Spatial Variation in Foliar Chemicals Within Radish (*Raphanus sativus*) Plants and Their Effects on Performance of *Spodoptera litura*

JITENDRA YADAV, CHING-WEN TAN, AND SHAW-YHI HWANG[1]

Department of Entomology, National Chung Hsing University, Taichung, Taiwan 402

ABSTRACT Foliar chemicals are variable within a plant and this may affect herbivore feeding preference. This study was carried out to quantify concentrations of primary (nitrogen, water, and total nonstructural carbohydrates) and secondary substances (sinigrin) in young and old leaves of *Raphanus sativus* L. and to evaluate performance and survival of a generalist herbivore *Spodoptera litura* F. feeding on them. Forty to 50-d-old *R. sativus* plants were used in both foliar chemical analysis and insect performance bioassays. Leaves located on the third to the sixth node from the base of the plant were defined as old leaves and the remaining leaves (from seventh node to the plant apex) of the plant were referred as young leaves in this study. All foliar chemicals except water differed significantly between young and old leaves. Moreover, young leaves were more nutritious but much more defended, based on sinigrin content, against *S. litura* than old leaves. Performance and survival of *S. litura* were reduced on young leaves as compared with old leaves. Male and female larval duration only differed significantly on young leaves. Female larval development time was longer than male development time on young leaves, but not on older leaves. Therefore, this study revealed that defenses in young leaves have differential effects upon male and female *S. litura*.

KEY WORDS foliar chemicals, spatial variation, performance, *Raphanus sativus*, *Spodoptera litura*

Plants are naturally equipped with various defensive traits against herbivory (Kessler and Baldwin 2002, Stamp 2003). These defensive traits include morphological and chemical characteristics which are specific to particular plant families or genera. Plant traits, ranging from metabolic processes to morphology, normally vary (Jones 1999). Phytochemicals, the metabolic plant traits, are more influential than morphological characteristics in determining herbivore host ranges (Schultz 1988).

Plants contain various defensive phytochemicals, such as alkaloids, steroids, phenolics, saponins, glucosinolates, tannins, resins, essential oils, and organic acids (Schoonhoven et al. 2005). Glucosinolates are the most prominent phytochemicals in Brassicaceae; but their profiles differ considerably among species within this plant family. For example, radish (*R. sativus* L.) possesses only 15 different glucosinolates out of the total 120 glucosinolates reported from Brassicaceae (Fahey et al. 2001). However, only few glucosinolate compounds have been identified for their defensive properties against herbivores. Sinigrin is the predominant glucosinolate in several Brassicaceae species (Kushad et al. 1999, Wennberg et al. 2006, Smallegange et al. 2007, Arany et al. 2008, Cartea et al. 2008) and its defensive role against herbivores has been documented (Shields and Mitchell 1995, Li et al.

2000, Gabrys and Tjallingii 2002). Although glucosinolates have been found to negatively affect performance of some generalist herbivores (Agrawal 2000, Li et al. 2000, Gols et al. 2008); studies on its separate effect on generalist male and female herbivores are lacking.

In addition to plant defensive chemicals, herbivore performance may also be affected by the levels of nutrients in plant tissues (Tabashnik and Slansky 1987). There are several reports of nutritional variation among plant parts (Alonso and Herrera 2000, Bittencourt-Rodrigues and Zucoloto 2005, Lambdon and Hassal 2005). Nitrogen and water are the two most important nutrients for many insect herbivores, and their relative content can strongly affect the overall nutritional quality of plant tissues for insect herbivores (Mattson 1980, Scriber and Slansky 1981, Schoonhoven et al. 2005, Chen et al. 2008).

Phytochemicals are regulated genetically, developmentally, and environmentally, and can, therefore, differ considerably within and among plant tissues (Orians and Jones 2001, Pavia et al. 2002, Donaldson et al. 2006). The causes for phytochemical variations within a plant that affect the expression of various plant traits relevant to herbivores is leaf phenology (Lindroth et al. 1987, Riipi et al. 2002), defense induction (Karban and Baldwin 1997), somatic mutations in meristematic tissues (Gill et al. 1995, Tuskan et al. 1996), and ontogenetic maturation (Boege and

Marquis 2005, Laitinen et al. 2005). Previous studies have found that glucosinolate concentrations tend to be higher in younger leaves (Lambdon and Hassal 2005, Shelton 2005, Reifenrath and Müller 2009). Spatial variation of phytochemicals within a plant potentially alters the ecological and evolutionary consequences of plant–herbivore interactions and also determines feeding niche of herbivores within a plant. Although young leaves are more nutritious than old leaves, generalist herbivores commonly prefer older foliage (De Boer 1999, Bluthgen and Metzner 2007). This preference is likely because of the higher concentrations of defensive chemicals typically found in younger leaves, which can act as toxins, deterrents or both. Numerous hypotheses have been postulated about why variation of such phytochemicals has been maintained within a plant and how it improves plant defense against herbivores. The optimal defense theory (ODT) postulates that defenses are allocated within a plant in proportion to risk of the plant part and value of it to plant fitness (Rhoades 1979). The ODT theory also predicts that defense allocation should change dynamically as fitness value and risk of attack of plant tissues change during plant development. However, only a relatively small number of studies have been carried out to measure spatial variation of foliar secondary compounds and nutrients within Brassicaceae plants and measured their effects on performance of male and female generalist herbivores.

This study was conducted to assess the spatial variations of foliar chemicals in radish plants and their effects on a generalist herbivore, *Spodoptera litura* F. The common cutworm, *S. litura* is a polyphagous insect pest widely distributed throughout Asia and Oceania (Venette et al. 2003) and the host plant spectrum of *S. litura* includes important agricultural crops in different plant families including Brassicaceae. Radish plant, *Raphanus sativus* L. is a fast-growing, herbaceous, and cosmopolitan crop of Brassicaceae, which is commonly attacked by *S. litura* in the field. The specific objectives of this study were to (1) quantify major foliar chemicals (nitrogen, total nonstructural carbohydrates, water, and sinigrin) of young and old leaves of radish plants, and (2) evaluate performance of the generalist herbivore, *S. litura* and its male and female on young and old leaves of radish plants.

Materials and Methods

Insects. Males and females *S. litura* were collected from colonies maintained at the Insect-plant laboratory, Department of Entomology, National Chung Hsing University, Taiwan, and were housed (10 pairs) in a glass cylinder (22 cm height × 14.5 cm diameter) lined with tissue paper for egg collection. The glass cylinder was placed at room temperature (25 ± 2°C) and paired adult insects were fed sugar solution. Sugar solution was freshly prepared each time by dissolving sugar until saturation into 500 ml distilled water and 300 ml beer with 6 g ascorbic acid, and 3 g hydroxybenzoic acid methyl ester (Sigma, Steinheim, Germany). Three cotton sticks (7 cm length × 1 cm

diameter) were soaked in sugar solution and were hung inside the glass cylinder to feed them. Eggs laid on tissue paper were collected and put inside a plastic rearing cup (250 ml, Alpha Plus Scientific Corporation, Taoyuan, Taiwan) containing a small moistened cotton ball. The rearing cup was placed inside a growth chamber (model A 414931206,Yuh Chuen Chiou Industries Ltd., Taiwan) under controlled conditions (25 ± 2°C, 65 ± 3% RH, 16:8 h L:D) for hatching. Hatched larvae were also reared under the same controlled conditions in the growth chamber and were fed on artificial diet. Artificial diet was prepared following a protocol developed by Gupta et al. (2005). First instars were reared aggregately in the same rearing cup. Second instars were transferred individually to a plastic tray with 30 compartments per tray, until pupation. Pupae were collected, sexed and both male and female were separately kept in plastic rearing cups (250 ml) until adult emergence. Ten pupae were usually placed in each cup. After emergence, males and females were paired again for oviposition to maintain a colony throughout this study.

Plants. Radish (*R. sativus*) seeds were purchased from a local seed company (Known-You Seed Company, Kuosheng, Taiwan) and were grown in a greenhouse under controlled environmental conditions (25 ± 3°C, 16:8 h L:D). Seeds were treated with water at 45°C for 30 min for surface sterilization. Seed were then sown in potting soil (MOS-010, Known-You Seed Company, Kuosheng, Taiwan) in 104-well plates (35 ml/well) and were watered daily. One-week-old seedlings (first true leaf stage) were transplanted in plastic pots (10.5 cm height × 12 cm diameter) filled with potting soil. The plants were watered every other day. Forty to 50-d-old plants that had 9–10 true leaves were used in this study. Plants were grown in four different lots at 10 d interval (100 plants per lot) to ensure availability of plants of approximately the same age throughout the bioassay. Leaves of two age classes were used in bioassays and for chemical analysis. 'Old leaves' were harvested from the third to the sixth node from the base of the plant. Basal leaves that had turned yellow were excluded from the study. 'Young leaves' were harvested from the seventh node to the plant apex (10th node).

Larval Performance Bioassay. A bioassay was conducted to evaluate total larval developmental time, duration of different instars, pupal weight, and mortality during different instars of *S. litura* reared on old and young leaves of *R. sativus*. All leaves from each test plant were removed using surgical scissors and classified as 'young' or 'old' as described above for use in the bioassay. The petiole of each leaf was placed into an Eppendorf microcentrifuge tube (2 ml, Bioscience Inc., Salt Lake City, UT) filled with water to maintain leaf turgor. The petiole was inserted through a hole drilled in the lid of the Eppendorf tube and the insertion point was sealed with parafilm (model Parafilm "M" laboratory film, Pechiney Plastic Packaging Inc., Chicago, IL) to prevent evaporation. Eppendorf tubes containing leaves were placed individually in an opaque plastic cup (15.1 cm height × 9.5 cm diameter,

Good Flag Biotechnology Corporation, Tauyang, Taiwan). An insect pin (number 5, 0.60 mm diameter, Foot Tsu Yong Stationery, Changhua, Taiwan) was used to make tiny holes in each plastic cup cover to ensure aeration. Eggs of *S. litura* were collected and placed individually on each leaf using a soft paint brush (bristle size 0.5 mm diameter) and an insect pin (number 5, 0.60 mm diameter). Eighty eggs were used in each treatment (old leaves and young leaves) and unhatched eggs during the bioassay were excluded from this study. Plastic cups with individual leaves infested with eggs were placed in the growth chamber ($25 \pm 2°C$, $65 \pm 3\%$ RH, 16:8 h L:D). Each leaf was removed from its cup and observed daily in the morning using a stereomicroscope to detect eggs and first to second instar larvae. The plastic cup was cleaned daily and leaves were replaced every 1–3 d to ensure the leaf material was fresh. During observation, larval developmental stage was recorded based on head capsule color and molting stage. First and second instars could be distinguished clearly by head capsule color while subsequent instars were distinguished based on its molting stage. Larval molt was recorded and used as the main criteria to determine the developmental stage of the larvae (third-last instar). The total development time of each larva was calculated as elapsed time from egg hatching to pupation. Immediately after pupation, pupae were transferred to a tissue paper-lined plastic cup (250 ml) and held for 3 d before sexing and weighing.

Relative Growth Rate (RGR) Bioassay. A bioassay was conducted to assess the RGR of *S. litura* during the fourth instar on old and young leaves of *R. sativus*. Relative growth rate of fourth instar of *S. litura* was calculated as mean dry weight gain per day on old and young leaves of *R. sativus* (40–50 d old). In this bioassay, only the third and the seventh leaves of test plants were used as young leaf and old leaf, respectively. Twelve fourth instar larvae were individually reared on either old or young leaves. Leaf turgor was maintained as described in larval performance bioassays. Fifty third instars at molting stage were selected from a mass of larvae grown on artificial diet. Larvae were placed individually in small petri dishes (1 cm height $\times$ 5.5 cm diameter, Alpha Plus Scientific Co., Taoyuan, Taiwan) and were observed three to four times a day for emerging fourth instar larvae. Newly emerged fourth instar larvae were collected and immediately weighed and placed individually on a leaf within the petri dish. At the same time, 10 newly emerged fourth instar larvae were first weighed, frozen at $-20°C$ for 24 h and then oven dried at 45°C for 1 wk (model YH 605 TW, Yuan, Hung, Instrument, Co., Ltd., Taiwan) to estimate water content. The difference in wet and dry weight of these 10 larvae was used to calculate an average percent water value for larvae, for use in RGR calculation. Larvae feeding on leaves were examined 3–4 times daily for signs of molting. Before molting, larvae were transferred individually to another small petri dish. As soon as fifth instars emerged, larvae were frozen immediately and

oven dried to calculate RGR following formula developed by Waldbauer (1968).

Foliar Chemical Analyses. Water content was quantified for leaf tissue of 10 randomly selected test plants. The third and the seventh leaves of these test plants were taken as representative of old and young leaves, respectively. Leaves were removed, placed in a plastic bag on ice and carried immediately to laboratory. The wet weight of each sample leaf was measured within 20 min. The leaves were then oven dried at 45°C for 1 wk and reweighed to calculate dry weight. Percent foliar water content was calculated using the wet and dry weight values of these leaves.

Ten sample plants of the same age as those used for insect performance bioassays were randomly selected and their old and young leaves were removed with surgical scissors, as above and brought to the laboratory. In the laboratory, the midvein of each was removed, after which the leaf was placed in a paper envelop and flash-frozen in liquid nitrogen for 30 min. Leaves were then freeze-dried (model GID 201B, Ulvac Kiko Inc., Yokohama, Japan) for 24 h, ground, and stored at $-20°C$ until needed for chemical analyses. Nitrogen, sinigrin and total nonstructural carbohydrates (TNC) content of sample leaves were quantified. Kjeldahl nitrogen was determined by a micro-Nesslerization technique (Lang 1958) after acid digestion of leaf samples (Parkinson and Allen 1975), using glycine p-toluenesulfonate (5.665% N, Sigma) as the standard. An enzymatic method was used to measure the TNC of each leaf sample. Extracts of TNC (starch plus soluble carbohydrates/sugars) were incubated with amyloglucosidase to completely hydrolyze starch before assaying for reducing sugars (Madsen 1997, Liao 2003). Old and young leaves of six sample plants were selected for sinigrin analysis using high-performance liquid chromatography (HPLC) (Tsao et al. 2002). Sinigrin was extracted from dried sample leaves powder using 50% acetonitrile (ACN) in water. A stock standard solution (1 mg/ml, wt:vol) of sinigrin in 50% ACN was prepared and then was diluted to various concentrations (0–100 $\mu g/ml$) to generate a calibration curve. A C18 reverse-phase column (5 μm, 15 cm $\times$ 4.6 mm, Hypersil gold, Thermo Fisher Inc., Waltham, MA) was used in the HPLC instrument. The detector was fixed at 228 nm (λ_{max}) for sinigrin. The binary mobile phase was composed of NH_4OAC (ammonium acetate, 0.025 M, pH 6.75) (A) and ACN (B). The flow rate was kept constant at 1.0 ml/min for a total run time of 10 min and the solvents were using 99% A/1% B (vol:vol). A 100 μl syringe (Gelman Instrument Co., Ann Arbor, MI) was used to inject each sample (20–25 μl) to the HPLC instrument. The HPLC instrument was equipped with a UV detector and Chrom Manager multisystem software, which was used in data acquisition and analysis. Sinigrin standards were used to draw a regression curve to obtain the regression equation used to calculate sinigrin concentration in the leaf samples.

Statistical Analysis. The data were analyzed using the procedure TTEST in SAS system 1999 for Windows, version 8 (SAS Institute Inc., Cary, NC). The

Table 1. Larval performance of *S. litura* on the two leaf age classes of *R. sativus*

|	Young leaf	Old leaf	P value
Larval development time (days)	39.46 ± 1.61	27.75 ± 0.73	<0.01
Pupal wt (mg)	229.66 ± 11.53	266.68 ± 8.75	<0.05
Mortality (%)	48	30	
Relative growth rate of fourth instar (mg/mg/d)	0.18 ± 0.03	0.65 ± 0.04	<0.01
Duration of fourth instar (days)	6.95 ± 0.61	2.94 ± 0.14	<0.01

t-test was used to compare total larval duration, pupal weight, RGR, instar duration, water, nitrogen, soluble carbohydrate, insoluble carbohydrate, total nonstructural carbohydrates, and sinigrin values between young and old leaves.

Results

Larval Performance. The performance of S. *litura* larvae was lower on young leaves than on old leaves, based on significant differences in all the parameters measured in this study (Table 1). Larval development time on young leaves was significantly longer (>1.5-fold) than on old leaves ($t = -7.73$; df = 37.9; $P < 0.0001$). Pupal weight was significantly higher when the larvae were reared on old leaves, compared with young leaves ($t = 2.59$; df = 58; $P = 0.0120$). The total mortality of larvae was higher on young leaves than on old leaves (30% on old leaves, 48% on young leaves) (Table 1). Moreover, larval duration during different instars except first instar was also significantly longer on young leaves than on old leaves (first instar, $t = -1.67$, df = 58; $P = 0.10$; second instar, $t = -3.58$, df = 43, $P < 0.05$; third instar, $t = -3.67$, df = 31.9, $P < 0.05$; fourth instar, $t = -4.55$, df = 29.2, $P < 0.01$; fifth instar, $t = -6.27$, df = 35.9, $P < 0.01$; sixth instar, $t = -7.59$, df = 39.1, $P < 0.01$) (Fig. 1). Most mortality of larvae occurred during first instar on both young and old leaves and mortality was also comparatively higher on
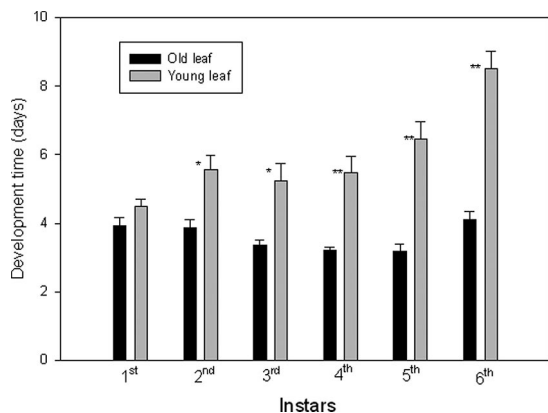
Fig. 1. Larval development time during different instars (mean ± SE) of S. *litura* on old (*n* = 32) and young (*n* = 28) leaves of *R. sativus*. Asterisks denote significant differences between leaf type. *$P < 0.05$. **$P < 0.01$.
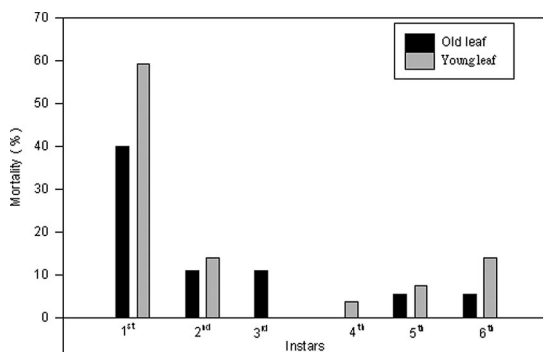
Fig. 2. Larval mortality rates for different instar S. *litura* on old and young *R. sativus* leaves.

young leaves during first instar (≈59% and 40% of total mortality on young leaves and old leaves, respectively) (Fig. 2). Some larvae failed to pupate and died with a rate of 14% on young leaves and 5.5% on old leaves.

Male and Female Larval Development Time. Male and female larval development time differed only on young leaves (Fig. 3). Development time on young leaves was significantly longer for female larvae than for male larvae ($t = -2.07$; df = 26; $P = 0.0487$), whereas no significant difference was observed between male and female larval development time on old leaves ($t = -1.62$; df = 29; $P = 0.1171$).

RGR. RGR and duration of fourth instars of S. *litura* differed on old and young leaves (Table 1). RGR was almost 3.5-fold greater on old leaves than on young leaves ($t = 8.98$; df = 18; $P < 0.0001$). Mean fourth instar duration was also significantly different, lasting 7 d on young leaves and 3 d on old leaves ($t = -6.37$; df = 10; $P < 0.0001$).

Foliar Chemistry. The nitrogen concentration in young leaves was almost 1.5 times greater than in old leaves ($t = -5.64$; df = 16; $P < 0.0001$) (Table 2). Water content did not differ between young and old leaves ($t = 0.63$; df = 18; $P = 0.5377$) (Table 2). Total TNC concentration was significantly higher in old leaves than in young leaves ($t = 2.80$; df = 15; $P = 0.0135$) (Table 2). Both soluble carbohydrate and insoluble carbohydrate levels were also significantly higher in old leaves than in young leaves (soluble carbohydrate, $t = 2.59$; df = 18; $P = 0.0185$, insoluble carbohydrate, $t = 2.76$; df = 8.91; $P = 0.0225$) (Table 2). Sinigrin concentration was significantly higher (≈1.7-fold) in young leaves than in old leaves ($t = -2.94$; df = 9; $P = 0.0165$) (Table 2).

Discussion

The content of most compounds measured in this study differed significantly between young and old leaves of *R. sativus*. In bioassay, the generalist caterpillar S. *litura* performed better on old leaves as compared with young leaves. Thus, the differences in primary and secondary metabolites in the two leaf age
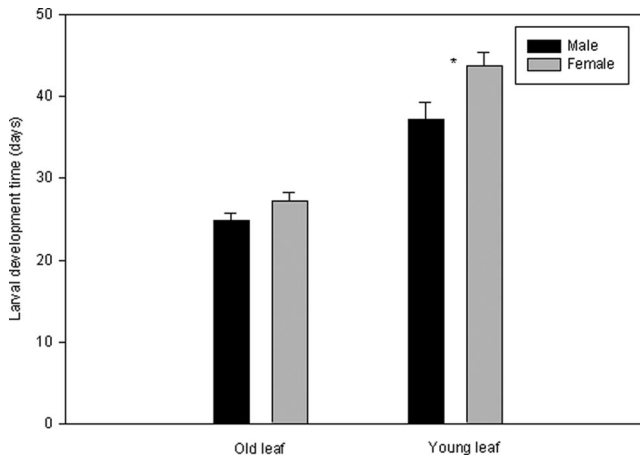
Fig. 3. Larval development time of male and female (mean $\pm$ SE) of *S. litura* on old (17 M, 14 F) and young (18 M, 10 F) leaves of *R. sativus*. Asterisks denote significant differences. *$P < 0.05$.

classes evidently contribute to differences in *S. litura* performance.

Young leaves are sites of rapid protein synthesis and other anabolic processes (Marschner 1995); therefore, they were expected to be a richer food source for insects. In this study, total nitrogen concentration in young leaves was almost 1.5 times higher than in old leaves. Past studies also support higher level of nitrogen in young leaves than in old leaves of Brassicaceae plants as well as trees (Ikonen 2002, Lambdon and Hassal 2005, Bluthgen and Metzner 2007). Nitrogen is a constitutive element of amino acids, the building blocks of proteins. Deficit in protein causes death of early instars, but in later instars, this deficit in protein may prolong growth of instars (Raubenheimer and Simpson 1999). In addition to nitrogen, water content can also affect leaf nutritional quality. When normal water content in foliage falls, its nutritional value decreases (Schoonhoven et al. 2005). However, previous studies are contradictory concerning water content level in old and young leaves of plants. Some studies report that water content is high in young leaves (Coley et al. 2006), whereas others report that water content is lower in young leaves (Reifenrath and Müller 2009).

Nonstructural carbohydrates are produced in tissue during photosynthesis. These carbohydrates are used either as energy sources for vegetative growth and development or as precursor in the biosynthesis of various chemicals in plant (Ralph et al. 1992). Results of this study revealed that total TNC concentration (i.e., sugar and starch) was significantly higher in old leaves than that in young leaves. In addition, insoluble carbohydrate (starch) was the dominant carbohydrate accounting for almost 63 and 67% of TNC in old and young leaves, respectively. Although TNC levels in plant tissue are used to reflect plant overall carbon supply status (Körner 2003, Shi et al. 2006), the caloric value of plants is considered less important to insects (Schoonhoven et al. 2005) because carbohydrates can also be synthesized from fats and/or amino acids by the insects themselves (Behmer 2009). Moreover, our results also showed that TNC to nitrogen ratio (TNC/N) was lower in young leaves (4.79) than that in old leaves (8.47). A high carbon to nitrogen ratio (C/N) in the leaves might indicate poor nutritional quality (Rasmann et al. 2009). Therefore, young leaves of *R. sativus* should have afforded better nutrition to caterpillars compared with old leaves in this study.

Our study clearly demonstrates higher concentration of a defensive chemical, sinigrin, in young leaves; with concentration was almost twice as high compared with old leaves. Previous studies have also found higher level of glucosinolates in young leaves (Lambdon and Hassal 2005, Shelton 2005). The young leaves may be photosynthetically more active compared with the old leaves and have longer future life span as well. Consequently, they could be expected to contribute significantly to future photosynthetic assimilation. Hence, they are more valuable for prospective plant fitness. Moreover, very young leaves are usually still lacking effective mechanical defenses (Harper 1989) and would experience much higher herbivory than old leaves within the same plant (Kursar and Coley 1991, Boege and Marquis 2006). Therefore, it would be adaptive for radish plants to allocate higher concen-

Table 2. Concentration (mean $\pm$ SE) of primary and secondary metabolites in the two leaf age classes of *R. sativus*

Dry weight (%)	Young leaf	Old leaf	*P* value
Nitrogen	4.75 ± 0.18	2.97 ± 0.28	<0.01
Water (%)	86.29 ± 1.18	87.35 ± 1.22	0.54
Insoluble carbohydrate	7.68 ± 0.44	9.43 ± 0.51	<0.05
Soluble carbohydrate	15.15 ± 0.27	15.93 ± 0.07	<0.05
Total nonstructural carbohydrate	22.83 ± 0.57	25.36 ± 0.62	<0.05
Sinigrin (μmol/g)	46.37 ± 4.98	27.15 ± 3.89	<0.05

Nitrogen ($n = 8$ plants for old leaves and 10 plants for young leaves). Water ($n = 10$ plants each for old and young leaves). Insoluble carbohydrate, soluble carbohydrate, and total nonstructural carbohydrate contents ($n = 8$ plants for old leaves and 9 plants for young leaves). Sinigrin ($n = 5$ plants for old leaves and 6 plants for young leaves).

tration of defensive compound to those parts where herbivore damage would cause maximum losses in term of their fitness.

Although young leaves had better nutritious conditions, our results show lower performance and higher mortality of *S. litura* on young leaves compared with old leaves. The difference in sinigrin concentrations between old and young leaves appears to be directly responsible for the markedly different performance of *S. litura* between these two leaf classes. Sinigrin and its hydrolyzed products have been reported as herbivore feeding deterrents (Shields and Mitchell 1995, Gabrys and Tjallingii 2002) and to be lethal to neonate larvae of generalist herbivores (Li et al. 2000).

The most interesting result in this study was the difference in development time of male and female larvae on young leaves. Female larvae usually consume more food than male larvae to meet nutrient requirements for future oogenesis (Slansky and Scriber 1985, Wheeler 1996). Therefore, the nutritional intake target may be different for male and female larvae. Higher concentration of defensive chemicals in young leaves may reduce feeding thereby prolonging development time of female *S. litura.* This is probably the first study to show stronger effects of defensive compounds against females than males in a generalist.

In summary, this study demonstrates the phenologic variation of major foliar compounds within *R. sativus.* Our study further shows that young leaves were more nutritious than old leaves, but are also more protected against the generalist, *S. litura.* This study also reveals that the defensive compound levels in young leaves have significantly different effects on the development time of male and female *S. litura.*

Acknowledgments

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