

INHIBITION OF FEEDING BY A GENERALIST INSECT DUE TO INCREASED VOLATILE LEAF TERPENES UNDER NITRATE-LIMITING CONDITIONS

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Abstract—Nitrogen-limited plants of *Heterotheca subaxillaris* accumulate greater quantities of leaf volatile terpenes than do nitrogen-rich plants. A series of feeding trials were performed to determine if such nitrate-limited plants are better defended against generalist-feeding insect herbivores. Soybean looper (*Pseudoplusia includens*) larvae were fed leaves from *H. subaxillaris* rosettes grown under high and low nitrate supply regimes. Larval consumption, growth, and survival declined as the leaf volatile terpene content increased. Larval consumption and growth were enhanced by higher plant nitrate supply and with increasing leaf age. The results suggest that the higher quantity of volatile terpenes in the leaves of nitrate-limited plants may better defend these leaves against generalist-feeding insects.

Key Words—*Heterotheca subaxillaris*, Asteraceae, *Pseudoplusia includens*, Lepidoptera, Noctuidae, volatile terpenes, nitrogen, herbivory.

INTRODUCTION

Plants growing under nitrogen-limiting conditions generally have a slower growth rate than those growing under nitrogen-rich conditions (Chapin, 1980). Comparable loss of leaf nitrogen to herbivores by nitrate-limited and nitrate-rich plants presumably has a greater impact on the growth of nitrogen-limited plants. Carbon supply does not limit plant growth under low nitrate conditions, and consequently, increased quantities of carbon-based defenses should be selected for as nitrate availability decreases (Janzen, 1974; McKey et al., 1978; Bryant et al., 1983; Coley et al., 1985; Mihaliak and Lincoln, 1985).

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Leaf mono- and sesquiterpenes are known to function as carbon-based chemical defenses against herbivores (Eisner, 1964; Rice et al., 1978; Mabry and Gill, 1979; Langenheim and Hall, 1983). Increased quantities of leaf mono- and sesquiterpenes occur as plant nitrate availability decreases (Mihaliak and Lincoln, 1985) and may provide greater defense against insect herbivores. The quantity of leaf volatiles is also known to decline with leaf age (Crankshaw and Langenheim, 1981; Mihaliak and Lincoln, 1985). This decrease coincides with the lower productivity and nitrogen content of older leaves (Mooney, 1972; Mihaliak and Lincoln, 1985). The high volatile terpene content of young leaves under nitrate-limiting conditions (Mihaliak and Lincoln, 1985) suggests a pattern of increased carbon allocation to the defense of those leaves which represent the largest relative proportion of the potential photosynthetic capacity of a plant. Plant allocation to defense should reflect leaf value (Rhoades, 1979), assuming that leaf value is measured by its relative contribution to future plant productivity and the cost of replacement (Mooney and Gulmon, 1982).

The objective of this study was to determine if the increased quantity of leaf mono- and sesquiterpenes under nitrate-limiting conditions better defends plants against a generalist insect herbivore. A further goal was to investigate whether patterns of insect consumption and growth are consistent with the prediction that a leaf which represents a high relative proportion of the photosynthetic capacity of a plant is better defended than a leaf which will contribute less to future plant productivity. Despite an often observed increase in herbivore consumption on leaves with a relatively low concentration of nitrogen (Mattson, 1980; Scriber and Slansky, 1981; Lincoln et al., 1982, 1986), we predict that insect consumption rates should decline as leaf terpene levels increase under nitrate-limiting conditions. Further, since the potential contribution of a leaf to plant productivity is greatest when a leaf is young, and decreases with leaf age, (Mooney and Gulmon, 1982), we expect that consumption and growth of herbivores will be less on these young leaves than on older, less defended leaves.

To test these predictions, we have performed feeding trials using the generalist lepidopteran *Pseudoplusia includens* (soybean looper) and young and mature leaves from *Heterotheca subaxillaris* rosettes grown under high and low nitrate supply regimes. *Pseudoplusia includens* is recognized as a polyphagous species (Kogan and Cope, 1974) and has been observed feeding on *H. subaxillaris* (personal observation).

METHODS AND MATERIALS

The plant species used in this experiment, *Heterotheca subaxillaris* (Lam. Britton and Rusby) (Asteraceae) (camphorweed), is a herbaceous annual/biennial (Awang and Monaco, 1978) which occurs in old fields, disturbed sites, and open sandy areas of the southeastern United States. Forty-one mono- and ses-

quiterpenes have been identified from the leaves of *H. subaxillaris* (Lincoln and Lawrence, 1984). *Pseudopiusia includens* (Walker) larvae used in the feeding trials were obtained from a laboratory colony which was maintained on a pinto bean-soy protein-wheat germ diet (Greene et al., 1976) in an environmental incubator under a thermophotoperiod of 14 hr light-10 hr dark (26°C-20°C).

The plants were grown in a greenhouse using seed germinated on filter paper in Petri dishes. Seedlings were transferred into a potting medium of perlite, vermiculite, and sand (1:1:1) within one week of germination and maintained under a photoperiod of 14 hr day-10 hr night using 1000-W coated metal halide and high-pressure sodium lamps as supplemental light (690 $\mu\text{mol}/\text{m}^2/\text{sec}$). The plants were watered on alternate days with either tap water or a nutrient solution. The nutrient solution contained either 0.5 mM or 5.0 mM nitrate and provided the nitrate-limited and nitrate-rich treatments, respectively. These solutions consisted of equimolar concentrations of KNO_3 and $\text{Ca}(\text{NO}_3)_2$. CaCl_2 and KCl were added to the nitrate-limited solution to maintain equal Ca^{2+} and K^+ concentrations. Both nutrient solutions contained 1.65 mM K_2HPO_4 , 1.35 mM KH_2PO_4 , 2.0 mM MgSO_4 , micronutrients, and chelated iron.

Feeding trials were performed by removing leaves from individual 12-week-old *H. subaxillaris* rosettes, enclosing the petiole in a folded moistened tissue, and then placing the larvae and leaf into a plastic Petri dish. All leaves and insects were kept in the environmental incubator throughout the feeding trial under the thermophotoperiod described above. A second matched leaf was removed from each plant and used to determine the leaf volatile terpene content, the leaf nitrogen content, and the leaf water content. No perceivable changes in the leaf volatile terpene or nitrogen content occur when leaves are detached from the plant.

Larvae were fed either an immature (1-week-old) or fully expanded (4-week-old) leaf from a *H. subaxillaris* plant grown under either the 0.5 mM or the 5.0 mM nitrate treatment. For comparison, feeding trials were also performed using 1-week-old leaves from soybean plants (*Glycine max* L. Merr. cv. Ransom) grown under the 5.0 mM nitrate treatment. Leaf age was determined by placing small rings over the petiole of newly emerging leaves. All of the feeding trials began with larvae which had recently molted to the fourth instar and had not begun to feed. The larvae were kept with the leaf until they either molted to the fifth instar or died. Seventy feeding trials were performed on *H. subaxillaris* leaves, and ten were performed using soybean leaves.

The initial fresh weight of each larva and leaf used in the feeding trials was recorded. At the end of the feeding trial, the leaf was dried and weighed, and larva was frozen and then dried to determine the final dry weight. The initial dry weight of the larvae was computed using a fresh weight-dry weight conversion calculated from matched larvae not used in the experiment. The initial dry weight of the leaves was calculated from a regression analysis of the fresh and dry weights of the matched leaves which were also used to determine leaf

terpene, nitrogen, and water content. The relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested food (ECI) (Waldbauer, 1968) were computed on a dry weight basis over the entire fourth-instar feeding period.

Leaf volatile terpene content was determined for the matched leaves using gas chromatography by adding a known amount of an internal standard (*n*-tetradecane) to a pentane extract of each leaf. After extraction, the sample was centrifuged to remove all leaf material and then concentrated to a volume of approximately 100 μ l under a stream of nitrogen. Analyses were performed using a gas chromatograph equipped with a SP-1000 fused silica capillary column (0.25 mm \times 30 m), a flame-ionization detector, and a digital integrator (temperature program from 50 to 175°C at a rate of 3°C/min with a 5-min initial and 15-min final isothermal period). Leaf volatile terpene contents were calculated without response factors for individual compounds.

The nitrogen content of the residual leaf material collected after pentane extraction was measured using a Hewlett-Packard Carbon-Hydrogen-Nitrogen Analyzer and a digital integrator. The nitrogen content of each sample was computed using a response curve generated with cystine (U.S. Department of Commerce, Bureau of Standards). Measured leaf nitrogen contents were not corrected for the materials extracted with pentane.

All statistical analyses were performed using the Statistical Analysis System, SAS Institute Inc.

RESULTS

The leaf volatile terpene content was higher under nitrogen-limiting conditions (\bar{X} = 4.73 mg/g, SD = 1.39) than under nitrogen-rich conditions (\bar{X} = 3.73 mg/g, SD = 2.38; F = 7.87, P < 0.01), and the leaf nitrogen content was reduced when plant nitrate availability was limited (Table 1; \bar{X} = 24.4 mg/g at high nitrate, \bar{X} = 18.5 mg/g at low nitrate; F = 19.6, P < 0.001). As leaves aged, reduced quantities of leaf volatile terpenes (F = 21.7, P < 0.001) and leaf nitrogen (F = 4.22, P < 0.05) occurred (Table 1). The volatile leaf terpene content changed less with leaf age under nitrate-rich conditions than under nitrate-limiting conditions (F = 5.61, P < 0.05 for nitrogen treatment by leaf age interaction). Under nitrate-limiting conditions, leaf nitrogen content was near the level suggested as the minimum necessary for larval growth (Fox and McCauley, 1977).

Larval consumption (RCR), growth (RGR), and survival were lower on the leaves of nitrate-limited plants (Table 1; nitrate treatment effect: RCR, F = 31.5, P < 0.001; RGR, F = 55.97, P < 0.001; survival, χ^2 = 16.6, P < 0.01). These leaves of nitrate-limited plants contain more volatile terpenes and

TABLE 1. CHARACTERISTICS OF LEAVES OF *Heterothea subaxillaris* AND SOYBEAN, AND PERFORMANCE OF *Pseudoplusia includens* LARVAE OVER ENTIRE FOURTH INSTAR^a

	0.5 mM Nitrate		5.0 mM Nitrate		Soybean
	Immature	Mature	Immature	Mature	
Leaf nitrogen content (mg N/g leaf)	20.5 ^{ab}	16.6 ^a	24.8 ^c	23.5 ^{bc}	34.8 ^d
Leaf volatile content (mg volatiles/g leaf)	6.25 ^a	3.22 ^b	4.02 ^b	3.03 ^b	
Leaf weight consumed (mg dry wt.)	6.1 ^a	13.3 ^a	27.0 ^b	48.9 ^c	10.8 ^a
Leaf tissue consumed* (% of total leaf)	25 ^a	44 ^{ab}	50 ^{bc}	67 ^c	
Relative consumption rate (mg consumed/mg larva)	8.9 ^a	16.9 ^{ab}	24.6 ^{bc}	31.6 ^c	7.6 ^a
Relative growth rate (mg growth/mg larva)	-0.28 ^a	-0.02 ^a	0.59 ^b	0.96 ^b	1.01 ^b
Conversion efficiency (mg growth/g eaten)	-47 ^a	-27 ^a	22 ^a	28 ^a	234 ^b
Insect survival (%)	14	38	78	89	100

^aLarvae were offered either immature (1-week-old) or mature (4-week-old) leaves from plants grown using nutrient solutions which contained either 0.5 mM or 5.0 mM nitrate. *Pseudoplusia includens* performance on 1-week-old leaves of soybean plants grown using 5.0 mM nitrate is given. Means in each row followed by the same letter are not significantly different (Tukey's studentized range test, $P < 0.05$, * arcsin square root transform).

less nitrogen than comparable leaves of nitrate-rich plants. The negative mean growth rate for larvae which fed on leaves of nitrate-limited plants is due to weight loss of some larvae prior to their death during the feeding trial. The RGR of the surviving larvae which were offered leaves from the 0.5 mM plants averaged 0.50 mg/mg larva over the fourth instar. Consumption and growth were reduced on immature leaves and increased on older leaves (leaf age effect: RCR, $F = 7.61$, $P < 0.01$; RGR, $F = 6.72$, $P < 0.01$).

Leaf volatile terpene content has a negative effect on the growth and consumption rate of *P. includens*, while larval growth was positively related to leaf nitrogen content (Table 2). The absence of a relationship between consumption and leaf nitrogen contrasts with the often observed compensatory feeding response of herbivores which encounter nitrogen-poor leaves (Mattson, 1980; Scriber and Slansky, 1981; Lincoln et al., 1982, 1986).

Leaf water content, measured as percent of leaf fresh weight, increased significantly under nitrate-rich conditions (for 0.5 mM plants, $\bar{X} = 75.3\%$, $SD = 3.2\%$; for 5.0 mM plants, $\bar{X} = 78.1\%$, $SD = 1.8\%$; $F = 11.6$, $P < 0.005$).

TABLE 2. EFFECT OF LEAF VOLATILE TERPENE CONTENT AND LEAF NITROGEN CONTENT ON RELATIVE GROWTH RATE AND RELATIVE CONSUMPTION RATE OF *P. includens* LARVAE OVER ENTIRE FOURTH INSTAR^a

	Leaf nitrogen content		Leaf volatile content	
	Slope	<i>P</i>	Slope	<i>P</i>
Relative growth rate	0.03	<0.05	-0.09	<0.01
Relative consumption rate	0.33	<0.19	-2.00	<0.006

^aMultiple regression analysis, *df* = 2, 67.

However, when added to a multiple-regression analysis with leaf volatile terpene and leaf nitrogen content, leaf water content did not significantly influence either RCR ($P < 0.44$) or RGR ($P < 0.56$) of the larvae.

The total amount of leaf tissue consumed by larvae was lowest on leaves from nitrate-limited plants (Table 1; $F = 31.5$, $P < 0.001$). Increased larval consumption on leaves from nitrate-rich plants resulted in both a greater absolute amount of leaf tissue being eaten and a larger proportion of each nitrate-rich leaf being consumed by the herbivores. Further, more leaf tissue was consumed by larvae feeding on mature leaves than on immature leaves.

The conversion efficiency (ECI) of larvae was significantly lower when offered leaves of nitrogen-limited plants than when offered nitrogen-rich leaves ($F = 5.03$, $P < 0.05$). Further, ECI was lower when larvae fed on *H. subaxillaris* leaves than on soybean leaves (Table 1). The growth rate of *P. includens* larvae on soybean leaves was not different from their growth rate on *H. subaxillaris* leaves grown under nitrate-rich conditions. However, a significant increase in consumption occurred when feeding on the *H. subaxillaris* leaves.

DISCUSSION

The higher leaf volatile terpene content combined with the reduced leaf nitrogen content of nitrate-limited plants resulted in decreased performance by a generalist herbivore. These results suggest that leaves of nitrate-limited plants may be better defended from generalist-feeding insect herbivores than leaves of nitrate-rich plants and that young leaves are better protected than older leaves.

The concentration of leaf nitrogen is usually considered to be a major determinant of insect herbivore feeding and nutrition (Mattson, 1980; Scriber and Slansky, 1981; Lincoln et al., 1982; Scriber, 1984). However, the results of this study and of previous investigations of the interactions between leaf nitrogen and carbon-based chemical defenses suggest that both nitrogen and allelochemicals are important factors in determining the nutritional quality of

plant tissue for herbivores (Lincoln et al., 1982; Redak and Cates, 1984; Lincoln, 1985; see, however, Morrow and Fox, 1980).

In the current study, leaf water content was lower under nitrate-limited conditions but failed to explain the variance in either RCR or RGR. Low leaf water content (ca. 60%) can suppress larval growth and nitrogen utilization efficiency (Scriber, 1977; Scriber and Slansky, 1981). However, slower larval growth due to variation in water content is not likely to occur on leaves from herbaceous plants (Scriber, 1977; Slansky and Feeny, 1977).

An abundance of leaf nonstructural carbohydrates appears to increase production and/or decrease turnover of volatile terpenes (Croteau et al., 1972; Croteau, 1984). Higher concentrations of nonstructural carbohydrates accumulate in leaves as plant nitrate availability declines (Chapin, 1980; Fritsch and Jung, 1984). Thus, the ecological hypothesis regarding increased carbon allocation to defense under nitrate-limiting conditions (Rundel, 1982; Coley et al., 1985; Mihaliak and Lincoln, 1985) and the current results are consistent with the hypothesized physiologic controls on plant terpene biosynthesis (i.e., increased availability of carbon for the production of volatile terpenes under nitrate-limiting conditions).

The results agree with the predictions of the leaf value hypothesis (Rhoades, 1979). Nitrogen-limited plants commonly have a slower growth rate than nitrogen-rich plants. Loss of leaf material by a nitrogen-limited plant should have a greater impact on growth than a comparable loss of leaf tissue by a nitrogen-rich plant. In the current study, less consumption occurred on the leaves of nitrate-limited plants than on leaves of nitrate-rich plants, and the quantity of leaf volatiles was highest under nitrate-limiting conditions. Thus, leaves which potentially contribute a high relative proportion to total plant productivity appear to be better defended. Similarly, the potential contribution to productivity is greatest in immature leaves and declines with leaf age. Young leaves contained higher levels of volatile terpenes, and consumption was reduced on these leaves.

The high leaf terpene content in leaves with a relatively low nitrogen content (i.e., those of nitrate-limited plants) increases the quantity of terpenes per unit nitrogen an insect must consume when feeding on these leaves. If leaf volatile terpenes are toxic, herbivores may be able to consume only small quantities of the immature leaves of nitrate-limited plants or, if the terpenes were repellent, the herbivore would move in search of another food source. In either case, the increased allocation to carbon-based defense by the slower growing, resource-limited plant (Coley et al., 1985; Mihaliak and Lincoln, 1985) would be successful in retaining leaf nitrogen.

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