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Response of Two *Pieris* (Lepidoptera: Pieridae) Species to Fertilization of a Host Plant

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Ying-Zhi Chen, Li Lin, Chih-Wei Wang, Chin-Chang Yeh, and Shaw-Yhi Hwang (2004) Response of two *Pieris* (Lepidoptera: Pieridae) species to fertilization of a host plant. *Zoological Studies* **43**(4): 778-786. In this study, we explored relationships among plant fertilization, plant phytochemical compositions, and the performance of *Pieris* butterfly larvae. Cabbages (*Brassica oleracea* var. *capitata* L.) were grown under 2 levels of nutrients, and their foliar chemistry (water, nitrogen, and total glucosinolates) was measured. Two species of cabbage white butterflies, *Pieris rapae crucivora* and *P. canidia canidia*, were reared on foliage from each of the 2 treatments to measure insect performance. Oviposition responses of *P. rapae crucivora* and *P. canidia canidia* to fertilized vs. unfertilized host plants were compared using choice tests. Foliar water and nitrogen contents increased with increased nutrient availability. Concentrations of total glucosinolates, however, decreased with increased nutrient availability. For the most part, insect performance varied significantly between the 2 nutrient treatments. Both insect species performed well on the fertilized treatments which contained high water and nitrogen, but low glucosinolate contents. Ovipositional female butterflies of both species were also found to prefer fertilized plants. Finally, our results also indicate that *P. rapae crucivora* performed relatively better on high-nutrient plants. http://www.sinica.edu.tw/zool/zoolstud/43.4/778.pdf

Key words: *Brassica oleracea*, *Pieris*, Plant chemistry, Feeding trials, Oviposition preference.

Host plant chemistry is considered to be one of the most important factors affecting the performance of herbivorous insects (e.g., Ehrlich and Raven 1964, Feeny 1975, Rhoades and Cates 1976, Berenbaum 1981, Lindroth et al. 1988, Denno et al. 1990, Barros and Zucoloto 1999, Fischer and Fiedler 2000). Host plant chemistry can influence host-plant selection, specialization, and the host range of insect herbivores (Denno et al. 1990). The quality and quantity of the host plant chemistry, in turn, change in response to intrinsic and extrinsic factors, such as genetic variations and environmental factors.

Environmental factors are known to modulate primary and secondary chemical profiles of host plants (Bryant et al. 1983, Herms and Mattson 1992). The physiology and biochemistry of a plant may be altered by environmental stress to a degree that the plant's nutritional value to herbivores changes. These phytochemical changes often affect the behavior and physiology of insects (Courtney and Kibota 1989). In some instances, the foliar nutritional and allelochemical changes may improve the host plant forage quality and can therefore be considered beneficial to herbivorous insects (Mattson and Haack 1987). However, a number of studies have revealed a substantial variety of insect species responses to environmentally induced phytochemical changes (Waring and Cobb 1992).

Among environmental factors, the presence and concentration of nitrogen (N) are considered to be two of the most important factors affecting the phytochemical production of host plants; the N content itself strongly affects the performance of herbivorous insects (Fischer and Fiedler 2000). For the most part, higher N levels in host plants increase developmental rates of insects (e.g.,

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Slansky and Feeny 1977, Tabashnik 1982, Taylor 1984, Myers 1985, Ohmart et al. 1985, Cates et al. 1987, Estiarte et al. 1994, Obermaier and Zwölfer 1999). In contrast, low N contents in host plants result in poor larval performance (Myers and Post 1981, Myers 1985, Cates et al. 1987, Taylor 1988, Clancy 1992). In some species, larvae compensate for lower N concentrations by increasing food consumption or nutrient utilization efficiency, or by concentrating their feeding to the most N-rich parts of plants (Slansky and Feeny 1977, Mattson 1980, Ravenscroft 1994, Obermaier and Zwölfer 1999, Wheeler and Halpern 1999). In addition, the ovipositional behavior of insects may also be associated with host-plant nutrient levels (Myers 1985, Hugentobler and Renwick 1995). Ovipositional female cabbage white butterflies, *Pieris rapae crucivora*, were shown to prefer fertilized plants with higher nitrogen and phosphorus contents (Myers 1985).

Relationships between cabbage white butterflies (*Pieris* spp.) and their host plants have been studied for many years (e.g., Feeny 1977, Finch and Ackley 1977, Wolfson 1980, Theunissen et al. 1985, Renwick and Radke 1985 1988, Huang and Renwick 1993). *Pieris* butterfly larvae feed on plants within the Cruciferae and a few related families (e.g., the Resedaceae and Capparidaceae). A common characteristic of these host plant families is the presence of glucosinolates. Glucosinolates have been shown to play a central role in the host selection behavior of a number of insect species, such as *Pieris* butterflies (Feeny 1977, Nielsen 1978), that specialize on these plant families. Generally, *Pieris* butterfly adult females use the glucosinolates or their hydrolysis products as positive signals for the recognition of suitable host plants during oviposition (Schoonhoven 1972, Feeny et al. 1983, Chew and Robbins 1984, Renwick and Radke 1985 1988, Huang and Renwick 1993 1994). Glucosinolates also play a role as feeding stimulants for larvae of cabbage white butterflies (Schoonhoven 1968, Dethier 1982).

Chemical fertilizers are extensively used in the production of cruciferous crops worldwide (Bakker and Berendse 1999). Past studies have indicated that fertilization affects the chemical composition (e.g., nitrogen and glucosinolate contents) of members of the Cruciferae (Nilsson 1988, Freyman et al. 1991, Zebarth et al. 1991). Yet, little is known about the effect of these phytochemical changes on insect herbivores that specialize on the Cruciferae. The objectives of this study were to evaluate the effect of fertilization on the chemical composition of cabbage plants and to assess the impact of such chemical variations on 2 *Pieris* butterflies. *Pieris rapae crucivora* and *P. canidia canidia*, are the only 2 cabbage butterflies in Taiwan. We predicted that fertilization of plants would change the nitrogen and glucosinolate contents of plants and also influence the performance of these 2 *Pieris* species.

MATERIALS AND METHODS

Plants

An important economic crop, the Chinese cabbage, *Brassica oleracea* var. *capitata* L., was used in this experiment. Fifty seedlings (1 wk old) were obtained from a local company and grown in a greenhouse. Each seedling was potted individually in a 4-L pot containing a 1:1 mixture of sand-topsoil (local silt-loam) and was randomly assigned to one of 2 treatments. Every 5 d, plants were given either 100 ml water alone or the same volume of water containing nitrogenous 20-20-20 (N-P-K) fertilizer (1/1000 Hyponex® 5, Marysville, OH). Additional water was given to all plants every 2 d or as necessary. The 2 treatments were planned to establish differences in leaf N levels. The plants were 6 wk old when used for the bioassays.

Insects

Twenty mated female butterflies of *P. rapae crucivora* were caught in the winter of 2001 (Jan.) on the campus of National Chung Hsing University, Taichung, Taiwan, at near sea level. Ten mated female *P. canidia canidia* were also caught at about the same time in southern Taiwan at an elevation of about 250 m. These 2 butterfly species were separately kept in screen cages (60 x 60 x 60 cm) in a greenhouse. Each cage was supplied with a vial of 10% sucrose solution containing yellow food coloring and a cotton wick to facilitate feeding (Renwick and Radke 1985 1988, Huang and Renwick 1993). One Chinese cabbage plant was also enclosed in each cage on which the butterflies could lay eggs. After hatching, neonate larvae were transferred to 250 ml rearing cups with cabbage foliage and grown in a Percival growth chamber (with a 16:8 h light: dark photoperiod) at a constant 22°C. Some of these neonate larvae were assigned to feeding studies, while others

were reared on cabbage foliage until adulthood for oviposition experiments.

Oviposition experiments

To evaluate the foliage quality of the host plants, we measured the ovipositional choice of adult butterflies. After pupation, pupae of both butterfly species were separated by sex (Richards 1940), and after eclosion, butterflies were transferred to greenhouse cages for mating and oviposition choice tests. Butterflies in the colony and bioassay cages were provided with vials of 10% sucrose solution containing yellow food coloring and a cotton wick to facilitate feeding. Supplementary lighting in the greenhouse (with a 16 h photophase) facilitated mating (Wolfson 1980). Nylon mesh cages (60 x 60 x 60 cm) were positioned in the greenhouse where they could receive sun from early morning until late afternoon. Five days after eclosion, 3 mated female butterflies were released into the cages, and 2 potted plants representing treatments (fertilized) and controls (unfertilized) were provided. Seventy-two hours later, plants were removed from the cages, and the eggs were counted. Eight replicates (in the choice test) were conducted for each butterfly species.

Feeding trials

Long- and short-term feeding trials were conducted to evaluate the feeding performance of *P. rapae crucivora* and *P. canidia canidia* on leaves from fertilized and unfertilized cabbage leaves. For experimental bioassays, insects were reared in a Percival growth chamber (with a 16:8 h light: dark photoperiod) at a constant 22°C.

Long-term feeding trials were conducted to assess the effects of foliage quality on insect development and growth over the entire larval feeding and pupal stages. Feeding trials began when eggs hatched in Feb. 2001. One newly hatched larva of each insect species was weighed and individually reared until pupation in a plastic rearing cup (250 ml) with leaves from either fertilized or unfertilized plants. Sixty individuals were reared per treatment per insect species (i.e., 60 x 2 x 2 = 240 larvae). Leaf material was changed every day to ensure freshness. To monitor the growth rates of insects over the entire larval period, larval weights were measured daily. For each individual, the following traits were measured: development times from hatching to pupation and adult eclosion, pupal and adult weights, forewing length, growth rate, and proportional weight loss at metamorphosis. Each pupa was weighed 3 d after pupation and placed in a rearing cup until adult emergence. Larval duration was calculated as the elapsed time from egg hatching to pupation. Pupal duration was calculated as the time between pupation and adult emergence. The individual growth rate of each larva used in the experiments was calculated according to the method of Gotthard et al. (1994): Growth rate = [ln (pupal weight) - ln (hatching weight)]/larval time. This relative growth rate indicates the mean weight gain per day. In addition, the proportional weight loss between pupation and adult eclosion was measured based on the formula of Gotthard et al. (1994): Proportional weight loss = 1 - (adult weight/pupal weight). Mean and standard errors were calculated for larval weights, pupal weights, adult weights, larval durations, pupal durations, forewing lengths, growth rates, and proportional weight losses for insects fed on foliage from differently treated plants. Additional leaf material from the test plants was collected during the bioassay to measure leaf water, nitrogen, and glucosinolate contents.

Short-term feeding trials were conducted to evaluate the foliar quality effects on growth rates, food consumption rates, and food processing efficiencies of 4th instar larvae of both insect species. Fifty newly hatched larvae from each insect species were grown on cabbage foliage in a Percival growth chamber (with a 16:8 h light: dark photoperiod) at a constant 22°C until molting to 4th instars. Each assay consisted of a newly molted and weighed larva placed into a rearing cup (250 ml) containing a leaf from a plant of one of the 2 different treatments (n = 30 replicates per insect species per plant treatment). Leaves were changed every 1~2 d or as necessary during the bioassay. Upon molting to 5th instars, larvae were frozen, oven-dried at 50°C for 1 wk, and re-weighed. Nutritional indices were calculated to evaluate insect growth, consumption, and food utilization efficiency (Hare 1998). These indices were calculated from standard formulas for approximate digestibility (AD = (Ingestion - Feces)/Ingestion), efficiency of conversion of digested food (ECD = Biomass gained/(Ingestion - Feces)), and efficiency of conversion of ingested food (ECI = Biomass gained/Ingestion) as described by Waldbauer (1968) and Hare (1998). Initial rather than average weights of the larvae were used to calculate the relative growth rate (RGR) and relative consumption rate (RCR) (Farrar et al. 1989). Initial dry weights of test

insects were estimated based on a wet-to-dry weight conversion factor (of 0.148) determined from 10 newly molted 4th instars for each insect species. Similarly, initial dry weights of leaves fed to insects were estimated by dry weight conversions (at 0.088 for fertilized plants and 0.107 for unfertilized plants) using foliage collected from each plant species at the time of the bioassay. Means and standard errors were calculated for the duration, relative growth rate (RGR), relative consumption rate (RCR), total consumption (TC), approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI) for insects fed foliage from differently treated plants. As with the long-term feeding study, additional leaf material from test plants was also collected during the bioassay for foliar water, nitrogen, and glucosinolate content measurements.

Foliar chemistry of plant materials

Concurrent with the insect feeding trials, additional foliage (similar leaves as used in the bioassays) was collected from plants used in the bioassays, then flash-frozen in liquid nitrogen, freeze-dried, ground, and stored in a freezer. Water, total nitrogen, and total glucosinolate contents were quantified for each foliar sample. Differences between the wet and dry weights of leaf samples were used to determine water contents. Foliar nitrogen contents were determined by a standard micro-Kjeldahl assay. Leaf samples were first digested in acid (Parkinson and Allen 1975), and nitrogen contents were quantified by a micro-Nesslerization technique (Lang 1958). Glycine *p*-toluenesulfonate (5.665% N) was used as the standard. Total glucosinolates were quantified based on a determination of the glucose released after myrosinase digestion and subsequent deproteinization (Saini and Wratten 1987). A glucose-peroxidase system was used with 4-aminophenazone as the oxygen acceptor. Means and standard errors for foliar water, total nitrogen, and total glucosinolate concentrations for each of the plant species were calculated.

Statistical analysis

For the bioassays, means and standard errors (s.e.) were calculated for the insect performance parameters (survival, duration, larval weight, pupal weight, growth rate, consumption rate, and food utilization efficiency) and plant chemistry. Student *t*-tests (PROC TTEST; SAS Institute 1989) were used to compare insect performances between fertilized and unfertilized host plants.

RESULTS

Host-plant quality

Leaves of the fertilized plants had higher water and nitrogen contents than those of unfertilized plants. The level of total glucosinolates, however, was lower in fertilized than in unfertilized plants (Table 1). To the naked human eye, the leaves of fertilized plants appeared darker green in color.

Oviposition experiments

Females of both butterfly species laid significantly more eggs on the fertilized compared to the unfertilized host plants. Female butterflies of *P. rapae crucivora* laid almost more than 5-fold more eggs on fertilized than on unfertilized cabbage plants (Table 2). Female *P. canidia canidia* also laid more eggs on the fertilized compared to unfertilized host plants (Table 2).

Table 1. Water, nitrogen, and glucosinolate contents of *B. oleracea* var. *capitata* (mean ± SE)

Treatment	Water (%)	Nitrogen (% dry weight)	Glucosinolates (% dry weight)
Fertilized	91.2 ± 0.7	3.39 ± 0.48	0.69 ± 0.27
Unfertilized	89.3 ± 1.2	1.95 ± 0.29	2.73 ± 0.44
t	-5.57	-10.81	-5.21
df	22	32	14
P	<0.001	<0.001	<0.001

Table 2. Mean number of eggs laid on fertilized and unfertilized control plants in oviposition experiment (mean ± SE)

Treatment	Number of eggs	
	P. rapae crucivora	*P. canidia canidia*
Fertilized	73.6 ± 48.5	67.8 ± 19.4
Unfertilized	14.8 ± 10.5	12.3 ± 7.0
t	-3.34	6.02
df	16	10
P	0.004	<0.001

Feeding trials

The results of long-term feeding trials indicated that, overall, caterpillars of both butterfly species grew faster on fertilized host plants. The growth rate of *P. rapae crucivora* was significantly higher on fertilized than on unfertilized plants (Table 3). Therefore, total development time (from hatching to adult) of this insect species was shorter on fertilized than on unfertilized plants (Table 3). Altogether, mean development time was about 3 d longer on unfertilized compared to fertilized *B. oleracea* var. *capitata*. Larval weight of *P. rapae crucivora* varied markedly between insects reared on fertilized and unfertilized cabbage plants; the range of variation was more than 1.5-fold from 6 d of age onward (Fig. 1). Pupal weight, adult weight, and forewing length of *P. rapae crucivora*, however, did not significantly differ between insects fed on fertilized or unfertilized plants (Table 3).

As with the results for *P. rapae crucivora*, the growth rate of *P. canidia canidia* was also significantly higher on fertilized than on unfertilized plants (Table 4). Total development time (from hatching to adult) was also less on fertilized than on unfertilized plants (Table 4). Overall, the mean development time was about 5 d longer on unfertilized compared to fertilized *B. oleracea* var. *capitata*. This was due to a longer larval duration, as the pupal duration did not vary significantly between these 2 treatments (Table 4). Likewise, larval weight of *P. canidia canidia* markedly varied between insects reared on fertilized and unfertilized cabbage plants; the range of variation was more than 4-fold from 6 d of age onward (Fig. 1). However, in contrast to the results for *P. rapae crucivora*, pupal weights and adult weights of *P. canidia canidia* differed significantly between fertilized and unfertilized host plants (Table 4). Both pupal and adult weights were lower for larvae which fed on fertilized plants (Table 4). Yet, forewing length of *P. canidia canidia* did not significantly differ between insects which fed on fertilized or unfertil-

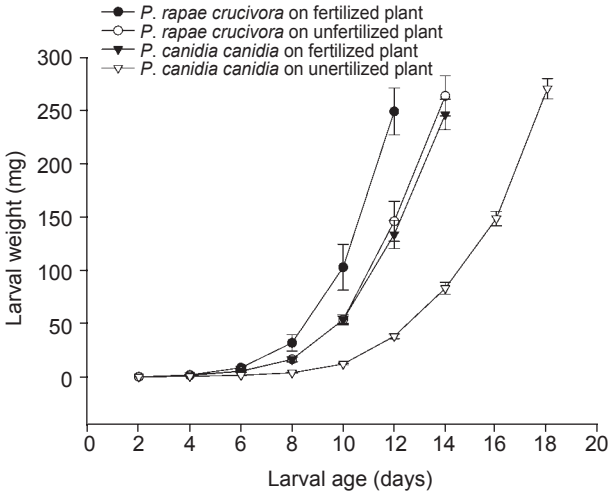

Fig 1. Growth of *P. rapae crucivora* and *P. canidia canidia* larvae on fertilized and unfertilized cabbage.
Each point represents the mean value for weights of 60 insects. Vertical lines indicate ± 1s.e.

Table 3. Performance of *P. rapae crucivora* on fertilized and unfertilized host plants (mean ± SE)

Treatment	Larval duration (days)	Pupal duration (days)	Development time (days)	Pupal weight (mg)	Adult weight (mg)	Forewing length (mm)	Growth rate (% / day)	Weight loss (%)
Fertilized	14.3 ± 0.7	8.6 ± 0.6	23.0 ± 1.1	191.1 ± 18.5	77.1 ± 10.0	27.1 ± 1.1	50.8 ± 2.6	59.6 ± 3.2
Unfertilized	16.9 ± 1.1	9.4 ± 0.9	26.3 ± 1.1	197.8 ± 17.5	79.9 ± 9.8	27.0 ± 0.9	43.5 ± 2.5	59.6 ± 3.1
t	12.43	4.75	14.48	1.72	1.28	-0.29	-13.07	0.01
df	63	60	79	82	79	85	70	80
P	＜0.001	＜0.001	＜0.001	0.089	0.203	0.767	＜0.001	0.988

Table 4. Performance of *P. canidia canidia* on fertilized and unfertilized host plants (mean ± SE)

Treatment	Larval duration (days)	Pupal duration (days)	Development time (days)	Pupal weight (mg)	Adult weight (mg)	Forewing length (mm)	Growth rate (% / day)	Weight loss (%)
Fertilized	14.8 ± 1.0	10.0 ± 0.6	24.8 ± 1.3	174.9 ± 18.7	65.7 ± 7.4	27.2 ± 1.2	48.6 ± 2.9	62.4 ± 1.6
Unfertilized	19.0 ± 0.6	10.6 ± 0.7	29.6 ± 1.2	205.1 ± 19.7	86.9 ± 7.6	27.5 ± 1.0	38.5 ± 0.9	57.5 ± 2.1
t	-11.34	-1.8	-7.33	-3	-5.44	-0.61	12.08	4.72
df	15	7	9	8	8	10	20	7
P	＜0.001	0.114	＜0.001	0.017	＜0.001	0.554	＜0.001	0.002

ized plants (Table 4).

Results of the short-term feeding trials revealed that the performance (durations, growth rates, and food processing efficiencies) of 4th-instar *P. rapae crucivora* varied substantially between fertilized and unfertilized host plants (Table 5). *Pieris rapae crucivora* larvae had higher growth rates (RGR), digestibility (AD), and conversion efficiencies (ECI) on fertilized than on unfertilized host plants. In contrast, the consumption rate (RCR) and total consumption (TC) were higher for insects which fed on unfertilized host plants. In addition, survivorship of 4th-instar *P. rapae crucivora* was higher for larvae which fed on fertilized than on unfertilized plants (Table 5). Generally, the performance of *P. rapae crucivora* in this short-term study paralleled that of insects in the long-term study, with faster growth on fertilized foliage and higher consumption on unfertilized foliage.

Performance (durations, growth rates, and food processing efficiencies) of 4th-instar *P. canidia canidia* also varied significantly between fertilized and unfertilized host plants (Table 6). *Pieris canidia canidia* larvae had higher digestibility (AD) and conversion efficiency (ECD and ECI) on fertilized than on unfertilized host plants. However, the growth rate (RGR), consumption rate (RCR), and total consumption (TC) were all higher for insects which fed on unfertilized host plants. Similar to the results for the 4th-instar *P. rapae crucivora* study, survivorship was also higher for larvae which fed on fertilized than on unfertilized plants (Table 6). Overall, the performance of *P. canidia canidia* in the short-term study partially paralleled that of insects in the long-term study, with higher consumption on unfertilized foliage.

DISCUSSION

In this study, we clearly demonstrated that fertilization significantly changed the phytochemical content of *B. oleracea* var. *capitata*. These phytochemical changes were further associated with changes in the performance of cabbage white butterfly larvae. Female butterflies of both species preferred to oviposit on foliage of fertilized plants, and caterpillars also grew faster on the fertilized foliage.

The literature indicates that foliar nitrogen content can increase from 50% to 220% after long-term fertilization (Slansky and Feeny 1977, Myers 1985, Estiarte et al. 1994), while leaf water concentration may also be elevated by up to 15% in

Table 5. Fourth-instar *P. rapae crucivora* performance (mean ± SE)

Treatment	Survival (%)	Duration (Days)	RGR (mg/mg/day)	RCR (mg/mg/day)	TC (mg)	AD (%)	ECD (%)	ECI (%)
Fertilized	100	2.7 ± 0.3	1.1 ± 0.2	5.1 ± 0.7	216.3 ± 23.3	45.0 ± 5.9	48.8 ± 11.2	21.4 ± 2.6
Unfertilized	80.4	3.5 ± 0.4	0.9 ± 0.2	6.8 ± 1.1	377.1 ± 43.8	21.8 ± 5.8	65.0 ± 20.5	13.1 ± 1.9
t		7.22	-3.17	5.68	13.8	-10.8	2.9	-11.67
df		34	39	25	24	23	28	33
P		＜0.001	0.003	＜0.001	＜0.001	＜0.001	0.007	＜0.001

RGR = relative growth rate；RCR = relative consumption rate；TC = total consumption；AD = approximate digestibility；ECD = efficiency of conversion of digested food；ECI = efficiency of conversion of ingested food.

Table 6. Fourth-instar *P. canidia canidia* performance (mean ± SE)

Treatment	Survival (%)	Duration (Days)	RGR (mg/mg/day)	RCR (mg/mg/day)	TC (mg)	AD (%)	ECD (%)	ECI (%)
Fertilized	80	3.1 ± 0.2	0.6 ± 0.1	2.8 ± 0.2	149.7 ± 8.6	63.1 ± 2.9	37.9 ± 3.7	23.8 ± 1.4
Unfertilized	60	3.2 ± 0.2	0.8 ± 0.1	5.9 ± 0.4	313.1 ± 13.1	52.9 ± 1.9	26.5 ± 1.1	14.0 ± 0.4
t		-1.39	-5.08	-14.52	-24.37	7.38	8.77	22.01
df		10	12	7	8	12	10	10
P		0.194	＜0.001	＜0.001	＜0.001	＜0.001	＜0.001	＜0.001

RGR = relative growth rate；RCR = relative consumption rate；TC = total consumption；AD = approximate digestibility；ECD = efficiency of conversion of digested food；ECI = efficiency of conversion of ingested food.

fertilized foliage (Wolfson 1980 1982). Results of our study revealed a similar pattern with nitrogen content increasing by 74% in fertilized plants; but water increased by only 2% in fertilized foliage. In contrast to the results of primary metabolites, concentrations of foliar secondary compounds decreased in fertilized plants. The main nitrogen-based secondary metabolites of *B. oleracea* var. *capitata* are glucosinolates. Our results indicated that concentrations of glucosinolates substantially decreased in fertilized foliage. Based on the carbon/nutrient balance hypothesis (Bryant et al. 1983, Tuomi et al. 1988), fertilized plants may have elevated vegetative growth and this increasing growth requires a larger proportion of available nitrogen (Karowe et al. 1997, Hemming and Lindroth 1999). Therefore, the production of nitrogen-based secondary compounds, such as glucosinolates, may decrease (Karowe et al. 1997). Overall, this study indicated that the addition of nutrients increased foliar nitrogen and water concentrations but decreased glucosinolate content.

Ovipositing butterflies have been shown to select food plants most suitable for their offspring's development (e.g., Rausher 1981, Myers 1985, Janz and Nylin 1997). According to our results, females of both butterfly species were able to discriminate between fertilized and unfertilized *B. oleracea* var. *capitata*. Female butterflies deposited a substantially larger number of eggs on leaves of fertilized plants. Previous studies also revealed similar patterns of female *P. rapae* oviposition preference for fertilized host plants (Wolfson 1980, Myers 1985). One reason for the attractiveness of these fertilized plants may be their chemical contents (Dethier 1982, Myers 1985, Brandhorst-Hubbard et al. 2001). Cabbage white butterflies were found to prefer ovipositing on plants with a better physiological status, which have foliage that is usually a greener color with higher water and nitrogen contents (Wolfson 1980, Myers 1985). Likewise, our results revealed that fertilized plants had increased water and nitrogen contents, and butterflies laid more eggs on these plants.

Secondary metabolites may also play an important role in regulating the oviposition behavior of cabbage white butterflies. Host plant glucosinolates are considered to be oviposition stimulants for different *Pieris* species (Huang and Renwick 1993, Huang et al. 1993 1994). Our results, however, indicated that both cabbage white butterfly species preferred to lay eggs on fertilized plants even though they had decreased glucosinolate concentrations. A possible explanation is that an optimal (or minimal) concentration of glucosinolates was reached in fertilized host plants, thus initiating the stimulating response for these 2 butterflies (Huang and Renwick 1993). Thus, these female butterflies seem to be able to integrate various olfactory and/or visual stimuli and decide to accept or reject a potential host plant as an ovipositional substrate.

Differences in the degree of fertilization also translated into marked treatment effects for caterpillar performance. In general, caterpillars of both butterfly species had increased growth rates and shorter development times when fed on more-nutritious foliage. In a related study, Myers (1985) demonstrated similar results of caterpillars of *P. rapae* growing faster when grown on the preferred fertilized plants. In addition, Myers (1985) also found that caterpillars attained a heavier weight when grown on fertilized foliage. Our results, however, revealed that caterpillars' final weights were lower when they fed on fertilized foliage. In response to low quality foods, herbivorous insects may have compensatory responses which include increased food consumption and nutrient utilization efficiency (Wheeler and Halpern 1999), that result in a final biomass comparable to larvae fed high-quality food (Slansky and Feeny 1977). Thus, our results suggest that compensatory feeding may occur in these 2 cabbage white butterflies in response to poor-quality (unfertilized) foliage.

Comparison of the performance of these 2 *Pieris* species revealed differences in their ability to effectively utilize host plant resources. For adults, the 2 *Pieris* species showed little difference in locating oviposition host plants. Patterns in response to changes in phytochemical concentrations were generally similar between larvae of these 2 *Pieris* species, i.e., both butterfly species grew faster on foliage of fertilized plants. However, larvae of *P. rapae crucivora* grew faster and larger than *P. canidia canidia* on fertilized foliage; but the 2 species showed little difference in growth when feeding on foliage of unfertilized plants. Our results indicate that food conversion efficiencies are similar between these 2 *Pieris* species, but *P. rapae crucivora* has a higher food intake. Therefore, *P. rapae crucivora* may have an increased competitive advantage in highly fertilized crop fields.

Although our results indicate an increase in performance and a choice of fertilized over unfertilized plants, the underlying mechanisms are not clear. This could have been due to either a decrease in foliar glucosinolate concentrations or

an increase in nitrogen contents. Caterpillars grown on high-glucosinolate foliage may need to convert much of their resources for growth to detoxifying the glucosinolates. Our results, however, are unable to distinguish between these 2 potential alternative explanations.

In conclusion, this research shows that fertilization can strongly affect host plant chemistry, and that the foliage of fertilized plants has increased nutrient and decreased allelochemical contents. It was also revealed that these phytochemical changes may affect the performance of insect herbivores specializing on these plants. Both *Pieris* species grew better and oviposited more eggs on fertilized foliage. Moreover, this study reveals that these 2 *Pieris* butterflies may respond differently to changes in host plant chemistry. Work now underway addresses the potential host preference and selection of these 2 *Pieris* butterflies among cultivated and wild host plants, and we are investigating the influence of host plant chemistry on the population dynamics of these 2 butterflies.

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