Host Pubescence and the Behavior and Performance of the Butterfly *Papilio Troilus* (Lepidoptera: Papilionidae)

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ABSTRACT We found that leaf pubescence in *Sassafras albidum* (Nuttall) Nees negatively influences the growth and survivorship of a lepidopteran herbivore, *Papilio troilus* (L.). *S. albidum* leaves varied greatly in pubescence among plants. In laboratory choice experiments, *P. troilus* larvae preferred to eat and adult females preferred to oviposit on nonpubescent *S. albidum* leaves. Larvae fed pubescent *S. albidum* had slower growth and higher mortality in early instars than larvae fed on nonpubescent leaves. These negative effects of pubescence on adult oviposition, larval growth rate, and larval survivorship suggest that the population size of *P. troilus* will reflect availability of nonpubescent hostplants. In field surveys, the frequency of pubescent *S. albidum* was significantly greater in open habitats created by harvesting pine forest than in hardwood forests, perhaps because of higher light and lower moisture in open areas. By changing the availability of quality food resources, forest management may have unintended negative impacts on this butterfly population.

KEY WORDS *Papilio troilus, Sassafras albidum*, host preference, landscape variation, oviposition

MANY PLANTS HAVE evolved morphological and chemical defenses that decrease herbivore growth rate and survivorship (e.g., Scriber and Slansky 1981, Crawley 1983, Ayres and MacLean 1987, Stamp and Bowers 1990, Matsuki and MacLean 1994). These defenses include allelochemicals (Denno and McClure 1983, Stamp and Yang 1996), and physical defenses such as leaf trichomes, which have been shown to decrease herbivore growth rate (Schillinger and Gallun 1968, Levin 1973, Johnson 1975, Webster 1975) and survivorship by inhibiting digestion, or by causing direct physical damage (Gilbert 1971). Several published reviews document the importance of plant defenses, including pubescence, in limiting herbivory (Levin 1973, 1976; Johnson 1975; Denno and McLure 1983; Stamp 1996).

In the eastern United States, naturally occurring *Sassafras albidum* (Nuttall) Nees vary greatly in leaf pubescence, from being totally smooth (no pubescence) to highly pubescent. If pubescence is a deterrent to *S. albidum*'s herbivores, then the distribution of high quality host, rather than the plant's distribution alone, will be of greatest consequence to the population biology of *S. albidum*'s herbivores. We conducted laboratory experiments to test for the effects of pubescence in *S. albidum* on oviposition, growth, and survival of the herbivore *Papilio troilus* (L.). We hypothesized that pubescence decreases survivorship and growth rate of *P. troilus* larvae and decreases oviposition by adult females.

Many herbivorous larvae have limited dispersal ability, and larval survivorship is related to the ability of adult females to find suitable host plants. Landscape-level variation in quality hosts may limit oviposition, or may lead to higher oviposition on low quality hosts. Qualitative plant traits may be linked to environmental variability (e.g., Myers and Bazely 1991). For example, the quantity and effectiveness of toxic allelochemicals may be related to light, temperature, and nutrients (Jones et al. 1979, Larsson et al. 1986, Bryant et al. 1987, Ayres 1993, Dudt and Shure 1994, Stamp and Yang 1996). Pubescence may be associated with levels of light and moisture (Johnson 1975, Ehrlinger et al. 1976, Ehrlinger and Mooney 1978, Donselman and Flint 1982).

To determine the distribution of suitable food plants for *P. troilus*, we measured landscape-level variation in naturally occurring *S. albidum* through surveys of three common habitats in the southeastern United States, open habitats created by harvesting pine trees, pine forest, and hardwood forest. Because some of the environmental changes associated with forest management (changes in light intensity and moisture) are also those known to affect pubescence in some plants (Ehrlinger and Mooney 1978, Donselman and Flint 1982), we studied landscape-level variation in host quality in the context of forest management. We hypothesized that habitat type influences the frequency of pubescence in *S. albidum.*

Materials and Methods

Study Site. We conducted this study at the Savannah River Site, SC. The site is managed for pine forest.

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Open areas created by harvesting pine permit light to reach ground level, permitting the establishment and growth of herbaceous vegetation. Because pine trees (*Pinus taeda* L., *P. palustris* Miller, and *P. elliotii* Englem) are planted at high densities, the canopy closes quickly and prevents light penetration to the forest floor. Hardwood forests were also common on the site, and they were composed of *Carya* sp., *Quercus* sp., *Liquidamber styracaflua* L., *Fagus grandifolia* Ehrhart, *Ulmus alata* Michaux, *Magnolia grandiflora* L., *Ilex opaca* Aiton, *Diospyrus virginiana* L., *Prunus serotina* Ehrhart, *Cornus florida* L., *Myrica cerifera* L., as well as *Pinus.* Pine plantations are planted on xeric upland sites, whereas hardwood forests typically occur in wet bottomlands and drainages.

The study species, *P. troilus* (Lepidoptera: Papilionidae) was common and was capable of moving through open and forested habitats (Haddad 1999). It has two larval host plants, *S. albidum* and *Lindera benzoin* (L.) Sprengel. *S. albidum* occurs in upland pine and hardwood habitats, old fields, and sandhills, and *L. benzoin* occurs exclusively in bottomland hardwoods (Workman and MacLeod 1990). *S. albidum* is clonal, and leaves within a clone vary little in pubescence (personal observation). In forest management, rapid pine growth and frequent understory burning cause the height of the typical *S. albidum* individual to be < 2 m.

Female Oviposition Experiment. We captured female *P. troilus* adults in the field in June 1995. We assumed that females encountered in the field had been inseminated. After capture with a hand held net, we placed individuals in a 6 by 9-cm glassine envelope. Within 2 h, we transported females to the laboratory and immediately placed them into an individual plastic container (30 cm diameter, 15 cm high) lined with a paper towel. At the perimeter of the round container, we positioned one leaf in each of four cardinal locations. In opposite positions we placed a smooth and pubescent leaf of *S. albidum.* In the two remaining positions, between the *S. albidum* leaves and opposite each other, we placed control leaves of *Fotinia* sp., a waxy, tough leaf that differed markedly in texture, reflectivity, and morphology from *S. albidum* leaves. We chose at random the cardinal position of the smooth leaf. We inserted leaves in water-filled floral tubes to prevent wilting, and replaced tubes with fresh leaves every second day.

An external light (75-W bulb) adjacent to each leaf was used to induce female oviposition. We positioned each light 60 cm from the container to maintain the temperature within the container at 30–35°C, the optimal egg laying temperature for *P. troilus* (Lederhouse et al. 1992). We held the ambient temperature constant at 21°C. We maintained lights on a continuous cycle of 4 h on and 4 h off. Only one light was on at a time; the light in one cardinal position was activated for 2 min, followed by the next clockwise position, which was lighted for 2 min. This rotation continued for the entire 4-h period.

Each of 26 females oviposited until it died, or until it did not lay eggs for five consecutive days. We fed females daily with a 1:4 honey:water solution. For each female, we recorded the number of eggs on each leaf. Eggs not laid on the leaves, but on the container near the leaves, were excluded from counts. We tested for differences in numbers of eggs laid on the three leaf types using a two-factor, subject by treatment analysis of variance (ANOVA).

Larval Growth Experiment. We used *P. troilus* eggs from the oviposition experiment for a larval growth and survivorship experiment. From each female, we used 10 eggs laid on smooth leaves and 10 laid on pubescent leaves. We removed eggs from leaves and assigned them at random to small plastic containers with either all smooth or all pubescent *S. albidum* leaves. Feeding continued until the larvae pupated or died.

We monitored containers daily for the stage of larval development, recorded the date of molting, and replaced leaves every second day. From these data, we assigned an indicator variable (1-survived, 0-died) for each larval stage. An ANOVA was conducted on the probability of survival to the next larval stage. We approximated growth rate as the number of days between instars.

Larval Food Choice Experiment. We conducted a second feeding experiment to determine larval preferences for smooth or pubescent leaves. We measured leaf choice for different individuals at each instar. We obtained larvae from two sources: (1) those we raised in the oviposition experiment in excess of the 20 used in the larval growth experiment, and (2) those we collected in the field.

We fed larvae from each of the five instars in individual boxes with similar quantities of smooth and pubescent *S. albidum.* For the first three instars, we quantified foraging rates on each leaf type by laying a leaf of known area on a grid of 2 by 2 mm squares and counting the number of consumed cells. The fourth and fifth instars typically consumed entire leaves in a 24-h period. We outlined leaves on graph paper before the experiment, such that the unconsumed leaf area, when placed within the traced area, could be subtracted from the total leaf area to estimate leaf area consumed. We replaced all leaves daily. We performed an ANOVA to analyze effects of leaf type on leaf area consumed among instars.

Landscape Distribution of Pubescence in *S. albidum.* To determine the prominence of pubescence in landscapes managed for pine forest, we conducted landscape-level field surveys on *S. albidum.* Surveys were conducted in three habitat types: open areas (pine harvested within 1–3 yr), 30- to 40-yr-old pine forest, and hardwood forests. For each of five different areas (each >10 ha) and for each of the habitat classes, pubescence in *S. albidum* was measured for plants encountered along linear transects that traversed the stand at 20-m intervals. The length and number of transects varied to fit within irregular habitat areas, and ended 15 m from the habitat boundary. We recorded leaf pubescence of the *S. albidum* stem nearest to points established at 15-m intervals along the transects. This interval avoided repeat counting of

Table 1. Oviposition preference by female *P. troilus*

Leaf type	n	Mean	SE
Smooth *S. albidum*	26	16.3	3.87
Pubescent *S. albidum*	26	2.0	0.78
Control (*Fotinia* sp.)	26	0.69	0.33
ANOVA		F	P
Females	26		
Within subjects			
Leaf type	2	17.08	0.001
Error	50		
Contrasts			
Smooth vs Pubescent	1	17.30	0.001
Error	25		
Pubescent vs Control	1	2.90	0.10
Error	25		

Analyses are a two-factor ANOVA, where female *P. troilus* are the subjects, and leaf type is the within subject factor.

stems from the same clone. In each stand, pubescence was recorded for 50 individual plants. Leaves were classified as pubescent or nonpubescent.

We surveyed stands in four regional blocks. There were one or two stands of each forest type within the block, and stands within a block were closer to each other (often adjacent stands) than to another block. Blocks were separated by 6–19 km. We analyzed the proportion of nonpubescent *S. albidum* as a two-factor ANOVA based on forest type and regional block.

Results

Female Oviposition Experiment. Female *P. troilus* oviposited more frequently on smooth *S. albidum* leaves (Table 1). Nearly three-fourths of all eggs were laid on smooth leaves, and differences in oviposition between smooth and pubescent were highly significant (Table 1). Oviposition did not differ significantly between pubescent *S. albidum* and control (*Fotinia* sp.) leaves (Table 1).

Larval Growth and Mortality Experiments. We assigned 130 and 105 *P. troilus* larvae to smooth and pubescent treatment groups, respectively. Of those feeding on smooth *S. albidum*, 21% (27 individuals) pupated. Only 7% (7 individuals) of those feeding on pubescent *S. albidum* pupated. Mortality was highest in the first and second instars (Table 2). Survivorship between the first and second instars was significantly higher on smooth *S. albidum* ($F = 14.15$; df $= 1, 233$; $P = 0.001$). Few (3) larvae died after the second instar.

Fig. 1. Proportion of *S. albidum* with smooth leaves (mean ± SE) in three common habitats at the Savannah River Site. Letters above the x-axis indicate groupings generated by a Tukey comparison of means.

We approximated development rates by measuring larval duration from the hatching date through pupation. Larvae fed smooth leaves averaged 35.4 d to pupation ($n = 27$; SE $= 0.9$), whereas larvae fed pubescent leaves averaged 40.6 d to pupation ($n = 5$; SE $= 0.9$). The number of days from hatching to pupation differed significantly (ANOVA; $F = 5.43$; df $= 1, 32$; $P = 0.03$).

Larval Food Choice Experiments. When given a choice between smooth and pubescent *S. albidum*, *P. troilus* larvae consumed 77 to 90% smooth leaves (Table 2). The proportion of smooth leaf area consumed did not differ significantly among instars. The quantity of *S. albidum* consumed (both smooth and pubescent leaves) increased exponentially in successive instars (Table 2), from a leaf area of 12.88 mm^2/d (SE $= 1.53$) in the first instar, to 1520 mm^2/d (SE $= 167$) in the fifth instar ($R^2 = 0.89$).

Landscape Distribution of Pubescence in *S. albidum*. The frequency of pubescent *S. albidum* leaves differed between habitat types and between region (full model: $F = 9.89$; df $= 5, 9$; $R^2 = 0.85$; $P = 0.002$). There were significantly more pubescent plants in open habitats than in hardwood forests (Fig. 1; $F = 5.82$, $P = 0.02$; Tukey comparison of means). Pubes-

Table 2. Survivorship, leaf choice, and leaf area consumed by larval *P. troilus*

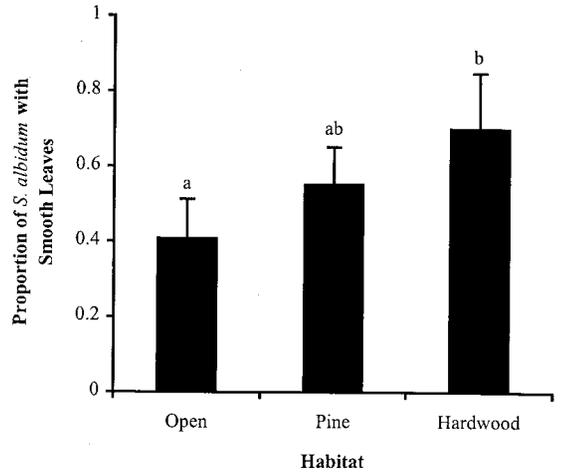| Instar	Survivorship on smooth *S. albidum*		Survivorship on pubescent *S. albidum*		Larval feeding experiments		
	n	Proportion remaining	n	Proportion remaining	n	Leaf choice − proportion smooth ± SE	Leaf area consumed (mm^2) ± SE
1	130	1	105	1	22	0.84 ± 0.06	12.9 ± 1.5
2	46	0.35	15	0.14	19	0.88 ± 0.04	75.5 ± 10.9
3	30	0.23	7	0.07	10	0.90 ± 0.03	219.5 ± 23.1
4	30	0.23	7	0.07	5	0.79 ± 0.10	472.5 ± 122.7
5	27	0.21	7	0.07	17	0.77 ± 0.07	1520.7 ± 167.0

cence was more similar within a regional block than among blocks ($F = 12.61$, $P = 0.001$).

Discussion

This study suggests that pubescence is an effective defense for *S. albidum* against herbivory. Larval survivorship of *P. troilus*, a key herbivore of *S. albidum*, is one-third less on pubescent than on smooth *S. albidum* leaves. These results corroborate other evidence for the deleterious effects of leaf hairs on herbivores (Schillinger and Gallun 1968, Levin 1973, Johnson 1975, Webster 1975, but see Matsuki and MacLean 1994).

Pubescence appears to be particularly effective in lowering larval survivorship in the first two instars (Table 2). Plant defenses that lower survivorship of early instar caterpillars are most beneficial to the plant, because, as Table 2 documents, the increase in plant material consumed is approximately exponential with larval development. In another study, we estimated the mean duration of each larval stage (N.M.H., unpublished data: first–6.7 d; second–5.5 d; third– 6.8 d; fourth–4.2 d; fifth–7.0 d). Larvae spend 60% of their lifespans in the final three instars, but consume 96% of their total food in those instars (Table 2).

Larvae clearly prefer smooth *S. albidum* (Table 2). This is suggested by both their higher mortality on pubescent leaves, and their consistent choice of smooth leaves as ≈77–90% of their diet. Larvae typically remain on the same plant until they pupate, so their food is determined primarily by adult females and their dispersal of eggs. Larval success is highly dependent on the ability of females to encounter high quality *S. albidum* and to discriminate between plants that vary in quality. In the laboratory, females discriminate strongly against pubescent *S. albidum.* Not only were the number of eggs on smooth *S. albidum* significantly greater than on pubescent and control leaves, the number of eggs laid on pubescent and control leaves did not differ significantly (Table 1).

Population success of *P. troilus* may be dependent on the ability of adult females to encounter high quality plants for oviposition in complex landscapes. Pubescence in open, upland habitats was significantly more common than in hardwood stands. The reasons for the differences in *S. albidum* pubescence are unknown, but may be related to two environmental characteristics that distinguish open habitats from hardwood forests, moisture and light. In the southeastern United States, pine forests are typically planted and harvested on upland soils, which are drier than bottomlands that contain hardwood forests. Lower moisture, as well as higher light availability caused by forest harvests, may contribute to the higher frequency of pubescence in open habitats. Interestingly, *S. albidum* grows most productively in open habitats created by forest harvests, where its leaf quality to *P. troilus* is lowest.

Intense habitat modification and management may have important consequences for the population dynamics of *P. troilus.* Although we did not collect data on *P. troilus* population sizes at the landscape level and have no direct observations of impacts of forest harvest on this species, the results of this study do suggest that forest management may alter the distribution of suitable resource for this butterfly. The maintenance of *P. troilus* populations may depend on the ability to find high quality host plants at the landscape scale.

This study demonstrates that leaf pubescence, a characteristic that varies greatly in *S. albidum*, has a significant, negative influence on *P. troilus* growth and survivorship. Adult female *P. troilus* are able to discriminate quality in larval hosts. However, forest management also appears to be creating large areas that are productive for low quality host plants. These findings suggest that forest harvests may have subtle and unintended consequences for insect populations in managed landscapes.

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