Effect of Light Intensity on *Brassica rapa* Chemistry and *Plutella xylostella* (Lepidoptera: Plutellidae) Life History Traits[1].

W.A. Johnson[2], J.R. Nechols, R.A. Cloyd, D. Rotenberg[3] and M.M., Kennelly[3]

Department of Entomology, Kansas State University, 123 Waters Hall, Manhattan, Kansas 66506 USA

J. Entomol. Sci. 47(4): 327-349 (October 2012)

Abstract Greenhouse studies were conducted to examine the effects of light intensity on pac choi (*Brassica rapa* L. var. *chinensis* cv. 'Mei Qing Choi') and the corresponding plant-mediated effects of light intensity on the diamondback moth (*Plutella xylostella* L.). Plants with and without diamondback moth larvae were exposed either to ambient light or shade in 4 experiments conducted at different times of the year, resulting in a range of light intensities. The plant parameters measured were shoot biomass, primary nutrients, and phenolic content of leaves; for diamondback moth, larval consumption, cohort development, and male and female body weights were measured. In the light treatments, plants tended to have higher levels of phenolics and exhibited greater shoot biomass with higher carbon:nitrogen ratios under ambient versus shade. However, diamondback moth responses were not correlated with any plant responses, with one exception. Higher concentrations of ferulic acid under higher light intensities were associated with lower adult male body weights. Larval consumption was not different among the 4 months. However, cohort development was more rapid on plants during August, which had higher light intensities, compared with July, where light intensity was lower. Based on the responses measured, growing pac choi in the greenhouse under different light intensities is unlikely to affect diamondback moths through changes in pac choi chemistry unless decreased male body weight confers a fitness cost. However, plant-mediated effects associated with changes in light intensity on *P. xylostella* survival should be evaluated.

Key Words diamondback moth, *Plutella xylostella*, *Brassica rapa*, light intensity, plant-mediated herbivore responses

Light is a fundamental requirement for plant growth and development. Both the quality and quantity of light influence the degree to which photosynthesis occurs (Aldrich and Bartok 1994). A reduction in light intensity tends to reduce the photosynthesis (Mooney and Gulmon 1982). A lower photosynthetic rate then decreases the stores of carbon available for the synthesis of secondary metabolites used for defense to the production of primary metabolites such as amino acids and proteins (Schoonhoven et al. 2005). 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 carbon is unavailable for producing secondary compounds, including those compounds that serve as plant defenses against insect herbivores and environmental stress (Bryant et al. 1983).

[1]Received 23 January; accepted for publication 26 April 2012.
[2]Corresponding author (email: wendyann@ksu.edu).
[3]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Light intensity can have an important influence on herbivore performance by altering leaf nitrogen and protein levels, both of which are essential for insect growth and development (Dudt and Shure 1994, Louda and Rodman 1996, Niesenbaum and Kluger 2006, Behmer 2009). Light intensity may also affect the accumulation of secondary compounds, primarily phenolic compounds, which function broadly to alleviate plant stress (Muth et al. 2008), or more specifically by providing a source of resistance to insect herbivores (reviewed by Dixon and Paiva 1995, Felton 1996, reviewed by Bassman 2004, Schijlen et al. 2004, Oh and Rajashekar 2009). For example, under high light intensities, elevated concentrations of the phenolic compound ferulic acid has been shown to be functionally linked to lignin in cell walls, resulting in increased leaf thickness (McKey 1979, Lewis and Sarkanen 1998). 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 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). For example, the phenolic compounds caffeic acid and sinapic acid have been shown to negatively affect herbivores (Felton 1996, Stout et al. 1998) either by causing direct toxicity or by reducing protein quality or availability during digestion (Cooper-Driver et al. 1977, Caldwell et al. 1983, 1998, 2003, Bieza and Lois 2001).

Despite evidence linking light intensity with plant quality and associated impacts on insect herbivores (Izaguirre et al. 2007), the specific responses of herbivores to changes in plant chemistry are variable (Mole and Waterman 1988, Shure and Wilson 1993). For example, little is known about how variation in the light environment influences the balance between phenolic and nutrient contents of plants (Ingersoll et al. 2010), and what the relative importance of each is with respect to population growth and performance of insect herbivores (Close and McArthur 2002).

The diamondback moth (*Plutella xylostella* L.) and one of its host plants, pac choi (*Brassica rapa* L.), were used in this study to explore effects of light environment on plant-insect interactions. The diamondback moth is a serious insect pest of worldwide importance on cruciferous crops (Liu et al. 2002, Talekar et al. 2003). Pac choi is a cruciferous plant grown widely as a food crop. Prior studies with pac choi have established that primary and secondary metabolism change in response to conditions in the growing environment (Zhao et al. 2007). Pac choi is also known to contain a high amount of phenolic compounds compared with other crucifers, which makes it an excellent choice for assessing the effects of phenolics on the diamondback moth (Harbaum et al. 2007, Lin and Harnly 2010). Therefore, the specific objectives of this research were to (1) examine the effects of light intensity on various components of pac choi chemistry and plant growth, and (2) assess whether light intensity effects on pac choi correspond to changes in selected measures of diamondback moth fitness. A corollary objective was to determine if diamondback moth larval feeding had an impact on pac choi chemistry. As such, effects of light intensity were compared using diamondback moth-infested and uninfested plants.

Materials and Methods

Plant material. Seeds of pac choi (*Brassica rapa* var. *chinensis* cv. 'Mei Qing Choi') were obtained from Johnny's Seeds (Winslow, ME) and germinated in plug flats (Hummert's International, Topeka, KS) containing a soil-less growing medium,

MetroMix 200 (Sungro, Alberta, Canada), consisting of Canadian sphagnum peat moss, vermiculite, perlite, a wetting agent, and trace amounts of N-P_2O_5-K_2O. Two-week-old seedlings were transplanted into 16 × 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 6 wks with a 24N:8P:16K (Miracle Grow, The Scotts Company LLC, Marysville, OH) solution.

Insects. A colony of diamondback moth 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 - 5 potted pac choi plants in a 0.60 × 0.60 × 0.91 m frame cage covered with 1.4 mm mesh screening in a greenhouse under natural daylight conditions and a temperature range of 20 - 23°C.

Experimental design. Four experiments were conducted in a greenhouse (6 × 7.3 m) at Kansas State University, 2 in the summer (28 June through 27 July, and 5 August through 4 September 2009) and 2 in winter-spring (25 January through 28 February, and 5 March through 10 April 2010). In all experiments, light treatment (ambient or shade cages) and herbivory (presence or absence of diamondback moth larvae) were the main effects arranged in a split-plot design, where light treatment was the whole-plot factor and herbivory was the randomly assigned factor. Whole- and subplot plot factors were arranged in a completely randomized design (CRD) with 5 replications (cage) for each main effect. Spacing between adjacent cages was 0.3 m. Each cage contained one whole-plot (16 plants) arranged in 4 rows of 4 plants. Plants in 2 of 4 rows were randomly assigned to be infested with diamondback moth, whereas the other 2 rows were left uninfested. This resulted in a total of 160 plants per experiment, which were distributed over 3 greenhouse benches, where each bench was 3.6 × 9.1 m. Experimental plants had an average of 10 leaves and were 6 wks old from time of germination.

To manipulate light intensity, cages were made by covering polyvinyl chloride (PVC) square frames (1.2 × 1.2 × 1.2 m) with plastic and/or cloth materials. For the ambient treatments, frames were covered with clear 3-mm polyethylene (DuraGreen, DuraGreen Marketing Inc. LLC, Mount Dora, FL), and for the shade treatments, the covering consisted of clear polyethylene plus 2 layers of 52% heavy white knit shade cloth (PAK Unlimited Inc., www.pacunlimited.com). To avoid insect movement between rows, plants were spaced so that foliage did not touch. Infestation was accomplished by individually transferring 20 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 because the larvae may drop from plants if disturbed.

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. However, only the light intensity measurements taken during daylight hours were used for data analysis. Light intensity measurements used in the July and August experiments were recorded from 0,600 AM through 2000 h, whereas measurements used in the February and March experiments were taken from 0,800 AM through 1800 h. Light intensity, measured as lumens/ft^2, was converted to lumens/m^2 by multiplying values by the constant 10.76 (Mechtly 2008). For all experiments, plants were exposed to light treatments for approx. 4 wks.

Plant chemical analyses. When pupation was observed on infested plants in all light cages, randomly chosen plants (2 from each infested row and 2 from each uninfested row for a total of 8 plants) were sampled from all light cages for chemical analyses. Analyses included total carbon to nitrogen ratio (C:N), percentage total protein, percentage moisture content, and total phenolic content. In addition, 7 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, pers. commun.). To obtain sufficient plant material for the phenolic analyses, 2 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 0,600 through 0,800 h in all experiments (You and Yang 2001). Although greenhouse light intensities varied during the 0,600 through 0,800 h 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. Rajashekar, pers. commun.). 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 approx. 1 wk until used in the analyses. For moisture content and C:N ratio, 2 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.

For the C:N analyses, 2 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 percentage protein values were based on multiplying percentage total N by the constant 6.25 (Fujihara et al. 2001). Percentage moisture content of leaf tissue was based on the difference between wet and dry weights of the leaf samples [(fresh weight – dry weight)/dry weight * 100].

Total phenolic content was analyzed using the modified Folin-Ciocalteu method (Pennycooke et al. 2005, Oh 2008, M-.M. Oh, pers. commun.). Modifications to this method included the use of 0.5 g of frozen leaf tissue, which was ground and combined with 3 mL of 80% (v/v) acetone, and 1 mL of this extract solution was then used for spectrophotometer absorbance reading (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 (FW) tissue.

The extraction of individual phenolics from the pac choi leaves is described by Nicolle et al. (2004) with minor modifications (Oh 2008). Modifications included the use of 1 g of frozen leaf tissue, which was ground and combined with 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 resuspended 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 then used in high performance liquid chromatography (HPLC) for identification of the selected phenolics. 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, KS State University (Manhattan, KS). Of the phenolics assayed, only chlorogenic, caffeic, ferulic and sinapic acids, and L-7-O-G were detected in the leaf samples. Specific phenolic data are reported in mg/100 mL of methanolic extract.

Shoot biomass and leaf area removed by diamondback moth larvae. Due to the time associated with processing samples, the remaining 4 infested and uninfested plants were sampled the next day after sampling for plant chemical analyses to obtain estimates of shoot biomass and percentage leaf area removed by diamondback moth larvae. Shoot biomass, from the crown up, was measured as fresh weight using an electronic balance to the nearest 0.001 g. To measure the leaf area removed by larval feeding (herbivory), digital photos were taken of 2 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 the total leaf area and total leaf area removed by diamondback moth larvae on 4 infested plants in each replicate (Dudt and Shure 1994, Lamari 2002).

Diamondback moth responses. To obtain body weights, pupae were carefully removed from the foliage of plants used for plant biomass and herbivory and placed individually in preweighed 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 16:8 (L:D) 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 diamondback moth was identified by the distinctive diamond-shaped pattern on the forewings, which is not present on females (Shirai 1993, Muhamad et al. 1994).

Herbivory (estimated by percent leaf area removed) was used to measure the effect of feeding larvae on plant responses across months and treatments. However, when pupae were collected in each month, counts of remaining larvae in each instar (recorded per plant to determine age-class distributions) were different between experiments. To avoid an age-class bias when comparing levels of herbivory between experiments, percentage 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 fertility treatments and across experiments. To compare herbivory on an individual basis, larval feeding equivalents were determined by dividing the percentage 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. These relative instar consumption values were derived from consumption based on *Erinnyis ello* L. (Lepidoptera: Sphingidae), which has similar instar consumption (Pratissoli et al. 2002). For *E. ello*, a defoliating caterpillar, 75% of larval consumption takes place during the last instar stage (Bellotti et al. 1992), which is similar to the diamondback moth. This procedure resulted in the following formula:

$$\mathbf{PC} = \frac{\mathbf{PLFR}}{\Sigma(\mathbf{IN} \times \mathbf{CR})}$$

where PC = percentage consumption per insect per plant, PLFR = percentage total leaf area removed, IN = number of each instar, and CR = standardized consumption values for each instar (3rd instar = 3, 4th instar = 8, and pupa = 16).

Because insect development is known to be affected by temperature (Precht et al. 1973, Wellington et al. 1999), comparisons of diamondback moth 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 (DD) as a standardized unit. The number of diamondback moth in each instar per plant per treatment was multiplied by the number of DD needed for that life stage to complete development based on data from Ansari et al. (2010) for diamondback moth feeding on *Brassica rapa* L. (Brassicales: Brassicaceae). Adult data were not included in calculations because samples were taken when pupae were present, before adult emergence occurred. The total number of DD was then summed and the average number DD for the cohort was computed as follows:

$$\mathbf{CDD} = \frac{\Sigma(\mathbf{IN} \times \mathbf{DD})}{\Sigma \mathbf{TI}}$$

where CDD = average cohort DD, IN = number of each instar, DD = the number of DD needed for that life stage to complete development (2nd instar = 200.8, 3rd instar = 138.3, 4th instar = 75.8, and pupa = 0), and TI = total number of instars present.

Statistical analysis. All data pertaining to light intensity, temperature, plant parameters, diamondback moth response variables, and diamondback moth life stage 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 month, light, and herbivory (plants with and without diamondback moth) 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. For variables having significant month x light interactions, means were then sliced to show significance whereas holding each main effect constant (SAS Institute 2002). Prior to analysis, percentage data were arcsin-transformed to normalize the data, including moisture content, protein content, and proportion of leaf tissue consumed per insect. All data presented are nontransformed.

The LS MEANS statement (SAS Institute 2002) and Fisher's Protected LSD were used to make pairwise treatment comparisons. To determine if light and plant parameters, and plant parameters and diamondback moth 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 College, PA). Then multiple regressions were performed in a step-wise backward elimination procedure (SAS Institute 2002) to remove the variables with the highest P values (nonsignificant) 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 4 infested plants in each light cage (replicate). Data were subjected to ANOVA using a general linear model with light intensity and month as main effects, and the two-way interaction of light × month. The LS MEANS statement (SAS Institute 2002) and Fisher's Protected LSD were used to make pairwise treatment comparisons. Means were then separated for multiple comparisons using the adjusted Tukey method. Correlations of light intensity and temperature with diamondback moth body weights and herbivory, within light treatments by month and across months, were conducted using PROC CORR test procedures (SAS Institute 2002).

Developmental data for diamondback moth were averaged over the 4 infested plants in each plot, and then subject to ANOVA using a general linear model with light and month as main effects, and the two-way interaction of light x month. The LS MEANS statement and Fisher's Protected LSD were used to make pairwise treatment comparisons. Means were then separated for multiple comparisons using the adjusted Tukey method (SAS Institute 2002).

Results

Light intensity and temperature. There was a significant light treatment *x* month interaction for light intensity and temperature (Table 1). Under both ambient and shade conditions, light intensity was significantly higher in August and February than July and March (Fig. 1). Slicing tests for the significant interaction revealed that light intensity was significantly greater under ambient (15,067 ± 280 lumens/m^2) than under shade (6,452 ± 276 lumens/m^2) conditions when data across months were held constant (P values < 0.001). Furthermore, when light intensity was combined across treatments, slicing tests resulted in significant differences between all months when light treatments were held constant (P values < 0.001). Therefore, light intensity was not only different between light treatments within each month but also among months. For temperature, slicing the interaction across months revealed that temperature was significantly different between both the ambient (23.3 ± 0.27°C) and shade (22.3 ± 0.27°C) treatments (P values < 0.001). However, when the interaction was sliced across light treatments, temperature was only significantly different between treatments in August (25.59 ± 0.23°C) and March (16.64 ± 0.22°C) (P values < 0.001), but not for July (26.34 ± 0.22°C) or February (22.43 ± 0.22°C) ($P \leq 0.05$) (Fig. 1).

Plant responses to light intensity. Data associated with plant responses for both diamondback moth-infested and uninfested plants were pooled because there was no significant effect or significant interactions with the main effect of herbivory on any of the plant responses ($P \geq 0.05$) (Tables 2 and 3).

The C:N ratio, protein content, shoot biomass, caffeic and ferulic acids, and L-7-O-G all had significant month × light interactions ($P \geq 0.05$) (Tables 2 and 3). Slicing tests for main effects (month and light) were used to determine the significance of each effect whereas holding the other constant. Slicing for the effect of light treatment on C:N ratio revealed that the effect was only significant in July ($P < 0.0001$), August ($P = 0.001$) and March ($P = 0.04$), but not for February ($P = 0.11$). Slicing for the effect of month on C:N ratio revealed that the effect was significant for both ambient ($P < 0.0001$) and shade ($P = 0.02$) conditions. Therefore, light treatments affected C:N ratio but was dependent on month, where significantly higher levels of C:N were found in plants under ambient conditions in July compared with other treatment × month interactions (Fig. 2). Slicing for the effect of light treatment on protein content showed

Table 1. Results from a mixed model ANOVA associated with light intensity (lumens/m²) and temperature (°C) data for the main factors of month, light treatment (LT), light replicate (LR), and the interaction of month × LT.

Factor	N	df	Light intensity		Temperature	
			F	*P*	*F*	*P*
Month**	40	3	65.47	<0.001*	924.54	<0.01*
LT†	20	1	478.77	<0.001*	6.69	0.06
LR‡	20	4	0.51	0.73	0.6	0.68
Month × LT	40	3	11.98	<0.001*	3.48	<0.001*

* Indicates significance at $P \leq 0.05$.

** July, August, February, and March experiments. Light and temperature variables correspond to 10 light cages × 4 months, $n = 40$.

† Ambient and shade treatment. Light and temperature variables correspond to 5 ambient or 5 shade cages × 4 months, $n = 20$.

‡ Five light cages per light treatment. Light and temperature variables correspond to 5 ambient or 5 shade cages × 4 months, $n = 20$.

that the effect was significant in July ($P < 0.0001$), August ($P = 0.005$) and February ($P = 0.01$), but not for March ($P = 0.47$). Slicing for the effect of month on protein content revealed that the effect was significant under both ambient ($P < 0.0001$) and shade ($P = 0.003$) conditions. Protein content was typically higher under shade conditions, and was significant for July and August (Fig. 2). Slicing for the effect of light treatment on shoot biomass revealed that the effect was significant in August ($P = 0.002$), February ($P = 0.0005$) and March ($P < 0.0001$), but not for July ($P = 0.26$). For the effect of month on shoot biomass, slicing revealed that the effect was significant under both ambient ($P = 0.002$) and shade ($P = 0.001$) conditions. For significant months, shoot biomass was significantly higher under ambient conditions (Fig. 2). Slicing for the effect of light treatment on ferulic acid showed that the effect was only significant in August ($P = 0.0006$) and February ($P = 0.007$), but not for July ($P = 0.74$) or March ($P = 0.56$). For the effect of month on ferulic acid, slicing revealed that the effect was significant only under ambient ($P < 0.0001$) but not shade ($P = 0.09$) conditions. For August and February, ferulic acid was significantly higher under ambient conditions compared with shade conditions and to the other months (Fig. 2). Slicing for the effect of light treatment on caffeic acid showed that the effect was only significant in July ($P = 0.05$), but not for the other months (P values ≥ 0.05). For the effect of month on caffeic acid, slicing revealed that the effect was significant only for shade ($P = 0.03$) but not ambient ($P = 0.07$) conditions. For July, caffeic acid was significantly higher under shade compared with ambient conditions (Fig. 2). Slicing for the effect of light treatment on L-7-O-G revealed that the effect was only significant in August ($P = 0.02$) and July ($P = 0.05$), but not for February ($P = 0.69$) or March ($P = 0.77$). Finally, for the effect of month on L-7-O-G, it was determined that the effect was significant for both ambient ($P = 0.01$) and shade ($P = 0.04$) conditions. However, L-7-O-G was not consistently different by light treatments in significant months (Fig. 2).

Plant response variables not having significant month × light interactions were significant for either main effect with the exception of chlorogenic acid, which was not

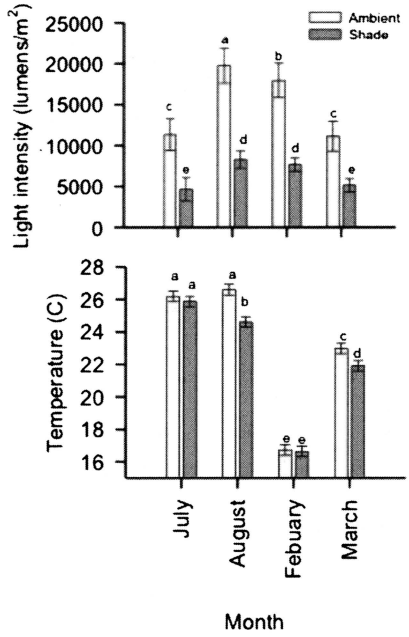

Fig. 1. Mean (± SEM) light intensity and temperature associated with ambient and shade treatments for each month (n = 5 reps) having significant month × light interactions. Data from inside light treatment cages (ambient and shade) with measurements taken at 30-min intervals using HOBO data loggers for the July, August, February, and March experiments. Means followed by common letter are not significantly different at $P \leq 0.05$ using Fisher's Protected LSD.

significant for any month or light effects (Table 2). The effect of light treatment was significant for total phenolic content, sinapic acid, and moisture content (Tables 2 and 3). Total phenolics were significantly higher under ambient (0.62 ± 0.01 GAE/g FW tissue) than shade (0.54 ± 0.01 GAE/g FW tissue) conditions when data were pooled across the 4 months ($P \leq 0.05$). However, the effect of month was also significant for total phenolic content (Table 2), where total phenolics were significantly higher in March (0.71 ± 0.02 GAE/g FW tissue) than July (0.32 ± 0.02 GAE/g FW tissue) and August (0.64 ± 0.02 GAE/g FW tissue) ($P < 0.0001$). Of the 4 phenolic compounds detected, only sinapic acid was significantly affected by light treatment across months (Table 3). The concentration of sinapic acid was significantly higher under ambient (7.74 ± 0.72 mg/100 mL extract) than under shade (3.14 ± 0.82 mg/100 mL extract) conditions for all months ($P \leq 0.05$). The mean percent moisture content was significantly higher in plants grown under shade conditions than those grown under ambient light, however, the differences in moisture content were too small to be considered meaningful to the study results.

Insect responses to plant chemistry. All diamondback moth responses were significantly affected by month, but light treatment had no effect (Table 4). Mean body

Table 2. Results from a mixed model ANOVA associated with plant response data for the main factors of month, light treatment (LT), light replicate (LR), herbivory (HB), and the interaction of month × LT.

Factor	N	df	Percentage C:N ratio		Percentage Protein		Total Phenolics□		Chlorogenic acid^		Caffeic acid^	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Month**	80	3	23.10	<0.001*	11.7	<0.001*	53.1	<0.001*	1.6	0.2	3.2	0.03*
Lt†	40	1	45.00	<0.01*	19.02	0.01*	11.3	<0.01*	4.2	0.1	0.1	0.8
LR‡	20	4	0.48	0.75	0.77	0.59	0.26	0.9	0.6	0.7	0.9	0.6
HB◊	40	1	0.27	0.60	0.52	0.47	1.22	0.27	1.78	0.18	1.21	0.27
Month × LT•	40	3	7.45	<0.01*	4.26	0.008*	0.93	0.43	0.5	0.7	2.9	0.04*

* Indicates significance at $P \leq 0.05$.

** July, August, February, and March experiments. Responses correspond to 10 light cages with diamondback moth and 10 light cages without diamondback moth × 4 months, $n = 80$.

† Ambient and shade treatment. Responses correspond to 10 light cages × 4 months, $n = 40$.

‡ Five light cages per light treatment. Responses correspond to 5 ambient or 5 shade cages × 4 months, $n = 20$.

◊ Herbivory refers to 5 replicates with diamondback moth and 5 replicates without diamondback moth × 4 months, $n = 40$.

• Responses correspond to 10 light cages with diamondback moth × 4 months, $n = 40$.

□ Total phenolic content is reported as gallic acid equivalents/g fresh weight of plant tissue.

^ Concentrations of specific phenolic compounds are reported as mg/100 mL of methanolic extract.

Table 3. Results from a mixed model ANOVA associated with plant response data for the main factors of month, light treatment (LT), light replicate (LR), herbivory (HB), and the interaction of month × LT.

Factor	N	df	Sinapic acid^		Ferulic acid^		L-7-O-G^		Percentage Moisture		Shoot Biomass	
			F	*P*	*F*	*P*	*F*	*F*	*P*	*F*	*F*	*P*
Month**	80	3	1.2	0.3	9.0	<0.01*	3.0	1.2	0.3	9.0	3.2	0.03*
Lt†	40	1	18.0	<0.01*	11.0	<0.01*	0.1	18.0	<0.01*	11.0	0.1	0.8
LR‡	20	4	1.2	0.3	1.7	0.15	0.9	1.2	0.3	1.7	0.9	0.6
HB◊	40	1	0.08	0.77	0.03	0.85	2.25	0.08	0.77	0.03	1.21	0.27
Month × LT•	40	3	0.9	0.4	4.5	0.007*	3.8	0.9	0.4	4.5	2.9	0.04*

* Indicates significance at $P \leq 0.05$.

** July, August, February, and March experiments. Responses correspond to 10 light cages with diamondback moth and 10 light cages without diamondback moth × 4 months, $n = 80$.

† Ambient and shade treatment. Responses correspond to 10 light cages × 4 months, $n = 40$.

‡ Five light cages per light treatment. Responses correspond to 5 ambient or 5 shade cages × 4 months, $n = 20$.

◊ Herbivory refers to 5 replicates with diamondback moth and 5 replicates without diamondback moth × 4 months, $n = 40$.

• Responses correspond to 10 light cages with diamondback moth × 4 months, $n = 40$.

^ Concentrations of specific phenolic compounds are reported as mg/100 mL of methanolic extract.

weights, by month, are presented in Table 5. For both male and female, pupal and adult weights were significantly higher (2-fold) in March than in the other 3 months. However, light intensity and temperature were not significantly correlated with body weights in the 4 months ($P \geq 0.05$).

In months where diamondback moth body weights were the highest, the concentration of ferulic acid was the lowest. Although ferulic acid and diamondback moth body weights were significantly different by months, regression analysis, with data averaged across months, showed a significant negative relationship associated with ferulic acid and adult male diamondback moth body weights in the ambient treatment ($F = 6.60$, df = 1, $P = 0.02$) but not the shade treatment ($F = 0.34$, df = 1, $P = 0.57$) (Fig. 3). In general, ferulic acid was higher in the ambient treatments with the exception of July (Fig. 2). Across months, there was a wide range of light intensities. When these data were combined, there was a significant positive relationship between the concentration of ferulic acid and light intensity under ambient light ($F = 18.42$, df = 1, $P = 0.001$), however, this relationship was not observed under the shade treatment ($F = 0.46$, df = 1, $P = 0.56$) (Fig. 4). In contrast to the males, there was no significant relationship between ferulic acid concentration and adult female diamondback moth body weights, either in the ambient or shade treatments (P values ≥ 0.05).

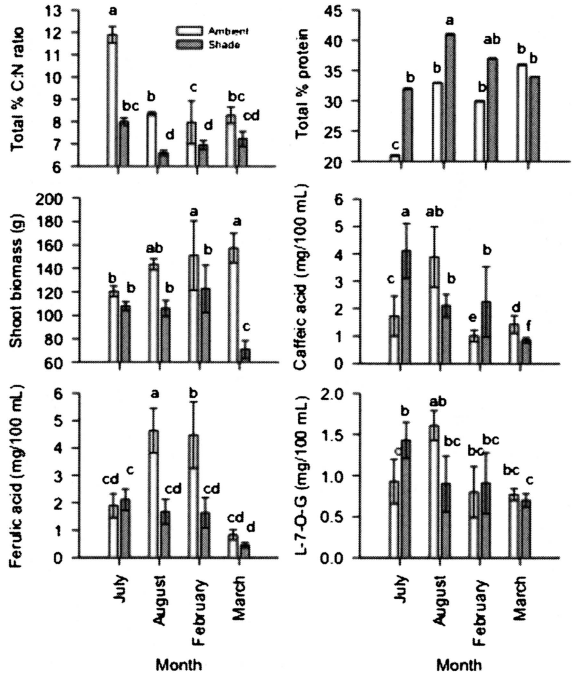

Fig. 2. Mean (± SEM) *Brassica rapa* responses in each month (July, August, February, and March) and ambient and shade light treatments having significant month × light interactions ($n = 5$ reps). Means followed by common letter are not significantly different at $P \leq 0.05$ using Fisher's Protected LSD.

Table 4. Results from a mixed model ANOVA associated with herbivory and diamondback moth (*P. xylostella*) body weights for the main factors of month, light treatment (LT), light replicate (LR), and the interaction of month × LT.

Factor	N	df	Percentage Leaf Area Removed♦		Female adult□		Male adult□		Female pupal□		Male pupal□	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Month**	40	3	24.61	<0.0001*	5.17	0.007*	19.42	<0.01*	6.54	0.002*	4.55	0.01*
LT†	40	1	2.2	0.15	0.33	0.57	3.01	0.09	0.6	0.42	0.01	0.92
LR‡	20	4	1.31	0.29	0.93	0.46	0.77	0.55	1.5	0.21	1.19	0.33
Month × LT◊	40	3	0.41	0.74	0.63	0.6	0.59	0.62	0.4	0.72	1.47	0.24

* Indicates significance at $P \leq 0.05$.

** July, August, February, and March experiments. Responses correspond to 10 light cages with diamondback moth × 4 months, $n = 40$.

† Ambient and shade treatment. Responses correspond to 10 light cages × 4 months, $n = 40$.

‡ Five light cages per light treatment. Responses correspond to 5 ambient or 5 shade cages × 4 months, $n = 20$.

◊ Responses correspond to 10 light cages with diamondback moth × 4 months, $n = 40$.

♦ Percentage leaf area removed based on total leaf area and leaf area removed by diamondback moth larval feeding per plant.

□ Body weights in mg.

Table 5. Mean (± SEM) male and female diamondback moth *Plutella xylostella* body weights and numbers recorded associated with each month. Data is pooled across ambient and shade treatments, for the July, August, February, and March experiments.

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

* Means followed by a common letter within a row are not significantly different between months. Significance was determined at $P \leq 0.05$ using Fisher's LSD.

** Represents the average number of diamondback moth used to determine mean body weights from 4 infested plants in each light cage across light treatments.

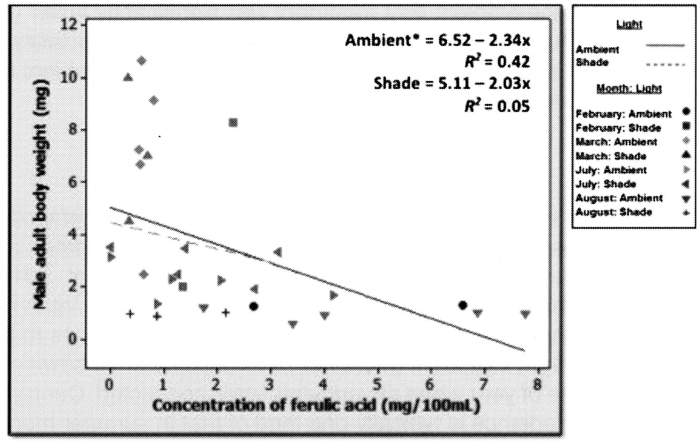

Fig. 3. Relationship of ambient (17 plants with male data) and shade (13 plants with male data) treatments between male adult *Plutella xylostella* body weight and concentration of ferulic acid in *Brassica rapa* pooled across the four months (July, August, February, and March). The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination (R^2). Starred equations are significant at $P \leq 0.05$.

Although sinapic acid concentrations were elevated under higher light intensities, concentrations did not correspond to the diamondback moth response variables. There were no other specific phenolic compounds, or combinations of phenolic compounds that were significantly correlated with diamondback moth fitness parameters.

The percentage leaf area removed was a direct measure of herbivory used in the analysis for treatment effects. Percentage leaf area removed was not significantly different by light treatment but was significantly different by month ($P < 0.0001$) (Table 4). Each month was different from each other with July plants experiencing significantly more leaf area removed by larvae (21.69 ± 3.15%), compared with August (16.31 ± 3.56%), than February (6.34 ± 1.12%), and March (2.11 ± 0.42%) (P values ≤ 0.05). However, because of differences in the diamondback moth age-class distribution across months, consumption, as larval feeding equivalents, was computed as an unbiased measure of herbivory. Analysis of the data showed that light treatment did not significantly affect larval feeding ($F = 1.06$, df = 1, $P = 0.30$), but month was significant ($F = 3.35$, df = 3, $P = 0.02$). Larval feeding was significantly greater in July (1.57 ± 0.78 feeding equivalents) compared with August (0.29 ± 0.06 feeding equivalents), February (0.08 ± 0.02 feeding equivalents), and March (0.01 ± 0.006 feeding equivalents). Larvae were exposed to the highest temperatures in July, but lower light intensities than August, indicating that greater feeding in July may have been temperature related. However, neither light intensity nor temperature were significantly correlated with diamondback moth larval feeding across months ($P = 0.52$, $r = -0.47$ and $P = 0.38$, $r = 0.61$, respectively).

A comparison of larval development (based on cumulative DD) between July and August, which had similar temperatures but different light intensities, revealed that the

rate of development in the ambient light treatment was significantly faster ($F = 23.88$, df = 1, $P \leq 0.0001$) in August (30.02 ± 4.67 DD) under higher light intensities (19,755 ± 2130 lumens/m^2) than in July (54.97 ± 4.91 DD), which had lower ambient light intensities (11,351 ± 1926 lumens/m^2).

Discussion

The effects of light intensity on plant primary and secondary metabolism, along with their impact on insect herbivores, have been minimally investigated associated with plant-insect interactions (Henriksson et al. 2003, Guerra et al. 2010). In this study, we showed for the first time that pac choi and diamondback moths responded to a relatively broad range of light intensities, and that these responses to light intensity resulted both from light treatment (more than a 2-fold difference between ambient and shade) and the time of year when experiments were conducted. 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). However, light intensities measured during July were relatively low compared with the other 3 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. In addition, they also tended to have a lower protein content compared with plants grown under shade. The increased levels of phenolics under higher light intensities may be a result of increased carbon, which occurred in this study, and is supported by the

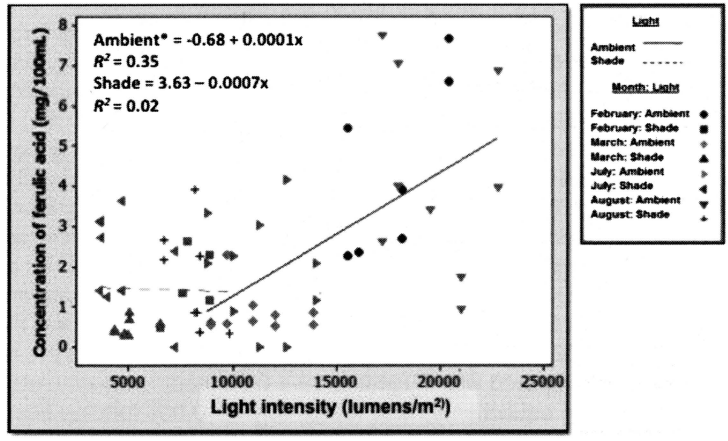

Fig. 4. Relationship of ambient and shade light intensity and concentration of ferulic acid in *Brassica rapa* pooled across the four months (July, August, February, and March). The best-fit relationships (linear) are shown as solid lines, along with line equations and coefficients of determination (R^2). Starred equations are significant at $P \leq 0.05$. ($n = 40$ reps).

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).

With respect to the diamondback moth, we hypothesized that if higher light intensity increased phenolic plant defenses, then diamondback moth fitness should be reduced on plants exposed to higher light intensities compared with those exposed to lower light intensities. However, results showed that despite relatively lower protein and higher phenolic contents in plants, neither development nor survival was adversely affected. In fact, development was significantly faster under higher light intensities in August compared with the lower intensities experienced in July. In some cases, herbivores feeding on plants deficient in protein and with a higher phenolic content may 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, consumption by diamondback moth larvae did not diminish in months with the highest light intensities. The overall findings suggest that high light intensity either had no adverse effect on diamondback moth 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 diamondback moth development, perhaps by acting as beneficial antioxidants. For example, certain phenolic compounds, such as chlorogenic acid, have been shown to increase the fitness of the tobacco budworm, *Heliothis virescens* F. (Lepidoptera: Noctuidae) by acting as an antioxidant in the midgut (Johnson and Felton 2001).

It is unclear whether light-mediated changes in phenolics affected diamondback moth body weights. Across months, male diamondback moth body weights were negatively correlated with concentrations of ferulic acid, which increased in pac choi plants under high light intensities. Thus, light intensity-induced increases in ferulic acid may be related to an adverse effect on male adult body weights. Although ferulic acid represents only a minor fraction of the total phenolic content in plants, it has been identified a compound involved in resistance against some herbivores (Argandona et al. 1980, Thackaray et al. 1990, Garcia-Conesa et al. 1999, Husken et al. 2005, Milkowski and Strack 2010). In contrast to the greenhouse experiments, results of an artificial diet study in which only the concentration of ferulic acid varied showed no dose-dependent effect on diamondback moth weight, development, or survival (Johnson 2011). Thus, if ferulic acid is functionally related to male diamondback moth body weight, the differences observed between the artificial diet study and the greenhouse experiments may be associated with interactions between ferulic acid and plant enzymes and/or proteins within the plant, indicating that interactions between phenolics and other plant components may be responsible for reducing male body weights (Stamp and Osier 1998, Garcia-Conesa et al. 1999, Miles 1999).

Whereas this study showed a relationship between ferulic acid and male diamondback moth body weight, there was no correlation between ferulic acid and female pupal or adult body weights. Why ferulic acid appeared to effect only males is unknown, but may be due to physiological and behavioral differences between male and female diamondback moth. For example, female Lepidoptera generally are heavier than males throughout their life cycle, and may also have variable nutritional needs due to differences in their reproductive physiology (Slansky and Scriber 1985, Zeng et al. 1997). For diamondback moth females to acquire the necessary resources for

egg production, they 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 diamondback moths should have experienced body weight changes at least equal to males unless they engaged in compensatory feeding to overcome effects of ferulic acid. Unfortunately in this study, no data are available to compare larval consumption between male and female diamondback moth. Regardless, male body weights are known to be important in migration and mate-finding (Shirai 1993, Muhamad et al. 1994), which may have an impact on population densities. Future studies should examine further the possibility of fitness costs to diamondback moth on plants grown under different light intensities by considering other important fitness traits, including survival, reproduction or oviposition.

Although the induction of phenolics in plants by light (bottom-up effect) has been demonstrated, production of phenolics may also be induced by feeding activities of herbivores (top-down effect) (Karban and Myers 1989, Smith 2005). Because this could have been a confounding factor, the experiments conducted in this study compared phenolic content in plants under various light intensities, with and without diamondback moth larvae, and showed that the presence of herbivores had no effect on any of the phenolic acids tested. However, other studies have demonstrated changes in phenolics resulting from diamondback moth larval feeding. For example, Caputo et al. (2006) and Ehlting et al. (2008) showed elevated total phenolic contents in *Arabidopsis* plants on which diamondback moth larvae fed. Widarto et al. (2006) reported that genes associated with biosynthesis of phenolics were induced in *Brassica rapa* leaves exposed to feeding by diamondback moth larvae. On the basis of these studies, it is unclear why differences were not observed in phenolics between diamondback moth-infested and uninfested plants. However, there are several possible explanations. Leaf phenolics were measured from the middle whorl to avoid leaf age bias, however, it is not known if diamondback moth larvae adjusted feeding sites based on variations in leaf phenolics. In fact, diamondback moth 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. Results may also be associated with sampling time for phenolic content, where tissue samples were taken between 0,600 and 0,800 h 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 0,800 h, which then fluctuates during the course of a day. It is possible that diamondback moth larvae actively fed during the day, and may have induced production of phenolics higher than those detected from samples taken during the morning hours. Widarto et al. (2006) detected induction of phenolic biosynthesis-related genes, which were measured in plant tissue that was exposed to diamondback moth larvae for less than 48 h In the current study, phenolic content was measured in plant tissue after pac choi had been exposed to larvae for over 7 ds. Plants may have responded to herbivory by producing phenolics only within 48 h of exposure, which may have prevented detectable differences in responses. Finally, the absence of a phenolic response to diamondback moth 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 can disperse 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).

When plants are grown under lower light intensity, their quality for herbivores may increase both as a result of decreased levels of phenolics used for plant defense (Mole and Waterman 1988) and increased protein content, which enhances nutritional quality (Mattson 1980). Under these conditions, larval consumption may increase, as was observed for the European corn borer, *Ostrinia nubilalis* Hübner, on field corn (Manuwoto and Scriber 1985). With respect to pac choi and diamondback moth, plants grown under shade conditions in the greenhouse typically contained higher levels of protein and lower levels of phenolics compared with those grown under ambient light. However, these differences in plant chemistry did not appear to influence consumption or other responses in *P. xylostella*. It is possible that differences in light intensity and subsequent changes in protein and phenolic contents may not have been large enough to exert an effect on diamondback moths.

There are practical implications of this study with respect to the production of pac choi. For example, seasonal differences in light intensity appear to be important with respect to changes in plant chemistry. Production of pac choi occurs in environments that vary in light intensity, such as open field, high tunnels, and greenhouses. Thus, producers should be aware that changes in plants related to light environment may affect crop nutrient content or secondary chemistry, although it is unclear whether these changes enhance protection against insect herbivores or increase susceptibility. Whereas lower light environments (occurring in greenhouses and high tunnels) did not affect leaf consumption by diamondback moths, rate of development was delayed. Delayed development under lower light conditions may be related to lower phenolic contents, which have been associated with negative effects on insect growth and development time in other plant systems (Isman and Duffey 1982, Dixon and Paiva 1995). However, experiments that compare diamondback development in different pac choi production environments, but without temperature bias, are needed.

Acknowledgments

The authors thank Dr. Channa Rajashekar and Dr. Myung-Min Oh for consultation and training concerning phytochemical analyses and for providing laboratory space, Dr. Kimberly Williams for use of greenhouse space and light intensity cages, and Kathy Lowe, Kansas State University Soil Testing Lab, and Cheryl Armendariz, Kansas State University Ruminant Nutrition Lab, for help in processing samples. The authors thank Dr. Brian McCornack for critically reviewing this manuscript, and also Dr. Anthony Joern and Dr. Nathan Nelson for reviewing a previous draft of this manuscript. This study was supported by USDA/IOP Program Award No. CS9979. This is Contribution Number 12-275-J of the Kansas State Agricultural Experiment Station.

References Cited

Aldrich, R. A. and J. W. Bartok Jr. 1994. Greenhouse engineering. Northeast Regional Agricultural Engineering Service. Ithaca, NY.

Ansari, S. M., T. Ahmad and H. Ali. 2010. Effect of Indian mustard on feeding, larval survival, and development of *P. xylostella* at constant temperatures. Entomol. Res. 40: 182-188.

Argandona, V. H., L. C. Luza, H. M. Niemeyer and L. J. Corcuera. 1980. Role of hydroxamic acids in the resistance of cereals to aphids. Phytochemistry 19: 1665-1668.

Bassman, J. H. 2004. Ecosystem consequences of enhanced solar ultraviolet radiation: secondary plant metabolites as mediators of multiple trophic interactions in terrestrial plant communities. Photochem. Photobiol. 79: 298-382.

Behmer, S. T. 2009. Insect herbivore nutrient regulation. Annu. Rev. Entomol. 54: 165-187.

Bellotti, A. C., B. V. Arias and O. L. Guzman. 1992. Biological control of the cassava hornworm *Erinnyis ello* (Lepidoptera: Sphingidae). Fla. Entomol. 754: 506-515.

Bieza, K. and R. Lois. 2001. An *Arabidopsis* mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. Plant Physiol. 126: 1105-1115.

Bryant, J. P., F. S. Chapin III and D. R. Klein. 1983. Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40: 357-368.

Caldwell, M. M., R. Robberecht and S. D. Flint. 1983. Internal filters: prospects for UV-acclimation in higher plants. Plant Physiol. 58: 445-450.

Caldwell, M. M., L. O. Bjorn, J. F. Bornmann, S. D. Flint, G. Kulandaivelu, A. H. Teramura and M. Tevini. 1998. Effect of increased solar UV-B on terrestrial ecosystems. Photochem. Photobiol. B: Biology 46: 40-52.

Caldwell, M. M., C. L. Ballare, J. F. Bornman, S. D. Flint, L. O. Bjorn, A. H. Teramura, G. Kulandaivelu and M. Tevini. 2003. Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors. Photochem. Photobiol. 2: 29-38.

Caputo, C., M. Rutitzky and C. L. Ballare. 2006. Solar ultraviolet-B radiation alters the attractiveness of *Arabidopsis* plants to diamondback moths (*Plutella xylostella* L.): impacts on oviposition and involvement of the jasmonic acid pathway. Oceologia 149: 81-90.

Close, D. C. and C. McArthur. 2002. Rethinking the role of many plant phenolics- protection from photodamage not herbivores? Oikos 99: 166-172.

Coley, P. D., J. P. Bryant and F. S. Chapin III. 1985. Resource availability and plant antiherbivore defense. Science 230(4728): 895-898.

Cooper-Driver, G., S. Finch and T. Swain. 1977. Seasonal variation in secondary plant compounds in relation to the palatability of *Pteridium aquilinum.* Biochem. Syst. Ecol. 5: 177-183.

Dixon, R. A. and N. L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7: 1085-1097.

Dudt, J. F. and D. J. Shure. 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. Ecology 75: 86-98.

Ehlting, J., S. G. Chowrira, N. Mattheus, D. S. Aeschliman, G.-I. Arimura and J. Bohlmann. 2008. Comparative transcriptome analysis of *Arabidopsis thaliana* infested by diamond back moth (*Plutella xylostella*) larvae reveals signatures of stress response, secondary metabolism, and signaling. BMC Genomics 9: 154.

Farmer, E. E. 2001. Surface-to-air signals. Nature 411: 854-856.

Felton, G. W. and S. S. Duffey. 1991. Protective action of midgut catalase in lepidopteran larvae against oxidative plant defenses. J. Chem. Ecol. 17: 1715-1732.

Felton, G. W. 1996. Nutritive quality of plant protein: sources of variation and insect herbivore responses. Arch. Insect Biochem. Physiol. 32: 107-130.

Foggo, A., S. Higgins, J. J. Wargent and R. A. Coleman. 2007. Tri-trophic consequences of UV-B exposure: plants, herbivores and parasitoids. Oecologia 154: 505-512.

Fujihara, S., A. Kasuga and Y. Aoyagi. 2001. Nitrogen-to-protein conversion factors for common vegetables in Japan. J. Food Sci. 66: 412-415.

Garcia-Conesa, M.-T., P. D. Wilson, G. W. Plump, J. Ralph and G. Williamson. 1999. Antioxidant properties of 4,4'-dihydroxy- 3,3′ –dimethoxy-β,β' –bicinnamic acid (8-8 –diferulic acid, non-cyclic form). J. Sci. Food Agric. 79: 379-384.

Gordon-Weeks, R. and J. A. Pickett. 2009. Role of natural products in nature: plant-insect interactions: part 3, Pp. 321-337. *In* B. Huang (ed.), Plant-environment interactions. Springer, Bonn, Germany.

Guerra, P. C., J. Becerra and E. Gianoli. 2010. Explaining differential herbivory in sun and shade: the case of *Aristotelia chilensis* saplings. Environ. Entomol. 4: 229-235.

Harbaum, B., E. M. Hubbermann, C. Wolff, R. Herges, Z. Zhu and K. Schwarz. 2007. Identification of flavonoids and hydroxycinnamic acids in pak choi varieties (*Brassica campestris* L. spp. *chinensis* var. *communis*) by HPLC-ESI-MS and NMR and their quantification by HPLC-DAD. J. Agric. Food Chem. 55: 8251-8260.

Henriksson, J., E. Haukioja, V. Ossipov, S. Ossipova, S. Sillanpaa, L. Kapari and K. Pihlaja. 2003. Effects of host shading on consumption and growth of the geometrid *Epirrita autumnata*: interactive roles of water, primary and secondary compounds. Oikos 103: 3-16.

Herms, D. A. and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol. 67: 283-335.

Husken, A., A. Baumert, D. Strack, H. C. Becker, C. Mollers and C. Milkowski. 2005. Reduction of sinapate ester content in transgenic oilseed rape (*Brassica napus*) by dsRNAi-based suppression of BnSGT1 gene expression. Mol. Breed. 16: 127-138.

Ingersoll, C. M., R. A. Niesenbaum, C. E. Weigle and J. H. Lehman. 2010. Total phenolics and individual phenolic acids vary with light environment in *Lindera benzoin.* Botany 88: 1007-1010.

Isman, M. B. and S. S. Duffey. 1982. Toxicity of tomato phenolic compounds to the fruitworm, *Heliothis zea.* Entomol. Exp. Appl. 31: 370-376.

Izaguirre, M. M., A. L. Scopel, I. T. Baldwin and C. L. Ballare. 2007. Convergent responses to stress. Solar ultraviolet-B radiation and *Manduca sexta* herbivory elicit overlapping transcriptional responses in field-grown plants of *Nicotiana longiflora.* Plant Physiol. 132: 1755-1767.

Johnson, K. S. and G. W. Felton. 2001. Plant phenolics as dietary antioxidants for herbivorous insects: a test with genetically modified tobacco. J. Chem. Ecol. 27: 2579-2597.

Johnson, W. A. 2011. Effects of light intensity and nitrogen source on pac choi (*Brassica rapa* L.), and interaction with the diamondback moth (*Plutella xylostella* L.). Ph.D. Diss. Kansas State Univer., Manhattan, KS.

Karban, R. and J. H. Myers. 1989. Induced plant responses to herbivory. Annu. Rev. Ecol. Syst. 20: 331-348.

Karban, R. and I. T. Baldwin. 1997. Induced responses to herbivory. Chicago Univer. Press, Chicago, IL.

Lamari, L. 2002. Assess: image analysis software for plant disease quantification V1.0. Am. Phytopath. Soc., St. Paul, MN.

Lavola, A., R. Julkunen-Tiitto, H. Roininen and P. Aphalo. 1998. Host-plant preference of an insect herbivore mediated by UV-B and CO_2 in relation to plant secondary metabolites- contrasting effects of (specialist and generalist) herbivores and natural enemies. Biochem. Syst. Ecol. 26: 1-12.

Lawton, J. H. and S. McNeill. 1979. Between the devil and the deep blue sea: on the problem of being a herbivore. Symp. British Ecol. Soc. 20: 223-244.

Lewis, N. G. and S. Sarkanen. 1998. Lignin and lignin biosynthesis. Am. Chem. Soc. Oxford Univer. Press, Washington, DC.

Li, J., T.-M. Ou-Lee, R. Raba, R. G. Amundson and R. L. Last. 1993. *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation. Plant Cell 5: 171-179.

Lin, L. Z. and J. M. Harnly. 2010. Phenolic component profiles of mustard greens, Yu Choy, and 15 other Brassica vegetables. Agric. Food Chem. 58: 6850-6857.

Liu, S.-S., F.-Z. Chen and M. P. Zalucki. 2002. Development and survival of the diamondback moth (Lepidoptera: Plutellidae) at constant and alternating temperatures. Environ. Entomol. 31: 221-231.

Louda, S. M. and J. E. Rodman. 1996. Insect herbivory as a major factor in the shade distribution of a native cruficer (*Cardamine cordifolia* A. Gray, bittercress). J. Ecol. 84: 229-237.

Manuwoto, S. and J. M. Scriber. 1985. Neonate larval survival of European corn borers, *Ostrinia nubilalis*, on high and low DIMBOA genotypes of maize: effects of light intensity and degree of insect inbreeding. Agric. Environ. 14: 221-236.

Mattson Jr., W. J. 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. 11: 119-161.

McKey, D. D. 1979. The distribution of secondary compounds within plants, Pp 55-133. *In* G.A. Rosenthal and D.H. Janzen (eds.), Herbivores: their interaction with secondary plant metabolites. Academic Press, New York, NY.

Mechtly, E. A. 2008. The international system of units, physical constants, and conversion factors. Publication NASA SP-7012. Scientific and Technical Information Office, NASA. Available online (verified 3/4/11): www.msis.jsc.nasa.gov.com.

Miles, P. W. 1999. Aphid saliva. Biol. Rev. Camb. Philos. Soc. 74: 41-85.

Milkowski, C. and D. Strack. 2010. Sinapate esters in Brassicaceous plants: biochemistry, molecular biology, evolution and metabolic engineering. Planta 232: 19-35.

Mole, S. and P. G. Waterman. 1988. Light-induced variation in phenolic levels in foliage of rainforest plants. II. Potential significance to herbivores. J. Chem. Ecol. 14: 23-34.

Mooney, H. A. and S. L. Gulmon. 1982. Constraints on leaf structure and function in reference to herbivory. Bioscience 32: 198-206.

Muhamad, O., R. Tsukuda, Y. Oki, K. Fujisaki and F. Nakasuji. 1994. Influences of wild crucifers on life history traits and flight ability of the diamondback moth, *Plutella xylostella.* Res. Popl. Ecol. 36: 53-62.

Muth, N. Z., E. C. Kluger, J. H. Levy and M. J. Edwards. 2008. Increased per capita herbivory in the shade: necessity, feedback, or luxury consumption? Ecoscience 15: 182-188.

Nicolle, C., A. Carnat, D. Fraisse, J. Lamaison, E. Rock, H. Michel, P. Amouroux and C. Remesy. 2004. Characterization and variation of antioxidant micronutrients in lettuce (*Lactuca sativa*). J. Sci. Food Agric. 84: 2061-2069.

Niesenbaum, R. A. and E. C. Kluger. 2006. When studying the effects of light on herbivory, should one consider temperature? The case of *Epimecis hortaria* F. (Lepidoptera: Geometridae) feeding on *Lindera benzoin* L. (Lauraceae). Environ. Entomol. 35: 600-606.

Oh, M. -.M. 2008. Plant adaptation and enhancement of phytochemicals in lettuce in response to environmental stresses. Ph.D. Diss. Kansas State Univer., Manhattan, KS.

Oh, M.-M. and C. B. Rajashekar. 2009. Antioxidant content of edible sprouts: effects of environmental shocks. J. Sci. Food Agric. 89: 2221-2227.

Ooi, P. A. C. 1979. Incidence of *P. xylostella* and its parasite in Malaysia. Malays. Appl. Biol. 8: 131-143.

Pennycooke, J. C., S. Cox and C. Stushnoff. 2005. Relationship of cold acclimation, total phenolic content and antioxidant capacity with chilling tolerance in petunia (*Petunia* × *hybrida*). Environ. Exp. Bot. 53: 225-232.

Pratissoli, D., J.-C. Zanuncio, R. Barros and H. Nonato de Oliveira. 2002. Leaf consumption and duration of instars of the cassava defoliator *Erinnyis ello* (Lepidoptera, Sphingidae). Rev. Bras. Entomol. 46: 251-254.

Precht, H., K. Bruck and K. Breuck. 1973. Temperature and life. Springer-Verlag, Bonn, Germany.

Sarfraz, R. M., L. M. Dosall, B. A. Keddie and J. H. Myers. 2011. Larval survival, host plant preferences and developmental responses of the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) on wild *Brassica* species. Entomol. Sci. 14: 20-30.

SAS Institute. 2002. SAS: Stat User's Guide, Version 9.1. SAS Institute, Cary, NC.

Schijlen, E. G. W., C. H. R. de Vos, A. J. van Tunen and A. G. Bovy. 2004. Modification of flavonoid biosynthesis in crop plants. Phytochem. 65: 2631-2648.

Schoonhoven, L. M., J. J. A. van Loon and M. Dicke. 2005. Insect-plant biology. Oxford Univer. Press, Oxford.

Shirai, Y. 1993. Factors influencing flight ability of male adults of the diamondback moth, *Plutella xylostella*, with special reference to temperature conditions during the larval stage. Appl. Entomol. Zool. (Jpn.) 28: 291-301.

Shure, D. J. and L. A. Wilson. 1993. Patch-size effects on phenolics in successional openings of the Southern Appalachians. Ecology 74: 55-67.

Slansky Jr., F. and J. M. Scriber. 1985. Food consumption and utilization, Pp 87-163. *In* G.A. Kerkutand L.I. Gilbert (eds.), Comprehensive insect physiology, biochemistry, and pharmacology vol. 4. Pergamon, New York, NY.

Smith, C. M. 2005. Plant resistance to arthropods. Springer, Dordrecht, the Netherlands.

Smith, H. 1982. Light quality, photoperception, and plant strategy. Annu. Rev. Plant Physiol. 33: 481-518.

Stamp, N. E. and T. L. Osier. 1998. Response for five insect herbivores to multiple allelochemicals under fluctuating temperatures. Entomol. Exp. Appl. 88: 81-96.

Stout, M. J., R. A. Brovont and S. S. Duffey. 1998. Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato. J. Chem. Ecol. 24: 945-963.

Talekar, N. S., F. C. Su and M. Y. Lin. 2003. How to produce safer leafy vegetables in net houses and net tunnels. Asian Vegetable Research and Development Center, Shanhua, Tainan, Taiwan.

Thackaray, D. J., S. D. Wratten, P. J. Edwards and H. M. Niemeyer. 1990. Resistance to the aphids *Sitobion avenae* and *Rhopalosiphum padi* in Gramineae in relation to hydroxamic acid levels. Ann. Appl. Biol. 116: 573-582.

Wellington, W. G., D. L. Johnson and D. J. Lactin. 1999. Weather and insects, Pp 313-353. *In* C.B. Huffakerand A.P. Guttierez (eds), Ecological Entomology. Wiley, New York, NY.

Widarto, H. T., E. van der Meijden, A. W. M. Lefeber, C. Erkelens, H. K. Kim, Y. H. Choi and R. Verpoorte. 2006. Metabolomic differentiation of *Brassica rapa* following herbivory by different insect instars using two dimensional nuclear magnetic resonance spectroscopy. J. Chem. Ecol. 32: 2417-2428.

Yamasaki, M. and K. Kikuzawa. 2003. Temporal and spatial variations in leaf herbivory within a canopy of *Fagus crenata.* Oecologia 137: 226-232.

You, M.-S. and G. Yang. 2001. Physiological stress reaction of Chinese cabbage, *Brassica chinensis*, infested by diamondback moth, *Plutella xylostella.* Entomol. Sci. 8: 131-140.

Zhao, X., T. Iwamoto and E. E. Carey. 2007. Antioxidant capacity of leafy vegetables as affected by high tunnel environment, fertilization and growth stage. J. Sci. Food Agric. 87: 2692-2699.

Zeng, F., S. Shu, Y. I. Park and S. B. Ramaswamy. 1997. Vitellogenin and egg production in the moth, *Heliothis virescens.* Arch. Insect Biochem. Physiol. 34: 287-300.