even without electricity, elevated temperatures in high tunnels allow for early spring planting and harvesting, and may also extend fall production of warm-season crops such as tomato (*Lycopersicon esculentum*) (Jett and Reed 2003).

High tunnels are well-adapted for growing crops year-round in the Midwest and are being adopted by producers in cooler climates such as the Northeast (Wein and Pritts 2009). High tunnel production is a practice in areas of the USA that rely on the transport of fruits and vegetables from warmer regions of the continent during the cooler seasons (Wein and Pritts 2009). However, as transportation costs continue to escalate, so has the interest in high tunnel production for local markets (Carey et al. 2009). Extending the growing season may provide revenue during unproductive periods and alleviates exorbitant out-of-season price premiums for consumers (Hunter 2010).

Protected environments, such as high tunnels and greenhouses, may alleviate problems associated with environmental conditions such as temperature, light and rain by providing a barrier (Knewtson 2008). The high tunnel environment is considered 'protected', and has been affiliated with a reduced-incidence of diseases and insect pests (Lamont et al. 2003). One advantage of a high tunnel is that certain fungal diseases, especially those spread by splashing water via rain and/or wind, are less problematic (Orzolek et al. 2004). Furthermore, it has been suggested that insect pests cause less damage in high tunnels than field production because crops are grown during cooler seasons when pests are less active (Moore 2007). However, the risk of insect attack under high tunnel compared to field production is still not well understood (Whitelam and Halliday 2007; Zhao et al. 2007).

High tunnels make it possible to overlap high-value cropping cycles within a single season (Harwood and Plucknett 1981). It has been reported that Kansas producers using high tunnels can have up to nine cropping cycles of certain vegetable varieties in a given year (Knewtson 2008). However, intensive cropping may deplete soil organic matter and lower rates of soil mineralization, resulting in decreased levels of available N for plants (Roorda van

Eysinga 1984; Lea and Morot-Gaudry 2001). Warm ambient air temperatures under high tunnels during cool-season months enhance crop maturity and need for available N (Waterer and Bantle 2000; Both et al. 2007). The amount of available N required for crop maturity may not be adequate if soils cannot undergo mineralization. Microbial activity associated with temperature-dependent mineralization is enhanced as soil temperature increases (Deenik 2006).

Since the high tunnel environment reduces light transmission, this may inadvertently impact growth and reproduction of plants (Antignus et al. 1996). Reduced light transmission may decrease photosynthesis and respiration, thus lowering assimilated carbon in leaves (Waring et al. 1985). Exposure to low light conditions may result in plants having high amino acid concentrations and low concentrations of carbon-based defensive compounds (Bryant et al. 1983; Matson and Waring 1984). As such, plants with high amino acid contents and low levels of defensive compounds may be more susceptible to insect herbivores (Prudic et al. 2005; Walter and Difonzo 2007). Plant chemical composition may be dependent on exposure to light (Parr and Bolwell 2000). Depending on the type of polyfilm covering used on high tunnels, reduced exposure of ultraviolet light may occur, resulting in the stimulation of biosynthetic pathways for producing flavonoid and phenolic compounds (Kulmann and Muller 2009). These compounds may therefore protect plants from photodamage caused by exposure to excessive light and insect herbivores (Muth et al. 2008; Anttila et al. 2010; Collins et al. 2010; Mazza et al. 2010). Use of high tunnels may provide an environment that impacts plant-insect interactions.

### *Organic Vegetable Production*

Besides the use of high tunnels, other production practices such as organic management may potentially impact plant-insect interactions. Organic production was defined in 1980 by the United States Department of Agriculture as producing crops without the use of synthetic

fertilizers, pesticides, and growth regulators (USDA 1980; 2000; 2002). Organic production of food crops has become popular, with North America representing the world's largest organic retail market (Willer and Kilcher 2009). In fact, retail sales of organic foods and beverages in the USA reached 20 billion dollars in 2007 (Haumann 2009). The demand for organic produce is high because consumers perceive the benefits of organic vegetables as having less impact on the environment and reducing exposure to pesticide residues (Greer and Diver 2000). Of the four million acres under organic production in the USA, approximately 98 thousand acres are in vegetable production (Delate et al. 2008). The number of certified organic vegetable farms is increasing as consumer demand for organic produce continues to increase, and implementation of federal guidelines has become more consistent (Baker et al. 2002; Haumann 2009). The transition to organic vegetable production is an important alternative to conventional production in regions where intense vegetable farming has created negative environmental impacts. For example, in California, concerns over water quality, due to nutrient enrichment associated with excessive application of synthetic nitrogen and phosphorus fertilizers, has resulted in a shift to organic procedures (Hartz 2010). In addition, California vegetable production has encountered a doubling in synthetic fertilizer costs since 2006 (Hartz 2010). As such, evaluating alternative fertilizers has become an important priority for vegetable producers (Kasim et al. 2011). Additionally, the shift to organic fertility practices has been linked to enhanced plant defense against herbivores.

In general, organic production systems are perceived as less susceptible to insects and diseases than conventional systems (Parrot and Marsden 2002). Because pest populations tend to be lower in organic production systems due to less pesticide use, this has resulted in an increase in natural enemy diversity (Phelan et al. 1996; Letourneau and Goldstein 2001; Altieri and

Nicholls 2003). Although experimental evidence is lacking, it has been suggested that plants grown under organic production systems are less susceptible to pest attack due to the high organic matter content in soils, which may increase the abundance and diversity of soil microbes (Hummel at el. 2002). Advocates of organic production claim that soils with high levels of organic matter have balanced concentrations of macro- and micro-nutrients, which promote naturally-occurring beneficial organisms that may improve plant vigor (Oehl et al. 2004; Hsu et al. 2009).

In conventional agriculture, use of synthetically-derived fertilizers and pesticides are typically permitted (Eicher 2003). Conventional systems have been shown to increase pest populations, possibly corresponding to plants having greater biomass and N-rich tissues (Johnson and Tabashnik 1999). Furthermore, it is suggested that excessive use of synthetic fertilizers may cause nutrient imbalances in soil, thus leading to lower plant vigor and susceptibility to insects and diseases (Phelan et al. 1996). Although previous studies have focused on fertilization practices that impact insect herbivores through changes in host plant chemistry (Hummel et al. 2002), the underlying mechanisms are complex and not well understood (Altieri and Nicholls 2003). Thus, a better understanding of the effects of fertility practices on plants and insect herbivores may lead to implementing pest management strategies that alleviate insect problems (Altieri and Nicholls 2003).

Organically certified producers must implement fertility practices based on guidelines of The National List of Allowed and Prohibited Substances as outlined by the National Organic Program (NOP) (USDA 2000). In order to certify farms as organic, producers must adhere to the NOP standards using materials approved for organic production systems and abide by the policies of their certifying agent (Bellows 2005). Some allowable sources of N include crop

rotations with legumes and application of plant- or animal-based materials (USDA 2000). These materials may include animal by-products such as manures, guano, blood meal, fish hydrolysate, and fish protein (Hadad and Anderson 2004). However, these practices may be expensive and often lack uniformity in available N (Gaskell and Smith 2007).

One of the goals of organic production is to maintain healthy soils by improving soil structure with organic matter (Gaskell et al. 2006). Organic matter (OM) is beneficial because, during decomposition, nutrients are released for plant uptake including N and phosphorous (P). High levels of OM can also reduce leaching of nutrients by adsorbing cations such as calcium and potassium, and enhance soil aggregation, which subsequently improves water-holding capacity (Gaskell et al. 2000). The temperature-dependent decomposition process of OM mineralization involves soil microorganisms that metabolize organic forms of C and N. During this process, excess N (beyond the requirement of the microorganisms) is released into the soil as ammonium ($NH_4^+$ - N), which can be further transformed by chemo-autotrophic bacteria through nitrification into nitrate ($NO_3^-$ - N). Plant roots readily take up both $NO_3^-$ - N and $NH_4^+$ - N (Larsson et al. 1992). Moreover, plants tend to grow and develop faster when both $NO_3^-$ and $NH_4^+$ - N are available because adsorption and assimilation results in a cation-anion balance within plants (Raven and Smith 1976). The difference in organic and conventional fertility is that conventional fertilizers typically supply high ratio of $NO_3^-$ - N to $NH_4^+$ - N that is readily available to plants (Delate et al. 2008), whereas organic fertility relies on a slow release of N by carbon sources through mineralization processes.

Excessive applications of inorganic forms of $NO_3^-$ - N and $NH_4^+$ - N may contaminate the environment (Comley 1987). Typically, environmental contamination is associated with nutrient enrichment of water supplies from applications of inorganic form of N and P (Mengel 1992). For

example, plants normally absorb less than 50 percent of the N applied in synthetic fertilizers with the remainder usually entering groundwater resources (Burow et al. 2008). In addition, methemoglobinemia (blue baby syndrome) is a blood disorder of infants that may be caused by high nitrate levels in groundwater aquifers (Comly 1987). Moreover, the Kansas Department of Health and Environment (KDHE) states that high levels of N and P from agricultural run-off into surface waters of Midwestern states enhance the growth of nuisance plants and algae in streams and reservoirs (KDHE 2004). Contamination by N and P in Midwestern states is further associated with nutrient enrichment in the Gulf of Mexico via the Mississippi River (KDHE 2004). For example, sections of the Gulf of Mexico are experiencing hypoxia or 'Dead Zones,' which may negatively impact aquatic life by reducing dissolved oxygen (KDHE 2004). The risk of contamination may be reduced by limiting applications or decreasing rates of synthetic fertilizers (Mengel 1992). It is not known, however, if contaminating levels of nutrients are associated with organic-use materials.

Fertility practices also impact plant-insect interactions by mediating changes in host plant quality (Letourneau and van Bruggen 2006). Specifically, the form of N in conventional and organic fertilizers can modify plant quality, thus influencing insect herbivores (Scriber 1984). Readily-available N associated with conventional fertilizers results in plants with high foliar N concentrations, which may increase susceptibility to insect herbivores (Mattson 1980; Prudic et al. 2005; Walter and Difonzo 2007). In addition, plants containing high tissue N may produce fewer C-based defensive compounds against herbivores compared to plants with limited tissue N (Le Bot et al. 2009). However, results from studies comparing the effects of conventional and organic N fertility on host plant quality and interactions with insect herbivores have been mixed (Beanland et al. 2003; Hsu et al. 2009). Morales et al. (2001) found that corn (*Zea mays*) plants

receiving high levels of N from a conventional fertilizer had higher populations of the aphid, *Rhopalosiphum maidis*, but had fewer populations of the fall armyworm, *Spodoptera frugiperda,* compared to plants receiving less N. It was suggested that differences in feeding behavior (phloem versus leaf chewer) may explain why higher aphid populations were found with higher N content, whereas fewer fall armyworms were presumed to be linked to the presence of N-related defensive compounds in the leaf tissue. Hsu et al. (2009) reported that concentrations of sinigrin (the most abundant defensive compound in *Brassica* plants) were higher in *Brassica oleraceae* plants receiving an organic fertilizer than in plants that received a synthetic fertilizer. Furthermore, Hsu et al. (2009) showed that cabbageworm (*Pieris rapae*) larvae developed faster on conventionally fertilized *B. oleraceae* than on plants receiving an organic fertilizer. It has been suggested that reduced development associated with the organic fertilizer may have been due to higher sinigrin concentrations (Hsu et al. 2009).

The quantity of N and the balance of soil macro- and micro-nutrients may also influence host plant quality for herbivores. Conventional fertilizers may provide higher levels of N but may also upset nutrient balances by decreasing soil buffering capacity or impact pH (Phelan et al. 1995). Beanland et al. (2003) compared soybean (*Glycine max*) shoot growth and herbivore responses at varying proportions of soil micro-nutrients. They found that soybean looper (*Pseudoplusia includens*), Mexican bean beetle (*Epilachna varivestis*), and the velvetbean caterpillar (*Anacarsia gemmatalis*) exhibited reduced developmental times on plants grown in a soil with adequate soil micro-nutrients compared to plants grown in soils deficient in micro-nutrients, specifically boron. When fertility provides a balance of macro- and micro-nutrients that are available to plants, both yield and protection from herbivores may be optimized (Beanland et al. 2003). Adequate soil nutrition is also important to plants that accumulate

elements such as zinc and nickel (Brooks 1988). It has been demonstrated that plants which accumulate these nutrients are less susceptible to certain herbivores, apparently due to their toxic effects (Coleman et al. 2005). Elements such as zinc have also displayed additive effects with other defense compounds against insect herbivores (Jhee et al. 2006).

### *Environment Effects and Host Plant Quality*

Light intensity and soil nutrition not only impact the production of secondary metabolites, but also primary plant metabolites such as protein and carbon (Coley et al. 1985; Jansson et al. 1991; Jansen and Stamp 1997; Close and McArthur 2002; Bassman 2004). For example, plants grown under shade conditions tend to have lower concentrations of C and C-based defenses (Dudt and Shure 1994), whereas plants grown in full sun typically have higher sugar concentrations as higher photosynthetic rates result in the production of more C (Rowe and Potter 2000). Furthermore, N-based defensive compounds may increase under low light intensity and enhanced soil nutrient availability (Folgarait and Davidson 1995). Carbon assimilation in plants also depends on available N, which influences leaf area development (Gastal and Lemaire 2002). When leaf area development is restricted, plants may increase the production of secondary metabolites (Penuelas and Estiarte 1997). Plant responses to stress (including C limitation) may include reallocation of primary metabolites (sugars and amino acids) to secondary metabolites (Coley et al. 1985), which may alleviate light stress from exposure to excessive ultraviolet B (UV-B) (280-320 nm) (Taiz and Zeiger 1998). Specifically, phenolic compounds may accumulate, thus protecting plant tissues by enhancing cell stability, or alleviating the formation of harmful free radicals (Taiz and Zeiger 1998; Jenkins and Brown 2007). Free radicals, such as singlet oxygen species and hydroxyl radicals, exert oxidative stress on cells by degrading lipids, proteins, and deoxyribose nucleic acid (DNA) (Close and McArthur

2002). Oxidative stress is enhanced by a lack of antioxidants, excessive formation of free radicals, or a combination of both. Compounds that counteract the presence of free radicals are those having antioxidant properties, which are capable of detoxifying free radicals via binding, thus inhibiting their ability to attract oxygen from plant proteins (Bassman 2004). A number of phenolic compounds are capable of functioning as antioxidants (Oh and Rajeshaker 2009). The protective properties of phenolics are well-understood, both in plant and animal systems (Parr and Bolwell 2000). However, the influence of light intensity and soil nutrition on the production and accumulation of phenolics in plants requires further study in order to understand plant-herbivore interactions under different growing environments. Environmental effects may mediate changes in plant growth and chemistry, thus impacting herbivore development and survival. There are a number of hypotheses that explain the potential influence of environmental factors on host plant chemistry. The plant stress hypothesis (White 1969) proposes that plants are more susceptible to herbivores when under stress due to a reduction in protein synthesis and presence of higher levels of amino acids in tissues, resulting in an increase in nutrient content and the production of fewer defensive compounds (Mattson and Haack 1987). Inbar et al. (2001) found that larval feeding and oviposition of the serpentine leafminer (*Liriomyza trifolii*), and larval growth rates of the corn earworm (*Heliothis zea*), were enhanced on vigorously-growing tomato (*Lycopersicon esculentum*) plants compared to plants that were mechanically damaged or deficient in water, fertilizer, or both. Increased herbivore performance on healthy, fast-growing plants is also supported by the plant vigor hypothesis, which proposes that larger, more vigorous plants may be more apparent and attractive to herbivores (Price 1991). This hypothesis is associated with herbivore guilds that interact closely with host plants such as leaf-gallers and shoot borers. Insects in these guilds tend to prefer vigorous plants to complete their life cycle.

Larger and structurally diverse plants may host a variety of herbivores, as well as tolerate damage from herbivory by compensating for tissue losses with enhanced growth (Schoonhoven et al. 2005). The resource availability hypothesis (Coley et al. 1985) suggests that the production of defensive compounds occurs when the costs are less than the benefits associated with increased protection against herbivores. This is similar to the plant stress hypothesis (White 1969), which encompasses the limitations of light intensity and soil nutrition, resulting in the production of secondary metabolites (White 1969). Additional hypotheses focus on the resources available for production of phenolic compounds, such as the C:N balance hypothesis (Bryant et al. 1983), which proposes that the allocation of defensive compounds depends on the relative availability of C and N resources associated with plant growth. This hypothesis indicates that increasing photosynthesis or decreasing available N may result in an increase in C-based defenses, whereas a decrease in photosynthesis or increase in N concentration would lead to an increase in N-based defenses (Bryant et al. 1983).

### *Light Intensity and Host Plant Quality*

Plants perceive the spectra of light in three distinct categories: ultraviolet (<400 nm), visible (400-700 nm), and far-red (700-800 nm) (Tevini and Teramura 1989; Rajapakse and Shahak 2007). Ultraviolet (UV) light, an energy-rich fraction of the solar spectrum, has been shown to affect plant-insect interactions by mediating primary and secondary metabolism (Bryant et al. 1983; Herms and Mattson 1992; Izaguirre et al. 2003; Kuhlmann and Muller 2009). UV light has been shown to influence insect consumption rates through effects on foliar chemical composition, although results vary based on the study system (Mole and Waterman 1988; Shure and Wilson 1993; Dudt and Shure 1994; Louda and Rodman 1996; Niesenbaum and Kluger 2006). Excessive levels of UV-B light can inhibit photosynthesis by damaging

chloroplasts, increasing leaf thickness, and stimulating the production of UV-absorbing phenolic compounds (Tevini and Teramura 1989). The quantity of UV-B light that plants receive depends on seasonal changes and geographic location. For instance, the seasonal solar angle determines the light path length through the atmosphere, and elevation and latitude also affect light path length and overall intensity (Jenkins and Brown 2007). In addition, UV-B light may also be deflected or scattered by atmospheric pollution, cloud cover, and surface reflection (McKenzie et al. 2003; Jenkins and Brown 2007). Plants grown inside high tunnels or greenhouses may experience reduced light exposure, which may influence both C assimilation and phenolic composition. Changes in plant chemistry due to light intensity may impact plant-herbivore interactions (Close and McArthur 2002; reviewed by Bassman 2004). However, there is little information pertaining to specific phenolic concentrations and impact on herbivores under varying light intensities (Mole and Waterman 1988; Close and McArthur 2002; Ingersoll et al. 2010).

Phenolics that have been shown to increase after exposure to UV light include flavonoids and hydroxycinnamic acids (Caldwell et al. 2003), which are compounds derived from cinnamic acid formed by phenylalanine ammonia-lyase in the shikimate acid pathway (Velasco and Mollers 1998; Hermann and Weaver 1999; Cartea et al. 2011). Visible light provides plants with the energy required for photosynthesis and C assimilation (Whitelam and Halliday 2007). In addition, visible light modulates several steps in phenolic metabolism, so that a higher phenolic content may be affiliated with exposure to high light intensities (Parr and Bolwell 2000).

An elevated carbon to nitrogen (C:N) ratio is associated with an increased photosynthetic rate in plant tissue, which may be due to exposure to high light intensity (Lea and Morot-Gaudry 2001). Leaves exposed to full sun may accumulate higher concentrations of sugars and proteins,

and also have a high moisture content due to an increase in the photosynthetic rate (Osisanya 1970; Nichols-Orians 1991). However, sugar and moisture content may stimulate insect feeding (Schoonhoven et al. 2005). For example, Rowe and Potter (2000) found that Japanese beetle (*Popillia japonica*) adults preferred feeding on rose (*Rosa* spp.) plants grown under sunlight compared to plants grown under shade. They suggested that beetle preference for sun plants may be associated with higher nutritional quality resulting from higher concentrations of amino acids and sugars (Rowe and Potter 2000). In contrast, some insects prefer to feed on plants grown in the shade. Caldwell et al. (2003) found that populations of thrips (*Caliothrips phaseoli*) on soybean (*Glycine max*), and leaf beetles (*Diabrotica speciosa*) on the annual weed, *Datura feroxon*, were lower on plants exposed to high light intensity. As such, plants grown under high light conditions may modulate defensive C-based phenolics differently than plants grown under shade (Karban and Myers 1989; Tegelberg et al. 2004). The light-dependent observations of herbivory on plants could be due to changes in phenolic content that vary because of light environment, either via differences in resource availability or induction by UV light (Ingersoll et al. 2010).

In addition to defense against herbivores, secondary metabolites function to protect plants from abiotic stress (Berenbaum and Zangerl 2008). The relationship between light intensity and phenolics has been extensively studied (Dudt and Shure 1994; Close and McArthur 2002; Bassman 2004; Izaguirre et al. 2007; Ingersoll et al. 2010). For example, Jansen and Stamp (1997) found that tomato (*Lycopersicon esculentum*) plants grown in full sun have higher concentrations of the phenolic compounds chlorogenic acid and rutin than plants grown in shade. These phenolic compounds may reduce growth, extend development time, and disrupt molting of insect herbivores (Isman and Duffey 1982). Likewise, Feeny (1970) reported that concentrations

of tannins in English oak (*Quercus robur*) were higher in leaves exposed to sun than shade leaves, which negatively impacted survival of the winter moth (*Operophtera brumata*). Phenolic compounds in the furanocoumarin class, which are present in the plant family Umbelliferae, are toxic to herbivores after activation by UV-A light (320-400 nm) (Sandberg and Berenbaum 1989). However, some insects have adapted to furanocoumarins by feeding inside rolled leaf tissue, which limits exposure to sunlight thus suppressing activation of furanocoumarins (Sandberg and Berenbaum 1989).

### *Soil Nutrition and Host Plant Quality*

The availability of soil nutrients and fertilizer applications may affect the chemical composition of plants (Klein and Blum 1990; Lea and Morot-Gaudry 2001; Gent 2002; Le Bot et al. 2009). According to the carbon-nutrient-balance hypothesis (Bryant et al. 1983), N fertilization should increase concentrations of some nutrients in leaves, whereas the concentration of C and C-based defense compounds should decrease (Wilkens et al. 1996). An increase in N will stimulate an increase in amino acid concentrations in plant leaves, and reduce the assimilation of carbohydrate via photosynthesis (Mooney and Gulmon 1982). A lack of C-based defense compounds may be due to insufficient C-sources for production (Bryant et al. 1983). Plants subject to N deficiency tend to accumulate C in tissues, which is then unavailable for the synthesis of amino acids or other N compounds. Instead, the excess C may be used for the production of C-based secondary metabolites (Rufty et al. 1988; Taiz and Zeiger 2002). Previous studies have demonstrated that low soil fertility is associated with an increased production of phenolic compounds (Slansky and Scriber 1985). Low soil fertility has been affiliated with enhanced concentrations of secondary metabolites when secondary compound accumulation is faster than the rate of increase in plant biomass (Koricheva 1999). Li et al. (2008) found that

total phenolic content was considerably lower as N fertilization increased for leaf mustard (*Brassica juncea*). Bryant et al. (1987) demonstrated that an increase in N fertilization resulted in a decrease in tannin and phenolic content in quaking aspen (*Populus tremuloides*) trees, which positively affected large aspen tortrix (*Choristoneura conflictana*) growth, compared to aspen tortix on trees with higher tannin and phenolic content, and lower N content that received no N fertilization (Bryant et al. 1987). Le Bot et al. (2009) reported that tomato (*Lycopersicon esculentum*) plants receiving low levels of $NO_3^-$ - N had elevated concentrations of chlorogenic acid and rutin, and higher concentrations of sugars compared to plants receiving high levels of $NO_3^-$ - N. It appeared that a higher N fertility inhibited secondary metabolite production, or perhaps secondary metabolite concentrations were diluted by the abundance of amino acids (Le Bot et al. 2009). Likewise, Chen et al. (2004) reported that leaves of fertilized cabbage (*Brassica oleracea* var. *capitata*) plants had higher moisture and N contents but lower levels of glucosinolates compared to the leaves of unfertilized plants. It was also noted that, in choice tests, females of two specialist cabbage butterfly species (*Pieris rapae crucivora* and *P. canidia canidia*) preferred to oviposit on the leaves of fertilized *Brassica oleracea* compared to unfertilized plants. Furthermore, caterpillars from both species that fed on fertilized plants developed faster than caterpillars feeding on unfertilized plants (Chen et al. 2004).

Soil nutrients other than N, when deficient, may also impact herbivores (Wright et al. 2010). For example, boron-deficient soils yielded *Brassica* plants with a higher phenolic content (Harborne 1980). In addition, Tolra et al. (2001) found that *Thlaspi caerulescens* (family Brassicaceae) had significantly lower concentrations of glucosinolates when grown in a soil deficient in Zn (Phelan et al. 1995; Phelan et al. 1996). Also, soybean plants grown in potassium (K)-deficient soil had higher soybean aphid (*Aphis glycines*) populations, possibly due to an

elevated N content and/or a higher concentration of amino acids (Walter and Difonzo 2007). These results may be associated with antagonistic effects of soil N and K, so that decreases in K may lead to increases in available N (Taiz and Zeiger 2002). Glucosinolates, which are sulfur-containing and known to stimulate the feeding of specialist herbivores in *Brassica* plants, depend on adequate sulfur (S) for production (Badenes-Perez et al. 2010). In fact, it has been reported that *B. vulgaris* plants receiving higher concentrations of sulfur via fertility practices, were more attractive to ovipositing DBM females, which enhanced its use as a trap crop to manage DBM populations. Sulfur fertility in this system is an example of how the plant growing environment can be modified to influence *Brassica*-DBM interactions.

# Chapter 2 - Effects of light intensity on pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') chemistry and interactions with the diamondback moth (*Plutella xylostella*)

## Introduction

Light is a fundamental requirement for plant growth and development. During photosynthesis, light energy is utilized to oxidize $H_2O$, resulting in the release of $O_2$ and the reduction of $CO_2$ to form glucose (Taiz and Zeiger 2002). Glucose, in the form of sucrose and starch, is used along with nitrogen to produce proteins for growth and development (Mooney and Gulmon 1982; Lewis and Sarkanen 1998). Sucrose and starch may also be allocated to produce C-based defense compounds, such as phenolics, tannins, and flavonoids, which may be used as protection against abiotic and biotic stresses (Dixon and Paiva 1995). The influence of light on the photosynthetic rate depends on the quality of light in the electromagnetic spectrum, duration of light energy over time, and intensity as measured by the quantity of wavelengths in the spectrum (Aldrich and Bartok 1994). As light intensity increases, greater numbers of photons strike chlorophyll pigments, which enhance the rate of photosynthesis (Whitelam and Halliday 2007). Increased light intensity is associated with greater carbon accumulation and higher carbon to nitrogen (C:N) ratios in leaf tissue (Osisanya 1970; Nichols-Orians 1991; Lea and Morot-Gaudry 2001). A reduction in light intensity may decrease the photosynthetic rate, reducing stores of carbon and diverting C from defense to the production of primary metabolites

(i.e., amino acid and proteins) (Mooney and Gulmon 1982; Schoonhoven et al. 2005). Plants exposed to shade conditions typically have limited C with less phenolic content (Koricheva et al. 1998; Henriksson et al. 2003). The theoretical basis for these shifts is the carbon:nitrogen (C:N) balance hypothesis, which predicts that when plants are exposed to lower levels of light, excess C is unavailable for producing secondary compounds, including those that serve as plant defenses against insect herbivores and environmental stress (Bryant et al. 1983). Plants containing relatively higher amounts of N in lower light conditions may be more nutritious for herbivores (Mattson 1980). Therefore, the light environment may determine plant nutritional content by regulating photosynthesis, the C:N ratio, and resources associated with plant defenses (Izaguirre et al. 2003; Young et al. 2005; Whitelam and Halliday 2007).

The accumulation of specific phenolic compounds, hydroxycinnamic acids, function to enhance cuticle thickness under high light intensities or function as antioxidants to alleviate plant stress (Muth et al. 2008). Both structurally and physiologically, these acids may have an important effect on herbivore feeding, growth and development and, thus, provide resistance in plants to insects (Dixon and Paiva 1995; Felton 1996; reviewed by Bassman 2004). For example, hydroxycinnmic acids such as caffeic, ferulic, and sinapic acids, have been reported to negatively affect herbivores (Felton 1996; Stout et al. 1998) by direct toxicity or indirectly by reducing protein quality or availability during digestion (Cooper-Driver et al. 1977; Caldwell et al. 1983, 1998, 2003; Dixon and Paiva 1995; Bieza and Lois 2001).

Similar to the hydroxycinnamic acids, chlorogenic acid is a phenolic compound that has been identified to function in defense against the western flower thrips (*Frankliniella occidentalis*) in chrysanthemum (*Dendranthema grandiflora*) (Leiss et al. 2009). In barley (*Hordeum vulgare*), increases in ferulic acid concentration have been reported to reduce

populations of the cereal aphid (*Schizaphis graminum*) (Argandona et al. 1980; Thackaray et al. 1990; Cabrera et al. 1995).

Light intensity has been shown to have an important influence on herbivore consumption rates and overall performance through changes in leaf nitrogen and protein levels, which are essential for insect growth and development (Mole and Waterman 1988; Shure and Wilson 1993; Dudt and Shure 1994; Louda and Rodman 1996; Niesenbaum and Kluger 2006; Behmer 2009). For example, *Epimecis hortaria* larvae consumed more leaves of the spicebush (*Lindera benzoin*) under shade conditions where percent N and soluble proteins were higher and leaf toughness was lower compared to plants grown in sun-exposed conditions (Muth et al. 2008). Likewise, a reduction in herbivory by sawfly larvae (Hymenoptera: Tenthredinidae) was observed on *Artistotelia chilensis* saplings in sun-exposed conditions compared to shade conditions, which was correlated with reduced leaf thickness in the shade (Guerra et al. 2010).

Although some experiments have been conducted to understand how varying the light environment influences the phenolic and nutrient contents in plants (Ingersoll et al. 2010), little is known about the importance of these changes in plant chemicals with respect to insect herbivores (Close and McArthur 2002), including the diamondback moth (*Plutella xylostella*) (DBM). In a field study using *Arabidopsis*, Caputo et al. (2006) found that larval feeding by DBM was three times greater under shade conditions than under ambient (high intensity) light. However, increased feeding under shade conditions did not correspond with a lower total phenolic content in *Arabidopsis*. Individual phenolics were not measured in the study. These findings suggest that differences in herbivory may be linked to plant nutritional quality, or a reduction of specific phenolic compounds that contribute to plant resistance. Therefore, evaluating concentrations of individual phenolics rather than measuring total phenolics alone,

may be more important in understanding mechanisms involved in plant-insect interactions (Bryant et al. 1983; Close and McArthur 2002).

Pac choi (*Brassica rapa*) is a crop commonly grown during spring and fall in the field and in protected environments such as high tunnels. Differences in the quantity and quality of light under these diverse growing conditions, coupled with important effects from light-mediated changes in plant chemistry and the herbivores that feed on them, indicate a need to determine if changes in pac choi chemistry are likely to occur under different light intensities that may affect the risk of DBM feeding.

Due to the lack of knowledge regarding light intensity effects on plant chemistry and the corresponding effects on herbivores, experiments may show that light intensity is an important factor in plant-herbivore relationships. Therefore, the specific objectives of this research were to 1) determine if effects of light intensity directly impact pac choi chemistry, including specific phenolic compounds, and growth, and 2) examine if light intensity effects on pac choi have a corresponding impact on selected measures of DBM fitness (such as pupal and adult body weights). A third, collorary, objective was to determine if DBM larval feeding, alone, had an impact on pac choi chemistry. Therefore, effects of light intensity were compared for DBM-infested and uninfested plants. Based on the literature (Coley et al. 1985), it was predicted that higher levels of total and specific phenolic compounds will occur under high light intensities. It was also predicted that structural and chemical changes in pac choi, mediated by increased phenolic production, will lead to less herbivory by DBM. Less herbivory could be associated with changes in plant chemistry, changes in larval development, or by differences in the number of feeding larvae.

# Materials and Methods

The study was conducted in a series of four experiments, conducted twice in summer: June 28-July 27 and August 5-September 4, 2009; and twice in winter/spring: January 25-February 28 and March 5-April 10, 2010.

### *Plant material*

Pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') (Johnny's Seeds; Winslow, ME) seeds were germinated in 7 x 12 plug flats (Hummert's Int; Topeka, KS) containing a soilless growing medium, MetroMix 200 (Sungro; Alberta, Canada), which consisted of Canadian sphagnum peat moss, vermiculite, perlite, a wetting agent, and trace amounts of NPK. Two-week-old seedlings were transplanted into 16 x 19 cm containers and grown under either ambient or shade conditions (see below). Plants were watered every other day and seedlings were fertilized weekly for six weeks with a 24N:8P:16K (Miracle Grow; The Scotts Company LLC, Marysville, OH) solution.

### *Insects*

A colony of diamondback moth (*Plutella xylostella* L.) (DBM) was established at Kansas State University (Manhattan, KS) from a shipment obtained from Benson Research (Carlisle, PA), which was originally collected (in 1988) in Geneva, NY, and maintained on a wheat germ and casein-based diet. The colony was maintained on 4 to 5 potted pac choi plants in a 0.60 x 0.60 x 0.91-m frame cage covered with 1.4 mm mesh screening in a greenhouse under natural daylight and a temperature range of 20-23°C.

***Experimental design***

The experiments were conducted in a greenhouse at Kansas State University (Manhattan, KS) using pac choi plants that were 6 weeks old from the time of seedling germination. Plants had an average of 10 leaves when the experiments were initiated. The design was a completely randomized design with the main factor light treatment (ambient and shade) and a split-plot factor of herbivory treatments (DBM present or absent) within each light treatment. In each of five replicate ambient and five replicate shade plots, eight pac choi plants were infested with DBM larvae and eight plants were left uninfested. Individual light plots were cages with polyvinyl chloride (PVC) square frames (1.2 x 1.2 x 1.2 m) that were covered with either clear 3-mm polyethylene (DuraGreen; DuraGreen Marketing Inc. LLC, Mount Dora, FL) for the ambient treatments, or clear polyethylene plus two layers of 52% heavy white knit shade cloth (PAK Unlimited Inc.; www.pacunlimited.com) for the shade treatments. Each plot contained 16 plants, arranged in four rows of 4 plants. Plants in 2 of the 4 rows were randomly assigned to be infested with DBM, whereas the other two rows were left uninfested. Rows of plants did not touch to avoid insect movement between rows. Infestation was accomplished by individually transferring twenty larvae from colony plants to the same leaf in the middle whorl of the experimental plants using a fine-tipped paintbrush. Care was taken not to touch plants after artificial infestation since the larvae may drop from plants if disturbed. There were a total of 160 plants used in each experiment, where light replicates were distributed over three 3.6 x 9.1 m greenhouse benches. Spacing on bench-tops between either side of the treatment cages was 0.3 m. For each main plot, a randomly chosen set of four infested and four uninfested plants were

sampled for N, C, protein, and phenolic content. The remaining set of four infested and four uninfested plants were used to measure plant biomass, herbivory, and DBM responses.

For each experiment, light intensity and temperature were measured inside each cage using HOBO data loggers (Onset; MicroDaq, Contoocook, NH) set at 30-min recording intervals. Only the light intensity measurements taken during daylight hours were used for analysis. Light intensity measurements during the July and August experiments were taken from 6:00 am-8:00 pm, whereas measurements during the February and March experiments were taken from 8:00 am-6:00 pm. Light intensity, measured as lumens/$ft^2$, was converted to lumens/$m^2$ by multiplying values by the constant 10.76 (Mechtly 2008).

***Plant sampling, and plant and herbivore responses***

When pupation was observed on DBM-infested plants in all light plots, the first set of plants was randomly sampled for chemical analysis. The analyses included total carbon to nitrogen ratio, percentage total protein, percentage moisture content, and total phenolic content. In addition, seven specific phenolic compounds were assayed: ferulic acid, sinapic acid, chlorogenic acid, caffeic acid, leutolin-7-O-glucoside (L-7-O-G), myricetin, and querticin. These specific compounds were selected based on prior experiments with pac choi and phenolic content (M-.M. Oh, personal communication). To obtain sufficient plant material for the phenolic analyses, two leaves were excised at the top of the petiole from the middle whorl (youngest, fully-expanded leaves) of each pac choi plant. To avoid bias from diel cycling of plant nutrients and phenolics, samples were taken only between 6:00-8:00 am in all experiments (You and Yang 2001). Although greenhouse light intensities varied during the 6:00-8:00 am sampling time between the summer and winter experiments, the differences in light intensity was not expected to impact the comparison of plant responses between experiments (C. B. Rajeshaker, personal

communication). To avoid a chemical response in plant tissue due to wounding from excising leaf tissue, leaf material was immediately frozen in liquid nitrogen and stored at -20°C for approximately 1 week until used in the analyses. For moisture content and C:N ratio, two additional leaves from the middle whorl of each plant were excised, weighed, placed in #2 brown paper bags, and stored at -20°C until used in the analyses. Due to the time associated with taking samples, the remaining four infested and uninfested plants were sampled the next day for estimates of shoot biomass and percentage of leaf area removed by DBM larvae (for infested plants). Larval instar counts and body weights of DBM pupa and adults were also obtained.

***Shoot biomass and leaf area removed by DBM***

Shoot biomass from the crown up, was measured as fresh weight to the nearest 0.001 mg. To measure the leaf area removed by larval feeding (herbivory), digital photos were taken of two leaves randomly-chosen from the middle whorl (youngest, fully expanded leaves). A photo-imaging analysis program, APS Assess (APS Press; St. Paul, MN), was used to quantify total leaf area and total leaf area removed by DBM larvae on four infested plants in each replicate (Dudt and Shure, 1994; Lamari 2002).

***DBM responses***

From plants used for plant biomass, herbivory, and life stage measurements, pupae were carefully removed from the foliage and placed individually in pre-weighed glass tubes with a cotton cap. Tubes were weighed to obtain pupal weights. Pupae were then stored in a growth chamber at 22°C and a 16:8 (L:D = hrs of light:dark) photoperiod until emergence. Adults were then sexed and individually weighed. Pupal and adult body weights were measured to the nearest 0.001 mg using an electronic balance. Male DBM were identified by the distinctive diamond-shape patterning on the forewings, which is not present on females (Shirai 1993; Muhamad et al.

1994). When pupae were collected, counts of remaining larvae in each instar were recorded per plant to determine age-class distributions. Age-classes were not significantly different between light treatments within experiments (see Appendix A), but they did differ among experiments. To avoid an age-class bias when comparing levels of herbivory in relation to light intensity according to light treatment and month, total leaf tissue consumed (estimated by percent leaf area removed) was converted to per capita consumption using estimated proportions of feeding, per instar and numbers in each age class, to derive a standard unit of feeding which could be compared between light treatments and across experiments To compare herbivory on an individual basis, larval feeding equivalents were determined by dividing the percentage of total leaf area consumed by the cumulative number of relative feeding equivalents. Feeding equivalents were obtained by multiplying the number of each instar counted on plants by the estimated relative proportion of leaf tissue required for each instar to complete development. This procedure resulted in the following formula: [percent consumption per insect per plant = percent total leaf area removed / (number of $3^{rd}$ instars x 3) + (number of $4^{th}$ instars x 8) + (number of pupae x16)]. These relative instar consumption values were derived from consumption rates for *Erinnyis ello* (Lepidoptera), which has four larval instars (Pratissoli et al. 2002).

Because insect development is known to be impacted by temperature (Precht et al. 1973; Wellington et al. 1999), comparisons of DBM developmental time were made between July and August only, which had similar temperatures but different light intensities. To accommodate the mixed age-classes, analyses were not done on days-to-complete-development because this number varied by instar and also may have been biased by differences in age-class distribution between treatments. Instead, rate of development was estimated using degree-days (D-D) as a

standardized unit. The D-D used in the analysis was not related to actual greenhouse temperatures in the experiments. The number of DBM in each instar per plant per treatment was multiplied by the number of D-D needed for that life stage to complete development based on data from Ansari et al. (2010) for DBM feeding on *Brassica rapa* at 25°C. The total number of D-D was then summed and the average number D-D for the cohort was computed as follows: [average cohort D-D = (number of $2^{nd}$ instars x 200.8 D-D) + (number of $3^{rd}$ instars x 138.31 D-D) + (number of $4^{th}$ instars x 75.81 D-D) + (number of pupae x 0 D-D) / total number of all instars present]. Adult data were not included in calculations because samples were taken when pupae were present, before adult emergence occurred.

***Plant chemistry analyses***

For N and C levels, two leaves per plant were dried in a forced air oven at 68°C for 72 h and then ground in a stainless steel Wiley mill to pass through a 20 mesh screen (Scientific Apparatus; Philadelphia, PA). Total N and C (both free and structural forms) were determined from the ground tissue using a dry combustion procedure (TruSpec CN, LECO Corporation; St. Joseph, MO) conducted at the Kansas State Soil Testing Laboratory (Manhattan, KS). The percent protein values were based on multiplying percent total N by the constant 6.25 (Fujihara et al. 2001). Percent moisture content of leaf tissue was based on the difference between wet and dry weights of the leaf samples [100 - (fresh weight – dry weight)].

Total phenolic content was analyzed using the modified Folin-Ciocalteu method (Pennycooke et al. 2005; Oh 2008; M-.M. Oh, personal communication). Approximately 0.5 g of frozen leaf tissue was macerated using a mortar and pestle. To extract the phenolics, 3 mL of 80% (v/v) acetone was added to the ground tissue. Then 1 mL of mixture was poured into a 1.5 mL microcentrifuge tube, which was then covered with aluminum to exclude light and left

overnight at 5°C. Tubes were centrifuged for 2 min at 112 RCF (relative centrifugal force). Then 50 ml of supernatant was pipetted into a new 1.5 mL tube and mixed with 135 µL $H_2O$, 750 µL of 1/10 dilution Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO), and 600 µL 7.5% (w/v) $Na_2CO_3$. Samples were vortexed for 10 sec and incubated for 15 min at 45°C in a water bath. Samples were allowed to cool to room temperature before reading absorbance at 765 nm (U-1100 spectrophotometer; Hitachi Ltd. Japan) (Pennycooke et al. 2005). A gallic acid standard curve was prepared using 1 mg/mL gallic acid (Acros Organics, Belgium) in 80% (v/v) acetone from a stock solution. Total phenolics were reported as gallic acid equivalents (GAE)/g fresh weight tissue.

The extraction of individual phenolics from the pac choi leaves is described by Nicolle et al. (2004) with minor modifications (Oh 2008), involving approximately 1 g of frozen leaf tissue ground by a mortar and pestle. Phenolics were then extracted using 25 mL of 70% methanol at 80°C for 1 min in a water bath. After agitation on a shaker plate for 1 h, the solution was filtered using No.1 paper (Whatman; UK). The filtered extract (9 mL) was then evaporated to dryness by speed vacuum (Savant SVC-100H Speed Vac Concentrator; Midland, MI) under reduced pressure at 43°C and then re-suspended in 5 mL of 70% methanol. The concentrated solution was filtered through a 0.45 µm ascrodisc filter (Millex, Millipore Corporation; Bedford, MA). A 5 µL aliquot of the extract was injected into a high performance liquid chromatography (HPLC) system equipped with an autosampler (SpectraSYSTEM AS1000: Thermo Separation Products; San Jose, CA), pump (HP 1050, Hewlett Packard; Palo Alto, CA), integrator (HP 3396, Hewlett Packard; Palo Alto, CA), and UV/VIS detector (Acutect 500, Thermo Separation Products; San Jose, CA). Column separation identified the selected phenolics (chlorogenic, caffeic, sinapic and ferulic acids, luetolin-7-O-glucoside, myricetin, and quercetin) at 60°C. Constituents of the

extract were subjected to column separation with eluent A [$H_2O$:$CH_3COOH$ = 338:1 (v/v)] and eluent B [$H_2O$:$C_4H_{10}O$:$CH_3COOH$ = 330:8:1 (v/v/v)] at a flow rate of 1.8 mL/ min. The gradient started at 20% B in A, which was held for 5 min, then increased to 100% B for 20 min. After equilibration (2 min at 100% B), the composition of the solution returned to the initial condition (20% B). Peaks for each phenolic compound were quantified and identified at 330 nm by comparing them with standard compounds of chlorogenic, p-coumaric, caffeic, sinapic and ferulic acids, and quercetin-3-O-glucoside (Sigma-Aldrich; St. Louis, MO) and luteolin-7-O-glucoside (Indofine Chemical Company, Inc.; Hillsborough, NJ). High performance liquid chromatography was performed by the Ruminant Nutrition Laboratory, Kansas State University (Manhattan, KS). Concentrations of specific phenolic compounds were reported in mg/100 mL. Of the phenolics assayed, only chlorogenic, caffeic, ferulic and sinapic acids, and L-7-O-G were detected in the leaf samples.

***Statistical analysis***

All data pertaining to light intensity, temperature, plant, DBM response variables, and DBM life stage data were analyzed using SAS Systems for Windows, Version 9.1 (SAS Institute 2002). Data were subjected to a mixed model analysis of variance (ANOVA) using the PROC MIXED procedure with experiment, light, and herbivore (plants with and without DBM) as the main effects, and light replicate as the random effect. Tests for significance were conducted for all main effects and for the two- and three-way interactions. Because there was significant variation in greenhouse conditions and plant/insect responses among experiments, results for each of the four experiments were analyzed separately. ANOVA results for each experiment are presented in Appendix B. Prior to analysis the percent data were arcsin-transformed to

normalize, including moisture content, protein content, and proportion of leaf tissue consumed per insect. All data presented are non-transformed.

The LS MEANS statement (SAS Institute 2002) and Fisher's Protected LSD were used to make pair-wise treatment comparisons. To determine if light and plant, and plant and DBM variables were correlated, multiple regression analyses were performed using the PROC REG procedure (SAS Institute 2002). Prior to fitting any regression models, and to determine the model that resulted in the lowest variance and highest regression coefficient ($R^2$), a best-subsets analysis was performed using MINITAB Version 14 (Minitab Inc., State Collage, PA). Then multiple regressions were performed in a step-wise backward elimination procedure (SAS Institute 2002) in order to remove the variables with the highest $P$ values (non-significant) until only those with $P \leq 0.05$ remained in the model. Relationships identified by multiple regressions were then assessed for best fit (linear or quadratic) in MINITAB using regression with fitted line plots. Significant relationships are presented with $P$ and $F$ values, along with coefficients of determination.

The proportion of leaf tissue consumed per insect was computed per plant, and then averaged for four infested plants in each plot (replicate). Data were subjected to ANOVA using a general linear model with light and experiment as main effects, and light x experiment in the two-way interaction. Means were separated using Tukey's method (SAS Institute 2002). Correlations of light intensity and temperature with DBM body weights and herbivory within light treatments by experiment and across experiments were conducted using PROC CORR test procedures (SAS Institute 2002).

Developmental data for DBM were averaged over the four infested plants in each plot, and then subjected to ANOVA using a general linear model with light and experiment as main effects, and light by experiment in the two-way interaction. Means were separated using Tukey's test procedures (SAS Institute 2002).

## Results

### *Light intensity and temperature*

Light intensity varied significantly by light treatment (ambient vs. shade) and experiment (month) (Table 2.1). There was also a significant light treatment by experiment interaction, indicating that differences between light treatments varied among experiments (Table 2.1). When data were combined across the four greenhouse experiments, light intensity was more than two-fold greater under ambient conditions than under shade conditions (Table 2.2). Under both ambient and shade conditions, light intensity was significantly higher in August and February than in July and March (Table 2.3).

Temperature was not significantly affected by light treatment (Table 2.1). However, temperature did vary significantly by experimental month, and there was a significant treatment by experiment interaction (Table 2.1). Differences among all experiments were significant (Table 2.4), where temperatures were the highest in July, followed by August, March, and February.

***Plant responses to light intensity***

There was no significant effect of herbivory on any of the plant responses, nor were there significant interactions for any of the plant responses ($P < 0.05$). Therefore, data associated with plant responses for DBM-infested and uninfested plants were pooled.

Several plant responses were influenced by light treatment, experiment, or both (Table 2.1). The C:N ratio and percent protein content were significantly affected by both light treatment and experimental month, and there was a significant light by experiment interaction (Table 2.1). In all experiments, the C:N ratio in pac choi leaves was higher under ambient conditions compared to shade; differences were significant in all experiments except February (Table 2.6). Moreover, the regression analysis revealed that C:N ratio was significantly negatively correlated with light intensity under shade ($F = 29.42$, $df = 1$, $P = 0.0001$), and ambient conditions ($F = 8.29$, $df = 1$, $P = 0.007$) across experiments (Figure 2-1). In contrast to C:N ratios, which were higher under ambient light, percent protein was significantly higher in the shade treatment for 3 of the 4 experiments (Table 2.6). Regression analysis also revealed that percent protein content was significantly positively correlated with light intensity under shade conditions ($F = 35.15$, $df = 1$, $P = 0.0001$), and was significantly negatively correlated with light intensity under ambient conditions ($F = 13.71$, $df = 1$, $P = 0.001$) across experiments (Figure 2-2).

Total phenolic content was significantly affected by both light treatment and experiment, but the interaction was not significant (Table 2.1). Total phenolics were significantly higher under ambient conditions than shade when data were pooled across the four experiments (Table 2.5). In addition, regression analysis using pooled data across experiments, revealed that total phenolic content was significantly positively correlated with light intensity under shade

conditions ($F$ = 5.81, $df$ = 1, $P$ = 0.02), but not ambient ($F$ = 3.04, $df$ = 1, $P$ = 0.09) (Figure 2-3). Despite the significant correlation in the shade treatment, the $R^2$ value explained only 18% of the variation.

Of the four phenolic compounds assayed, sinapic and ferulic acid were significantly affected by light treatment; ferulic acid also varied significantly with experiment (Table 2.1). The concentration of sinapic acid was significantly higher under ambient light than in the shade in all experiments (Table 2.5). Ferulic acid also was significantly higher under ambient compared to shade conditions, but only for the August and February experiments which had higher light intensities than the March and July experiments (Table 2.6).

Average shoot biomass was significantly affected by light treatment and by the interaction of light by experiment (Table 2.1). In three of the four experiments, shoot biomass was slightly but significantly higher under ambient conditions than under shade conditions (Table 2.6). The mean percent moisture content was significantly higher in plants grown under shade conditions than in those grown under ambient light; however, the differences in moisture content were too small to be considered biologically relevant.

***Insect responses to plant chemistry***

All DBM responses were significantly affected by experiment, but light treatment had no effect (Table 2.1). Mean body weights, by experiment, are presented in Table 2.7. For both males and females, pupal and adult weights were significantly higher (two-fold) in March than in the other three experiment months. However, light intensity and temperature were not significantly correlated with body weights in the four experiments.

In the experiments where DBM body weights were the highest, the concentration of ferulic acid was the lowest. Although ferulic acid and DBM body weights were significantly different by experiments, regression analysis, with data averaged across experiments, showed a significant negative relationship associated with ferulic acid and adult male DBM body weights in the ambient treatments ($F = 6.60$, $df = 1$, $P = 0.02$) but not shade ($F = 0.34$, $df = 1$, $P = 0.57$) (Figure 2-4). In general, ferulic acid was higher in the ambient treatments (Table 2.6). Although light intensities were different by experiment, data averaged across experiments showed a significant positive relationship regarding concentration ferulic acid and light intensity under ambient ($F = 18.42$, $df = 1$, $P = 0.001$) but not shade treatment ($F = 0.46$, $df = 1$, $P = 0.56$) (Figure 2-5). In contrast, there was no significant relationship associated with ferulic acid concentration and adult female DBM body weights in either the ambient or shade treatments. Although sinapic acid concentrations were elevated under higher light intensities, concentrations did not relate to DBM response variables. There were no other specific phenolic compounds, or combinations of phenolic compounds that were significantly correlated with DBM fitness parameters.

To determine if ferulic acid concentration had a functional effect on male body weights, an artificial diet study using increasing concentrations of ferulic acid in diets was conducted in two experiments under controlled laboratory conditions (see Appendix C for Materials and Methods). There was a significant difference in percent survival of DBM among the ferulic acid treatment (concentration) in the first experiment ($F = 2.35$, $df = 4$, $P \leq 0.05$), and in the second experiment ($F = 10.65$, $df = 4$, $P \leq 0.001$). In the first experiment, percent survival was significantly lower in the 5 mg/g ferulic acid treatment, compared to the other ferulic acid and control treatments. In the second experiment, percent survival was lowest at 7 mg/g ferulic acid,

compared to the other ferulic acid and control treatments. However, sample sizes were small and significant differences were not consistent between the two experiments.

The percentage of leaf area removed was the direct measure of herbivory used in the analysis for treatment effects (Table 2.1). However, because of differences in DBM age-class distribution across experiments, consumption as larval feeding equivalents was computed as an unbiased measure of herbivory (refer to Materials and Methods). Analysis of the data showed that light treatment did not significantly affect larval feeding ($F = 1.06$, $df = 1$, $P = 0.30$), but experiment was significant ($F = 3.35$, $df = 3$, $P = 0.02$). Larval feeding was significantly greater in July (1.57 ± 0.78 feeding equivalents) compared to August (0.29 ± 0.06 feeding equivalents), February (0.08 ± 0.02 feeding equivalents), and March (0.01 ± 0.006 feeding equivalents). Light intensity and temperature were not significantly correlated with DBM larval feeding across experiments ($P = 0.52$, $r = -0.47$ and $P = 0.38$, $r = 0.61$, respectively).

A comparison of larval development (based on cumulative D-D) between July and August, which had similar temperatures but different light intensities, revealed that the rate of development was significantly faster ($F = 23.88$, $df = 1$, $P < 0.0001$) in August (30.02 ± 4.67 D-D) under high light intensities than in July (54.97 ± 4.91 D-D) which had low light intensities.

## Discussion

This study is the first to demonstrate that variations in light intensity may influence pac choi-DBM interactions through changes in plant chemistry. The greenhouse experiments were conducted under natural light conditions, resulting in variation by experiment and within light

treatments within an experiment. During the winter months, greenhouse irradiance is typically one third of that in summer months at latitudes near 40°N (Manhattan, KS: 39°N) due to low sun angles and shorter day-lengths (Aldrich and Bartok 1994). In this study, light intensities measured during the July experiment were relatively low compared to the other three months. This may be attributed to extensive cloud cover for July (wdl.agron.ksu.edu/monthly), which can reduce light intensity by 90% (Smith 1982).

Pac choi plants exposed to high light intensities generally had elevated levels of total phenolic content, as well as high concentrations of ferulic and sinapic acids. The increased levels of phenolics under higher light intensities may be a result of increased carbon (C) which occurred in this study, and is supported by the resource availability hypothesis (Coley et al. 1985). These findings are consistent with other studies in which high light intensity was correlated with enhanced levels of total phenolics (Dudt and Shure 1994; Ingersoll et al. 2010), and high levels of ferulic acid and sinapic acids (Li et al. 1993). High levels of phenolics in plant tissues may represent a defense against multiple types of stresses (Dixon and Paiva 1995; Close and McArthur 2002; Schijlen et al. 2004; Oh and Rajeshaker 2009). For example, elevated concentrations of ferulic acid under high light intensities have been shown to be functionally-linked to lignin in cell walls, resulting in increased leaf thickness (McKey 1979; Lewis and Sarkanen 1998). Furthermore, thicker leaves may protect plants from depleting C reserves when light radiation exceeds plant photosynthetic capacity (Coley et al. 1985; Herms and Mattson 1992; Lavola et al. 1998; Izaguirre et al. 2007). In addition to their role in mitigating photodamage, light-associated increases in plant phenolic content may negatively affect growth, development, and consumption rates of certain herbivores (Mole and Waterman 1988; Yamasaki and Kikuzawa 2003; Foggo et al. 2007).

In addition to induction of phenolics by light (bottom-up), the accumulation of phenolic content in plants may be induced by herbivore feeding (top-down) (Karban and Myers 1989; Smith 2005). As such, this could have been a confounding factor with respect to the effects of light intensity on phenolic production in pac choi. However, a comparison of the phenolic content in plants, with and without DBM larvae, revealed that none of the phenolic acids tested differed between the two herbivore treatments. In contrast, other studies have demonstrated changes in phenolics due to DBM larval feeding. For example, Caputo et al. (2006) and Ehlting et al. (2008) demonstrated that elevated total phenolic contents in *Arabidopsis* were induced by DBM larval feeding. In another study, Widarto et al. (2006) reported that genes associated with biosynthesis of phenolics were induced in *Brassica rapa* leaves exposed to feeding by DBM larvae.

There may be several explanations for the similarities in phenolic content of pac choi between infested and uninfested plants, which differs from other studies with DBM. First, the method used for analyzing phenolic content may not have accurately measured levels of phenolics in pac choi leaves. The Folin-Ciocalteau method is appropriate for determining phenolic activity in plants but is limited in assessing the quantity of total phenolics because the presence of alkaloids and proteins in plant tissue can interfere with absorbance readings (Appel 1993). Second, leaf phenolics were measured from the middle whorl to avoid leaf age bias; however, it is not known if DBM adjusted choice in feeding sites based on variations in leaf phenolics. In fact, DBM larvae tend to aggregate and feed in the inner whorl of *Brassica* plants where changes in phenolics may have occurred (Ooi 1979), but leaves of the inner whorl were not sampled in the current study. Third, results may also have been associated with sampling time for phenolic content, where tissue samples were taken between 6:00-8:00 am to avoid

fluctuations caused by diel cycling of phenolics. The sampling time was chosen based on findings from You and Yang (2001), which determined that a representative baseline for phenolic content in *Brassica chinensis* can be detected in plants around 8:00 am, which then fluctuates during the course of a day. It is possible that DBM larvae actively fed during day, and may have induced production of phenolics higher than those detected from samples taken during morning hours. Fourth, Widarto et al. (2006) detected induction of phenolic biosynthesis-related genes, which were measured in plant tissue that was exposed to DBM larvae for less than 48 hrs. In the current study, phenolic content was measured in plant tissue after pac choi had been exposed to larvae for over 7 days. Plants may have responded to herbviory by producing phenolics only within 48 hrs of exposure, which may have prevented detection. Finally, the absence of any phenolic response to DBM feeding may be associated with close spatial proximity of infested and uninfested plants. Volatile signals emitted in response to herbivore feeding may include methyl jasmonate, methyl salicylate, and ethylene (Farmer 2001), which may have dispersed between rows of infested and uninfested plants. This may have resulted in similarities in phenolic content between adjacent plants. In fact, plant volatiles emitted by larval feeding have been shown to stimulate the phenylpropanoid pathway in nearby uninfested plants (Karban and Baldwin 1997; Gordon-Weeks and Pickett 2009). Although volatile profiles for pac choi are unknown, ferulic and sinapic acid esters present in pac choi have been identified as volatile signal components in many other plant species (Lewis and Sarkanen 1998).

The C:N ratios were significantly higher in plants under high light intensities compared to plants exposed to low light intensity, which substantiates other studies where a higher C content was associated with exposure to high light (Aldrich and Bartok 1994; Koricheva et al. 1998; Henriksson et al. 2003). In the current study under high light intensities, plants with

greater C:N ratios also tended to have a lower protein content compared to plants grown under shade. Under low light intensity, the C:N ratio was lower than under high light intensity, which suggests a higher N content for production of proteins under low light intensity. A higher N content under low light may be associated with elevated levels of protein detected in plants under shade. Plants with higher phenolic contents in high light intensity tended to contain lower protein levels. This trend is further supported by the protein competition model, where protein and phenolic synthesis compete for the same biosynthetic precursor compound, phenylalanine, indicating that production of proteins and phenolics are inversely correlated (Jones and Hartley 1999).

It would be expected that if high light intensity increased phenolic plant defenses against DBM, then DBM fitness traits would be less affected under low light conditions. Decreases in plant protein associated with high light intensities may reduce the nutritional quality of plants for insect herbivores (Mattson 1980). These effects may be compounded by increases in phenolics in high light (Felton 1996). For example, herbivores feeding on plants deficient in protein, and also with a higher phenolic content, may need to consume more plant tissue to compensate for the combined negative effects associated with reduced protein quantity and quality (Lawton and McNeill 1979; Isman and Duffey 1982; Felton and Duffey 1991. However, increased total herbivory under low light has also been demonstrated. For example, Manuwoto and Scriber (1985) found that low light intensity resulted in increased per capita consumption rates by the European corn borer, *Ostrinia nubilalis*, on field corn. It was concluded that increased feeding was more likely mediated by higher leaf N concentrations than by lower concentrations of defensive compounds. In another study, Muth et al. (2008) reported that leaves of *Lindera benzoin* grown in shade contained higher amounts of protein and less C compared to plants

exposed to sun; they were also associated with higher amounts of herbivory from caterpillars of *Epimecis hortaria*. In the current study, larval consumption by DBM was not reduced under high light (plants having less protein and higher a phenolic content), compared to plants under low light (plants having higher protein and lower phenolic content) in any of the experiments. Furthermore, DBM development was significantly faster under higher light intensity associated with the August experiment compared to the lower light intensity experienced in the July experiment. This suggests that high light intensity either had no adverse effect on DBM or potentially even improved the quality of pac choi as a food source. It is possible that combinations of phenolic compounds that increased under high light had a positive effect on DBM development. For example, certain phenolic compounds, such as chlorogenic acid, have been shown to increase the fitness of the tobacco budworm *Heliothis virescens* by acting as an antioxidant in the midgut (Johnson and Felton 2001).

For DBM, it is unclear whether light-mediated changes in phenolics affected DBM body weights. Across experiments, male DBM body weights were negatively correlated with concentrations of ferulic acid, which increased under high light intensities. Together, these data suggest that light intensity-induced increases in ferulic acid in pac choi may have adversely affected male body weights. However, ferulic acid represents only a small fraction of the total phenolic content in plants, but it has been identified as being involved in resistance against herbivores (Argandona et al. 1980; Thackaray et al. 1990; Garcia-Conesa et al. 1999; Husken et al. 2005; Milkowski and Strack 2010). Interestingly, results of an artificial diet study in which only the concentration of ferulic acid varied showed no dose-dependent effect on DBM weight, development, or survival. Thus, if ferulic acid is functionally related to male DBM body weight, the differences observed between the artificial diet study and the greenhouse experiment may be

associated with interactions between ferulic acid and plant enzymes and/or proteins within the plant, indicating that interactions between phenolics and plant proteins may have contributed directly or indirectly to the observed effect on male body weight (Stamp and Osier 1998; Garcia-Conesa et al. 1999; Miles 1999).

Adding to the complexity, certain enzymes, such as polyphenoloxidase, mediate oxidation of ferulic acid esters to quinones, which exert toxic effects on the insect midgut (Ni et al. 2001). Negative effects on insect midgut from exposure to polyphenoloxidase, in combination with phenolic compounds, have been shown to occur in the cereal aphid (*Rhopalosiphum padi*) feeding on wheat (*Triticum aestivum*), and tobacco hornworm (*Manduca sexta*) feeding on tobacco (*Nicotiana attenuata*) (Havlickova et al. 1996; Havlickova et al. 1998; Haltischke et al. 2001). Furthermore, exogenous application of ferulic acid to wheat plants experienced less feeding by the grain aphid (*Sitobion avenae*) (Leszczynski et al. 1995). An ester of ferulic acid present in cell walls, feruloyl quinic acid, has been identified as a defense compound in corn (*Zea mays*), which was correlated with reduced feeding and lower larval body weights of the corn borer, *Sesamia nanagriodes* (Santiago et al. 2006). In the current study, larval consumption was significantly higher in July when concentrations of ferulic acid in pac choi were lowest in both light treatments.

Although ferulic acid may be associated with male DBM adult body weights, there was no correlation between ferulic acid and female pupal or adult body weights. Therefore, it is unclear why the effect of ferulic acid was specific to males in the greenhouse experiments; however, physiological and behavioral differences between males and females may be responsible. For example, female Lepidoptera generally are heavier than males throughout their life cycle, and may have variable nutritional needs due to differences in their reproductive

physiology (Slansky and Scriber 1985; Zeng et al. 1997). For DBM to acquire the necessary resources for egg production, females must obtain and then retain larger amounts of stored proteins during larval feeding than males (Sarfraz et al. 2011). It is possible that with greater nutritional demands, female DBM should have experienced body weight changes at least equal to males unless they engaged in compensatory feeding to overcome effects of ferulic acid. Unfortunately, no data are available to compare larval consumption between male and female DBM in this study. Regardless, male body weights are typically correlated with migration and mate-finding (Shirai 1993; Muhamad et al. 1994). This suggests that high light intensities may negatively impact population growth of DBM by reducing male weights, thus reducing feeding damage on pac choi. Future studies should examine further the possibility of fitness costs to DBM on plants grown under different light intensities by considering other important fitness traits, including development, survival, sex ratio, and reproduction or oviposition.

As in many studies, all factors could not be included in the current study. With respect to pac choi responses and relationships with DBM, future studies might include measuring photosynthetically active radiation (PAR), which is used to determine chlorophyll levels, which may be more appropriate to assess plant responses to light (Oh 2008). Likewise, in terms of host plant quality for DBM, it may be important to determine amino acid levels in plant tissue because these are likely associated with larval development (Mattson 1980). Furthermore, regression models showing the important relationships associated with the data sets resulted in low coefficients of determination ($R^2$), 42% for male DBM body weights and ferulic acid (Figure 2-4), and 35% for ferulic acid and light intensity (Figure 2-5). Low coefficient values suggest that there may be other plant components present that were important regarding relationships with DBM. For example, it may have been useful to know if leaf thickness or surface waxes

changed, thus affecting DBM feeding under the different light treatments. Guerra et al. (2010) found that *Aristotelia chilensis* had thicker leaves and less damage from DBM larval feeding when grown under high light intensities compared to plants grown under low light intensities. Moreover, it has been demonstrated that high levels of phenolic compounds can mediate increases in leaf thickness.

Another limitation of the current study was not including glucosinolates in the analysis of pac choi chemistry, which was due to constraints in the amount of plant tissue needed for another chemical analysis. Glucosinolates are N- and sulfur-containing compounds that are prevalent in *Brassica* spp., and may provide chemical defenses against generalist, as well as specialist herbivores (Halkier and Gershenzon 2006). However, low concentrations of glucosinolates have been shown to actually stimulate feeding of DBM larvae (Louda and Mole 1992; Louda and Rodman 1996). Thus, any differences in N content under ambient light compared to shade may have altered pac choi glucosinolate levels, differentially affecting male and female larval feeding behavior.

This study was conducted using DBM larvae maintained in a colony originally obtained from Benson Research (Carlisle, PA) in 2009. The commercial supplier's colony originated from a population collected in Geneva, NY in 1988, and was maintained on a wheat germ and casein-based diet. The long-term effects of continuous rearing of DBM are unknown and may have resulted in a lowering of the colony fitness. Studies using wild populations of DBM may be warranted to determine if similar results would be obtained.

In summary, both pac choi and DBM exhibited responses that varied with changes in light intensity and experiment. Light intensity appears to have altered phenolic and protein contents, both in the presence and absence of DBM. Evidence associated with the impact of light

intensity on DBM is limited, but it appears that light intensity may mediate changes in pac choi phenolic content, specifically ferulic acid, which may indirectly influence male DBM body weights. Identifying ferulic acid as a potential defense compound against DBM helps focus research efforts concerning host plant resistance against DBM. In general, results from the current study supported the findings of other investigators, which suggest that light intensity is an important environmental factor that may alter both primary and secondary plant metabolism with the potential to impact herbivores (Muth et al. 2008; Ingersoll et al. 2010).

There are also some practical implications of this study associated with the production of pac choi. For example, seasonal differences in light intensity appear to be important with respect to changes in plant chemistry. Because pac choi typically has two cropping cycles (spring and fall in temperate regions) and is grown in environments that vary in light intensity, such as open field, high tunnels, and greenhouses, producers should be cognizant of the fact that changes in plants may affect crop nutrient content. As such, pac choi crops grown in protected environments may be subject to more damage from insects if leaves contain higher protein contents, leading to extensive feeding damage (Mattson 1980). A reduction in plant resistance chemicals in some growing environments (i.e., low light) may increase the crop risk to herbivory. However, it is unclear whether different plant production environments enhance protection against insect herbivores or increase suceptibility. Light intensities in the greenhouse are generally much lower than field conditions; therefore, it is difficult to extrapolate the results from the current study to other crop production settings (for example, see Appendix D for results from a field and high tunnel study in which light intensity data were collected). However, differences in crop risk may be more likely when comparisons are made of pac choi grown in high tunnels or greenhouses versus an open field environment due to the substantial differences in light intensity.

# Tables and Figures

**Table 2.1 Analysis of variance (ANOVA) values for light intensity, temperature, *Brassica rapa* and *Plutella xylostella* response variables, by experiment, light treatment, light treatment replicate, and the two-way interaction between experiment and light treatment. Values in bold are significant ($P \leq 0.05$ using Fisher's Protected LSD).**

| | *Experiment*[1] | | | | *Light Treatment*[2] | | | | *Light Replicate*[3] | | | | *Experiment x Light Treatment*[4] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Environmental Variables*** | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* |
| Light intensity (lumens/$m^2$) | 40 | 3 | **65.47** | **<0.0001** | 20 | 1 | **478.77** | **<0.0001** | 20 | 4 | 0.51 | 0.73 | 80 | 3 | **11.98** | **<0.0001** |
| Temperature (°C) | 40 | 3 | **924.54** | **<0.01** | 20 | 1 | 6.69 | 0.06 | 20 | 4 | 0.60 | 0.68 | 80 | 3 | **3.48** | **<0.001** |
| ***Plant Response Variables*** | | | | | | | | | | | | | | | | |
| % C:N ratio | 80 | 3 | **23.17** | **<0.001** | 40 | 1 | **45.00** | **<0.01** | 20 | 4 | 0.48 | 0.75 | 80 | 3 | **7.45** | **<0.01** |
| % Protein | 80 | 3 | **11.79** | **<0.001** | 40 | 1 | **19.02** | **0.01** | 20 | 4 | 0.77 | 0.59 | 80 | 3 | **4.26** | **0.008** |
| Total phenolic content[5] | 80 | 3 | **53.14** | **<0.001** | 40 | 1 | **11.25** | **<0.01** | 20 | 4 | 0.26 | 0.90 | 80 | 3 | 0.93 | 0.430 |
| Chlorogenic acid[6] | 80 | 3 | 1.64 | 0.190 | 40 | 1 | 4.20 | 0.10 | 20 | 4 | 0.63 | 0.66 | 80 | 3 | 0.45 | 0.710 |
| Caffeic acid[6] | 80 | 3 | **3.17** | **0.030** | 40 | 1 | 0.06 | 0.82 | 20 | 4 | 0.85 | 0.56 | 80 | 3 | **2.85** | **0.040** |
| Sinapic acid[6] | 80 | 3 | 1.19 | 0.320 | 40 | 1 | **18.14** | **<0.01** | 20 | 4 | 1.19 | 0.32 | 80 | 3 | 0.91 | 0.440 |
| Ferulic acid[6] | 80 | 3 | **9.02** | **<0.01** | 40 | 1 | **10.95** | **<0.01** | 20 | 4 | 1.70 | 0.15 | 80 | 3 | **4.52** | **0.007** |
| L-7-O-G[6] | 80 | 3 | **2.97** | **0.040** | 40 | 1 | 0.14 | 0.70 | 20 | 4 | 0.87 | 0.49 | 80 | 3 | **3.80** | **0.010** |
| % Moisture content | 80 | 3 | 0.37 | 0.770 | 40 | 1 | **5.41** | **0.02** | 20 | 4 | **2.46** | **0.05** | 80 | 3 | 1.71 | 0.170 |
| Shoot biomass (g) | 80 | 3 | 2.33 | 0.080 | 40 | 1 | **41.99** | **<0.01** | 20 | 4 | 0.99 | 0.42 | 80 | 3 | **6.38** | **<0.01** |
| ***Insect Response Variables*** | | | | | | | | | | | | | | | | |
| Female adult weight (mg) | 40 | 3 | **5.17** | **0.007** | 40 | 1 | 0.33 | 0.57 | 20 | 4 | 0.93 | 0.46 | 80 | 3 | 0.63 | 0.600 |
| Male adult weight (mg) | 40 | 3 | **19.42** | **<0.01** | 40 | 1 | 3.01 | 0.09 | 20 | 4 | 0.77 | 0.55 | 80 | 3 | 0.59 | 0.620 |
| Female pupal weight (mg) | 40 | 3 | **6.54** | **0.002** | 40 | 1 | 0.66 | 0.42 | 20 | 4 | 1.55 | 0.21 | 80 | 3 | 0.44 | 0.720 |
| Male pupal weight (mg) | 40 | 3 | **4.55** | **0.010** | 40 | 1 | 0.01 | 0.92 | 20 | 4 | 1.19 | 0.33 | 80 | 3 | 1.47 | 0.240 |
| % Leaf Area Removed | 40 | 3 | **24.61** | **<0.0001** | 40 | 1 | 2.20 | 0.15 | 20 | 4 | 1.31 | 0.29 | 80 | 3 | 0.41 | 0.740 |

[1] July, August, February, and March experiments. Environmental variables correspond to 10 light cages x 4 experiments, N = 40. Plant response variables correspond to 10 light cages with DBM and 10 light cages without DBM x 4 experiments, N = 80. Insect response variables correspond to 10 light cages with DBM x 4 experiments, N = 40.
[2] Ambient and shade treatment. Environmental variables correspond to 5 ambient or 5 shade cages x 4 experiments, N = 20. Plant and insect responses correspond to10 light cages x 4 experiments, N = 40.
[3] Five light cages per light treatment. Environment, plant, and insect variables correspond to 5 ambient or 5 shade cages x 4 experiments, N = 20.
[4] Plant response variables correspond to 10 light cages with DBM x 4 experiments, N = 80.
[5] Total phenolic content is reported as gallic acid equivalents/g fresh weight of plant tissue.
[6] Concentrations of specific phenolic compounds are reported as mg/ 100 mL of methanolic extract.

**Table 2.2. Mean (± SE) light intensity and temperature inside light treatment cages (ambient and shade) by light treatment, pooled over the four experiments (July, August, February, and March).**

| | Light Intensity (lumens/$m^2$)[1] |
|---|---|
| Ambient | 15067 ± 280 a[2] |
| Shade | 6452 ± 276 b |
| | **Temperature (°C)** |
| Ambient | 23.3 ± 0.27 a |
| Shade | 22.3 ± 0.27 a |

[1] N = 20, 5 light cages per light treatment x 4 experiments.
[2] Means followed by a common letter are not significantly different at $P \leq 0.05$ (Fisher's protected LSD test).

**Table 2.3. Mean (± SE) light intensity inside light treatment cages (ambient and shade), with data pooled for the four experiments (July, August, February, and March).**

| | Light Intensity (lumens/$m^2$)[1] | |
|---|---|---|
| | **Ambient** | **Shade** |
| July | 11351 ± 1926 Aa[2,3] | 4659 ± 1431 Ba |
| August | 19755 ± 2130 Ab | 8306 ± 1032 Bb |
| February | 17969 ± 2087 Ac | 7682 ± 807 Bb |
| March | 11136 ± 1829 Aa | 5143 ± 796 Bc |

[1] N = 5.
[2] Means followed by a common upper case common letter within a row are not significantly different at $P \leq 0.05$ (Fisher's protected LSD test).
[3] Means followed by a common lower case letter within a column are not significantly different at $P \leq 0.05$ (Fisher's protected LSD test).

**Table 2.4. Mean (± SE) temperature inside greenhouse cages by experiment (July, August, February, and March), with pooled data for ambient and shade treatments.**

| | Temperature (°C)[1] |
|---|---|
| July | 26.34 ± 0.22 a[2] |
| August | 25.59 ± 0.23 b |
| February | 16.64 ± 0.22 d |
| March | 22.43 ± 0.22 c |

[1] N = 10, 5 ambient plus 5 shade cages. Temperature is based on an average of measurements taken at 30-min intervals using HOBO data loggers for the duration of experiments.
[2] Means followed by a common letter are not significantly different at $P \leq 0.05$ (Fisher's protected LSD test).

**Table 2.5. Mean (± SE) concentrations for total phenolics and sinapic acid in *Brassica rapa* by ambient and shade treatment, pooled across the four experiments (July, August, February, March).**

| *Plant Response Variables*[1] | *Ambient* | *Shade* |
|---|---|---|
| Total phenolic content[3] | 0.62 ± 0.01 A[2] | 0.54 ± 0.01 B |
| Sinapic acid[4] | 7.74 ± 0.72 A | 3.14 ± 0.82 B |

[1] For each response N = 10, 5 replicates with and without *Plutella xylostella* combined.
[2] Means followed by common upper case letter within a row indicate a significant difference between ambient and shade means. Significance was determined at $P \leq 0.05$ using Fisher's Protected LSD.
[3] Total phenolic content is reported as gallic acid equivalents/g fresh weight of plant tissue.
[4] Concentration of sinapic acid is reported as mg/100 mL of methanolic extract.

**Table 2.6. Mean (± SE) *Brassica rapa* responses to differences in ambient and shade treatment by experiment (July, August, February, and March).**

| | *July* | | *August* | | *February* | | *March* | |
|---|---|---|---|---|---|---|---|---|
| ***Plant Response Variables*** [1] | **Ambient** | **Shade** | **Ambient** | **Shade** | **Ambient** | **Shade** | **Ambient** | **Shade** |
| Total % C:N ratio | 11.90 ± 0.37 Aa[2,3] | 8.00 ± 0.16 Ba | 8.36 ± 0.07 Ab | 6.60 ± 0.11 Bb | 7.97 ± 0.96 Ab | 6.96 ± 0.19 Ab | 8.29 ± 0.36 Ab | 7.23 ± 0.33 Bab |
| Total % protein | 21 ± 0.5 Bb | 32 ± 0.4 Ab | 33 ± 0.4 Ba | 41 ± 0.8 Aa | 30 ± 0.7 Ba | 37 ± 1.2 Aa | 36 ± 4.8 Aa | 34 ± 0.6 Ab |
| Caffeic acid[4] | 1.73 ± 0.73 Bb | 4.11 ± 1.0 Aa | 3.89 ± 1.1 Aa | 2.11 ± 0.41 Aab | 1.01 ± 0.21 Ab | 2.25 ± 1.28 Aab | 1.43 ± 0.32 Ab | 0.85 ± 0.09 Ab |
| Ferulic acid[4] | 1.89 ± 0.44 Ab | 2.11 ± 0.39 Aa | 4.63 ± 0.81 Aa | 1.67 ± 0.45 Bab | 4.47 ± 1.21 Aa | 1.63 ± 0.55 Bab | 0.83 ± 0.18 Ab | 0.45 ± 0.09 Ab |
| L-7-O-G[4] | 0.93 ± 0.27 Bb | 1.43 ± 0.22 Aa | 1.61 ± 0.18 Aa | 0.90 ± 0.34 Bbc | 0.80 ± 0.31 Ab | 0.91 ± 0.37 Aabc | 0.77 ± 0.07 Ab | 0.70 ± 0.08 Ac |
| Shoot biomass (g) | 120.4 ± 4.45 Ab | 107.9 ± 3.60 Aa | 143.4 ± 4.7 Aab | 105.9 ± 6.76 Ba | 151.6 ± 29.5 Aa | 122.6 ± 20.1 Ba | 157.4 ± 12.5 Aa | 71.0 ± 7.6 Bb |

[1] For each response N = 10, 5 cages per light treatment, plants with and without *Plutella xylostella* combined.
[2] Upper case letters indicate a significant difference between ambient and shade treatments, within rows, within months. Significance was determined at $P \leq 0.05$ using Fisher's Protected LSD.
[3] Lower case letters within rows indicate significant differences within each light treatment, pooled for experiments. Significance was determined at $P \leq 0.05$ using Fisher's Protected LSD.
[4] Concentrations of specific phenolic compounds are reported as mg/100 mL of methanolic extract.

**Table 2.7. Mean (± SE) *Plutella xylostella* (diamondback moth, DBM) female and male body weights, pooled across ambient and shade treatments, by experiment (July, August, February, and March).**

| *Insect Body Weights (mg)* | *July* | | *August* | | *February* | | *March* | |
|---|---|---|---|---|---|---|---|---|
| Female pupal weight | 7.23 ± 0.60 b[2] | 75[1] | 6.63 ± 0.18 b | 74 | 6.30 ± 1.63 b | 55 | 14.40 ± 2.24 a | 25 |
| Male pupal weight | 5.62 ± 0.28 b | 77 | 4.54 ± 0.20 b | 95 | 6.21 ± 1.50 b | 58 | 9.50 ± 1.44 a | 31 |
| Female adult weight | 4.73 ± 0.36 b | 73 | 2.47 ± 0.14 b | 70 | 4.72 ± 1.15 b | 51 | 9.87 ± 2.04 a | 23 |
| Male adult weight | 2.54 ± 0.24 b | 72 | 0.90 ± 0.05 b | 86 | 2.79 ± 0.81 b | 57 | 7.97 ± 1.14 a | 28 |

[1] N represents the average number of DBM used to determine mean body weights from 4 infested plants in each light cage, across light treatments.
[2] Means followed by common lower case letter within a row indicate a significant difference between experiments. Significance was determined at $P \leq 0.05$ using Fisher's Protected LSD.

**Table 2.8. Mean (± SE) percent survival of *Plutella xylostella* in artificial diet study, by experiment.**

| *Ferulic Acid Concentration (g/mL)* [1] | *Experiment 1* | | | *Experiment 2* | | |
|---|---|---|---|---|---|---|
| 0 | 100 | A[3] | 15 [2] | 55 | A | 11 |
| 1 | 86 | A | 13 | 60 | A | 12 |
| 3 | 100 | A | 15 | 65 | A | 13 |
| 5 | 40 | B | 6 | 65 | A | 13 |
| 7 | 93 | A | 14 | 25 | B | 5 |

[1] Diet treatments are presented as mg ferulic acid/g of prepared diet (refer to Appendix C for Materials and Methods).
[2] N = surviving number of larvae remaining at the end of each experiment that begin with 15 larvae per treatment in the first experiment and 20 larvae per treatment in the second experiment.
[3] Means followed by the same letter within a column are not significantly different at $P \leq 0.05$.

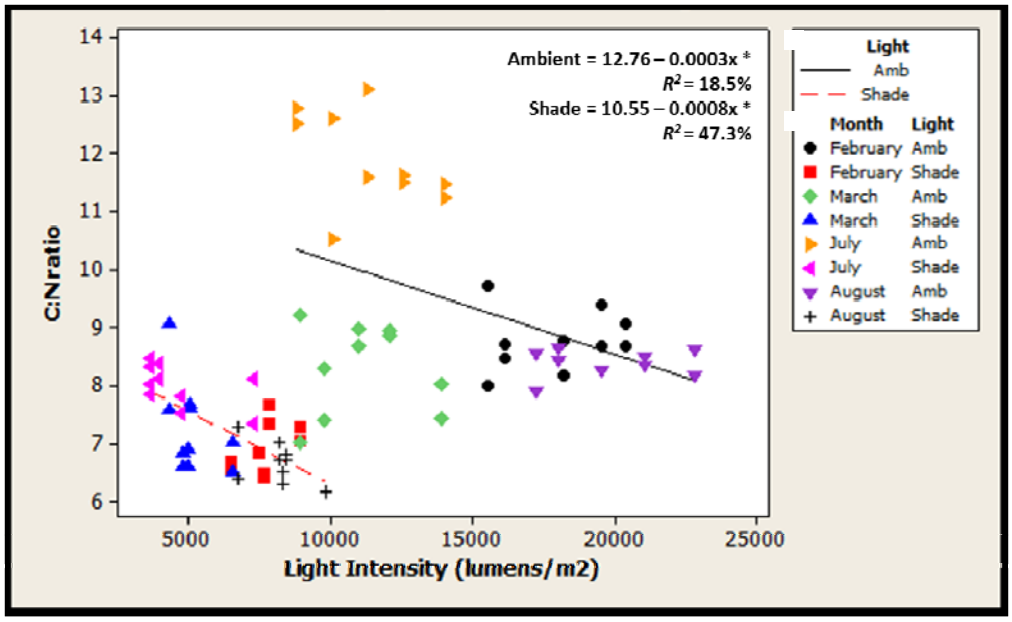


**Figure 2-1. Relationship of light intensity associated with ambient (amb) and shade treatments (N = 40) and carbon:nitrogen (C:N) ratios in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Equations for ambient and shade were significant at $P \leq 0.05$.**

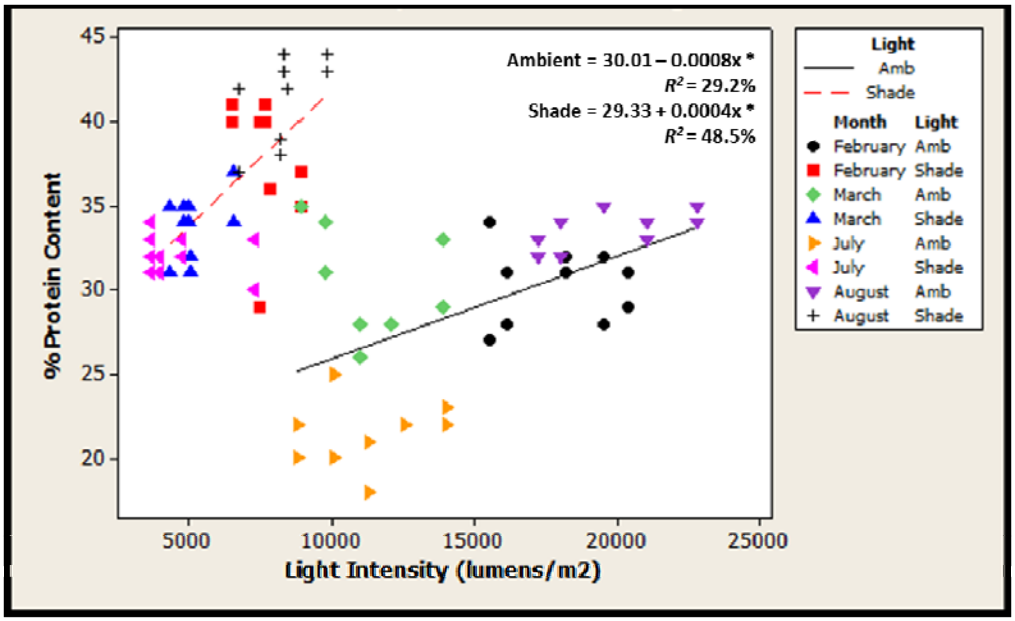


**Figure 2-2. Relationship of light intensity associated with ambient (amb) and shade treatments (N = 40) and protein content in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Equations for ambient and shade were significant at $P \leq 0.05$.**

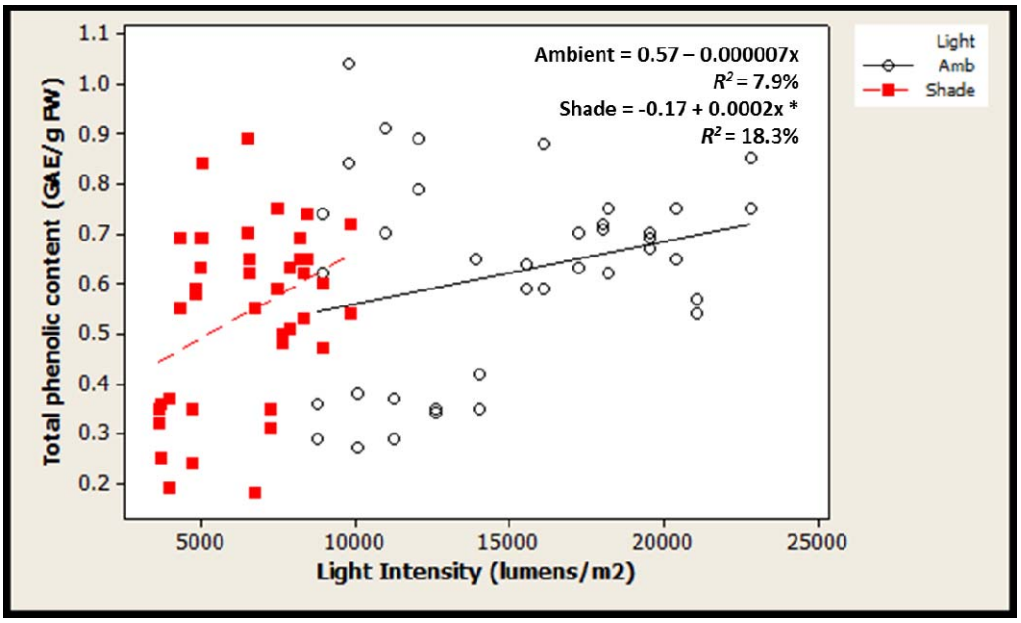


**Figure 2-3. Relationship of light intensity associated with ambient (amb) and shade treatments (N = 40) and total phenolic content in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Starred equations are significant at $P \leq 0.05$.**

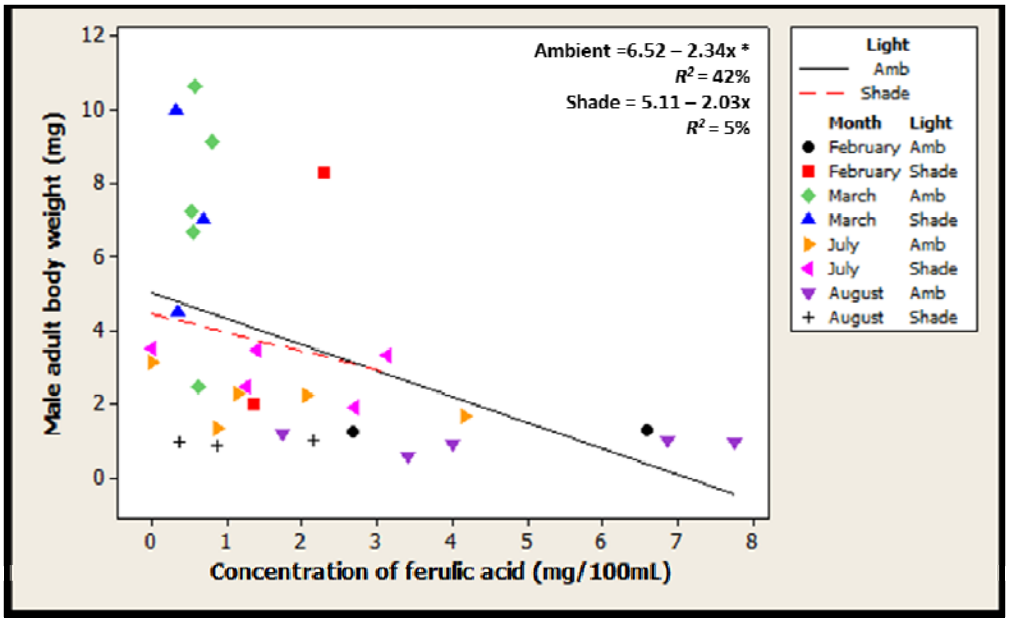


**Figure 2-4. Relationship of ambient (N = 17 plants with male data) and shade (N = 13 plants with male data) treatments between male adult *Plutella xylostella* body weight and concentration of ferulic acid in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Starred equations are significant at $P \leq 0.05$.**

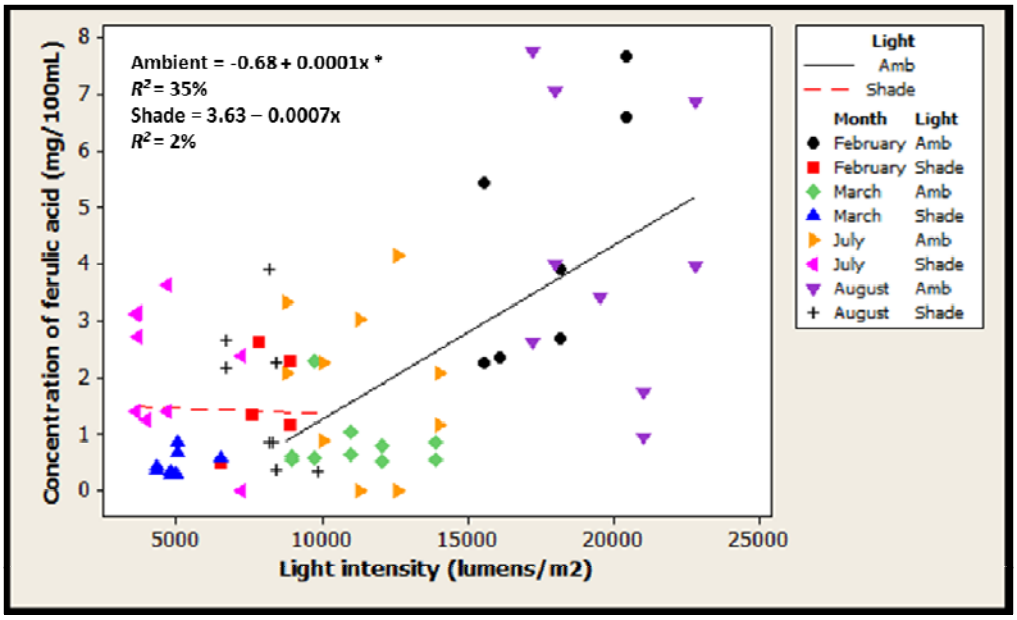


**Figure 2-5. Relationship of ambient (amb) and shade light intensity and concentration of ferulic acid in *Brassica rapa* pooled for the four experiments. The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination ($R^2$). Starred equations are significant at $P \leq 0.05$. N = 40 plants used in analysis.**

# Chapter 3 - Effects of nitrogen source on pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') chemistry and interactions with the diamondback moth (*Plutella xylostella*)

## Introduction

Nitrogen (N) is one of the most important nutrients that crops require for growth and development, where N is used to produce proteins and amino acids in plant tissue (Raven et al. 1976; Jones 1998). To ensure that adequate supplies of N are present, supplements are provided through fertilization. However, because the form of N differs with the type of fertilizer used, its availability to crops varies. Organic fertilizers, which are derived from natural sources, some of which are approved for use in certified organic production systems, typically release N more slowly than conventional fertilizers because the N is organically-bound and requires mineralization (conversion to a mineral) before plant uptake can occur. However, synthetically-derived fertilizers (e.g., sodium nitrates, ammonium sulphate, ammonium salts) provide N in forms such as nitrate ($NO_3^-$ - N) and ammonium ($NH_4^+$ - N), which are readily available for uptake by plant roots (Mengel 1992). The apparent benefit to plants of rapid uptake of N may be offset due to a decreased soil buffering capacity, which is the ability to cope with additional hydrogen ions without a change in pH (Phelan et al. 1995; Phelan et al. 1996; Phelan 2004). For example, applying inorganic sources of N may influence uptake of other soil nutrients such as calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), and potassium ($K^+$) (Larsson et al. 1992). More specifically,

plants receiving inorganic $NO_3^-$ - N may experience greater uptake of $Ca^{2+}$ and $Mg^{2+}$, whereas plants provided with inorganic $NH_4^+$ - N tend to experience greater uptake of $K^+$ (Mengel and Kirkby 1987). Furthermore, applications of inorganic $NH_4^+$ -based fertilizers may reduce water and $Ca^{2+}$ uptake, reduce carbon contents in plant tissue, and potentially decrease plant growth (Jones 1998).

The availability of N supplied by fertilizers is known to influence plant composition (Penuelas and Estiarte 1997). For example, increasing the availability of soil N to plants increases the ratio of nitrogen to carbon in plant tissue, which may increase production of protein and N-based defensive compounds in plant tissues (Folgarait and Davidson 1995). However, decreasing soil N may limit protein production in plants and stimulate the production of carbon (C)-based defensive compounds (Penuelas and Estiarte 1997). Thus, fertility practices can mediate a plant's ability to counteract biotic and abiotic stresses in the environment (Inbar et al. 2001; Le Bot et al. 2009). A major plant stress factor is attack by insect herbivores.

Fertility practices that alter plant chemistry have been shown to impact plant-insect interactions (Altieri et al. 2005; Letourneau and van Bruggen 2006). Specifically, effects on herbivory due to fertilization are associated with plant nutritional quality and the production of plant chemical defenses (Rosenthal and Janzen 1979; Scriber and Slansky 1981; van Bruggen 1995; Behmer et al. 2002; Lee et al. 2003; Behmer 2009; Leiss et al. 2009). In organic production systems, soil fertility practices have been shown experimentally to enhance plant resistance against insects, potentially by enhancing C-based defenses and limiting levels of N in tissues (Howard 1947; Eigenbrode and Pimental 1988; Luna 1988; Phelan et al. 1995; Geisler 1998; Brandt and Molgaard 2001; Parrot and Marsden 2002). For example, caterpillar populations (*Pieris rapae, Plutella xylostella,* and *Diacrisia virginica*) were lower on plants

(*Brassica oleracea)* receiving cow manure or sewage sludge than on those receiving a synthetic fertilizer (Culliney and Pimentel 1986).

Carbon-based defensive compounds, such as phenolics, may not accumulate in plant tissues if C reserves are insufficient (Feeny 1970; Bryant et al. 1983; Zhao et al. 2007). Carbon reserves include starch stored in plastids and sucrose stored in the cytosol (Taiz and Zeiger 2002). If N is limited due to inadequate fertility, this may modify photosynthetic rates, and shift the partitioning of C between starch and sucrose, as well as the distribution of C to shoot and root sinks (Rufty et al. 1992). An increase in N will yield an increase in amino acid concentrations in leaves, while reducing the assimilation of carbohydrates via photosynthesis (Bryant et al. 1983; Wilkens et al. 1996). Therefore, a lack of C-based defense compounds in plants receiving adequate N may be due to insufficient C-sources for production, as described by the C:N balance hypothesis (Bryant et al. 1983). As such, any modifications to plant chemistry may impact plant-insect interactions (Altieri et al. 1998; Altieri et al. 2005; Letourneau and van Bruggen 2006). By affecting certain parameters of plant chemistry, fertility may influence herbivore performance including, moisture content, N content (proteins and amino acids), and defensive compounds (Rosenthal and Janzen 1979; Scriber and Slansky 1981; van Bruggen 1995; Behmer et al. 2002; Lee et al. 2003; Behmer 2009; Leiss et al. 2009).

Similar to other insects, fertilization practices have been shown to impact the diamondback moth (*Plutella xylostella*) (DBM), which is a major insect pest of *Brassica* crops (Sarfraz et al. 2011). For example, unfertilized cabbage (*Brassica napus*) plants were shown to experience higher levels of herbivory from DBM larvae compared to fertilized plants (Sarfraz et al. 2009). However, unfertilized plants contained DBM larvae with lower larval body weights, compared to fertilized plants (Sarfraz et al. 2009). This suggests that lower larval body weights

due to feeding on unfertilized cabbage may be due to the high levels of C-based defensive compounds (Sarfraz et al. 2009; 2010b).

For DBM, both population abundance and oviposition preferences have been shown to be influenced by fertility practices. For example, Staley et al. (2010) found that DBM populations increased on *Brassica* crops receiving high (200 ppm N/hectare) and low (100 ppm N/hectare) rates of ammonium nitrate, compared to plants receiving high (200 ppm N/hectare) and low (100 ppm N/hectare) rates of an organic-based fertilizer, in this case, plant-based manure or chicken manure pellets. In another study, Sarfraz et al. (2010b) reported that increased sulfur (S) availability to *B. napus* plants increased DBM female oviposition, presumably due to the presence of S-based sinigrin, which is a volatile compound that elicits oviposition in DBM (a bottom-up effect). Elevated concentrations of foliar sulfur (S) were detected after exposure to feeding by DBM larvae on *B. napus*, whereas the concentration of N declined after feeding (a top-down effect) (Sarfraz et al. 2009).

Although these studies demonstrate that fertility may affect *Brassica*-DBM interactions, it is not clear whether fertilizer inputs induce changes in the production of primary metabolites or phenolic compounds that may impact DBM fitness and survival (Jansson et al. 1991; Hummel et al. 2002; Sarfraz et al. 2009; Sarfraz et al. 2010b; Staley et al. 2010). Moreover, few studies have compared the effects of fertilizer type (organic-based versus synthetically-derived) on plant chemistry and herbivory (Oelhaf 1978; Chen et al. 2004; reviewed by Kristiansen and Merfield 2006; Zehnder et al. 2007). Understanding the effects of an organic-based versus synthetically-derived fertilizer on pac choi-DBM interactions may lead to implementing pest management strategies that alleviate insect outbreaks (Altieri and Nicholls 2003; Fageria 2005; Staley et al. 2010) and maximize crop production.

This study compared N sources from an approved-for-organic-use fish hydrolysate (fish waste enzymatically broken-down into peptides) with a conventional fertilizer derived from inorganic salts, which was formulated to have a similar nutrient content to the fish hydrolysate except for N composition. For the fish hydrolysate, the organically-bound N requires mineralization prior to plant uptake, whereas N from the conventional fertilizer is readily available to plants (Kristinsson and Rasco 2000). Based on previous literature (Bryant et al. 1983; Coley et al. 1985), it was predicted that plants receiving the fish hydrolysate fertilizer should have less available N compared to plants receiving the synthetically-derived fertilizer. A lower level of available N associated with the organic treatment should yield plants with higher leaf C, higher phenolic content, lower shoot biomass, and lower body weights and survival rates of DBM compared to plants receiving the conventional fertilizer.

This research addresses several knowledge gaps, including DBM responses to total and specific phenolic contents (Sarfraz et al. 2009; Sarfraz et al. 2010a, Staley et al. 2010), effects of organic and conventional fertilizers on pac choi chemistry (Zhao et al. 2007), and impact of macro- and micro-nutrients provided by fertilizers on pac choi-DBM interactions (Dale 1988a; Coleman et al. 2005; Schoonhoven et al. 2005). The specific objectives were to 1) assess the direct effect of an organic and conventional fertilizer on pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') chemistry and growth, 2) determine the corresponding effects on DBM development and survival, and 3) evaluate effects of DBM larval feeding on pac choi under organic and conventional fertility regimes.

## Materials and Methods

This study involved two greenhouse experiments, conducted from May 1-June 5, 2010, and October 29-December 6, 2010 to determine the effects of an organic and conventional fertilizer on pac choi nutrient content, phenolic content, shoot biomass, plant height, leaf length, leaf number, and various life history parameters of DBM.

### *Plant material and insect colony*

Pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') seeds (Johnny's Seeds; Winslow, ME) were germinated in 7 x 12 plug flats (11 x 21 cm tray, Hummert's Int.; Topeka, KS) containing a soilless growing medium, MetroMix 200 (MetroMix, Sungro; Alberta, Canada) that consisted of Canadian sphagnum peat moss, vermiculite, perlite, a wetting agent, and trace amounts of N:P:K. Seventy-two, two-week-old seedlings were transplanted into 16 cm black plastic containers with a soilless growing medium, Natures All Organic Potting Soil (Natures, SunGro; Alberta, Canada) consisting of Canadian sphagnum peat moss, composted bark, compost and pumice.

A colony of DBM was established at Kansas State University (Manhattan, KS) using a shipment obtained from Benson Research (Carlisle, PA) in 2009. The commercial supplier's colony originated from a population collected in Geneva, NY in 1988, and was maintained on a wheat germ and casein-based diet. The colony was maintained on four to five potted pac choi plants in a 0.60 x 0.60 x 0.91 m frame cage covered with 1.4 mm mesh screening in a greenhouse under natural light and temperature between 20-23°C.

### *Experimental design and environmental conditions*

In each experiment, sixty-four pac choi transplants were arranged in a randomized complete block design with eight blocks, and eight containers (potted plants) per block, using two greenhouse benches (3.6 x 9.1 m) where plants received natural lighting for each experiment. In order to maintain uniform growing medium moisture across the blocks, eight additional pac choi transplants were placed at the corner of each bench, which served as indicator containers to determine when watering was needed. Pac choi plants received 750 mL of deionized water when the gravimetric weight of the indicator containers decreased by 30% from container capacity (Black 1965; Altimimi 2010) in adjacent blocks. Temperature and relative humidity were recorded using a HOBO data logger (Onset: MicroDaq; Contoocook, NH) set at 30-min intervals.

### *Fertility treatment*

Four of the eight plants within each replicate (block) were randomly assigned to receive either an organic or conventional fertilizer treatment. The organic fertilizer was a soluble fish hydrolysate (Neptune's Harvest; Gloucester, MA), diluted from the label 2.2:4.3:0.3 (N:P:K) ratio to 167 ppm N. This concentration of N was selected based on previous greenhouse experiments associated with optimal N rates and pac choi yield (Altimimi 2010). Three 100 mL diluted hydrolysate samples were submitted for nutrient testing [total N, total P by K persulfate digest, $NO_3^-$-N and $NH_4^+$-N in filtered samples by colorimetric analysis, and phosphorus, potassium, magnesium, sulfur, iron, sodium, and chloride in filtered samples analyzed by inductively coupled plasma spectroscopy] by the Soil Testing Laboratory at Kansas State University (Manhattan, KS). The final concentration of fish hydrolysate composition, based on test results, is shown in Table 3.1. For fish hydrolysate samples, total N was 227.7 mg/L with

98.4 % $NH_4^+$ - N and 1.6 % $NO_3^-$ - N. Based on these results, the conventional soluble fertilizer was formulated using inorganic salts similar to the fish hydrolysate, with the exception of N composition, which is presented in Table 3.2. The N composition in the conventional fertilizer was 261.9 mg/L total N with 22.9% $NH_4^+$ - N and 77.1% $NO_3^-$ - N (K.A. Williams, personal communication). Although the organic and conventional fertilizers were developed to be as similar as possible, the conventional fertilizer contained 34 mg/L more N than the average amount of fish hydrolysate, which ranged from 202-253 mg/L N. Three fertilizer applications for each experiment in regards to either conventional or organic treatment supplied a total of 210-230 mg of N per container. The conventional fertilizer was prepared at the start of each experiment by dissolving the inorganic salts in 32 L of deionized water and storing this solution in a 227 L black plastic container for the duration of each experiment. Plants were watered prior to adding the fertilizer with 750 mL of deionized water. For the organic fertilizer treatment, 45 mL of fish hydrolysate concentrate was added to 9 L of deionized water and stirred to dilute to the target concentration of 167 ppm N. Then, 330 mL of diluted hydrolysate was applied to each container. For the conventional fertilizer treatment, 330 mL of prepared fertilizer solution was applied to each container. There were three fertilizer applications, 1) at transplant, 2) seven days post-transplant, and 3) 14 days post-transplant.

***Herbivore treatment***

Of the remaining four plants in each block (two per fertilizer treatment), one organic and one conventionally fertilized plant was infested with twenty, second instar DBM larvae seven days after transplanting, while the remaining organic and conventionally fertilized plants were not infested. Inoculation involved the individual transfer of larvae from colony plants to the same leaf in the middle whorl of experimental plants using a fine-tipped paintbrush. Based on prior

observations with pac choi, exposure to twenty feeding larvae yields adequate residual leaf tissue for chemical analyses. To confine larvae to plants, the artificially inoculated plants were caged using 0.6 x 0.6 m nylon (2 mm mesh), which was fastened around the base of each infested pac choi plant with wood clothes pins.

When adult emergence began, moths were collected daily using the 'bellows method' (see Appendix E). Adults were then sexed and individually weighed to the nearest 0.001 mg using an electronic balance. Male DBM were identified by the distinctive diamond-shape patterning on the forewings, which is not present on females (Shirai 1993; Muhamad et al. 1994). Experiments were terminated at a point in time so that 1$^{st}$ instars could not emerge from eggs and begin feeding, potentially impacting the study results. At the time of the final plant sample, which commenced when DBM eggs appeared on plants where DBM had mated before collection, counts of each larval instar as well as pupae were taken. Age-classes were not significantly different between light treatments within each experiment (see Appendix A).

***Leachate analysis***

Seven days after the first fertilizer application, two organic and two conventionally treated plants from each block were randomly chosen for initial leachate and plant chemical analyses. Approximately 10 days after the third fertilizer application, final sampling of leachates, plant chemistry, and shoot biomass were conducted. Initial and final leachate samples were processed to assess electrical conductivity (EC), pH, and macro-nutrient levels in the growing medium. Electrical conductivity is a measure of nutrients or dissolved salts in the leachate solution, measured as mhos/cm, where a high EC value indicates a higher concentration of nutrients (Cavins et al. 2008). Containers were sampled using a pour-through method (Cavins et al. 2008), where containers were irrigated with 750 mL of deionized water and allowed to

equilibrate for 1-h over a 30 cm diameter clear plastic saucer. After equilibration, another 500 mL of deionized water was added to the containers in order to collect 60 mL of leachate from the saucers. Leachate samples were stored in 100 mL polyethylene vials (Fisher Scientific LLC; Denver, CO). Electrical conductivity and pH were measured for each leachate sample (32 initial samples and 32 final samples) using a hand-held meter (Hanna Instruments, Model HI98129: Hummert's Int.; Topeka, KS). Leachate samples were submitted to the Kansas State University Soil Testing Laboratory (Manhattan, KS) for analysis of total N, total P, and soluble K concentrations (ppm) in each sample. After digestion with K persulfate reagent in an autoclave, samples were analyzed using a Technicon AutoAnalyzer II for P content. An Alpkem RFA (Alpkem Corporation; Clackamas, OR) was used to quantify $NO_3^-$ - N in the digested samples using the cadmium reduction method. Potassium content was determined by filtering the samples through #642 filter paper prior to analysis using a Flame Atomic Absorption Spectrophotometer 3110 (Perkin Elmer Corporation; Norwalk, CT).

***Plant chemical analysis***

Shoot biomass from the crown up, was measured as fresh weight to the nearest 0.001 mg. Plant height and leaf length (cm) of one leaf in the middle whorl were recorded, along with the number of leaves for all plants. For phenolic content analysis, two leaves were excised from the middle whorl (youngest, fully expanded leaves) of pac choi plants. To avoid bias from diel cycling of plant nutrients and phenolics, samples were taken only between 6:00-8:00 am for both experiments (You and Yang 2001). Although greenhouse light intensities varied during the 6:00-8:00 am sampling time between both experiments, the difference in light intensity was not expected to impact the comparison of plant responses between experiments (C. B. Rajeshaker, personal communication). To avoid a chemical response in plant tissue due to wounding from

excising leaf tissue, leaf material was immediately frozen in liquid nitrogen and stored at -20°C for approximately 1 week until used in the analyses. For moisture content and C:N ratio, two additional leaves from the middle whorl of each plant were excised, weighed, placed in #2 brown paper bags, and stored at -20°C until analyzed. For C and N levels, leaf material was dried in a forced air oven set at 68°C for 72 h, then ground in a stainless steel Wiley mill to pass through a 20 mesh screen (Scientific Apparatus; Philadelphia, PA). Total % N and % C (both free and structural forms) were assessed from the ground tissue using a dry combustion procedure conducted by the Kansas State Soil Testing Laboratory (Manhattan, KS) via a TruSpec CN analyzer (LECO Corporation; St. Joseph, MO). Concentration of leaf total % P, K, Ca, Mg, S, Fe, Cu, Mn, and Zn from ground tissue were analyzed by an inductively coupled plasma spectrometer (SPECTRO Analytical Instruments; Kleve Germany) after nitric acid digestion. Moisture content of the leaf tissue was assessed based on the difference between wet and dry weights of the leaf samples. Total phenolic content was determined using the modified Folin-Ciocalteu method (Pennycooke et al. 2005; Oh 2008) with assays of 30 samples per run. The modifications were that approximately 0.5 g of frozen leaf tissue was macerated using a mortar and pestle. In order to extract the phenolics, 3 mL of 80% (v/v) acetone was added to the ground tissue. Then, 1 mL of mixture was poured into a 1.5 mL microcentrifuge tube (Fisher Scientific; Denver, CO), which was then covered with aluminum foil and left overnight at 5°C. Thirty tubes were centrifuged for 2 min at 112 RCF (relative centrifugal force). Then 50 mL of supernatant was pipetted into a new 1.5 mL tube and mixed with 135 µL $H_2O$, 750 µL of 1/10 dilution Folin-Ciocalteu reagent (Sigma-Aldrich; St. Louis, MO), and 600 µL 7.5% (w/v) $Na_2CO_3$. Samples were vortexed for 10 sec and incubated at 45°C in a water bath for 15 min. Samples were allowed to cool to room temperature before reading the absorbance at 765 nm (U-1100

spectrophotometer; Hitachi Ltd. Japan) (Pennycooke et al. 2005). A gallic acid standard curve was prepared using 1 mg/mL gallic acid in 80% (v/v) acetone stock solution. Total phenolics were reported as gallic acid equivalents (GAE)/g fresh weight tissue. One absorbance reading was taken for each plant sample.

The process of extracting individual phenolics from the pac choi leaves was based initially on the methods described by Nicolle et al. (2004) with minor modifications (Oh and Rajashekar 2009), which were that approximately 1 g of frozen leaf tissue was ground using a mortar and pestle. Phenolics were then extracted using 25 mL of 70% methanol at 80°C for 1 min. After agitation on a shaker plate for 1-h, the solution was processed through filter paper (No. 1, Whatman; Whatman Place, UK). The filtered extract (9 mL) was then evaporated to dryness using a speed vacuum (Savant SVC-100H Speed Vac Concentrator; Midland, MI) under reduced pressure at 43°C and then re-suspended in 5 mL of 70% methanol. The concentrated solution was filtered through a 0.45 μm ascrodisc filter (Millex, Millipore Corporation; Bedford, MA). A 5 μL aliquot of the extract was injected into a high performance liquid chromatography (HPLC) system equipped with an autosampler (SpectraSYSTEM AS1000, Thermo Separation Products; San Jose, CA), pump (HP 1050, Hewlett Packard; Palo Alto, CA), integrator (HP 3396, Hewlett Packard; Palo Alto, CA), and UV/VIS detector (Acutect 500, Thermo Separation Products; San Jose, CA). Column separation identified the chosen phenolics (chlorogenic, caffeic, sinapic and ferulic acid, luetolin-7-O-glucoside, myricetin, and quercetin) at 60°C. These compounds were selected for the individual phenolic assay because they have been identified as phenolics in pac choi that respond to changes in light intensity (Oh and Rajashekar 2009). Constituents of the extract were subjected to column separation with eluent A [$H_2O$:$CH_3COOH$ = 338:1 (v/v)] and eluent B [$H_2O$:$C_4H_{10}O$:$CH_3COOH$ = 330:8:1 (v/v/v)] at a flow rate of 1.8 mL/

min. The gradient started at 20% B in A, which was maintained for 5 min, then increased to 100% B for 20 min. After equilibration (2 min at 100% B), the composition of the solution returned to the initial condition (20% B). Peaks from the select phenolics were identified and quantified at 330 nm by comparing with standard compounds: chlorogenic, p-coumaric, caffeic, sinapic and ferulic acids, and quercetin-3-O-glucoside (Sigma-Aldrich; St. Louis, MO) and luteolin-7-O-glucoside (Indofine Chemical Company, Inc.; Hillsborough, NJ). High performance liquid chromatography analysis was performed by the Ruminant Nutrition Laboratory at Kansas State University (Manhattan, KS). Specific phenolics were reported in mg/100 mL.

***Herbivory and herbivore response***

The amount of herbivory was measured based on percent leaf area removed by feeding larvae on the infested plants. For the herbivory measurement, digital images were taken of two leaves from the middle whorl (youngest, fully expanded leaves) using a camera (PowerShot SD1000, Canon; Tokyo, Japan). A photo-imaging analysis program, APS Assess version 2.0 (APS Press; St. Paul, MN), was used to quantify total leaf area and total leaf area removed by DBM larva (Dudt and Shure, 1994; Lamari 2002). To compare herbivory on an individual basis, larval feeding equivalents were determined by dividing the percentage of total leaf area consumed by the cumulative number of relative feeding equivalents. Feeding equivalents were obtained by multiplying the number of each instar counted on plants by the estimated relative proportion of leaf tissue required for each instar to complete development. This procedure resulted in the following formula: [percent consumption per insect per plant = percent total leaf area removed / (number of $3^{rd}$ instars x 3) + (number of $4^{th}$ instars x 8) + (number of pupae x16)]. These relative instar consumption values were derived from *Erinnyis ello*, which also has four larval instars (Pratissoli et al. 2002).

To determine if DBM developmental time differed between the fertility treatments, the number of collected DBM adults during the experiments and remaining number in each instar on plants at the end of each experiment were multiplied by the number of degree days (D-D) needed for that life stage to complete development based on data from Ansari et al. (2010) for DBM feeding on *Brassica rapa* at 25°C. Degree-days were used to standardize development data, but are not related to actual greenhouse temperatures in the experiments. The total number of D-D was then summed and the average D-D for the cohort was computed as follows: [average cohort D-D = (number of $3^{rd}$ instars x 221.75 D-D) + (number of $4^{th}$ instars x 159.25 DD) + (number of pupae x 83.44 D-D) + (number of adults x 0 D-D) / total number of all stages present]. Second instars were not present on plants at the end of each experiment; therefore, they were not included in the analysis. Percent survival was determined as the sum of emerged adults and remaining larvae on plants at the end of each experiment divided by the initial number of larvae (20 per plant).

***Statistical analysis***

All data pertaining to leachate, plant and insect responses were analyzed using SAS Systems for Windows, Version 9.1 (SAS Institute 2002). Data were subjected to a mixed model analysis of variance (ANOVA) using the PROC MIXED procedure (SAS Institute 2002) with experiment (spring and fall), fertility (organic and conventional), and herbivory (plants with and without DBM), as the main effects, and block as the random effect. For plant and insect responses, tests for significance were conducted for all main effects and for any two-way interactions. For leachate variables, tests for significance were conducted for experiment and fertility effects, and for the interaction of experiment and fertility. In addition, leachate variables were subjected to analysis for the main effect of sampling time and the interaction of experiment

and fertility and sampling time. Because there was significant variation in variables between experiments, results for the spring and fall experiments were analyzed separately, then compared. Greenhouse environmental variables associated with light intensity, temperature, and relative humidity were also analyzed between experiments using an unpaired Students's t-test. Leachate N, P, K, and EC values were $\log_{10}$ transformed to normalize the data before analyses were performed. Percentage data for plants (leaf tissue N, C, P, K, Ca, Mg, S, moisture content) and DBM (consumption as larval feeding equivalents and cohort survival) were arcsin transformed prior to analysis to normalize the data. Light intensity, measured as lumens/$ft^2$, was converted to lumens/$m^2$ by multiplying values by the constant 10.76 (Mechtly 2008). All data presented are non-transformed.

The LS MEANS statement (SAS Institute 2002) and Fisher's Protected LSD were used to make pair-wise treatment comparisons. To determine if experiment and fertility were correlated with various plant and insect responses, multiple regression analyses were performed using the PROC REG procedure (SAS Institute 2002). Prior to fitting the regression models, elimination of extraneous variables from the large data sets was accomplished with a best-subsets analysis using MINITAB version 14 (Minitab Inc., State College, PA) (Dallal 2007). Multiple regressions applied a step-wise, backward elimination procedure (SAS Institute 2002), which removes the variables with the highest *P* values until only those with $P \leq 0.05$ remain in the model. Multiple regression results were assessed for best fit (linear or quadratic) in MINITAB using fitted line plots.

Development, larval consumption, and survival data were averaged for two infested plants for either fertility treatment in each block and subjected to ANOVA using a general linear model with fertility and experiment as main effects, block as a random factor, and the interaction

of fertility and experiment. Means for the treatment variables were separated using Tukey test procedures (SAS Institute 2002).

## Results

### *Greenhouse conditions*

Greenhouse environmental conditions (light intensity, temperature, and relative humidity) were significantly higher in the spring experiment than in the fall experiment (Table 3.3). Greenhouse conditions were significantly different between experiments, including light intensity ($t$ = -8.69, $df$ = 856, $P \leq 0.001$), temperature ($t$ = -18.62, $df$ = 2613, $P \leq 0.001$), and percent relative humidity ($t$ = -39.78, $df$ = 2613, $P \leq 0.001$).

### *Leachate variables*

There were no significant differences in leachate N, P, K, pH, and EC between the fertility treatments (Table 3.4). However, leachate N, P, K, pH, and EC were significantly affected by sampling time (Table 3.4), where all variables were significantly higher at pre-sample (prior to introducing DBM) compared to the post-sample (end of experiment). For leachate P the pre- and post-sample concentrations (mean ± SE) were 30.8 ± 1.7 ppm and 18 ± 2.5 ppm, respectively. For leachate N, K, pH, and EC, there was a significant experiment by sampling time interaction (Table 3.4), which indicates that the magnitude of difference between the pre- and post-samples was not the same in the spring and fall experiments. Mean concentrations for leachate N, K, pH, and EC are presented in Table 3.5. For leachate N, there

was a 77% [(pre-post/pre)*100] reduction from pre- to post-sampling in the spring experiment, whereas the reduction in the fall experiment was 50% (Table 3.5).

***Plant response variables***

There were no significant differences in the plant response variables between the fertility treatments in either experiment (Table 3.6), with the exception of percent leaf P and concentration of p-coumarin (Table 3.6). In both experiments, the percentage of leaf P was significantly higher for the conventional fertility treatment (0.71 ± 0.02 %) compared to the organic fertility treatment (0.63 ± 0.02 %). The concentration of p-coumarin was significantly higher in the organic fertility treatment (12.93 ± 1.18 mg/100 mL) compared to the conventional treatment (6.25 ± 0.58 mg/100 mL). However, detectable concentrations of p-coumarin were found in only 19 of 32 samples in the spring experiment and 6 of 32 samples in the fall experiment (Table 3.7). The interaction of experiment by fertility interaction was not significant for plant response variables, implying that the differences in plant responses between the fertility treatments were similar for the two experiments.

In general, percent leaf C, P, K, Ca, Mg, S, and ppm Cu, Mn, and Zn were higher in the fall experiment than the spring experiment, whereas ppm Fe, plant height, shoot biomass, and leaf number, were all generally lower in the fall experiment (Tables 3.8 and 3.9). Concentrations of specific phenolics were higher in the fall experiment compared to spring, whereas total phenolic content was higher in the spring experiment than fall (Table 3.7).

***Herbivory and DBM response variables***

Herbivory had a significant effect on percent leaf Ca, percent leaf Mg, and shoot biomass (Table 3.6). In both experiments the percentage of leaf Ca and leaf Mg was significantly higher

in plants with DBM than in plants without DBM (Table 3.9). However, shoot biomass was significantly greater in plants without DBM compared to plants with DBM (Table 3.9).

DBM development (based on cumulative D-D) was significantly affected by the fertility treatments (Table 3.10), with cohort development faster on plants receiving the conventional fertility treatment compared to plants receiving the organic treatment (Table 3.11). DBM development was also significantly different between experiments (Table 3.10), where development was faster in the fall experiment (73.55 ± 9.02 D-D) than the spring experiment (112.0 ± 20.46 D-D). The interaction of experiment by fertility was not significant (Table 3.10). Moreover, larval feeding equivalents were not significantly different between experiments, fertility treatments, or the interaction of experiment by fertility (Table 3.12).

The proportion of DBMs in each life stage did not differ between fertility treatments within an experiment (refer to Appendix A). Therefore, percent survival could be compared directly within experiments. The percent survival was significantly affected by fertility treatment (Table 3.10). Specifically, for both the spring and fall experiments, percent survival was significantly higher on plants receiving the conventional fertility treatment compared to plants receiving the organic fertility treatment (Table 3.11). Although percent survival was significantly affected by experiment (Table 3.10), the interaction of experiment by fertility was not significant ($F = 1.35$, $df = 1$, $P = 0.25$). Besides fertility treatment, the regression analysis revealed that DBM survival varied significantly with leaf Mg; survival was negatively related to leaf Mg at concentrations >0.6% in the fall experiment ($F = 6.79$, $df = 1$, $P = 0.02$) but was not significant in the spring experiment ($F = 4.07$, $df = 1$, $P = 0.06$). Changes in leaf Mg explained a very high percentage of variation in survival for the fall experiment ($R^2 = 97\%$) and almost half ($R^2 = 44\%$) in the spring experiment (Figure 3-1).

Fertility treatments did not significantly affect male or female DBM body weights (Table 3.10). However, female body weights were significantly different between experiments (Table 3.10), where adult females were almost twice as heavy in the spring experiment compared to the fall experiment (Table 3.12). Regression analysis revealed that female DBM body weights were significantly negatively correlated with leaf Ca under the organic fertility treatment in the spring experiment ($F = 9.40$, $df = 1$, $P = 0.03$) but not the conventional fertility treatment ($F = 2.53$, $df = 1$, $P = 0.16$) (Figure 3-2). DBM body weights were not significant for the interaction of experiment by fertility treatment.

## Discussion

In this study, the type of fertilizer used had no effect on any of the leachate variables and had relatively little effect on pac choi chemistry. With respect to plant responses, percent leaf phosphorus (P) was significantly higher in the conventional fertility treatment compared to the organic treatment in both experiments. In some plants, it has been shown that high levels of leaf P can limit the production of phenolics due to phosphorylation of P on leaf carbon (C), thus limiting available C for use in accumulating phenolics (Jones and Hartley 1999). However, there were no differences in any phenolic content between the fertility treatments and no differences in plant growth responses. These results differ from those of Zhao et al. (2009) in which they examined nutrient and phenolic contents in greenhouse-grown pac choi (*Brassica rapa* cv. *chinensis* 'Mei Qing Choi') in an experiment comparing an organic fish hydrolysate fertilizer with a conventional fertilizer (inorganic salt solution formulated similar to fish hydrolysate).

Unlike the current study, they found increased phenolic contents in plants that received the fish hydrolysate fertilizer compared to plants that received the conventional treatment. However, Zhao et al. (2009) added a slow-release compost amendment to the fish hydrolysate treatment, which may have immobilized N provided by the fish hydrolysate. Moreover, they used a different phenolic assay method, so results may not be comparable with other studies. In addition, levels of some leaf nutrients, such as leaf P, were approximately 10-fold greater than those found in the current study. Other factors which may explain the differences in plant responses between the two studies are that Zhao et al. (2009) applied a higher rate of N and applied fertilizer more often than in the current study.

Based on the C:N balance hypothesis (Bryant et al. 1983), it was predicted that leaf C and phenolic content would increase when N resources were limited for plant growth and development. In this study, the organic fertility treatment was designed to create a limited N supply for pac choi plants relative to the conventional treatment. However, based on the plant responses it was not apparent that N was actually a limiting factor in the organic treatment, nor were there differences in leaf C and phenolic content between the treatments. The similarities between the fertilizer treatments in this study may possibly be attributed to rapid mineralization of organically-bound N, which is common for animal-based fertilizers (Hartz and Johnstone 2006). Moreover, stable greenhouse temperatures (20-25°C) may have contributed to the lack of differences in available N between fertility treatments by promoting rapid enzymatic hydrolysis (catalytic decomposition) of amino acids in the organic fertility treatment. This process releases N as a by-product and may have negated any differences in N availability between treatments (Hartz and Johnstone 2006). Other greenhouse studies have reported similar plant chemistries

and growth responses in plants receiving fish-based and synthetically-based fertilizers (Aung and Flick 1980; Emino 1981; Hartz and Johnstone 2006).

In some field studies, differences in plant chemistry and/or plant growth responses have been observed between organic and conventional fertilizer treatments (Eigenbrode and Pimental 1988; Phelan et al. 1995; Brandt and Molgaard 2001; Gale et al. 2006). Fertilizer effects in field studies that were not observed in the current study could be related to the growing medium because field soils include beneficial microbes that may be responsible for breaking down organic matter into C and N (Kramer et al. 2002; Delate et al. 2008). Differences may also include variations in N availability depending on the type of organic fertilizer used. For example, Sousa et al. (2005) evaluated fertilizer types and growth of *Brassica oleracea*, and found increased levels of phenolics in plants treated with an organic fertilizer, based on sheep manure, compared to plants receiving a conventional fertilizer based on ammonium nitrate. Differences between available N in fish hydrolysate and sheep manure may explain these contrasting results, thus validating the need for evaluating N availability of multiple types of organic fertilizers.

In this study, herbivory impacted plant chemistry. Herbivory from DBM larvae modified levels of leaf Ca and Mg in both experiments, which were significantly higher in plants with DBM than those without DBM. Increased levels of calcium in plants have been shown to induce the formation of reactive oxygen species (ROS) that may negatively affect herbivores (Bolwer and Fluhr 2000; Kolupaev et al. 2008). Moreover, Ca also enhances the production of antioxidants such as superoxide-dismutase (SOD) and nitric oxide (NO) against herbivores (Rodriguez-Serrano et al. 2009). Similar to Ca, Mg levels in plants help to alleviate ROS caused by stress from herbivory (Lecourieux et al. 2006; Cakmak and Kirkby 2008). Furthermore, Mg is

widely used to signal the initiation of defenses against chewing herbivores, including the *LOX*, *MAPK*, and *HPL* defense pathways (Smith 2005). Because Ca and Mg are associated with plant defenses against stress, it is expected that levels would increase in response to DBM herbivory. This prediction is supported by the current study in which both Ca and Mg occurred at higher levels in infested pac choi plants than in uninfested plants. Likewise, Sarfraz et al. (2009) found significantly higher percentages of Ca and Mg in the leaf tissues of *Brassica napus* that had been infested with DBM compared to uninfested plants. With respect to insect defense, Sarfraz et al. (2009) found that plants with higher levels of Ca and Mg were associated with longer developmental times and shorter adult lifespans of DBM. However, in the current study, with the exception of female body weight, which was negatively correlated with Ca in the organic fertility treatment, no other factor negatively affected DBM that was correlated specifically with Ca or Mg.

Although there were no differences in the concentration of Ca between the organic and conventional fertility treatments, the apparent effect of Ca on female DBM body weight was only observed in the organic treatment, in which Ca levels explained 72% of the variation in female body weight, suggesting that Ca may have interacted with other plant components (e.g., defense proteins or various enzymes) to affect body weight. Because a correlation between Ca and body weight was not detected in the conventional fertility treatment, it is possible that different plant chemicals and/or concentrations were produced under the two treatments, and that any interactions with Ca differed in ways that modified its effect on DBM.

It is unclear why there was a correlation between Ca and body weight for female DBM but not males; however, this may be related to sex-related differences in DBM feeding behavior.

Sarfraz et al. (2009) observed differences in male and female DBM development and attributed these differences to unequal consumption rates; female larvae typically consumed more leaf tissue than males. As such, in the current study, if female DMB larvae consumed more tissue to obtain required quantities of protein, they may have ingested greater amounts of Ca, which negatively affected body weight. For example, velvetbean caterpillar (*Anticarsia gemmatilis*) larvae compensated for reduced nutrient levels in a diluted diet with caffeine by increasing consumption rates, although inadvertently the higher concentration of ingested caffeine interfered with food utilization, reduced growth, and lowered survival (Slansky and Wheeler 1992). Enhanced consumption may occur in female caterpillars, which generally are heavier than males, because they have variable nutritional requirements due to differences in their reproductive physiology (Slansky and Scriber 1985; Telang et al. 2001).

In both experiments, DBM had a significantly higher survival, and developed faster, on plants treated with conventional fertilizer. Although larval consumption was not affected by fertility treatments, the findings suggest that plant factors associated with the organic fertility treatment were less suitable for DBM compared to the conventional treatment. At this point, it is difficult to explain why there were differences in development and survival. However, the level of P in pac choi leaves was significantly lower for the organic fertility treatment. Although DBM development was not correlated with leaf P in the current study, P availability during larval development may be an important factor influencing insect life history traits (Visanuvimol and Bertram 2011). For example, decreased dietary P has been shown to reduce the growth rate of *Manduca sexta* larvae (Clancy and King 1993; Perkins et al. 2004). Alternatively, treatment differences in other plant components, such as amino acid composition and concentration, may have affected the nutritional quality of pac choi for DBM. Finally, chelating agents, fish-derived

enzymes, and minerals (such as boron) are all known to modify plant defenses (Phelan et al. 1995), so any differences in these chemicals could have been a factor.

The current study contributes information that may be useful to producers. First, both fertility treatments appeared to provide comparable N sources for growth of pac choi. When implementing an organic fertility program, producers may choose fish-based fertilizers that deliver N to plants similar to conventional fertilizers, which is different than slower-release plant-based fertilizers and compost amendments. As such, future research evaluating of multiple organic-use materials is warranted. Second, it appears that the effect of the organic fertility treatment negatively impacted DBM development and survival. Although general predictions regarding pac choi susceptibility to DBM feeding under organic fertility programs compared to conventional fertility programs cannot be made at present, further studies may demonstrate that crop susceptibility changes based on the type of fertilizer used. The possibility that the fish hydrolysate fertilizer induced pac choi defenses against DBM should be examined further by focusing on leaf P levels or other components associated with the hydrolysate formulation not measured in this study.

# Tables and Figures

**Table 3.1. Final concentration of nutrients applied to *Brassica rapa* containers from three applications of Neptune's Harvest fish hydrolysate (Neptune's Harvest; Gloucester, MA).**

| Total nutrients applied per pot | |
|---|---|
| **Nutrient** | **mg/L** |
| Total N[1] | 227.7 ± 14.8 |
| $NO_3$-N | 0.5 |
| $NH_4$-N | 30.0 |
| P | 100.4 |
| K | 49.7 |
| Ca | 100.7 |
| Mg | 11.5 |
| $SO_4$-S | 56.1 |
| Fe | 0.1 |
| Na | 76.9 |
| Cl | 32.3 |

[1] Total N is shown as mean ± SE to demonstrate the variability of N from three test samples of diluted fish hydrolyate.

**Table 3.2. Final concentration of nutrients applied to *Brassica rapa* containers from three applications of an inorganic salt solution, formulated to match the fish hydrolysate (Neptune's Harvest; Gloucester, MA).**

| Total nutrients applied per pot | |
|---|---|
| **Inorganic nutrients** | **mg/L** |
| Total N | 261.9 |
| $NO_3$-N | 201.9 |
| $NH_4$-N | 60.0 |
| Total P | 116.5 |
| Total K | 60.7 |
| $KNO_3$ | 156.7 |
| $Ca(NO_3)_2$ | 1055.6 |
| $MgSO_4$ | 57.8 |
| FeETDA | 2.2 |
| NaCl | 53.8 |
| $(NH_4)_2HPO_4$ | 285.2 |
| $NaH_2PO_4$ | 339.5 |

**Table 3.3. Mean (± SE) differences in greenhouse light intensity, temperature, and relative humidity for the spring and fall experiments.**

| *Environment* | Spring | Fall |
|---|---|---|
| Light intensity[1] | 18324 ± 376 A[3] | 12825 ± 505 B |
| Temperature °C[2] | 25 ± 0.1 A | 21.19 ± 0.1 B |
| % Relative humidity[2] | 59.34 ± 0.49 A | 33.06 ± 0.3 B |

[1] Light intensity measured in lumens/$m^2$; N = 856; only data from day-light hours used for analysis. Data were recorded at 30-min intervals for duration of experiments using HOBO data loggers.
[2] N = 2613; data from day and night hours used for analysis recorded from HOBO data loggers at 30-min intervals for each experiment.
[3] Means followed by common letter within a row are not significantly different, as determined by an unpaired Students's t-test at $P \leq 0.05$.

**Table 3.4. Analysis of variance (ANOVA) values for block (1-8), experiment (spring and fall), fertility (conventional and organic), and sample time (pre-sample: prior to DBM introduction, and post-sample: end of experiment), and the experiment by sample time interaction associated with the leachate variables. Values in bold are significant ($P \leq 0.05$).**

| | *Block* | | | *Experiment* | | | *Fertility* | | | *Sample Time* | | | *Experiment*Sample Time* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Leachate Variables*** [1] | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* |
| N (ppm) | 7 | 0.76 | 0.62 | 1 | 0.01 | 0.93 | 1 | 0.02 | 0.89 | 1 | 65.56 | **<0.001** | 1 | 6.45 | **0.01** |
| P (ppm) | 7 | 0.65 | 0.71 | 1 | 0.001 | 0.98 | 1 | 0.66 | 0.41 | 1 | 57.47 | **<0.001** | 1 | 3.58 | 0.06 |
| K (ppm) | 7 | 1.1 | 0.37 | 1 | 0.01 | 0.92 | 1 | 0.37 | 0.54 | 1 | 94.42 | **<0.001** | 1 | 6.59 | **0.01** |
| pH | 7 | 1.99 | 0.06 | 1 | 4.71 | **0.03** | 1 | 0.22 | 0.64 | 1 | 138.28 | **<0.001** | 1 | 11.95 | **<0.001** |
| EC[2] | 7 | 3.57 | **0.001** | 1 | 95.29 | **<0.001** | 1 | 0.52 | 0.47 | 1 | 48.54 | **<0.001** | 1 | 22.41 | **<0.001** |

[1] N = 126; 4 containers per fertility treatment in each of 8 blocks at two sampling times for two experiments.
[2] Reported in mhos/cm.

**Table 3.5**. **Mean (± SE) leachate variables in *Brassica rapa* containers at pre-sampling (7 days after first fertilizer application) and post-sampling (7 days after third fertilizer application).**[1]

| | Pre-sampling | | Post-sampling | |
|---|---|---|---|---|
| ***Leachate Variables*** | ***Spring*** | ***Fall*** | ***Spring*** | ***Fall*** |
| Total N (ppm) | 160.5 ± 12.5 A[2] | 134.3 ± 14.3 A | 38.5 ± 9.3 B | 66.9 ± 14.3 B |
| Total K (ppm) | 110.9 ± 5.8 A | 94.5 ± 8.6 A | 20.4 ± 4.8 B | 38.5 ± 8.3 B |
| pH | 5.8 ± 0.05 C | 5.8 ± 0.02 C | 6.5 ± 0.07 A | 6.29 ± 0.02 B |
| Electrical conductivity (EC)[3] | 3154.5 ± 112.8 A | 887.5 ± 75.5 C | 1268.9 ± 164.0 B | 774.0 ± 80.9 C |

[1] N = 126; 4 containers per fertility treatment in each of 8 blocks at two sampling times for two experiments.
[2] Means followed by different letter within a row and experiment are significantly different. Significance was determined at $P \leq 0.05$ (Fisher's Protected LSD).
[3] Reported in mhos/cm.

**Table 3.6. Analysis of variance (ANOVA) values for *Brassica rapa* response variables for block (1-8), experiment (spring and fall), fertility treatment (conventional and organic), and herbivore (with and without *Plutella xylostella*). Values in bold are significant at $P \leq 0.05$ (as determined by Fisher's Protected LSD).**

| | *Block*[1] | | | | *Experiment*[2] | | | | *Fertility*[3] | | | | *Herbivore*[4] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Plant Response Variables*** | *N*[5] | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* |
| Leaf % N | 63 | 7 | 1.06 | 0.4 | 63 | 1 | 23.05 | <0.0001 | 63 | 1 | 0.4 | 0.53 | 63 | 1 | 0.01 | 0.92 |
| Leaf% C | 62 | 7 | 1 | 0.44 | 62 | 1 | **113.69** | **<0.001** | 62 | 1 | 0.89 | 0.34 | 62 | 1 | 0.21 | 0.64 |
| Leaf % P | 62 | 7 | 0.57 | 0.77 | 62 | 1 | **55.49** | **<0.001** | 62 | 1 | **4.29** | **0.04** | 62 | 1 | 2.46 | 0.12 |
| Leaf % K | 62 | 7 | 0.88 | 0.53 | 62 | 1 | **47.13** | **<0.001** | 62 | 1 | 0.32 | 0.57 | 62 | 1 | 1.43 | 0.23 |
| Leaf % Ca | 62 | 7 | 0.69 | 0.68 | 62 | 1 | **22.1** | **<0.001** | 62 | 1 | 0.16 | 0.69 | 62 | 1 | **5.72** | **0.02** |
| Leaf % Mg | 62 | 7 | 0.18 | 0.98 | 62 | 1 | **4.27** | **0.04** | 62 | 1 | 0.07 | 0.79 | 62 | 1 | **3.72** | **0.05** |
| Leaf % S | 62 | 7 | 1.49 | 0.19 | 62 | 1 | **9.52** | **0.003** | 62 | 1 | 0.28 | 0.6 | 62 | 1 | 1.27 | 0.26 |
| Leaf Cu (ppm) | 62 | 7 | **4.78** | **<0.001** | 62 | 1 | **53.32** | **<0.001** | 62 | 1 | 0.11 | 0.73 | 62 | 1 | 0.02 | 0.9 |
| Leaf Fe (ppm) | 62 | 7 | 1.94 | 0.08 | 62 | 1 | **7.26** | **0.009** | 62 | 1 | 1.67 | 0.2 | 62 | 1 | 0.44 | 0.51 |
| Leaf Mn (ppm) | 62 | 7 | **2.24** | **0.04** | 62 | 1 | **23.94** | **<0.001** | 62 | 1 | 0.54 | 0.22 | 62 | 1 | 1.7 | 0.19 |
| Leaf Zn (ppm) | 62 | 7 | 1.31 | 0.26 | 62 | 1 | **88.79** | **<0.001** | 62 | 1 | 2.33 | 0.13 | 62 | 1 | 0.55 | 0.46 |
| Plant height (cm) | 63 | 7 | 0.001 | 1 | 63 | 1 | **14.32** | **<0.001** | 63 | 1 | 1.44 | 0.23 | 63 | 1 | 1.79 | 0.18 |
| Leaf number | 63 | 7 | 0.001 | 1 | 63 | 1 | **66.11** | **<0.001** | 63 | 1 | 0.1 | 0.75 | 63 | 1 | **12.07** | **0.001** |
| Leaf length (cm) | 63 | 7 | 0.001 | 1 | 63 | 1 | 0.07 | 0.78 | 63 | 1 | 0.78 | 0.38 | 63 | 1 | **5.81** | **0.01** |
| Shoot biomass (g) | 63 | 7 | 0.06 | 0.99 | 63 | 1 | **48.3** | **<0.001** | 63 | 1 | 0.001 | 0.98 | 63 | 1 | **7.13** | **0.01** |
| % Moisture content | 62 | 7 | **2.13** | **0.05** | 62 | 1 | 1.86 | 0.17 | 62 | 1 | 0.12 | 0.73 | 62 | 1 | 0.26 | 0.61 |
| Total phenolic content[6] | 61 | 7 | 0.43 | 0.87 | 61 | 1 | **39.59** | **<0.001** | 61 | 1 | 0.12 | 0.72 | 61 | 1 | 0.21 | 0.64 |
| Chlorogenic acid[7] | 58 | 7 | 0.001 | 1 | 58 | 1 | **22.34** | **<0.001** | 58 | 1 | 0.28 | 0.6 | 58 | 1 | 0.29 | 0.59 |
| p-coumarin[7] | 25 | 7 | **4.11** | **0.01** | 25 | 1 | **73.86** | **<0.001** | 25 | 1 | **25.56** | **<0.001** | 25 | 1 | 1.71 | 0.21 |
| Sinapic acid[7] | 54 | 7 | 0.66 | 0.7 | 54 | 1 | **4.31** | **0.04** | 54 | 1 | 0.52 | 0.47 | 54 | 1 | 1.08 | 0.3 |

[1] N = 8 blocks.
[2] Spring and fall experiments.
[3] Fertility treatments were organic fish hydrolysate and conventional fertilizer.
[4] Herbivore treatments included plants with and without *Plutella xylostella* larvae.
[5] Number of plants used in analysis.
[6] Total phenolic content is reported as gallic acid equivalents/g fresh weight of plant tissue.
[7] Concentrations of specific phenolic compounds are reported as mg/100 mL of methanolic extract.

**Table 3.7. Mean (± SE) total and specific phenolics in *Brassica rapa* for spring and fall experiments. Means followed by common letter within a row are not significantly different between experiments as determined by Fisher's Protected LSD at $P \leq 0.05$.**

| *Phenolic Variables* | Spring | | Fall | |
|---|---|---|---|---|
| Total phenolics[2] | 0.44 ± 0.15 A | 32 [1] | 0.24 ± 0.04 B | 30 |
| Chlorogenic acid[3] | 0.73 ± 0.43 A | 31 | 1.73 ± 1.06 B | 28 |
| p-coumarin[3,4] | 2.98 ± 1.97 A | 19 | 11.59 ± 5.37 B | 6 |
| Sinapic acid[3] | 0.80 ± 0.67 A | 32 | 3.19 ± 5.53 B | 29 |

[1] N represents number of plants having detectable phenolic levels from a total of 32 plants sampled; 4 plants in 8 blocks.
[2] Total phenolics measured in (GAE)/g FW plant tissue.
[3] Reported in mg/100mL.
[4] p-coumarin was significantly different between the organic and conventional fertility treatments ($P \leq 0.05$).

**Table 3.8. Mean (± SE) differences in *Brassica rapa* response variables between the spring and fall experiments across plants with and without *Plutella xylostella*.**

| *Plant Variables*[1] | Spring | Fall |
|---|---|---|
| Total % N | 3.32 ± 0.18 B[2] | 4.54 ± 0.18 A |
| Total % C | 39.06 ± 0.15 B | 41.56 ± 0.17 A |
| Leaf % P[3] | 0.52 ± 0.03 B | 0.72 ± 0.02 A |
| Leaf % K | 1.37 ± 0.10 B | 2.80 ± 0.16 A |
| Leaf S (ppm) | 0.56 ± 0.04 B | 1.27 ± 0.05 A |
| Leaf Cu (ppm) | 0.73 ± 0.14 B | 3.52 ± 0.40 A |
| Leaf Fe (ppm) | 166.9 ± 10.14 A | 88.43 ± 3.12 B |
| Leaf Mn (ppm) | 114.96 ± 6.5 B | 171.51 ± 10.84 A |
| Leaf Zn (ppm) | 41.80 ± 2.33 B | 74.74 ± 2.57 A |
| % Moisture content | 96.44 ± 0.14 A | 96.23 ± 0.10 B |

[1] N = 62 plants used in analysis.
[2] Means followed by a different upper case letter within a row and experiment are significantly different. Significance was determined at $P \leq 0.05$ (Fisher's Protected LSD).
[5] Indicates there was a significant difference between the organic and conventional fertility treatments ($P \leq 0.05$).

**Table 3.9. Mean (± SE) differences in selected *Brassica rapa* response variables between the spring and fall experiments and between plants with and without *Plutella xylostella*.**

| | Spring | | | Fall | | |
|---|---|---|---|---|---|---|
| **Plant Variables** | ***No Herbivore*** | ***Herbivore*** | **N[1]** | ***No Herbivore*** | ***Herbivore*** | **N** |
| Leaf % Ca | 0.46 ± 0.40 Bb[2] | 0.66 ± 0.28 Aa | 15 | 1.10 ± 0.24 Ba | 1.24 ± 0.19 Aa | 16 |
| Leaf % Mg | 0.54 ± 0.29 Bb | 0.67 ± 0.16 Aa | 15 | 0.70 ± 0.18 Ba | 0.81 ± 0.17 Aa | 16 |
| Plant height (cm) | 14.68 ± 1.30 Aab | 14.43 ± 1.67 Aa | 16 | 13.50 ± 1.36 Ab | 13.12 ± 1.25 Abc | 16 |
| Leaf number | 19.50 ± 3.77 Aa | 16.87 ± 3.26 Ab | 16 | 13.93 ± 1.48 Ac | 12.31 ± 1.44 Ac | 16 |
| Leaf length (cm) | 14.18 ± 1.93 Aa | 12.68 ± 2.27 Ab | 16 | 13.87 ± 2.12 Aab | 12.75 ± 1.61 Ab | 16 |
| Shoot biomass (g) | 141.93 ± 39.86 Aa | 119.93 ± 33.68 Bb | 16 | 92.72 ± 20.67 Ac | 82.15 ± 17.30 Bc | 16 |

[1] N = number of plants used in analysis.
[2] Means followed by different upper case letter indicates a significant difference in herbivore treatment within an experiment, where lower case letters within a row across experiments indicates a significant difference within treatments, between experiments. Significance was determined at $P \leq 0.05$ (Fisher's Protected LSD).

**Table 3.10. Analysis of variance (ANOVA) values for *Plutella xylostella* response variables for block (1-8), experiment (spring and fall), fertility treatment (organic and conventional), and the experiment by fertility interaction. Values in bold are significant as determined by Fisher's Protected LSD at $P \leq 0.05$.**

| | *Block* | | | | *Experiment* | | | | *Fertility* | | | | *Experiment*Fertility* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Insect Variables*** | *N*[1] | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* |
| Male body weight | 28 | 7 | 0.001 | 1 | 28 | 1 | 3.1 | 0.09 | 28 | 1 | 0.05 | 0.82 | 28 | 1 | 4.03 | 0.06 |
| Female body weight | 31 | 7 | 0.2 | 0.98 | 31 | 1 | 12.43 | **0.002** | 31 | 1 | 1.78 | 0.19 | 31 | 1 | 0.15 | 0.7 |
| % Survival[2] | 31 | 7 | 0.58 | 0.76 | 31 | 1 | 5.37 | **0.02** | 31 | 1 | 5.4 | **0.02** | 31 | 1 | 1.35 | 0.25 |
| Development per plant[3] | 31 | 7 | 1.56 | 0.2 | 31 | 1 | 17.13 | **0.0005** | 31 | 1 | 4.6 | **0.04** | 31 | 1 | 2.49 | 0.13 |
| Larval feeding equivalent[4] | 31 | 7 | 0.49 | 0.83 | 31 | 1 | 1.14 | 0.29 | 31 | 1 | 0.05 | 0.81 | 31 | 1 | 2.73 | 0.11 |

[1] Number of plants used in analysis to determine insect responses.
[2] Percent survival is based on collected adults over the course of the experiments and remaining life stages present on plants at the end of experiments divided by the number of initial larvae.
[3] Development is based on counts of life stages per plant and cumulative degree days required to complete larval stages to adult.
[4] Larval feeding equivalents are based on the summation of counts of instars with their proportion of total consumption and percent leaf area removed per plant for the relative proportions of consumption for each instar.

**Table 3.11. Mean (± SE) *Plutella xylostella* cohort development and survival per plant between organic and conventional fertility treatments across experiments (spring and fall).**

| ***Insect Variables***[1] | **Conventional** | **Organic** |
|---|---|---|
| Development per plant[2] | 83.9 ± 8.9 B[4] | 105.3 ± 6.63 A |
| % Survival[3] | 64 ± 0.04 A | 46 ± 0.05 B |

[1] N = insect data collected from 16 plants per treatment, per experiment.
[2] Development is based on cumulative number of degree days required for completion of larval stages to adult. Calculated as: [average cohort D-D = (number of 3rd instars x 221.75 D-D) + (number of 4th instars x 159.25 DD) + (number of pupae x 83.44 D-D) + (number of adults x 0 D-D) / total number of all stages present].
[3] Percent survival per plant is based on adults collected over the course of the experiments and remaining larvae present on plants at the end of the experiments divided by the initial number of larvae (20 larva/plant).
[4] Means followed by common letter within a row are not significantly different between experiments as determined by Fisher's Protected LSD at $P \leq 0.05$.

**Table 3.12. Mean (± SE) *Plutella xylostella* body weights and larval feeding equivalents between the spring and fall experiments across fertility (organic and conventional) treatments.**

| *Insect Variables* | Spring | N[3] | Fall | N |
|---|---|---|---|---|
| Female body weight[1] | 4.87 ± 1.10 A[2] | 62 | 2.73 ± 1.50 B | 52 |
| Male body weight[1] | 3.38 ± 0.85 A | 89 | 2.67 ± 1.72 A | 47 |
| Larval feeding equivalent[4] | 0.24 ± 0.04 A | 16 | 0.71 ± 0.11 A | 16 |

[1] Body weights reported in mg.
[2] Means followed by common letter within a row are not significantly different between experiments as determined by Fisher's Protected LSD at $P \leq 0.05$.
[3] N represents the number of insects collected in each experiment, and number of plants used for analysis of larval feeding equivalents.
[4] The larval feeding equivalent per plant was determined by multiplying the number of each instar counted on plants by the estimated relative proportion of leaf tissue required for each instar to complete development, divided by the total leaf tissue consumed.

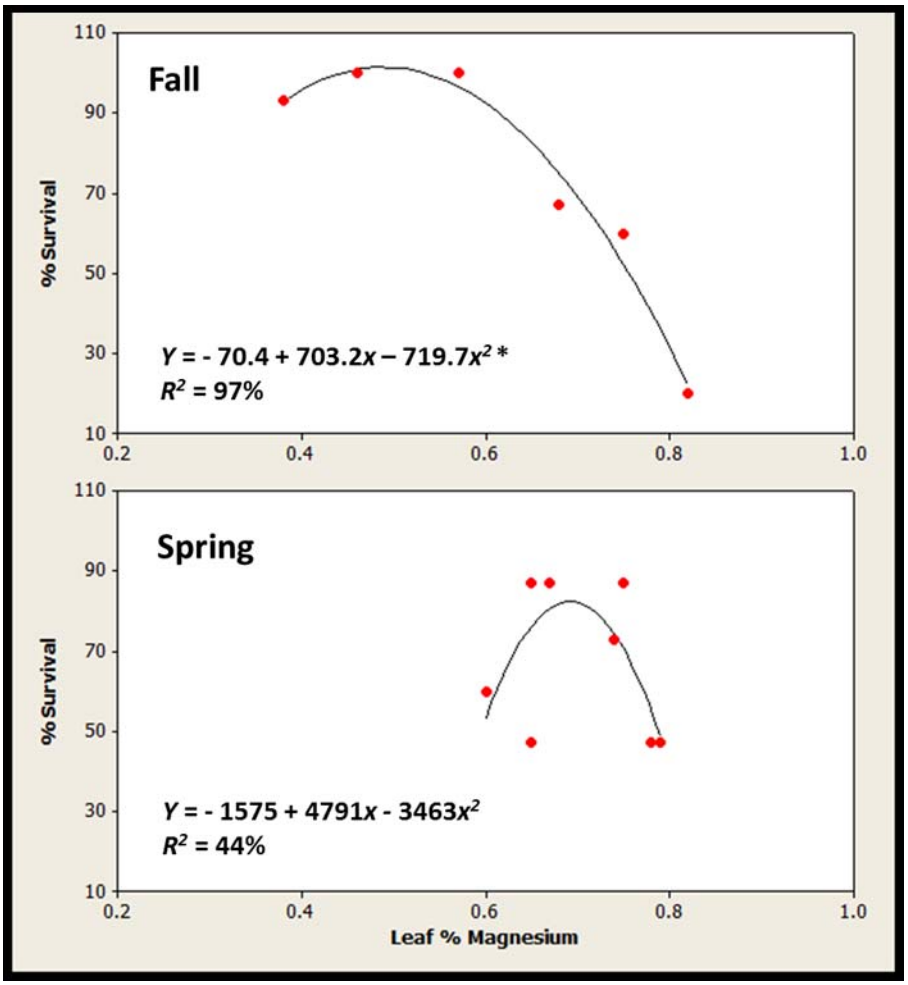


**Figure 3-1. Relationship between percent (%) survival of *Plutella xylostella* and percent (%) magnesium in *Brassica rapa* leaves for the spring and fall experiments. The best-fit relationships (quadratic) are shown, along with line equations and coefficients of determination. Starred relationships are significant at $P \leq 0.05$. Points represent survival per block (N = 4) used in the analysis.**

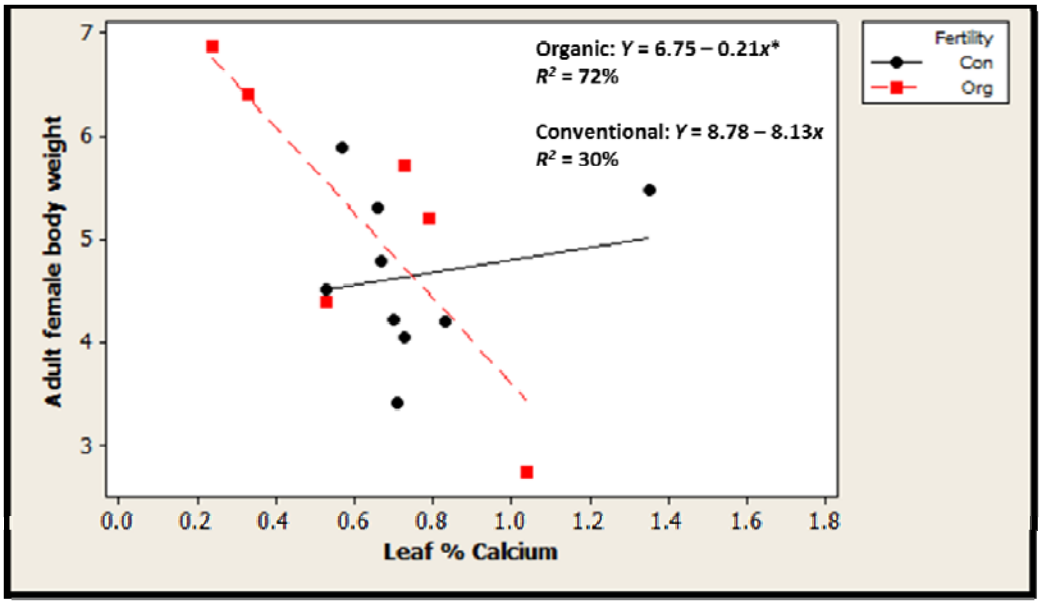


**Figure 3-2. Relationship between adult female body weight (mg) and percent (%) leaf calcium in *Brassica rapa* for the spring experiment associated with the organic (org) fertility treatment (N = 6 plants with female data) and conventional (con) fertility treatment (N = 8 plants with female data). The best-fit relationships (linear) are shown, along with line equations and coefficients of determination. Starred relationships are significant at $P \leq 0.05$.**

# Chapter 4 - Summary and Conclusions

## Summary

The goal of this research was to determine if two environmental factors, light intensity and nitrogen (N) source, affected the production of pac choi (*Brassica rapa* var. *chinensis*) primary and secondary metabolites, and also if they indirectly impacted diamondback moth (*Plutella xylostella*) (DBM) performance. Results of these studies demonstrated that both light intensity and nitrogen (N) source had some influence on the measured plant and DBM variables.

In the first study, plants with and without DBM were exposed to a range of light intensities via ambient and shade treatments conducted at four different times of the year: July, August, February and March. Pac choi and insect responses were compared between herbivore treatment, light treatment, and experiments. The plant responses measured in this study did not differ whether or not they were exposed to DBM larval feeding, suggesting that herbivory did not affect plant chemistry. Under the high light intensity treatment (mean ± SE across the four experiments: 15067 ± 280) lumens/$m^2$), plants exhibited greater shoot biomass, carbon and phenolic content, and the specific phenolic compound, ferulic acid. Higher concentrations of ferulic acid (>3 mg/100 ml methanolic extract) in pac choi were negatively correlated with adult male body weight and may have contributed to reduced body weight. Moreover, DBM development was faster under high light intensity. Plants exposed to lower light intensities (mean ± SE across the four experiments: 6452 ± 276 lumens/$m^2$) under shade conditions typically experienced greater larval consumption from DBM larvae. This result was not due to a

temperature-dependent effect. Shade plants had a significantly lower total phenolic content, and generally higher protein, compared to plants grown under high light intensity.

In the second study, applications of an organic-use fish hydrolysate fertilizer were compared to a conventional fertilizer to determine whether N source affects pac choi chemistry and growth, and if plant responses influenced DBM fitness parameters. There were no significant effects associated with the fertility treatments on growing medium leachate or pac choi nutrient and phenolic contents, with the exception of percent leaf phosphorus (P), which was significantly higher in the conventional fertility treatment. The presence of DBM on plants also had an effect on plant chemistry. Specifically, leaf Ca and Mg were significantly higher in pac choi plants infested with DBM compared to uninfested plants. Among infested plants, variation in these plant nutrients appeared to affect DBM fitness as leaf Ca was negatively correlated with female DBM body weights in both experiments (spring and fall), but only for the organic fertility treatment. In addition, DBM survival was negatively correlated with levels of leaf Mg for both fertility treatments in the fall experiment only. The type of fertility treatment used affected DBM development and survival. DBM survival was greater and development was faster on pac choi plants receiving the conventional fertilizer compared to the organic fertilizer. Results from these studies are summarized using a conceptual model presented in Figure 4-1.

## Conclusions

Results from the light intensity study are supported by findings of other investigators, which implicates light intensity as an important environmental factor capable of altering both primary and secondary plant metabolism (Muth et al. 2008; Ingersoll 2010). More specifically,

this study demonstrated that variation in light intensity, at least within the annual and light treatment ranges in the greenhouse, can affect pac choi chemistry. Evidence concerning the impact of light intensity on DBM is limited, but results from the current study indicate that light intensity may lead to changes in pac choi phenolic content, specifically the concentration of ferulic acid, which may indirectly influence male DBM body weights. The elevated concentration of ferulic acid under high light intensity corresponded to reduced male DBM body weights. Reduced body weights may affect DBM populations by limiting migration and mate-finding (Muhamad et al. 1994; Shirai 1995). As such, it may be possible that pac choi grown in protected environments under low light intensities could experience higher populations of DBM compared to pac choi grown under high light intensity. The range of light intensities tested under greenhouse conditions are much lower than light intensities found under field conditions (refer to Appendix D); however, a recent field study found that the numbers of DBM on *Brassica oleracea* in high tunnels was significantly greater (16 ± 1.5 average DBM per tunnel plot) than those in open-field plots (4.7 ± 0.7 average DBM per field plot) (Sarah Thompson, unpublished data). However, other factors associated with high tunnels, such as protection from weather, may explain the higher DBM densities. Pac choi crops grown in protected environments may be subject to more damage from DBM if population abundance is greater, but the light intensity study also found that larval consumption was not significantly different between light treatments, regardless of changes in protein and phenolic contents.

From the N source study, the organic fertility treatment yielded lower survival and reduced development of DBM compared to conventional fertility treatment. Organic fertility resulted in lower levels of leaf P, which may have promoted lower DBM fitness. However, lower fitness was not correlated with any differences in plant chemistry between fertility treatments.

Under the organic fertility treatment, higher levels of leaf Ca were associated with lower female body weights, but it is unclear whether changes in Ca would or would not influence the risk of DBM in pac choi crops. As previously stated, reduced body weights tend to reduce DBM populations. Reduced populations, having lower survival and slower development, indicates that pac choi grown with organic fertilizer could experience less susceptibility to DBM feeding compared to pac choi grown with conventional fertilizers.

This research was based on several hypotheses explaining plant defenses against insect herbivory, including the production of defensive compounds. The resource availability hypothesis (Coley et al. 1995) was supported by the light intensity study. This hypothesis states that increases in light, increases leaf C and allocation of leaf C to phenolic compounds. This did occur in pac choi within the range of light intensity that was evaluated. However, it is unclear if increases in phenolic compounds enhanced defenses against DBM. The C:N balance hypothesis, which is tied to assumptions of the resource availability hypothesis, suggests that limitations in available N for plants increases leaf C and allocation of C to phenolic compounds. The C:N balance hypothesis was not supported by the N source study. However, the fertility treatments used may not have limited N enough to test the C:N balance hypothesis, where neither leaf C nor phenolics differed between the fertility treatments. The underlying mechanisms responsible for the trends found in the N source study, including effects on DBM survival and development, are unclear and require further investigation. For example, qualitative or quantitative differences that account for decreased development and survival of DBM in the organic treatment between fertility treatments need to be examined. When such investigations are done, they may provide better evidence for the mechanism(s) involved with respect to DBM, thus contributing to the test of hypotheses related to plant defense against herbivores in general.

Although total and specific phenolic content in plants has been shown to be important in some plant-insect interactions, the results of these studies failed to demonstrate that phenolic levels in pac choi significantly affect DBM survival and development. This finding has been demonstrated in other systems. For example, increased levels of phenolics in tobacco (*Nicotiana tabacum*) had no effect on *Manduca sexta* development and survival (Bi et al. 1997; Johnson and Felton 2001). In the current study, it was predicted that herbivory from DBM larvae would increase phenolic content more so than plants not exposed to larvae. Although this prediction was not supported by the current study, several studies have also failed to demonstrate significant effects of plant phenolics on insect herbivores (Ayers et al. 1997; Johnson and Felton 2001). Regardless, it was demonstrated that both high light intensity and organic fertility treatments may have directly or indirectly affected DBM on pac choi.

This is the first study to demonstrate that concentrations of ferulic acid were increased by high light intensity, and that this phenolic compound appeared to negatively affect male DBM fitness. The results also raise interesting questions about if and how ferulic acid interacts with other phytochemicals to exert its effect and why female body weight was not affected. This is also the first study to show that pac choi receiving an organic fertilizer negatively impacted survival and development of DBM. This is an important finding because proponents of organic approaches often claim benefits for crop production and positive effects on natural enemy abundance (Phelan et al. 1996; Letourneau and Goldstein 2001; Altieri and Nicholls 2003), but rarely demonstrate direct effects on pests. As well, this study, in relation to others, indicates that the different sources of nitrogen and other components found within organic fertilizers affect both crop and pest responses.

The studies demonstrated that the effect of herbivory on plant responses may vary depending on the environment. In the light intensity study, herbivory did not affect pac choi chemistry, whereas in the N source/fertility study the presence of DBM was associated with increased levels of leaf Ca and Mg. Pac choi responses to light intensity may have obscured a response to herbivore feeding, but this is a speculation which requires further examination. Regarding N source, an absence of nutrient stress may have enabled pac choi to respond to DBM herbivory. This supports the photo-inhibition hypothesis described by Close and McArthur (2002), which suggests that plants will respond to environmental variables that reduce photosynthetic activities before responding to herbivore attack.

This research also extends what is known about the effects of plant nutrients on DBM and, in general, contributes to the literature concerning other insect herbivores. Higher Ca levels in plants had been reported to delay development time and lower adult lifespan of DBM (Sarfraz 2009); the current research adds body weight in females to the list of traits adversely affected by Ca concentration. Negative effects of Ca have also been documented for a wide range of herbivores (Molano-Flores 2001; Korth et al. 2006). More broadly, Ca and Mg are associated with multiple stress responses and signaling networks in plants associated with physical environmental as well as herbivore stress (Cakmak and Kirkby 2008).

## Limitations

The research in this dissertation has identified several trends with respect to pac choi and DBM interactions in response to environmental factors. However, the comprehensive approach

taken, logistical problems encountered, and the outcome of the data limit the conclusions that can be drawn from the two studies. Future investigations are therefore needed. This section outlines some of the limitations and caveats associated with the light intensity and fertility studies.

For the light intensity study, phenolic content was significantly higher under high light intensities compared to low light intensities, but was not directly correlated with light intensity. Since the production and/or accumulation of phenolics are affiliated with protection against any phytotoxic effects of light, the intensity of light in this greenhouse study may not have been sufficient enough to exert an increase in total phenolic content in plants, or may not have been determined by measuring light intensity alone. To assess whether phenolic content is correlated with light intensity, it may be more appropriate to measure the quality of light rather than quantifying light intensity. A method that may be used to determine plant responses to light would be to measure PAR (photosynthetically active radiation) on pac choi. This would provide a quantitative measure of the light wavelengths associated with photosynthesis in plants. Furthermore, determining pac choi exposure to ultraviolet (UV) light under varying light treatments may elucidate the results of the current study because UV illumination has been shown to increase plant resistance to herbivory (Caldwell et al. 2003; Stratmann 2003; Niesenbaum et al. 2006; Izaguirre et al. 2007).

Not all plant variables that respond to changes in light or are associated with defense against herbivores were measured in this study (Merrill 1983; Lasa et al. 2000; Morales et al. 2001). For example, a significant negative relationship between male DBM body weight and ferulic acid resulted in low coefficients of determination ($R^2$ = 42%). The low coefficient value indicates that other plant and/or environmental factors may be important in influencing male body weights. For example, plant factors, such as ascorbic acid and β-carotene, are antioxidants

essential to insect diets (Schmitz-Eiberger and Noga 2001). These antioxidants allow herbivores to counteract the negative effects of consumed phenolic compounds (Johnson 2005; Goggin et al. 2010). In fact, pac choi contains naturally high levels of ascorbic acid, which have been shown to increase in concentration under high light intensities (Rochfort et al. 2006). Assessing total antioxidant and specific levels of absorbic acid and β-carotene in pac choi may help explain the results obtained in the current research. It may also have been beneficial to determine the total antioxidant content (including ascorbic acid) of pac choi, which is accomplished using an ABTS [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)] colorimetric assay rather than the Folin-Ciocalteu method, which only detects 60% of antioxidant content (Pennycooke et al. 2005).

Other plant attributes, including leaf thickness and surface waxes of leaves, are known to affect plant-insect interactions and may be important factors to assess under varying light intensities. Guerra et al. (2010) found that *Aristotelia chilensis* had thicker leaves and less feeding damage from DBM larvae when grown under high light intensities compared to plants grown under low light intensities. Moreover, it has been shown that high levels of phenolics in leaf tissues increase leaf thickness, which may impact caterpillar feeding (Kakani et al. 2003; Rajapakse and Shahak 2007; Xu and Sullivan 2010).

For the N source study, the fertility treatments did not result in differences in leachate or plant N, suggesting that available N was not different enough between the treatments. However, additional measurements, including the amount of organic matter in the growing medium, C:N ratio in the fish hydrolysate, levels of $NH_4^+$ - N and $NO_3^-$ - N in the growing medium and plant tissue, mineralization rates, and plant available N after 7, 14, and 28 days post application may help elucidate the results of the study (Gale et al. 2006). These measurements may determine how, when, and what form of N is released from fertilizers and its availability for plant uptake.

For example, if the fish hydrolysate contained a relatively low ratio of C to N, then it would be expected that the fertilizer would release nitrogen faster (Vandermeer 1995). Fish-based fertilizers, which typically have a relatively low C:N ratio (between 4:1-8:1), are readily decomposed by microorganisms compared to fertilizers with a higher C:N ratio (Gale et al. 2006). Plant-based compost amendments tend to decompose and release N slowly, because they typically have a C:N ratio of 15:1 and higher (>15:1). As such, evaluating multiple fertilizer types, including compost amendments, may provide larger ranges of available N (Klein and Blum 1990; Haukioja et al. 1998; Lea and Morot-Gaudry 2001; Gent 2002; Le Bot et al. 2009; Wright et al. 2010). Modifications to the experimental approach that may promote differences include using several rates of N (low, medium, and high) of the organic and conventional fertilizers. Because the response to fertility treatments was similar in the current study, it may be important to examine multiple types of fertilizer materials, including compost amendments.

Other factors that could have been assessed to determine N limitation in plants include amino acid concentrations in leaf tissues. The effects of available N on nutrient content have been assessed by quantifying the specific amino acid, glutamine, in leaf tissues. Increased glutamine levels correspond to increased N because specific levels of glutamine catalyze the transformation of inorganic N to useable forms (Plaxton and McManus 2006). In fact, glutamine levels are considered to be the main factor responsible for the shift from primary to secondary metabolism in plants (Plaxton and McManus 2006).

Measuring chlorophyll content is another approach to determine the extent of N limitations in plants, as chlorophyll constitutes a majority of the N content in plants (Plaxton and McManus 2006). Changes in chlorophyll content in leaf tissue can be measured without destructive tissue sampling, using a hand-held chlorophyll flurometer, thus allowing for repeated

measures sampling, useful for determining the temporal changes in N as plants mature. In addition, studies to detect spatial variations in N, such as inner versus outer leaf whorls of pac choi, may improve our understanding of DBM feeding behaviors associated with spatial variations in N contents.

Another limitation of the N source study was not including glucosinolates in the analysis associated with pac choi chemistry, due to constraints on the amount of plant tissue required for more analyses. Glucosinolates are N- and S-containing compounds prevalent in *Brassica* spp., and known to deter generalist herbivores while stimulating feeding and oviposition in specialist herbivores such as DBM (Halkier and Gershenzon 2006). Even low concentrations of glucosinolates have been shown to stimulate feeding of DBM larvae (Louda and Mole 1992; Louda and Rodman 1996). As such, addressing the limitations, previously mentioned, in future studies may determine how light and N source affect other plant parameters that may directly or indirectly influence DBM.

## Suggestions for Future Research

Future research should concentrate on levels of ferulic acid, Ca, and Mg, which negatively impacted DBM life history traits and are also known to vary based on pac choi genotype. In fact, there are over 35 varieties of pac choi that vary in levels of Ca, Mg, and ferulic acid (Harbaum et al. 2007; Hanson et al. 2009). Characterizing DBM fitness to varietal ranges in Ca, Mg, and ferulic acid may help identify host plant resistance traits against DBM. Moreover, varietal differences in waxiness and plant size, along with nutrient levels, may also contribute to host plant resistance. Conducting varietal studies under protected environments with variable light intensities or studies using different N sources may determine important environment by

genotype interactions that inhibit DBM feeding. For example, Hsu et al. (2009) found that increases in the glucosinolate compound, sinigrin, occurred in one of two cabbage (*Brassica oleracea*) cultivars receiving an organic fertilizer, which negatively impacted the oviposition preference of *Pieris rapae crucivora*, but not for plants receiving a conventional fertilizer. Studies associated with determining natural resistance in pac choi varieties may allow producers to utilize specific pac choi cultivars that are less susceptible to DBM feeding.

Field studies evaluating light intensity and N source need to be conducted to determine if similar trends occur for pac choi and DBM in natural settings. The use of organically-managed field soil may be associated with resistance against herbivores, possibly due to levels of organic matter, soil buffering capacity, and presence of soil microbes (Reese 1979; Brodbeck et al. 1990; Phelan et al. 1995; Herms 2002; Staley et al. 2010), which are affiliated with improved plant vigor (Hadas and Rosenberg 1992; Pilbeam and Kirkby 1992; Hadas and Kautsky 1994; Oehl et al. 2004; Hsu et al. 2009). During the course of this research it was determined that evaluating DBM in the field without caging plants was not possible. However, the use of cages would have confounded the effects of light. Controlling for temperature, a key variable affecting development and survival, represents another major challenge when considering field work. However, future research could include caged feeding studies in the greenhouse under controlled environmental conditions using field soil (Phelan et al. 1996; Phelan 2004). It also may be possible to evaluate higher, near-field level, light intensities by conducting experiments in environmental growth chambers. Such studies may be enhanced by evaluating multiple types of organic fertilizers, manures, and compost amendments that provide a range of N to plants (Hartz 2000; Leite et al. 2007). Further testing is also needed to identify components of the fish hydrolysate fertilizer that contributed to the negative effects on DBM life history traits. A better

understanding of the fish hydrolysate components would supplement the findings from this research.

Beyond the assessment of light- and N source-mediated changes associated with host plant quality on the DBM, future research should consider indirect effects on natural enemies, such as predators and parasitoids. Direct and indirect tri-trophic effects on natural enemies is well-documented (reviewed by Price et al. 1980) and, along with host plant resistance, could lead to a more integrated management strategy for DBM. Relative to the present research, natural enemies may be influenced by the higher levels of Ca and Mg in pac choi in regards to their ability to locate DBM larvae. As an example, some natural enemies locate caterpillar hosts by means of volatile chemical cues released from herbivore-damaged plants, often by Ca- and Mg-associated signaling (Turlings et al. 1995; Winz and Baldwin 2001).

In addition, under the organic fertility treatment, the lower P levels consumed by DBM larvae could have an effect on the success of parasitoids since P can be a limiting factor for development in many insects (Visanuvimol and Bertram 2011). Regardless of the specific plant factor(s) involved, under the organic fertility treatment the rates of DBM development and survival were reduced, which could have consequence for natural enemies. For example, while natural enemies may benefit from prolonged exposure to hosts, they may also be negatively affected by lower host/prey abundance or by ingesting plant-derived chemicals in their DBM hosts/prey. DBM natural enemies, especially parasitoids, could be negatively impacted by the lower DBM body weights corresponding to higher concentrations of ferulic acid in pac choi under high light intensity, and higher levels of leaf Ca in the organic fertility treatment. Low body weights have been previously shown to reduce host quality for parasitoids (Ode 2006).

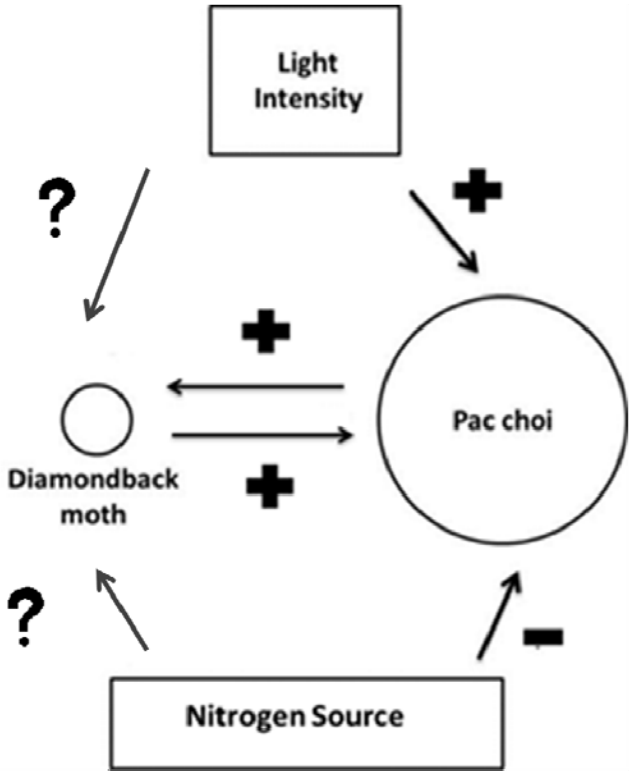


**Figure 4-1. A conceptual model summarizing study results, with arrows indicating examined relationships, + indicating a relationship was detected, – indicating a relationship was not detected, and ? indicating an unknown relationship. For the light intensity study, it was determined that 1) increases in light intensity were associated with higher phenolic contents and shoot biomass, and lower protein contents in pac choi (*Brassica rapa*) and 2) higher ferulic acid content was associated with lower male diamondback moth (*Plutella xylostella*) body weights. For the nitrogen source study, it was determined that 3) there were no significant differences between organic and conventional nitrogen sources on *B. rapa* chemistry (except levels of leaf P) or plant growth parameters; however 4) *P. xylostella* larval feeding was associated with high percent leaf calcium and magnesium contents, and survival and development of larva were negatively impacted by the organic fertility treatment. It is still not known if light intensity or nitrogen source directly affects *P. xylostella.***

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# Appendices

# Appendix A - Final *Plutella xylostella* age-class distributions associated with light intensity and nitrogen source studies.

**Table A.1. Differences in *Plutella xylostella* age-class distributions (means ± SE) on *Brassica rapa* plants in July, August, February, and March.**

| *Lifestage Classes*[1] | *July* | *August* | *February* | *March* |
|---|---|---|---|---|
| 2nd instar | 0 | 0.07 ± 0.05 B[2] | 0.47 ± 0.16 A | 0.40 ± 0.10 A |
| 3rd instar | 1.07 ± 0.26 B | 0.40 ± 0.11 B | 2.05 ± 0.27 A | 2.50 ± 0.21 A |
| 4th instar | 6.27 ± 0.64 A | 5.95 ± 2.12 B | 5.35 ± 0.54 B | 5.10 ± 0.47 B |
| Pupa | 4.25 ± 0.69 B | 7.27 ± 0.70 A | 2.42 ± 0.41 B | 1.79 ± 0.80 B |

[1] Average count of larval instars and pupae per plant recorded during final plant sampling for half of infested plants in each main plot. There were a total of 40 plants per month used in analysis and 20 larvae initialy inoculated onto each plant.
[2] Means followed by different letter within a row are significantly different between experiments. Significance was determined at $P \leq 0.05$ using Tukey's method.

**Table A.2. Differences in *Plutella xylostella* age-class distributions (means ± SE) on *Brassica rapa* plants for the spring and fall experiments.**

| *Lifestage Classes*[1] | *Spring* | *Fall* |
|---|---|---|
| 3rd instar | 2.80 ± 0.43 A[2] | 1.06 ± 0.33 B |
| 4th instar | 5.06 ± 0.58 A | 2.87 ± 0.49 B |
| Pupa | 1.50 ± 0.57 B | 4.31 ± 0.58 A |
| Adult | 2.37 ± 0.68 B | 7.93 ± 0.60 A |

[1] Average count of larval instars and pupae per plant recorded during final plant sampling and adult counts were made daily from the initation of adult emergence until completion of the experiments.There were a total of 32 plants per experiment used in analysis and 20 larvae initialy inoculated onto each plant .
[2] Means followed by different letters within a row are significantly different between spring and fall experiments. Significance was determined at $P \leq 0.05$ using Tukey's method.

# Appendix B - Tables of light intensity analysis of variance (ANOVA) for each light intensity experiment.

**Table B.1. July Experiment. Results of analysis of variance (ANOVA) for light intensity and temperature, plant (*Brassica rapa*) response variables, and insect (*Plutella xylostella*) response variables, by light (ambient and shade treatments), light replicate (5 replicates each of ambient and shade treatment), herbivore (plants with and without *P. xylostella*). Values in bold are significant ($P \leq 0.05$).**

| | *Light* [1] | | | | *Light Replicate* [2] | | | | *Herbivore* [3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Environmental Variables*** | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* |
| Light Intensity (lumens/$m^2$) | 34.37 | **<0.001** | 10 | 1 | 0.67 | 0.50 | 5 | 4 | | | | |
| Temperature (°C) | 28.93 | **<0.001** | 10 | 1 | 0.82 | 0.37 | 5 | 4 | | | | |
| ***Plant Response Variables*** | | | | | | | | | | | | |
| C:N Ratio | 155.67 | **<0.001** | 20 | 1 | 0.73 | 0.61 | 5 | 4 | 0.35 | 0.57 | 10 | 1 |
| % Protein | 270.03 | **<0.001** | 20 | 1 | 1.47 | 0.27 | 5 | 4 | 0.39 | 0.54 | 10 | 1 |
| Phenolic content (GAE/g FW) | 3.29 | 0.09 | 20 | 1 | 1.46 | 0.27 | 5 | 4 | 19.35 | **<0.001** | 10 | 1 |
| Chlorogenic Acid (mg/g FW) | 0.19 | 0.67 | 20 | 1 | 1.05 | 0.42 | 5 | 4 | 0.24 | 0.63 | 10 | 1 |
| Caffeic Acid (mg/g FW) | 5.46 | **0.04** | 20 | 1 | 2.51 | 0.10 | 5 | 4 | 1.38 | 0.26 | 10 | 1 |
| Sinapic Acid (mg/g FW) | 1.63 | 0.22 | 20 | 1 | 0.82 | 0.54 | 5 | 4 | 0.10 | 0.76 | 10 | 1 |
| Ferulic Acid (mg/g FW) | 0.12 | 0.73 | 20 | 1 | 0.57 | 0.68 | 5 | 4 | 1.06 | 0.32 | 10 | 1 |
| L-7-O-G (mg/g FW) | 2.32 | 0.15 | 20 | 1 | 0.94 | 0.47 | 5 | 4 | 1.09 | 0.31 | 10 | 1 |
| Moisture Content | 1.87 | 0.24 | 20 | 1 | 2.07 | 0.24 | 5 | 4 | 0.00 | 1.00 | 10 | 1 |
| Biomass (g) | 6.78 | **0.05** | 20 | 1 | 0.20 | 0.92 | 5 | 4 | 4.32 | 0.07 | 10 | 1 |
| ***Insect Response Variables*** | | | | | | | | | | | | |
| Male adult weight (mg) | 2.77 | 0.17 | 10 | 1 | 0.72 | 0.61 | 5 | 4 | | | | |
| Male pupal weight (mg) | 0.85 | 0.40 | 10 | 1 | 0.26 | 0.88 | 5 | 4 | | | | |
| Female pupal weight (mg) | 3.07 | 0.15 | 10 | 1 | 3.53 | 0.12 | 5 | 4 | | | | |
| Female adult weight (mg) | 4.20 | 0.10 | 10 | 1 | 1.46 | 0.36 | 5 | 4 | | | | |

[1] Light intensity and temperature correspond to 10 light treatments used in analysis, N = 10. Plant variables correspond to 10 light treatments with *P. xylostella* and without *P. xylostella*, N =20. Insect variables correspond to 10 light treatments with *P. xylostella*, N = 10.
[2] Light intensity and temperature correspond to 5 ambient or 5 shade treatments used in analysis, N = 5. Plant and insect responses correspond to 5 ambient or 5 shade treatments, N = 5.
[3] Plant response variables correspond to 10 treatments with *P. xylostella*, N = 10.

**Table B.2. August Experiment. Results of analysis of variance (ANOVA) for light intensity and temperature, plant (*Brassica rapa*) response variables, and insect (*Plutella xylostella*) response variables, by light (ambient and shade treatments), light replicate (5 replicates each of ambient and shade treatment), herbivore (plants with and without *P. xylostella*). Values in bold are significant ($P \leq 0.05$).**

| | *Light* [1] | | | | *Light Replicate* [2] | | | | *Herbivore* [3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Environmental Variables*** | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* |
| Light Intensity (lumens/$m^2$) | 298.27 | **<0.001** | 10 | 1 | 0.93 | 0.19 | 5 | 4 | | | | |
| Temperature (°C) | 11.74 | **0.004** | 10 | 1 | 1.89 | 0.16 | 5 | 4 | | | | |
| ***Plant Response Variables*** | | | | | | | | | | | | |
| C:N Ratio | 148.40 | **<0.001** | 20 | 1 | 1.22 | 0.35 | 5 | 4 | 0.04 | 0.83 | 10 | 1 |
| % Protein | 39.49 | **0.002** | 20 | 1 | 0.41 | 0.79 | 5 | 4 | 0.12 | 0.73 | 10 | 1 |
| Phenolic content (GAE/g FW) | 2.66 | 0.17 | 20 | 1 | 1.25 | 0.41 | 5 | 4 | 2.99 | 0.12 | 10 | 1 |
| Chlorogenic Acid (mg/g FW) | 1.89 | 0.24 | 20 | 1 | 2.05 | 0.27 | 5 | 4 | 2.58 | 0.16 | 10 | 1 |
| Caffeic Acid (mg/g FW) | 1.98 | 0.25 | 20 | 1 | 1.98 | 0.33 | 5 | 4 | 2.41 | 0.17 | 10 | 1 |
| Sinapic Acid (mg/g FW) | 10.46 | **0.002** | 20 | 1 | 6.22 | **0.008** | 5 | 4 | 10.60 | **0.008** | 10 | 1 |
| Ferulic Acid (mg/g FW) | 12.99 | **0.002** | 20 | 1 | 1.97 | 0.17 | 5 | 4 | 0.63 | 0.44 | 10 | 1 |
| L-7-O-G (mg/g FW) | 3.69 | 0.12 | 20 | 1 | 0.52 | 0.72 | 5 | 4 | 3.27 | 0.11 | 10 | 1 |
| Moisture Content | 0.02 | 0.88 | 20 | 1 | 0.90 | 0.53 | 5 | 4 | 0.00 | 0.99 | 10 | 1 |
| Biomass (g) | 26.47 | **0.006** | 20 | 1 | 3.29 | 0.13 | 5 | 4 | 1.19 | 0.30 | 10 | 1 |
| ***Insect Response Variables*** | | | | | | | | | | | | |
| Male adult weight (mg) | 0.18 | 0.69 | 10 | 1 | 0.15 | 0.95 | 5 | 4 | | | | |
| Male pupal weight (mg) | 0.27 | 0.63 | 10 | 1 | 4.02 | 0.10 | 5 | 4 | | | | |
| Female pupal weight (mg) | 0.06 | 0.82 | 10 | 1 | 0.58 | 0.70 | 5 | 4 | | | | |
| Female adult weight (mg) | 0.01 | 0.98 | 10 | 1 | 3.16 | 0.18 | 5 | 4 | | | | |

[1] Light intensity and temperature correspond to 10 light treatments used in analysis, N = 10. Plant variables correspond to 10 light treatments with *P. xylostella* and without *P. xylostella*, N =20. Insect variables correspond to 10 light treatments with *P. xylostella*, N = 10.
[2] Light intensity and temperature correspond to 5 ambient or 5 shade treatments used in analysis, N = 5. Plant and insect responses correspond to 5 ambient or 5 shade treatments, N = 5.
[3] Plant response variables correspond to 10 treatments with *P. xylostella*, N = 10.

**Table B.3. February Experiment. Results of analysis of variance (ANOVA) for light intensity and temperature, plant (*Brassica rapa*) response variables, and insect (*Plutella xylostella*) response variables, by light (ambient and shade treatments), light replicate (5 replicates each of ambient and shade treatment), herbivore (plants with and without *P. xylostella*). Values in bold are significant ($P \leq 0.05$).**

| | *Light* [1] | | | | *Light Replicate* [2] | | | | *Herbivore* [3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Environmental Variables*** | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* |
| Light Intensity (lumens/m$^2$) | 64.43 | **<0.001** | 10 | 1 | 0.99 | **0.06** | 5 | 1 | | | | |
| Temperature (°C) | 0.14 | 0.71 | 10 | 1 | 9.96 | **<0.001** | 5 | 1 | | | | |
| ***Plant Response Variables*** | | | | | | | | | | | | |
| C:N Ratio | 1.31 | 0.31 | 20 | 1 | 0.77 | 0.59 | 5 | 1 | 0.54 | 0.48 | 10 | 1 |
| % Protein | 28.14 | **<0.001** | 20 | 1 | 1.19 | 0.36 | 5 | 1 | 0.13 | 0.72 | 10 | 1 |
| Phenolic content (GAE/g FW) | 1.04 | 0.36 | 20 | 1 | 0.90 | 0.53 | 5 | 1 | 0.58 | 0.47 | 10 | 1 |
| Chlorogenic Acid (mg/g FW) | 0.01 | 0.92 | 20 | 1 | 1.43 | 0.40 | 5 | 1 | 0.08 | 0.80 | 10 | 1 |
| Caffeic Acid (mg/g FW) | 0.66 | 0.47 | 20 | 1 | 0.85 | 0.57 | 5 | 1 | 0.40 | 0.59 | 10 | 1 |
| Sinapic Acid (mg/g FW) | 13.13 | **0.008** | 20 | 1 | 1.59 | 0.27 | 5 | 1 | 0.02 | 0.90 | 10 | 1 |
| Ferulic Acid (mg/g FW) | 3.85 | 0.18 | 20 | 1 | 3.90 | 0.20 | 5 | 1 | 0.51 | 0.54 | 10 | 1 |
| L-7-O-G (mg/g FW) | 0.01 | 0.91 | 20 | 1 | 5.31 | **0.02** | 5 | 1 | 0.72 | 0.42 | 10 | 1 |
| Moisture Content | 10.73 | **0.03** | 20 | 1 | 0.83 | 0.57 | 5 | 1 | 13.85 | **0.009** | 10 | 1 |
| Biomass (g) | 0.77 | 0.41 | 20 | 1 | 2.70 | 0.13 | 5 | 1 | 2.21 | 0.18 | 10 | 1 |
| ***Insect Response Variables*** | | | | | | | | | | | | |
| Male adult weight (mg) | 2.17 | 0.23 | 10 | 1 | 0.02 | 0.99 | 5 | 1 | | | | |
| Male pupal weight (mg) | 2.31 | 0.22 | 10 | 1 | 0.001 | 0.99 | 5 | 1 | | | | |
| Female pupal weight (mg) | 4.46 | 0.12 | 10 | 1 | 0.13 | 0.95 | 5 | 1 | | | | |
| Female adult weight (mg) | 0.16 | 0.71 | 10 | 1 | 0.001 | 0.99 | 5 | 1 | | | | |

[1] Light intensity and temperature correspond to 10 light treatments used in analysis, N = 10. Plant variables correspond to 10 light treatments with *P. xylostella* and without *P. xylostella*, N =20. Insect variables correspond to 10 light treatments with *P. xylostella*, N = 10.
[2] Light intensity and temperature correspond to 5 ambient or 5 shade treatments used in analysis, N = 5. Plant and insect responses correspond to 5 ambient or 5 shade treatments, N = 5.
[3] Plant response variables correspond to 10 treatments with *P. xylostella*, N = 10.

**Table B.4. March Experiment. Results of analysis of variance (ANOVA) for light intensity and temperature, plant (*Brassica rapa*) response variables, and insect (*Plutella xylostella*) response variables, by light (ambient and shade treatments), light replicate (5 replicates each of ambient and shade treatment), herbivore (plants with and without *P. xylostella*). Values in bold are significant ($P \leq 0.05$).**

| | *Light* [1] | | | | *Light Replicate* [2] | | | | *Herbivore* [3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Environmental Variables*** | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* | *F* | *P* | *N* | *df* |
| Light Intensity (lumens/$m^2$) | 89.87 | **<0.001** | 10 | 1 | 0.22 | 0.92 | 5 | 4 | | | | |
| Temperature (°C) | 1.84 | 0.19 | 10 | 1 | 0.33 | 0.30 | 5 | 4 | | | | |
| ***Plant Response Variable*** | | | | | | | | | | | | |
| C:N Ratio | 11.02 | **0.006** | 20 | 1 | 2.45 | 0.10 | 5 | 4 | 0.22 | 0.64 | 10 | 1 |
| % Protein | 0.14 | 0.71 | 20 | 1 | 0.70 | 0.60 | 5 | 4 | 0.79 | 0.39 | 10 | 1 |
| Phenolic content (GAE/g FW) | 3.02 | 0.15 | 20 | 1 | 0.35 | 0.83 | 5 | 4 | 4.05 | 0.07 | 10 | 1 |
| Chlorogenic Acid (mg/g FW) | 19.45 | **0.008** | 20 | 1 | 0.37 | 0.82 | 5 | 4 | 0.16 | 0.69 | 10 | 1 |
| Caffeic Acid (mg/g FW) | 3.41 | 0.08 | 20 | 1 | 0.31 | 0.86 | 5 | 4 | 1.23 | 0.28 | 10 | 1 |
| Sinapic Acid (mg/g FW) | 17.40 | **0.001** | 20 | 1 | 0.47 | 0.75 | 5 | 4 | 0.17 | 0.68 | 10 | 1 |
| Ferulic Acid (mg/g FW) | 3.37 | 0.09 | 20 | 1 | 1.25 | 0.34 | 5 | 4 | 1.18 | 0.29 | 10 | 1 |
| L-7-O-G (mg/g FW) | 0.47 | 0.53 | 20 | 1 | 0.41 | 0.79 | 5 | 4 | 0.001 | 0.97 | 10 | 1 |
| Water Content | 1.42 | 0.29 | 20 | 1 | 0.39 | 0.80 | 5 | 4 | 0.00 | 1.00 | 10 | 1 |
| Biomass (g) | 44.90 | **0.002** | 20 | 1 | 0.89 | 0.54 | 5 | 4 | 0.001 | 0.97 | 10 | 1 |
| ***Insect Response Variables*** | | | | | | | | | | | | |
| Male adult weight (mg) | 10.53 | **0.04** | 10 | 1 | 25.30 | **0.01** | 5 | 1 | | | | |
| Male pupal weight (mg) | 0.62 | 0.48 | 10 | 1 | 0.70 | 0.64 | 5 | 1 | | | | |
| Female pupal weight (mg) | 0.76 | 0.47 | 10 | 1 | 4.06 | 0.35 | 5 | 1 | | | | |
| Female adult weight (mg) | 0.63 | 0.57 | 10 | 1 | 1.30 | 0.55 | 5 | 1 | | | | |

[1] Light intensity and temperature correspond to 10 light treatments used in analysis, N = 10. Plant variables correspond to 10 light treatments with *P. xylostella* and without *P. xylostella*, N =20. Insect variables correspond to 10 light treatments with *P. xylostella*, N = 10.
[2] Light intensity and temperature correspond to 5 ambient or 5 shade treatments used in analysis, N = 5. Plant and insect responses correspond to 5 ambient or 5 shade treatments, N = 5.
[3] Plant response variables correspond to 10 treatments with *P. xylostella*, N = 10.

# Appendix C - Effect of ferulic acid on *Plutella xylostella* in artificial diet study.

Based on results from the greenhouse light intensity study, an artificial diet study was conducted to determine the effect of varying ferulic acid concentrations on male diamondback moth (*Plutella xylostella*) (DBM) larvae, and also to assess whether high concentrations of ferulic acid would negatively impact female DBM. The diet study also provided an opportunity to evaluate measures of DBM fitness not measured in the light intensity study. To further elucidate the potential effects of ferulic acid on DBM, two experiments were conducted to modify the concentrations of ferulic acid that larvae were exposed to (Coleman et al. 2005).

## *Materials and Methods*

An artificial diet specific for rearing DBM was obtained from Bio-Serve, product #F9441B (Frenchtown, NJ). The exact diet was product # F9441B. The components of this diet are proprietary information, but are comprised primarily of soy-protein and the antibiotic, aureomycin. Diets were independently prepared in a concentration series (0, 1, 3, 5, and 7 mg/g of ferulic acid) with 15 replicate diet cups for each ferulic acid concentration in the first experiment and 20 replicate cups in the second experiment. The replicate number was increased in the second experiment to enhance the sample sizes for male and female DBM body weights.

Crystalline ferulic acid (4-hydroxy-3-methoxycinnamic acid) powder (Fisher Scientific; Denver, CO) was solubilized before adding into the prepared diet. For the concentrations of 1 and 3 mg/g ferulic acid, the powder was solubilized in 1 mL of ethanol, whereas the 5 and 7 mg/g ferulic acid concentrations were solubilized in 3 mL of ethanol and evaporated to 1 mL. Ferulic acid solutions were vortexed in a 10 mL vial until dissolved. A control with 1 mL of

ethanol and no ferulic acid was also included. The DBM diet was prepared by adding 2 g of agar to 88 mL of cold $H_2O$. The agar and $H_2O$ were allowed to reach boiling point on a hot-plate, then immediately removed. The agar solution was then poured into a glass mixing bowl, where 27 g of dry diet powder and solubilized ferulic acid was mixed in using an egg-beater, yielding a total of 0.1 L of prepared diet. Before the diet solidified, 5 mL was syringe injected into 30 mL, clear polyethylene creamer cups (Bio-Serve; Frenchtown, NJ) and allowed to dry for 10 min. For each ferulic acid concentration, an extra 5 mL was injected into a 50 mL Falcon tube, which was used to assay for total phenolic content, and ensured that ferulic acid concentrations increased in the treatment series.

Experiments were arranged in a completely randomized design. In the first experiment, 75 diet cups were placed onto five Styrofoam trays, with three rows of five cups per tray. In the second experiment, 100 diet cups were placed onto six Styrofoam trays. Experiment 1 was conducted from January 24 to February 3, 2011, and experiment 2 was performed from February 9 to February 26, 2011.

A colony of DBM obtained from Benson Research (Carlisle, PA) was maintained in a 1.4 mm mesh cage (0.60 x 0.60 x 0.91 m) on four to five pac choi plants under greenhouse conditions (20-23°C) and exposed to natural day-light. Second larval instars were transferred from the colony using a paintbrush and placed into each diet cup. Cups were then sealed with white paper caps (Bio-Serve; Frenchtown, NJ), and then cup trays were placed into a growth chamber set at 20 ± 1°C with a 12:12 (L:D HR) photoperiod.

Total phenolic content of the diets were analyzed using the Folin-Ciocalteu method (Pennycooke et al. 2005). Three samples per diet treatment were analyzed for a total of 15 samples associated with each experiment. Approximately 1 g of diet was mixed with 3 mL of

80% (v/v) acetone by vortexing for 1 min. For each sample, 1 mL of mixture was poured into a 1.5 mL microcentrifuge tube (Fisher Scientific; Denver, CO), which was then covered with aluminum and stored overnight at 5°C. The 15 samples were then centrifuged for 2 min at 112 RCF (relative centrifugal force). Fifty mL of supernatant was then pipetted into a new 1.5 mL tube and mixed with 135 µL $H_2O$, 750 µL of 1/10 dilution Folin-Ciocalteu reagent (Sigma-Aldrich; St. Louis, MO), and 600 µL 7.5% (w/v) $Na_2CO_3$. Samples were vortexed for 10 sec and incubated at 45°C in a water bath for 15 min. Samples were allowed to cool to room temperature before reading the absorbance at 765 nm (U-1100 spectrophotometer; Hitachi Ltd. Japan) (Pennycooke et al. 2005). A gallic acid standard curve was prepared using 1 mg/mL gallic acid (Acros Organics; Belgium) in 80% (v/v) acetone) stock solution. Total phenolics were reported as gallic acid equivalents (GAE)/g of medium.

Diet cups were checked daily to record the days to pupation and adult emergence. After adult emergence, individual diet cups were transferred to a freezer set at -20°C for 2-3 min to incapacitate insects long enough to determine sex and weight to the nearest 0.001 mg. Male DBM were identified by the distinctive diamond-shaped pattern on the forewings, which is not present on females (Shirai 1993; Muhamad et al. 1994). In addition, percent survival was determined for each treatment as the number of live moths per treatment divided by the total initial number of larvae per treatment multiplied by 100.

***Statistical Analysis***

Data pertaining to days to pupation, adult emergence, percent surival, and body weights were analyzed using SAS Systems for Windows, Version 9.1 (SAS Institute 2002). For each experiment, data were subjected to ANOVA using a general linear model by ferulic acid concentration. Means were separated using Tukey's test procedures (SAS Institute 2002). To

normalize percent data, survival was arcsin transformed prior to analysis. Because there was significant variation in survival among ferulic acid concentrations, results for each experiment are presented (non-transformed) in Chapter 2 (Table 2.8).

# Appendix D - Effect of light intensity on pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') total phenolic content under tunnel and field conditions.

Similar to the greenhouse light intensity study, a field trial was conducted to evaluate the effects of light intensity on phenolic content in pac choi under field conditions in open plots and under low tunnels during spring (April 5-June 26) and fall (September 8-October 6), 2009, at the Rocky Ford Research Station, Manhattan, KS. Low tunnels were used to represent conditions similar to high tunnels, which were not available for use (Figure D-1).

### *Materials and Methods*

*Experimental design.* Six experimental low tunnels (3.0 m x 1.5 mx 1.2 m) were arranged in a randomized complete block design with 6 replicates of field and low tunnel plots with 1.2 m spacing between plots.

*Tunnel and irrigation design.* Low tunnels (Figure D-1) consisted of 1.2 cm metal dowels and polyvinyl chloride pipe (1.9 cm) frames (1.2 x 3.6 m) covered with clear 6.0 mm polyethylene (DuraGreen: DuraGreen Marketing Inc. LLC; Mount Dora, FL). Specifications associated with materials and construction are presented in Figure D-2. The irrigation system consisted of drip-tape lines, attached to a 1.9 cm mainline, which extended the length of each planted row. The mainline was attached using a 250 psi pressure valve, 20 mesh filter, and timer, to an existing water supply.

*Plants.* Four-hundred and eighty, greenhouse-grown, four-week-old pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') plants were transplanted into field and low tunnel plots.

Each plot consisted of 4 rows spaced 0.3 m apart with 10 plants per row spaced 0.3 m apart. End row plants were not used but served to buffer any edge-effects. HOBO data loggers (Onset; MicroDaq, Contoocook, NH) were placed in the middle of each field and tunnel plot and used to record temperature and light intensity. Readings were taken at 1-h intervals for the duration of each of the two experiments.

*Sampling for plant chemistry*. Plants were sampled for total phenolic in the leaves (see Chapter 2, Materials and Methods for total phenolic content assay). Three weeks after transplanting, six plants in each plot were randomly chosen for analysis. Two leaves were removed from the middle whorl and immediately frozen in liquid nitrogen. Leaf samples were then stored in a freezer set at -20°C for one month until analysis.

*Statistical analysis*. Data for light intensity, temperature, and total phenolic content were subjected to an analysis of variance (ANOVA) using the PROC GLM procedure in SAS Systems for Windows, version 9.1 (SAS Institute Inc. 2002) with plot (open field and low tunnel) as the main effect. A Fisher's Protected LSD test was used to make mean comparisons.

## *Results and Discussion*

Table D.1 presents results associated with light intensity, temperature, and total phenolic content for plants grown in the open field and low tunnel plots for spring and fall experiments, along with light intensities associated with the greenhouse study. Light intensities in open field plots were significantly higher than low tunnel plots in both experiments (Table D.1). In the spring experiment, pac choi in the field plots had a significantly higher total phenolic content than pac choi in the tunnel plots (Table D.1). In the fall experiment, pac choi grown in field plots had a significantly higher total phenolic content than pac choi grown in the tunnel plots (Table D.1). Light intensities under ambient conditions in the greenhouse study were approximately half

of that found under the lowest light intensity recorded in the field study. Phenolic content of pac choi was higher in both light treatments in the greenhouse experiment compared to pac choi in the field study.

For the spring and fall field experiments, the phenolic content of pac choi was higher in open field plots under high light intensity compared to low tunnel plots under low light, which suggests that higher light intensities correspond to higher phenolic content. Based on findings in other studies, phenolic contents should increase with increasing light intensity (Mole and Waterman 1988; Shure and Wilson 1993; Dudt and Shure 1994; Louda and Rodman 1996; Niesenbaum and Kluger 2006). However, at lower light intensities associated with the greenhouse experiment, phenolic contents in pac choi were higher than pac choi grown under open field and low tunnel plots. This demonstrates that light intensity may not be influencing phenolic contents in pac choi grown under field conditions (open field or low tunnels). Other environmental factors affiliated with the field and greenhouse settings may have directly or indirectly contributed to the lower phenolics in the field, such as soil moisture, humidity, or light quality (Dixon and Paiva 1995; Kolkmann and Muller 2009).

**Table D.1.** Light intensity, temperature, and total phenolic content (mean ± SE) in *Brassica rapa* grown in open field and low tunnel plots for spring and fall experiments, along with means associated with pac choi grown under different light treatments (ambient and shade) in a greenhouse study.

| | *Spring Experiment* | | *Fall Experiment* | | *Greenhouse Study*[4] | |
|---|---|---|---|---|---|---|
| ***Variables*** | **Open Field** | **Low Tunnel** | **Open Field** | **Low Tunnel** | **Ambient** | **Shade** |
| **Light intensity**[1] | 47,892.7 ± 2722.3 A [3] | 35,260.5 ± 7187.6 B | 54,843.7 ± 2517.8 A | 49,377.6 ± 6897.1 B | 15,053 ± 677 | 6,445 ± 290 |
| **Temperature** | 28.4 ± 0.34 A | 28.8 ± 0.33 A | 18.81 ± 0.17 A | 16.8 ± 1.74 B | 22.2 ± 0.82 | 23.2 ± 0.96 |
| **Total phenolic content**[2] | 0.47 ± 0.10 A | 0.36 ± 0.08 B | 0.51 ± 0.18 A | 0.37 ± 0.07 B | 0.63 ± 0.03 | 0.54 ± 0.02 |

Light intensity (lumens/$m^2$) and temperature (°C) are averages of 6 low tunnel and 6 open field plots, where measurements were recorded at 1-hr intervals using HOBO data loggers. Only light intensity values recorded during daylight hours were used in the analysis.

[2] Total phenolic content is reported as (GAE)/g FW of plant tissue. There were a total of 6 plants per plot used in the analysis.

[3] Means for the open field and the low tunnel plots followed by the same upper case letter are not significantly different within a row by experiment $P \leq 0.05$ based on Fisher's Protected LSD test.

[4] Greenhouse data from light intensity study (refer to Chapter 2). Average light intensities (lumens/$m^2$) for ambient and shade light treatments across the four experiments. Only data recorded during daylight hours were used for the analysis. Average temperature (°C) for ambient and shade treatments across the four experiments. Data were recorded at 30-min intervals using HOBO data loggers.

**Figure D-1. Low tunnels and adjacent field plots consisted of four rows of ten plants each used to determine the effects of light intensity on *Brassica rapa* phenolic content.**

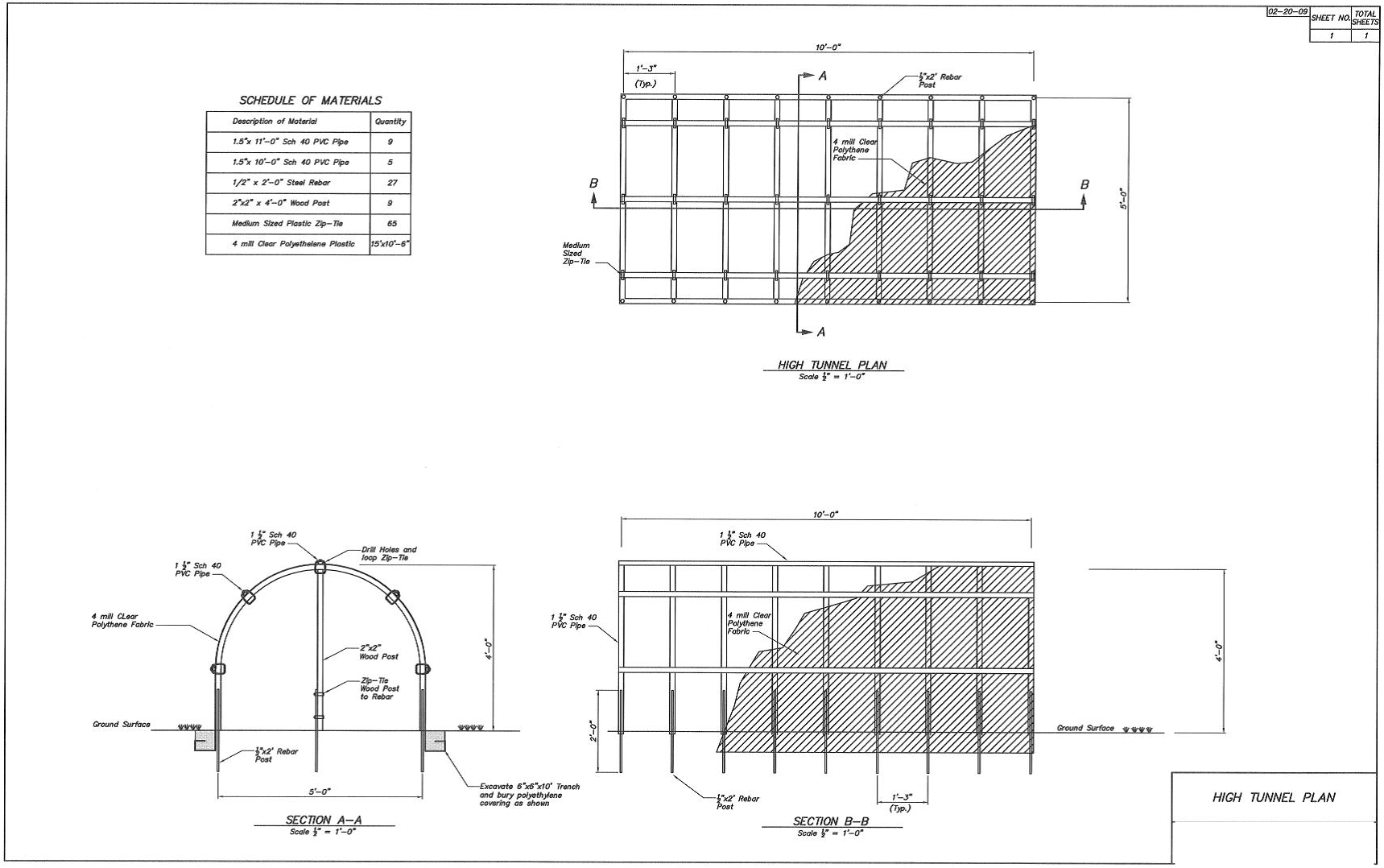


**Figure D-2. Design specifications for low tunnel construction.**

# Appendix E - 'Bellows method' for collecting adult *Plutella xylostella* moths.

A foot pump (23.8 cm x 33.7 cm x 7.6 cm) (Airhead; Target, Minneapolis, MN) was used to construct a moth-collecting device (Figure E-1). The foot pump used high-volume bellows to inflate or deflate at 910 MPa air pressure. The pump was connected to a 137.2 cm hose and valve adaptor. Polyethylene (5 cm x 0.60 m) tubing was connected to the valve adaptor. The free-end of the tubing was covered with nylon 16 mesh and glued (Gorilla Glue; Target, Minneapolis, MN) in place. The bottom of a clear polyethylene vial (20 ml) was cut to fit inside the plastic tubing, which was then glued in place. Inserting the hose and vial into the insect cages allowed for the removal of adult moths without disturbing or allowing escape of other *Plutella xylostella.* With the deflating action of the foot pump, adult *P. xylostella* moths were vacuumed into the vial with mesh screening. Finally, this action temporarily disoriented adult moth (W. Johnson personal observations), thus easing transfer into collection vials.

**Figure E-1. Foot pump device used to collect adult *Plutella xylostella* moths from cages.**