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PHYTOCHEMICAL BASIS FOR HOST PLANT SELECTION BY GENERALIST AND SPECIALIST SWALLOWTAIL BUTTERFLIES

presented by

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of the requirements for

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PHYTOCHEMICAL BASIS FOR HOST PLANT SELECTION BY GENERALIST AND SPECIALIST SWALLOWTAIL BUTTERFLIES

By

Cheryl R. Frankfater

A THESIS

Submitted to
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ABSTRACT

PHYTOCHEMICAL BASIS FOR HOST PLANT SELECTION BY GENERALIST AND SPECIALIST SWALLOWTAIL BUTTERFLIES

By

Cheryl Frankfater

Swallowtail butterflies species show clear patterns of oviposition preference among plants in the field and in the laboratory. Oligophagous butterflies, such as *Papilio troilus* and *P. palamedes*, oviposit on a select group of lauraceaous plants. In contrast, *P. glaucus*, a polyphagous swallowtail butterfly, will deposit eggs in a consistent preference hierarchy on trees from several families. Oviposition preferences are maintained by the presence of oviposition stimulants and deterrents in the foliage of host and non-host plants, detected by chemoreceptors located on the front tarsi of butterflies. Polar extracts and fractions from the preferred host plants of *P. troilus* and *P. palamedes* sprayed on to various substrates have been shown to stimulate oviposition relative to the controls. However, in addition to contact chemical stimuli, other sensory cues, such as color, odor and texture may also be important in eliciting maximum oviposition. Rejection of non-host plants also has a chemical basis. *P. glaucus* avoid ovipositing on host leaves sprayed with polar extracts from a non-host tree species. The experimental results decisively show that phytochemical stimuli detected through tarsal chemoreceptors governs host plant selection by these swallowtail butterflies.

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INTRODUCTION

Preface

The geographic range of papilionid species (Lepidoptera) is determined by their successful adaptation to various biotic and abiotic conditions. Thermal constraints, geographic barriers, predation, competition and host affiliation all affect current distribution patterns of swallowtail species. The ability of different papilionid species to recognize, accept and survive on various plant species, paramount to their successful radiation across latitudes and ecological niches, hinges on physiological recognition, behavioral selection and biochemical detoxification of the host plant allelochemicals. While vision and olfaction play a role in host location by gravid female swallowtails, the final decision whether or not to oviposit rests on chemical stimuli perceived through gustatory sensilla on the foretarsi (Feeny *et al.* 1988, Roessingh *et al.* 1991, Nishida 1995). The amount and ratio of oviposition stimulants and deterrents in foliage provide the basis for the relative preferences of swallowtails for their host plants (Honda and Hayashi 1995). The research presented here focuses on assessing the relative importance of leaf chemistry for oviposition for several swallowtail species. Both oviposition stimulants and deterrents will be studied.

In the lab, female swallowtail butterflies, whether generalist or specialist, show a consistent hierarchy of oviposition preference among host plants. Given the choice between leaves of seven species in an oviposition bioassay, *Papilio glaucus*, a highly polyphagous swallowtail, deposits eggs with greatest frequency on tulip tree, hop tree, white ash and black cherry, relatively fewer eggs on spicebush, and fewest on *Rhamnus* spp. and quaking aspen (Scriber unpubl., see Table 1). Swallowtails specializing on Lauraceous plants, such as *P. troilus* and *P. palamedes*, also show consistent oviposition preferences, placing greater than 70% of their eggs on these hosts in a 7-choice array (Table 2). In 3-choice bioassays, *P. troilus* females put the majority or their eggs on spicebush and sassafras, and lay notably fewer eggs on redbay, whereas *P. palamedes* lay eggs predominantly on redbay, with fewest on sassafras, and basically reject spicebush. (Lederhouse *et al.* 1992, see also Table 3). Over evolutionary time, natural selection on host choice and chemical constraints imposed by physiological adaptations provided the basis for current oviposition acceptance patterns.

Ancestral host-affiliations

Ancestral host-affiliations have guided the formation of modern swallowtail-plant associations. Feeny's chemical facilitation hypothesis (1991) suggests that adaptations to host plant chemistry may pave the way for host shifts to chemically similar taxa. It is believed that the ancestor of section III *Papilio* predominantly fed on Rutaceae before the disappearance of many of its more semi-tropical representatives from the middle latitudes during global cooling (Scriber 1995). Rutaceae-feeding may have preadapted several *Papilio* species (such as *Papilio machaon*) to utilization of the Umbelliferae and

Compositae, since these three families share several chemical constituents (Dethier 1941). *Papilio glaucus* provides an example of a species that may have "escaped" chemical constraints and has broadened its host range to include families of many temperate plants (Scriber *et al.* 1991a).

Several pieces of evidence support the hypothesis that Rutaceae is the ancestral host family of both the *glaucus* and *troilus* lineages (section III), two closely related monophyletic taxa (Figure 1). The survivorship of the *glaucus* group caterpillars on hoptree (*Ptelea trifoliata*, Rutaceae) is notable. More than 60% of all first instar caterpillars from 6 of the 8 *glaucus* group species survived to the second instar on hoptree. Because *troilus* group larvae refuse to initiate feeding on plants outside the Lauraceae, presumably due to a lack of feeding stimulants, Scriber *et al.* (1991a) could not assess the detoxification ability of those caterpillars on rutaceous plants. The shared use of Rutaceae by many species in sections III, IV and II of the tribe Papilionini (Scriber 1984, 1996) as well as some species in the Graphiini and Troidini also suggests ancestral origins of rutaceous feeding. Additionally, natural oviposition by *P. glaucus* has been observed on Rutaceae and Lauraceae (Scriber *et al.* 1991a).

Similar kinds of evidence points to Lauraceae as a possible ancestral host family of the two groups. 4 *glaucus* group species, plus 2 *troilus* group species show 60% or more survival of first instars on sassafras (*Sassafras albidum*, Lauraceae). Section V *Papilio* as well as other clades within the Papilionini and Leptocircini also utilize plants in this family (Scriber *et al.* 1991a). Both the Rutaceae and Lauraceae have in common the production of benzylisoquinoline alkaloids, essential oils and coumarins (Berenbaum

1995). Behavioral recognition and metabolic detoxification mechanisms may have facilitated shifts to current hosts with underlying chemical similarities.

Ancestral adaptations to Rutaceae and Lauraceae chemistry conceivably placed *Papilio troilus* on a path for utilization of modern, temperate-zone Lauraceae. Polyphagy of the contemporary *glaucus* group is less easily explained. However, the Rutaceae, Magnoliaceae (tulip tree is the preferred host plant of *P. glaucus*) and Lauraceae families are conspicuously united by the production of essential oils, lignans, mono- and sesquiterpenes, and to a lesser extent, phenylpropanoids (Berenbaum 1995).

Butterfly species may still behaviorally recognize and oviposit on ancestral hosts. Allozyme electrophoresis on the *glaucus* species group indicates that the tiger swallowtail species *P. canadensis* and *P. glaucus*, previously considered subspecies (Hagen *et al.* 1991), have most recently diverged from each other in comparison with the other *glaucus* group species. In lab bioassays, both *P. glaucus* and *P. canadensis* lay a substantial number of eggs on tulip tree, a favorite host of *P. glaucus*, although essentially all *P. canadensis* larvae fail to survive on the foliage. *P. canadensis* females laid a striking average of 43% of the eggs on tulip tree in a 3 choice bioassay between tulip tree, black cherry and quaking aspen despite the fact that *P. canadensis* larvae can survive and develop successfully only on the latter two hosts (Scriber *et al.* 1991b, Bossart and Scriber 1995). The majority of *P. canadensis* never encounter tulip tree in their range, except at their southern border. The common ancestor of *P. glaucus* and *P. canadensis* likely utilized plants in the family Magnoliaceae before the two subspecies diverged geographically and physiologically, and *P. canadensis* appears to have retained the ability to recognize and accept this host.

Evolution of oviposition preference

In *Papilio*, oviposition preference at least generally reflects larval performance in terms of growth rate, pupal weight, and survivorship. For the most part, females will avoid oviposition on plants toxic to their progeny. *P. canadensis* populations that encounter tulip tree at its northernmost limit in Central Michigan tend to show a decreased preference for oviposition on this plant compared to their counterparts from northern Michigan, Canada and Alaska that do not naturally contact tulip tree (Bossart and Scriber 1995). Singer *et al.* (1988) found that the degree of preference a female has for one of two host plants is significantly positively correlated with the mean larval weight of her offspring on that host after 10 days. Similarly, Bossart (1993) found that the average relative growth rate of *P. glaucus* larvae on tulip tree positively correlated with the mother's preference for tulip tree in a 2-choice bioassay with tulip tree and sweetbay. Under experimental conditions, *Euphydryas chalcedona* butterflies preferred to oviposit on the more nutritious of their two host plants (Williams, 1983).

However, discrepancies exist between oviposition preference and larval performance on potential host plants in the field. The general trend of *P. canadensis* females to avoid tulip tree in the lower latitudes appears inconsistent with the total inability of the larvae to detoxify the foliage. These findings suggest that the ability to avoid tulip tree is evolving slowly in populations that encounter it (Bossart and Scriber 1995). In the field, *E. chalcedona* butterflies oviposit on both *D. aurantiacus* and *S.*

californica, regardless of their differing nutritional value to the larvae. *S. californica,* the less abundant and less persistent host, often thrives in shadier habitats than *D. aurantiacus* where *E. chalcedona* butterflies rarely fly. Other selection pressures in addition to larval performance regulate host plant selection in the field.

A multitude of ecological factors may direct the evolution of oviposition preference (Strong 1988 series, Bernays and Chapman 1994a). Parasitism, for example, may take a heavy toll on larval survival and levels may vary with host plant (Damman and Feeny 1988, Ohsaki and Sato 1994). Although all 3 *Pieris* species studied by Ohsaki and Sato (1994) developed best on the cultivated cabbage species in the laboratory, two species preferred to oviposit on other plants that allowed escape from parasitism although larval performance suffered slightly. Smiley (1978) noted that *Heliconius melpomene* utilized only 1 out of the 5 of the plants that supported greatest caterpillar survivorship and fitness in the lab, ovipositing exclusively on the species that offered protection from parasitoids. Interspecific competition with the gypsy moth, *Lymantria dispar*, for quaking aspen foliage as well as an increased parasitism rate by generalized tachinids in gypsy moth infested quaking aspen stands may have facilitated the shift to gypsy moth-free white ash (Scriber and Redman 1993).

The abundance and availability of potential host plants affect evolution of oviposition strategies (Bossart and Scriber 1995, Wiklund 1984). Sweetbay (*Magnolia virginiana*) is the only common host plant encountered by *Papilio glaucus* populations throughout most of Florida (Scriber 1986). Florida *P. glaucus* females showed an increased preference for sweetbay relative to females from populations in Georgia and

Ohio that rarely or never encounter sweetbay. The imposed "ecological monophagy" (Scriber 1986) probably accounts for the documented regional specialization in oviposition preference (Bossart and Scriber 1995).

Thompson and Pellmyr (1991) suggest several other explanations for oviposition choice seemingly at odds with larval performance in the laboratory. For example, the preferred plant may be rare; a plant may be a recent addition to a habitat and there has not been ample time to evolve an avoidance mechanism; the habitat may be unfavorable for ovipositing females or larval growth; females may oviposit on more "toxic" plants that allow for larval sequestration although slower growth ensues; and females may search for clumped hosts in species that require consumption of several individuals to complete development. Rausher (1995) hypothesized that tradeoffs in larval and adult fitness may account for counterintuitive oviposition behavior of females *Battus philenor* females. In northern Mexico, *B. philenor* mothers preferentially search in sunny, rather than shady habitats, although the larval survival was much lower in sunny habitats and the rate of pupal parasitism was much higher. Rausher (1995) suggests that the abundance of orb-weaving spiders in shady habitats may increase adult mortality or that thermoregulatory constraints decrease oviposition efficiency in shady habitats although neither alternative has been tested.

Oviposition preference evolves in the context of other dynamic life history traits and phenology. For example, cold areas that offer a notably shorter developmental window between frosts may select for delayed emergence, delayed mating or delayed egg-laying that facilitate the host shift to phenologically late and nutritious white ash noted in populations of *Papilio canadensis* from northern Michigan. In addition, a short developmental window may also select for adaptations such as larger egg and first instar size and pupation at lower mass to insure fast completion of development (Scriber 1994) such as has been observed in Alaskan populations (Ayers and Scriber 1994).

The interaction of thermal degree day restrictions coupled with the number of generations possible in a given area may further complicate evolution of oviposition preference. Gravid females may show increased specificity for nutritious host plants in locations that allow barely enough time to complete the maximal number of generations. Host specificity may relax in latitudes where there is abundant time for completion of n generations, but not enough time for completion of n+1 generations. The overall effect may be alternating bands of greater and lesser host specificity (Nylin 1988, Scriber and Lederhouse 1992).

Evolution may affect host selection on several levels, from interspecific discrimination to selection of a particular oviposition site on a given plant individual. However, many proximal factors also affect the oviposition decisions of females.

Proximate factors

Circumstantial factors impacting the life of the adult female influence her choice of oviposition sites. For example, the proximity and location of nectar sources strongly influences the oviposition patterns of butterflies (Murphy 1983, Grossmueller and Lederhouse 1987). Despite the presence of sufficient tulip tree foliage during both generations of *P. glaucus,* females only visited and oviposited in the area during the second flight when the thistles were in bloom (Grossmueller and Lederhouse 1987). Females of another butterfly species, *Euphydryas chalcedona*, may avoid certain sites due to harassment by males attempting to mate (Williams 1983). The internal motivation of a female also influences her oviposition selectivity. In the case of *Battus philenor,* the size of the egg cluster is influenced by time since the last oviposition opportunity and host plant characteristics (Rausher 1995). In a no-choice situation, *Pieris rapae* will oviposit on *Iberis amara* after an initial delay, a plant it normally avoids. Bossart (1993) similarly found a negative correlation between host specificity and egg load, and a positive correlation between age (reflecting a decline in egg load) and host specificity (see also Scriber 1993). The time and energy budget of a female, coupled with her motivational state will influence oviposition decisions (Underwood 1994).

Host finding mechanisms

The size and surrounding vegetation of a plant affects its apparency to ovipositing females. Masumoto *et al.* (1993) observed that *Anthocharis scolymus* females approach taller host plants with greater frequency, particularly when the surrounding vegetation was sparse. Numbers of eggs and larvae were greatest on taller hosts, but decreased as the density of surrounding conspecific plants and other vegetation increased (Masumoto *et al.* 1993). Monarch butterflies (Oyeyele and Zalucki 1990) and parsnip webworms are also inclined to oviposit on taller plants (Zangerl and Berenbaum 1992).

Ovipositing butterflies use visual and chemical cues to lead them to the appropriate larval host plants. Rausher (1978) observed that female *Battus philenor* preferentially alight on leaves of a particular shape, regardless of whether or not the plant is a host plant, indicating that discrimination of leaf shape occurs at a distance. *Papilio demoleus* shows a positive orientational response to glass-screened cut branches of a host (*Citrus limettioides*) and a non-host (*Gossypium hirsutum*) indicating a visual attraction to general foliar characteristics (Saxena and Goyal 1978).

Butterflies show intrinsic behavioral responses to various colors (Ilse 1937, Saxena and Goyal 1978, Kolb and Scherer 1982, Traynier 1984). For example, certain colors or wavelengths may elicit either a feeding or drumming response (Ilse 1937, Kolb and Scherer 1982). In an experiment where discs were illuminated with monochromatic light at various wavelengths, wavelengths between 542-578 nm elicited the greatest number of drumming events by *Pieris brassicae* females. When the discs were sprayed with sinigrin to elicit oviposition, the greatest number of eggs were laid discs illuminated under 522-554 nm. These wavelengths compose the yellow-green region of the spectrum (Kolb and Scherer 1982). Gravid *P. demoleus* females were observed to fly toward yellowish-green and green muslin patches (Saxena and Goyal 1978). In his experiments on learning, Traynier (1984) noted inherent preferences of *P. rapae* for oviposition on green-colored substrates, and even particular shades of green.

Olfactory cues both serve to attract and to arouse the ovipositional urge in butterflies. In a wind tunnel, two polyphagous moths, *Heliothis virescens* and *Trichoplusia ni*, were attracted by odors from several plant species (Bernays and Chapman 1994). *Papilio demoleus* showed a significant orientational preference to a *Citrus* branch placed outside the nylon net wall of their cage relative to a glass-screened branch on the opposite side. Some abdominal curling and oviposition occurred on the net adjacent to the unscreened plant without any contact with the foliage, indicating that odor also serves in part to stimulate oviposition in the presence of visual cues (Saxena and Goyal 1978). Containers of host-plant volatiles elicited more landings by *Papilio polyxenes* on a contact-stimulant treated model plant relative to control cages lacking the volatile stimuli. In these studies, the presence of volatiles increased the overall activity of the butterflies, as measured by "flutter bouts" (Feeny *et al.* 1989). *Manduca sexta* were observed to fly toward an odor source and cease flight upon its removal. Volatile compounds may not only serve as an orientation cue, but as a physiological primer stimulating flight and oviposition in the presence of additional cues (Bernays and Chapman 1994).

Upon alighting on a plant, a gravid female perceives contact chemical cues through tarsal chemoreceptors that influence her final decision whether or not to oviposit (Feeny *et al.* 1988, Nishida and Fukami 1989, Honda 1990, Nishida *et al.* 1990, Roessingh *et al.* 1991, Sachdev-Gupta 1993). The presence of oviposition stimulants and relative lack of deterrents is crucial in the stimulation of oviposition. However, at this level, the perception of the nutritional state of a plant (Myers 1985), color (Myers 1985, Robertson 1987) and even phenological cues (Rausher and Papaj 1983, Damman and Feeny 1988) also influence her final decision. Information gained from sampling many conspecific plants allows the butterfly to discriminate between several individuals after alighting (Traynier 1986).

A sensory synergism of acceptable visual and chemical cues leads to the greatest likelihood of oviposition. For example, although *Pieris brassicae* females will land and drum on discs illuminated with yellow-green light, oviposition will only occur in the presence of a sinigrin solution (Kolb and Scherer 1982). Odor, color and contact chemical stimuli elicit the greatest number of orientation, abdominal curling and oviposition reponses by *Papilio demoleus*, and the absence of one or more of these cues reduces the frequencies of these behaviors (Saxena and Goyal 1978).

The ability of butterflies to learn may enable them to link chemical cues with physical cues (Traynier 1986, van Loon *et al.*1992). *Battus philenor* forms a search image that can be altered by experience. Early in the season, broad leaved *Aristolochia reticulata* predominate. Frequent encounters with *A. reticulata* reinforces the broad leaf search image, enabling the butterflies to efficiently locate other *A. reticulata* plants. Later in the season, *A. reticulata* foliage increases in toughness and declines in nutritional value while narrow leafed *A. serpentaria* remains nutritious and able to support larval development. A corresponding increase in landings on narrow-leaved foliage can be observed after the maturation of *A. reticulata.* Papaj and Rausher (1987) also demonstrated the ability of naive *B. philenor* females to learn to land on large young plants in preference to large old plants after given the opportunity to land on plants in an experimental enclosure. *Pieris rapae* approach and lay more eggs on nitrogen fertilized plants than controls (Myers 1985, Wolfson 1980). Myers (1985) found that leaf greenness and egg count were correlated and that greener plants had a higher concentration of nitrogen in their leaves. In all four cases (*Battus philenor*, *Eurytides marcellus*, *Papilio xuthus*, *Pieris rapae*) the larvae benefited from the oviposition decisions of the female, implicating a nutritive or chemical link between plant phenotype and differential oviposition.

The fundamental importance of phytochemistry

The balance of stimulants and deterrents in a leaf determine whether or not a butterfly will oviposit. In the case of *P. rapae*, the acceptability of bean seedlings (a non-host) depended on the ratio of the stimulant compound to deterrent compound introduced systemically into the plant. However, at very high stimulant levels, increasing the concentration of the deterrent did not impede oviposition (Renwick and Huang 1994). Differential sensitivity to stimulants and deterrents by closely related species of butterflies provides the basis for differential host ranges in several *Pieris* and *Papilio* species. The deterrent phellamurin found in the rutaceous plant *Phellodendron amurense* strongly inhibits oviposition by *Papilio protenor*, while is only partly inhibitory to *Papilio xuthus* at natural concentrations (Honda and Hayashi 1994). The dissimilar sensitivities of the two species to this compound provide the basis for marginal acceptance of *Phellodendron amurense* by *P. xuthus* and its complete rejection by *P. protenor* in the field (Honda and Hayashi, 1995). Honda and Hayashi (1995) note that in several *Papilio* species, such as *P. xuthus*, *P. protenor* and *P. machaon*, oviposition stimulants and deterrents are commonly flavonoids or their derivatives. They hypothesize that flavonoids originally deterred oviposition by *Papilio* species, but that sensorial adaptation to the compounds allowed the colonization of the foliage containing them. Based on feeding trials with many insects, Bernays and Chapman (1994a) have concluded that oligophagous insects are deterred by more compounds than polyphagous insects, and that polyphagous feeders are less sensitive to deterrents. This scenario may also explain host acceptance by generalist and specialist ovipositing swallowtail butterflies as well.

Several compounds with very similar structures often have ovipositional activity, lending support to the chemical facilitation hypothesis proposed by Dethier (1941) and Feeny (1991). In the pierid system, for example, indolyl and aromatic glucosinolates appear more stimulatory than their aliphatic counterparts (Renwick and Huang 1994). Closely related *Papilio* species may share sensitivity to the same family of chemical compounds and may have used such compounds as a bridge in their radiation onto different plant taxa. Additionally, host plants of a particular species may have in common several characteristic compounds and a lack of strong deterrents. Elucidation of the oviposition stimulants and deterrents in the various *Papilio* host plants will clarify the mechanisms for host acceptance and host shifts, thereby providing clues to the evolutionary relationships between different swallowtail species. I have chosen to make the characterization of oviposition stimulants and deterrents for several species of swallowtails the focus of my research.

In my thesis, I investigate the role of host chemistry in stimulating oviposition in two oligophagous butterflies, *P. troilus* and *P. palamedes*. Also, I present evidence for the presence of oviposition-deterring compounds in a non-host of the polyphagous butterfly *Papilio glaucus*. My thesis reinforces the fundamental importance of leaf chemistry to host plant selection by specialized and generalized *Papilio*.

Table 1. Average lifetime percentage of eggs distributed across a 7-choice array of plant species for different populations of *Papilio glaucus*. All data compiled from the spring and summer of 1994. From Scriber *et al.* unpublished.

Papilio glaucus	Spicebush LAURACEAE	Black cherry ROSACEAE	Quaking aspen SALICACEAE	Tulip tree MAGNOLIACEAE	*Rhamnus* spp. RHAMNACEAE	Hop tree RUTACEAE	White ash OLEACEAE	Females (n)
Michigan								
Ingham Co.	**13.2** ± 3.4	**18.0** ± 3.9	**9.8** ± 4.0	**22.6** ± 0.9	**5.0** ± 2.4	**11.8** ± 1.9	**19.7** ± 7.2	**5**
Eaton Co.	**7.5** ± 1.7	**8.2** ± 2.2	**2.9** ± 1.6	**43.0** ± 6.6	**0.2** ± 0.2	**17.4** ± 5.6	**18.8** ± 5.8	7
Ohio								
Gallia Co.	**9.1** ± 2.0	**10.6** ± 2.0	**5.0** ± 1.3	**39.9** ± 6.9	**2.0** ± 0.6	**25.6** ± 5.6	**7.8** ± 1.5	**17**
Lawrence Co.	**6.9** ± 3.7	**32.9** ± 19.1	**1.1** ± 1.1	**28.5** ± 10.7	**20.7** ± 19.4	**6.4** ± 3.7	**3.6** ± 1.9	**3**
Georgia								
Clark Co. (Aug.)	**8.0** ± 1.1	**8.7** ± 1.3	**2.1** ± 0.4	**32.9** ± 3.6	**1.1** ± 0.3	**26.8** ± 2.9	**20.1** ± 3.0	**39**
Clark Co. (Sept.)	**6.5** ± 1.3	**7.3** ± 1.3	**4.6** ± 1.3	**32.9** ± 3.7	**3.0** ± 1.5	**27.7** ± 3.1	**17.9** ± 2.6	**34**
Florida								
Highlands Co.	**12.2** ± 2.4	**12.6** ± 1.9	**6.5** ± 2.2	**31.2** ± 3.3	**5.4** ± 1.8	**13.0** ± 2.0	**19.1** ± 2.8	**6**

Table 2. Average lifetime percentage of eggs distributed across a 7-choice array of plant species for different populations of *Papilio troilus and P. palamedes*. All data compiled from the spring and summer of 1994. From Scriber *et al.* unpublished.

Papilio troilus	Spicebush **LAURACEAE**	Black cherry **ROSACEAE**	Quaking aspen **SALICACEAE**	Tulip tree **MAGNOLIACEAE**	*Rhamnus* spp. **RHAMNACEAE**	Hop tree **RUTACEAE**	White ash **OLEACEAE**	Females (n)
Ohio								
Gallia Co.	**89.5** ± 7.1	**5.2** ± 5.2	**0.6** ± 0.6	**0**	**2.4** ± 2.4	**2.3** ± 2.3	**0**	**3**
Lawrence Co.	**89.0** ± 6.7	**0.9** ± 0.9	**0**	**0.5** ± 0.5	**2.1** ± 2.1	**4.1** ± 3.5	**3.3** ± 3.0	**6**
Papilio palamedes								
Florida								
Highlands Co.	**87.5**	**7.1**	**0**	**0**	**0**	**0**	**5.4**	**1**

Table 2. Average lifetime percentage of eggs distributed across a 7-choice array of plant species for different populations of *Papilio troilus and P. palamedes*. All data compiled from the spring and summer of 1994. From Scriber *et al.* unpublished.

Papilio troilus	Spicebush **LAURACEAE**	Black cherry **ROSACEAE**	Quaking aspen **SALICACEAE**	Tulip tree **MAGNOLIACEAE**	*Rhamnus* spp. **RHAMNACEAE**	Hop tree **RUTACEAE**	White ash **OLEACEAE**	Females (n)
Ohio								
Gallia Co.	**89.5** ± 7.1	**5.2** ± 5.2	**0.6** ± 0.6	**0**	**2.4** ± 2.4	**2.3** ± 2.3	**0**	**3**
Lawrence Co.	**89.0** ± 6.7	**0.9** ± 0.9	**0**	**0.5** ± 0.5	**2.1** ± 2.1	**4.1** ± 3.5	**3.3** ± 3.0	**6**
Papilio palamedes								
Florida								
Highlands Co.	**87.5**	**7.1**	**0**	**0**	**0**	**0**	**5.4**	**1**

Table 3. Average lifetime percentage of eggs distributed on three Lauraceous host plants by *Papilio troilus* and *Papilio palamedes*. Data compiled between the years 1991-1994. After Scriber *et al.* unpublished.

Papilio troilus

LOCATION	REDBAY	SASSAFRAS	SPICEBUSH	FEMALES (n)
Michigan	**15.8** ± 5.8	**44.4** ± 7.3	**39.8** ± 7.4	**17**
Ohio				
Gallia Co.	**13.3** ± 6.3	**47.5** ± 13.5	**39.2** ± 16.6	**19**
Lawrence Co.	**20.6** ± 2.3	**57.2** ± 8.6	**22.2** ± 6.4	**9**
Georgia				
Echols Co.	**30.6** ± 11.5	**23.6** ± 12.2	**45.7** ± 11.6	**7**
Florida				
Highlands Co.*	**45.9** ± 16.6	**29.9** ± 5.2	**24.3** ± 21.2	**6**

Papilio palamedes

Georgia				
Echols Co.	**67.7** ± 32.3	**28.3** ± 28.3	**4.0** ± 4.0	**3**
Florida				
Highlands Co.*	**74.7** ± 4.8	**13.8** ± 3.5	**11.5** ± 3.2	**10**

* Redbay is the only Lauraceae species in this group of hosts that occurs in the southern 2/3 of Florida.

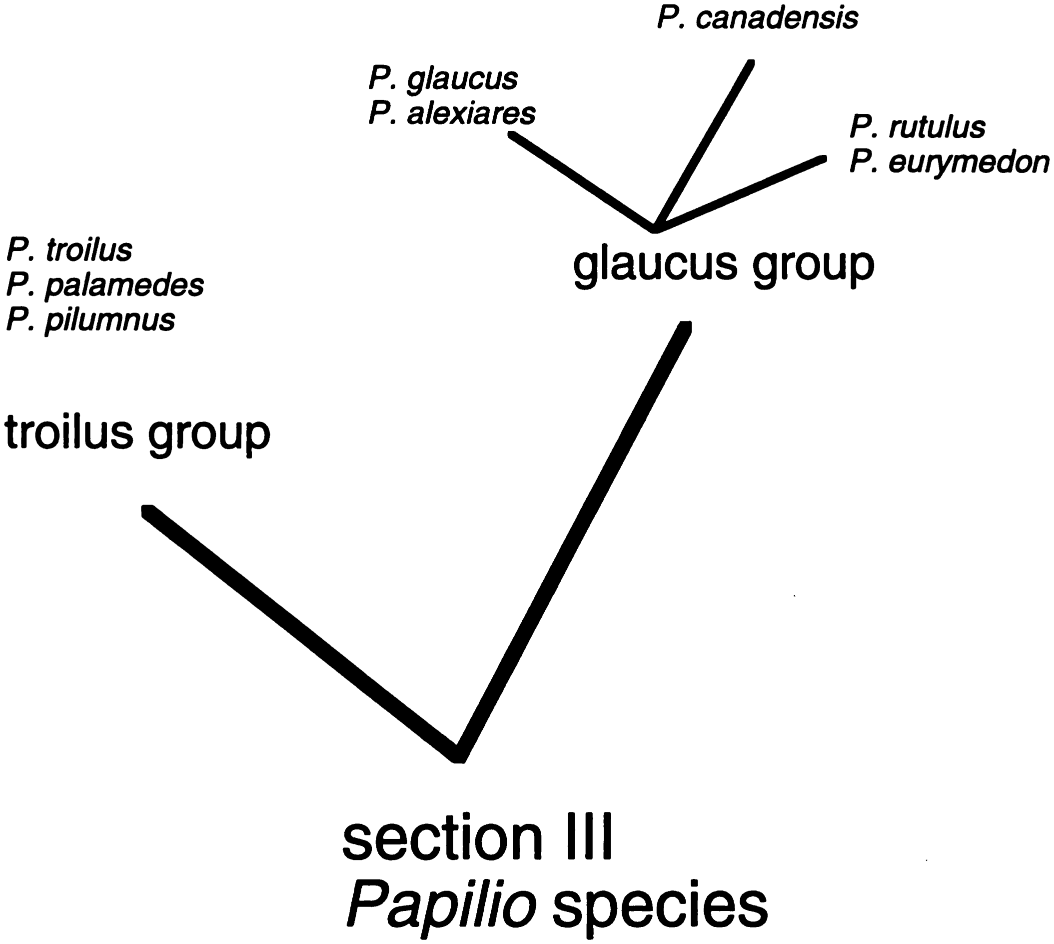

Figure 1. Consensus taxonomy for monophyletic section III *Papilio*. From Scriber *et al.* 1991.

CHAPTER 1

Chemical basis for host recognition by two oligophagous swallowtail butterflies, *Papilio troilus* and *Papilio palamedes* (Lepidoptera: Papilionidae)

Abstract

Females of *Papilio troilus* and *P. palamedes*, two closely related swallowtail butterflies, oviposit almost exclusively on a few woody plant species in the family Lauraceae. Dependence on chemical oviposition elicitors found only in the foliage of the host plants may explain the behavioral fidelity shown by the two oligophagous butterflies. The role of host plant chemistry in stimulating oviposition was assessed by extracting the preferred host foliage of the respective butterfly species, spraying the extracts and fractions on various substrates, and assessing oviposition relative to controls. *P. troilus* and *P. palamedes* were stimulated to oviposit on filter paper or non-host leaves sprayed with polar extracts of their primary host plants. However, oviposition was enhanced on non-host leaves relative to filter paper, perhaps due to a combination of visual, tactile and olfactory cues that are characteristic of leaf surfaces.

Introduction

Host plant affiliations primarily determine the geographic range of lepidopterans. The swallowtail butterflies *P. palamedes* and *P. troilus* recognize a very restricted range of plant taxa as hosts, ovipositing exclusively on a few species within the Lauraceae. *P.*

palamedes inhabits the eastern coastal states from southern Virginia south through Florida and west toward Texas and Mexico, closely corresponding to the geographic distribution of redbay (*Persea borbonia*), their predominant host in the field. The range of *P. troilus* includes that of spicebush (*Lindera benzoin*) and sassafras *(Sassafras albidum*), ranging from Canada to Florida east of the Mississippi River and occurring in sympatry with *P. palamedes* in southern Florida (Lederhouse *et al.* 1992). *P. troilus* utilizes redbay only in southern Florida, outside the habitat of spicebush and sassafras (Nitao *et al.* 1991).

Results of laboratory oviposition bioassays conform to patterns of host use in the field. The two specialist species accurately place their eggs on lauraceous foliage when placed in an array with 7 other non-host leaves in small bioassay containers (see Table 2, introduction). *P. palamedes* and *P. troilus* also exhibit the relative preferences among lauraceous host species. In bioassays with leaves from 3 lauraceous species, *P. palamedes* females deposit the majority of their eggs on redbay, and oviposit to a lesser extent on spicebush and sassafras. *P. troilus* females oviposit mainly on spicebush and sassafras, and place fewer eggs on redbay (Lederhouse *et al.* 1992, see Table 3, chapter 1).

Behavioral responses to compounds distributed throughout a plant community account for the patterns of host use observed in the field and laboratory. The ratio of chemical stimulants and deterrents found in foliage determine the range of plant species accepted for oviposition by the two specialist swallowtail butterflies (Honda and Hayashi 1995). Commonly, mixtures of stimulant compounds found within host plants can act in combination to synergistically elicit oviposition in swallowtail butterflies (Ohsugi *et al.* 1985, Feeny *et al.* 1988, Nishida and Fukami 1989, Honda 1990, Sachdev-Gupta *et al.* 1993). The stimulant mixture generally contains compounds ubiquitous to many plant families as well as those unique to a particular species (Nishida 1995).

Behavioral reliance on compounds characteristic of a particular plant taxa may account, in part, for the ovipositional specificity of oligophagous swallowtail species (Ohsugi *et al* 1985, Feeny *et al* 1988, Nishida and Fukami 1989, Honda 1990, Sachdev-Gupta *et al* 1993). For example, the oviposition stimulants of the swallowtails *Atrophaneura alcinous* and *Battus philenor* were characterized as a mixture of aristolochic acids and various sugars. Aristolochic acids are found predominantly in plants within the *Aristolochia* (Aristolochiaceae), and their indispensability in eliciting oviposition probably accounts for the sole use of plants in that genus by the two species. A physiological dependence on a few compounds exclusive to the family Lauraceae for oviposition stimulus may underlie the strong host recognition of *P. troilus* and *P. palamedes* both in the field and in multi-choice bioassay arenas in the lab. With that rationale, I focused on the chemical extraction and fractionation of oviposition stimulants found in spicebush and redbay, the respective favorite host plants of *P. troilus* and *P. palamedes*.

Materials and Methods

General bioassay procedure:

Field-caught or hand paired, lab-reared females were stored at 18°C until their use in the bioassay. Storage at that temperature increased the lifespan of the females relative to butterflies maintained at room temperature. Each bioassay arena consisted of 1 round, transparent plastic container (26 cm in diameter x 9 cm) lined with a paper towel at the bottom. Oviposition substrates, consisting of either filter paper or non-host leaves treated with host extracts, were placed along the sides of the container equally spaced from each other. The petioles of the leaves were inserted into waterpics to maintain leaf turgor. A single female occupied each bioassay arena, where she encountered all treatments and the control substrate simultaneously. Bioassay containers were stacked on turntables that rotated once every six minutes and were illuminated on one side with incandescent lights (see Scriber 1993). Once a day, egg counts on the oviposition substrates were tallied and fresh treatments and controls were supplied. Eggs laid greater than 2.5 cm away from a substrate (the approximate distance between a female's foretarsi and curled ovipositor), or equal distances between two treatments, were scored as "container." Females were fed a 20% honey solution once daily. Females were allowed to oviposit for several days, and analysis was performed on the percentage of total eggs laid on each substrate over the course of the bioassay.

Extractions:

Extraction and fractionation of Spicebush

In 1994, fresh, intact spicebush leaves (130.7 grams) collected from Kalamazoo county, Michigan were extracted for 1 minute with chloroform (3x), then with methanol (3x) in an extraction column. The procedure yielded 0.8 g of chloroform extract and 8.3

g of methanol extract.

A modified extraction procedure was used in 1995. Freeze-dried, ground spicebush leaves (120.5 g) collected from Gallia County, Ohio were extracted sequentially with 3 solvents. The leaf powder was extracted first with 700 ml of hexane for 5 minutes, then soaked (> 6 hours, 2x) in 700 ml of hexane. The procedure was repeated with ethyl acetate and methanol. The residues were dried *in vacuo* at 40°C. Subsequent procedures focused on the methanol extract, which yielded 20.3 grams of dried powder. An aliquot (10.2 g) of the methanol extract was redissolved in 200 ml of 20% methanol in a flask during agitation with a stir bar. The solution was extracted (3x) with 50 ml of ethyl acetate, distributing 8.0 g of material into the aqueous phase and 2.1 g into the ethyl acetate phase. The ethyl acetate-soluble material was dried under a vacuum at 40°C, and the aqueous phase was stored frozen at -20°C until use in the bioassays.

Extraction and fractionation of Redbay

In 1995, 72.9 g of freeze-dried, milled redbay leaves collected from Highland County, Florida were extracted with ca. 400 ml of each solvent, following the protocol for the 1995 extraction of spicebush. The procedure yielded 16.4 g of vacuum-dried methanol extract. The crude methanol extract (5.7 g) was partitioned between water and ethyl acetate as above, distributing 1.1 and 4.6 g into the organic-phase and aqueous-phase, respectively.

An additional 56.3 g of the redbay leaf powder stored at -80°C was extracted later in the summer to give 13.7 g of methanol extract. A portion of this material (6.9 g) was partitioned as above between ethyl acetate and water, yielding 1.6 and 5.3 g of material, respectively.

Bioassays:

4-choice filter paper bioassay (Figure 2)

A leaf area meter was used to measure the average surface area of 65 freshly picked spicebush leaves weighing a total of 22.4 grams. Aliquots of each extract from 1994 (average g extract/cm^2) were redissolved in their original extraction solvent and pipetted onto filter paper triangles of known surface area. The solvent was allowed to evaporate off of the paper strips before their inclusion in the bioassay. Four filter paper triangles, treated with either the methanol extract, the chloroform extract, both extracts, and solvent only were placed in each bioassay arena.

5-choice filter paper bioassay with spicebush leaf (Figure 4A)

Using spicebush extracts and fractions of leaf samples from 1995, gram leaf equivalents (average gram extract/leaf, hereafter abbreviated GLE) of the crude methanol extract and the water fraction were sprayed evenly onto both sides of filter paper triangles using a chromatographic sprayer. Approximately four times the GLE of the ethyl acetate fraction was misted onto the filter paper triangles due to a calculation error. However, this mistake did not significantly alter the results of the bioassay (see Figure 4A). Within one oviposition chamber, each female could contact all 5 oviposition substrates: 3 filter paper triangles (coated respectively with the crude methanol extract, the ethyl acetate fraction and the water fraction) an untreated filter paper triangle, and a spicebush leaf with its petiole inserted into an aquapic to maintain leaf turgor.

3-choice and 5-choice leaf bioassays (Figures 3, 4B and 5)

GLE of the crude extracts and fractions were sprayed separately onto both sides of tulip tree leaves using a chromatographic sprayer. Tulip tree leaves were chosen as a substrate because it is not a host plant of *P. troilus* or *P. palamedes* and it was thought that recognition of a leaf surface would enhance the accuracy of oviposition on the treatment choices. The 3-choice leaf bioassay with *P. troilus* consisted of a tulip tree treated with the 1994 methanol extract, an untreated tulip leaf, and a spicebush leaf. In the 5-choice bioassay, oviposition preference of *P. troilus* was tested on the 1995 crude methanol extract, the ethyl acetate and water fractions and untreated tulip tree and spicebush leaves. In the case of *P. palamedes*, redbay extracts and fractions were used, and 1 or 2 redbay leaves collected from plants in the greenhouse served as the positive control instead of spicebush.

Statistical analysis:

The percentage of eggs laid by individual females on each treatment throughout the duration of the bioassay was calculated. The percentages were arcsine transformed prior to their analysis to normalize the variances. A standard two-way analysis of variance (ANOVA) for unreplicated data was performed, with treatments and butterflies as the main effects (Sokal and Rohlf 1981). The term "unreplicated" refers to the fact that only 1 butterfly occupied each block, or bioassay arena. If treatment was found to be significant, a one-way ANOVA was performed on the arcsine transformed percentages, excluding the “container” category to maintain independence between treatments.

Specific significant differences between treatments could then be characterized with the Student-Newman-Keuls test (SuperANOVA 1993).

Results

In the 4-choice bioassay with *P. troilus* (Figure 2), two-way ANOVA showed significant differences between treatments ($F_4 = 6.60$, $p = 0.0015$), but not butterflies ($F_9 = 0.01$, $p = 1.0$). Filter-paper triangles coated with the combined chloroform + methanol extracts had high ovipositional activity, receiving 46% of all eggs laid, significantly more than the control and the other two treatments according to a one-way ANOVA ($p < 0.01$, Figure 1). Filter paper triangles coated with either the methanol or chloroform extract alone were not found to be significantly different from the control. Twenty-four percent of the eggs were scattered randomly around the oviposition container, and were included in the "container" category.

The 3-choice leaf bioassay (Figure 3) demonstrated the effect of the cues from the oviposition substrate on ovipositional selectivity. Only 5% of the eggs were laid off of the intended substrates on the sides and bottoms of the assay chamber. In the 2-way ANOVA, just the treatment category was found to be significant ($F_2 = 14.051$, $p < 0.0001$). A 1-way ANOVA revealed that *P. troilus* females laid significantly more eggs on tulip tree leaves treated with the methanol extract (48%) and intact spicebush leaves (36%) than tulip tree leaves sprayed with methanol solvent only ($p < 0.01$).

In the presence of an intact spicebush leaf, *P. troilus* female laid negligible amounts of eggs on filter paper triangles sprayed with spicebush extracts in the 5-choice assay (Figure 4A). 89% of the eggs were laid on the spicebush leaf, while significantly less (9%) were laid on the other filter paper substrates together ($p < 0.01$, 1-way ANOVA). In contrast, tulip tree leaves sprayed with spicebush extracts proved more stimulatory than both the tulip tree leaf control and the spicebush leaf itself (Figure 4B). Only 10% of the eggs were placed on spicebush compared to 30% on leaves containing the crude methanol extract, 30% on leaves treated with the ethyl acetate fraction, 18% on leaves treated with the aqueous fraction and 12% on the untreated tulip tree leaves. A one-way ANOVA revealed that the crude methanol extract and the ethyl acetate fraction received significantly more eggs than did the untreated spicebush and tulip tree leaves ($p < 0.05$).

In the case of *P. palamedes* as well, tulip tree leaves sprayed with the crude redbay extracts received more eggs than redbay leaves (Figure 5). Only the crude methanol extract was found to be significantly different from the untreated redbay and tulip tree controls by a one-way ANOVA ($p < 0.01$). In both *P. troilus* and *P. palamedes* tulip tree assays, the butterflies laid comparable percentages of eggs on the untreated tulip tree leaves and their preferred host leaf, and laid eggs with greatest frequency on tulip tree leaves coated with host extracts. The results of all the bioassays demonstrate that host plant chemicals stimulate oviposition by *P. troilus* and *P. palamedes*.

Discussion

Leaf chemistry clearly plays a role in host recognition by *P. troilus* and *P. palamedes*. In the *P. troilus* four-choice filter paper bioassay (Figure 2), the chloroform and methanol extract-treated filter paper triangles received more eggs than the untreated filter paper. The filter paper treated with both extracts received 46% of the eggs laid, significantly more than any other substrate, due to either a synergism or increased dosage of the oviposition stimulants. Results from the leaf bioassays (Figures 3, 4B and 5) also strongly implicate a role for chemical stimulants in oviposition. Tulip tree leaves sprayed with the methanolic extract of the preferred host and its fractions received significantly more eggs from the respective *Papilio* species than the untreated tulip tree leaf and even the host leaf itself.

However, chemical cues are not solely responsible for host recognition. In the *P. troilus* four-choice filter paper bioassay, a significant percentage of eggs was distributed around the bioassay arena, away from the filter paper substrates. A lack of cues vital to the recognition of a host surface may account for this seemingly indiscriminant dispersion of eggs. Off-substrate oviposition declined substantially in the 3-choice and 5-choice all-leaf assays, most likely due to the perception of general leaf cues such as color, texture and odor that characterizes host tissue (Figures 4B and 5). A comparison of the two 5-choice bioassays with *P. troilus* (Figure 4) dramatically demonstrates the importance of physical cues in host recognition. In the presence of an intact spicebush leaf, *P. troilus* females laid negligible amounts of eggs on extract-treated filter paper. However, when sprayed onto the surface of tulip tree leaves, *P. troilus* females preferred the extracts to the spicebush leaf itself.

The role of physical cues in host recognition was demonstrated by Städler (1974) who found that the eastern spruce budworm, *Choristoneura fumiferana*, preferred to oviposit on paper in the form of coniferous needles to flat sheets of the same material. Grant and Langevin (1994) also have shown that although conifer-feeding *Choristoneura* species laid significantly more eggs on the hexane extract-treated filter paper compared to the control, they preferred to oviposit on fake conifer foliage to extract-treated filter paper. *Pieris brassicae* demonstrates "wavelength specific behavior," by flying down and drumming on discs illuminated by light only in the 522-554 nm (yellow-green to green) range (Kolb and Scherer 1982). Similarly, Saxena and Goyal (1978) found that *Papilio demoleus* butterflies showed a positive orientational response to the glass-screened leaves of both lime (host plant) and cotton (non host) relative to the blank wall on the opposite side of their cage. Since the leaves of the two plants differ in shape, the butterflies may be attracted to general plant architecture or leaf color. Using muslin patches colored with various dyes, Saxena and Goyal (1978) then demonstrated a maximum attraction for green or yellow-green colors. In my experiment, filter paper triangles treated with methanol or ethyl acetate extracts had a vibrant green color, perhaps cuing oviposition in the four-choice filter paper assay. Since both *P. troilus* and *P. palamedes* laid approximately equal amounts of eggs on the untreated tulip tree leaf and their true host leaf, shape does not appear to be a distinguishing cue at close proximity.

A gestalt of visual and chemical cues greatly boosts the oviposition response in comparison with either cue by itself (Saxena and Goyal 1978, Traynier 1986). Ground disks illuminated with light of yellow-green wavelengths could elicit drumming behavior of *Pieris brassicae*, but never oviposition. Disks sprayed with sinigrin, an oviposition stimulant of *P. brassicae*, and illuminated with white light, were completely ignored. However, when both stimuli were present together, significant oviposition occurred (Kolb and Scherer 1982). Similarly, moistened yellow-green muslin treated with an ethanol extract from the *Citrus* host plants elicited more visits, abdominal curling, and oviposition by *Papilio demoleus* than either stimulus alone. The addition of host odor in the form of an ether extract further increased all three behavioral responses (Saxena and Goyal 1978).

Tactile cues also play a role in host acceptance, particularly in moths (Ramaswamy 1994). In my experiments, the chloroform extract-coated and chloroform + methanol extract coated-filter paper may have had a more waxy texture than either the untreated or methanol extract-treated filter paper, as leaf surface waxes are soluble in chloroform (Figure 1). A smooth texture in combination with chemical stimulants found in either extract may have caused more eggs to be distributed on the triangles treated with both extracts. However, Shorey (1964) found that *Trichoplusia ni* did not lay significantly more eggs on wax paper than blotting paper, although the proportion of eggs deposited on each substrate changed with the relative humidity. Oviposition stimulants dissolved in the wax layer (Bernays and Chapman 1994, Renwick and Huang 1994) could also account for oviposition on the chloroform extract-treated filter paper.

It is intriguing that both *P. troilus* and *P. palamedes* laid significantly more eggs on tulip tree leaves sprayed with certain host extracts than on their actual host leaf. It is possible that extracts applied to the surface of the non-host leaf have an increased functional dosage due to their accessibility to the tarsal chemoreceptors, unmasked by a surface layer of wax. In the confines of the plastic container, saturation of the air by leaf volatiles would also make it difficult for the butterflies to rely on olfactory cues to guide them to their true host leaf. Alternatively, a synergism between tulip tree leaf chemistry and the host plant extracts may have occurred.

An idiosyncracy of both of the 5-choice leaf bioassays, but not the 3-choice bioassay, was that the tulip tree control leaves received as many eggs as did the lauraceous host leaves. The redbay leaves taken from potted trees in the greenhouse were yellowish and of poor quality and may explain this unusual trend in the bioassay with *P. palamedes*. Alternately, the phenomenon may be due to “spillover” oviposition from neighboring extract coated leaves. The resinous extracts applied to the surface of the tulip tree leaves may have adhered to the tarsi of the butterflies. The large, continuous leaf surface area of the tulip tree leaf coupled with the closer proximity of all the leaves in the 5-choice assays may have exacerbated the problem of "spillover" oviposition in these arenas. Additional bioassay errors may have resulted from chemical changes in the tulip tree leaves due to their removal from trees, the synergism of tulip tree chemistry with that of the host plant extracts and altered permeability of the tulip tree leaves due to solvent effects. However, in other bioassays that used both a solvent-treated and untreated control leaf (see Chapter 2), no significant differences between the two were detected.

Although several factors are responsible for the recognition of a substrate as a host, as evidenced by the results here, leaf chemistry does dictate inter- and intraspecific oviposition decisions (Myers 1985, Nishida and Fukami 1989, Feeny 1991, Roessingh *et al.* 1991, Sachdev-Gupta *et al.* 1993, Honda and Hayashi 1995). Reliance on compounds unique to particular plants may keep related butterfly taxa faithful to chemically similar groups of plants. Elucidation of oviposition stimulants can permit parsimonious deductions about taxonomic relationships within the genus *Papilio* and their host affiliations past and future.

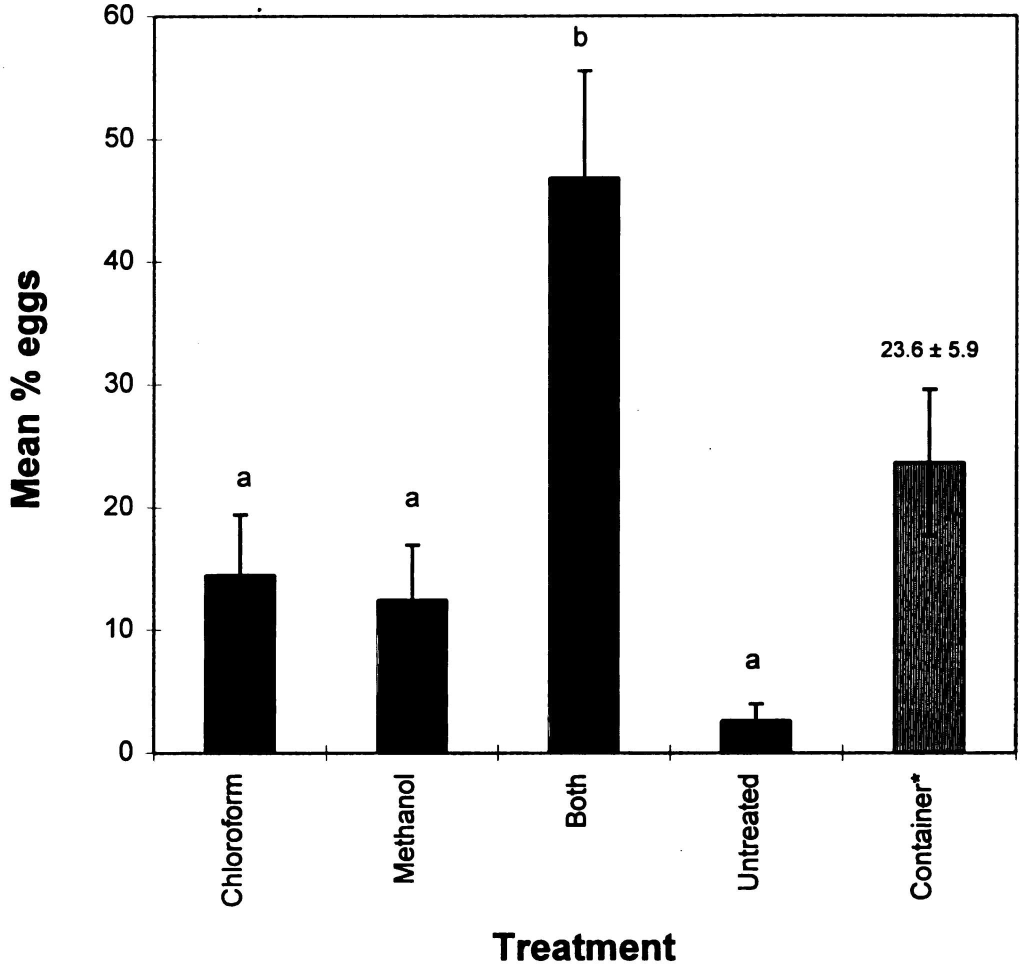

Figure 2. Percentage of eggs (mean ± S.E.) laid by *Papilio troilus* females (n = 6) on each of 4 choices: filter paper triangles coated with methanol extract (methanol), chloroform extract (chloroform), a combination of both extracts (both) and an untreated filter paper triangle (untreated). Extracts were made from fresh spicebush leaves. Significant differences between means ($p < 0.01$) are shown for arcsine transformed percentages.

*Container refers to the percentage of eggs (mean ± S. E.) laid off the filter paper substrate onto the sides and bottoms of the bioassay arena. It was excluded from the 1-way ANOVA and SNK test that determined the significance differences shown here.

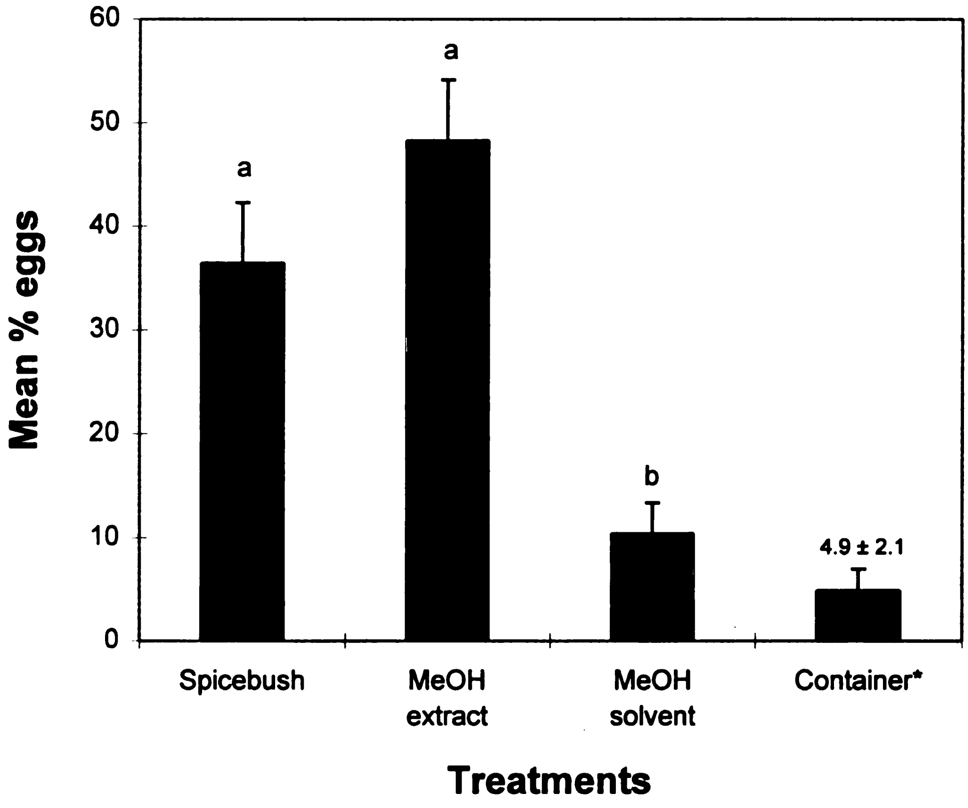

Figure 3. Percentage of eggs (mean ± S. E.) laid by *Papilio troilus* females (n=10) on each of three choices: an untreated spicebush leaf, a tulip tree leaf coated with the methanol extract of spicebush and a tulilp tree leaf sprayed with methanol only. Significant differences between means ($p < 0.01$) are shown for arcsine transformed percentages.

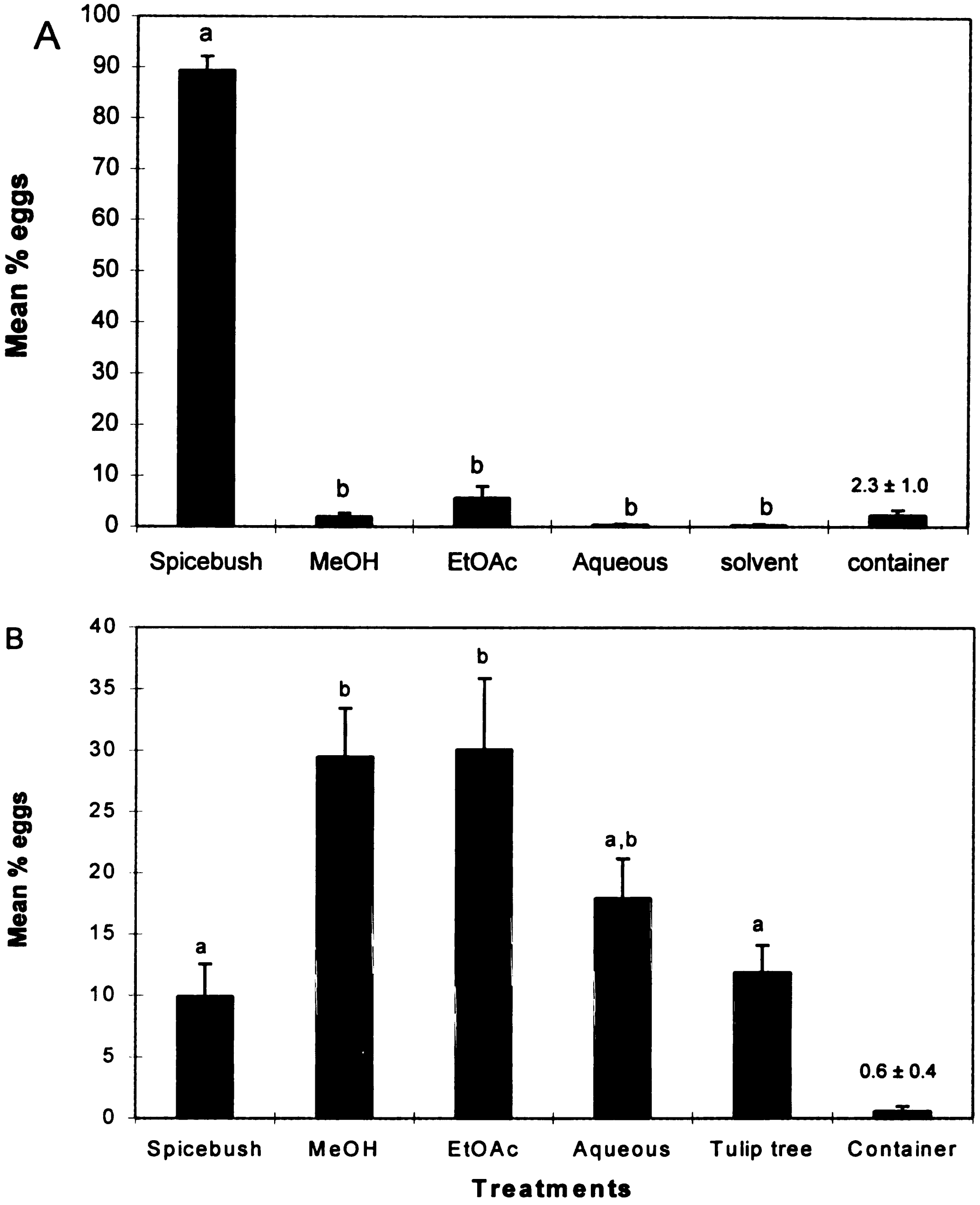

Figure 4. Percentage of eggs (mean S. E.) laid by *Papilio troilus* females on each of 5 choices: a spicebush leaf, substrate sprayed with crude methanol extract (MeOH), substrate sprayed with the ethyl acetate fraction (EtOAc), substrate sprayed with the aqueous fraction (Aqueous) and an untreated substrate. Significant differences between means ($p < 0.05$) are shown for arcsine transformed percentages. A. The substrates are filter paper triangles (n = 32 females). B. The substrates are tulip tree leaves (n = 16 females).

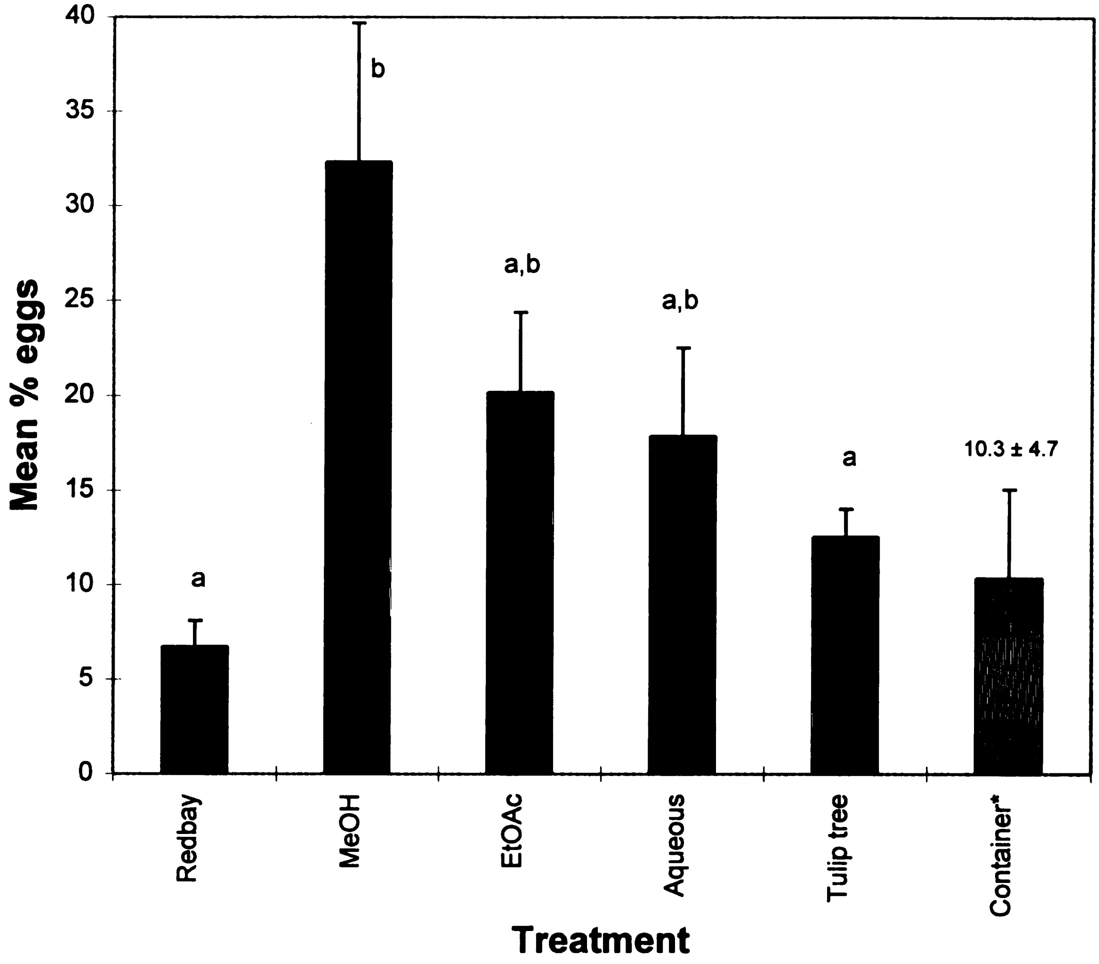

Figure 5. Percentage of eggs (mean ± S. E.) laid by *Papilio palamedes* females (n = 9) on each of 5 choices: an untreated redbay leaf, a tulip tree leaf sprayed with methanol extract, a tulip tree leaf sprayed with the ethyl acetate fraction, a tulip tree leaf sprayed with the aqueous fraction, and an untreated tulip tree leaf. Redbay leaves were taken from potted greenhouse trees and were yellow and of poor quality. Significant differences between means ($p < 0.05$) are shown for arcsine transformed percentages.

CHAPTER 2

Chemical extracts from a non-host, *Persea borbonia*, deter oviposition by *Papilio glaucus*, a polyphagous swallowtail

Abstract

The ability to perceive and respond to phytochemicals that reliably indicate the suitability of a potential host plant confers a selective advantage to ovipositing female swallowtail butterflies. *Papilio glaucus* females do not oviposit on redbay (*Persea borbonia*: Lauraceae), a non-host that does not support larval development and is commonly found in habitats alongside their principal host plant, *Magnolia virginiana*, in central Florida. I tested the hypothesis that deterrent compounds present in the leaves of redbay mediate its rejection by *P. glaucus*. Florida populations of *P. glaucus* did not oviposit on host leaves sprayed with the methanol extract of redbay foliage, although they accepted solvent treated and untreated tulip tree leaves in 3 choice bioassays. Additionally, tulip tree leaves sprayed with methanolic extracts of redbay also deterred oviposition by *P. glaucus* females from Ohio, Kentucky and Pennsylvania, although these populations do not naturally encounter redbay. Clearly, deterrent compounds found within this non-host are the basis of its rejection by *P. glaucus*. The evolutionary scenarios may that may account for this behavior are addressed.

Introduction

Papilio glaucus, *Papilio palamedes* and *Papilio troilus* occupy the same habitat patches in central and southern Florida. Although *P. glaucus* will oviposit and feed on plants from over a dozen plant families (Scriber *et al.* 1991), sweetbay *(Magnolia virginiana)* is the only host plant found in the region (Scriber 1986, see Table 4). *P. palamedes* and *P. troilus* oviposit and feed exclusively on redbay (*Persea borbonia*), as the other potential lauraceous hosts, sassafras and spicebush, do not extend that far south (Nitao *et al.* 1991). Redbay and sweetbay grow in intermixed stands in the hammock habitat, often with interdigitating branches, and all three swallowtail species are common in those areas when nectar sources are in bloom. *P. glaucus* does not oviposit on redbay (Scriber unpubl.), and the foliage is toxic to the larvae (Scriber *et al.* 1991).

Several hypotheses have been suggested to account for the vast host breadth of polyphagous Lepidoptera such as *P. glaucus*. Both oligophagous and polyphagous Lepidoptera can detect and process a variety of chemical stimuli through their peripheral nervous system via chemoreceptive sensilla (Blaney and Simmonds 1990, Roessingh *et al.* 1991, Schoonhoven *et al.* 1992). The central nervous system of the polyphagous butterfly may simply recognize a broader range of chemical stimuli as "host" than oligophagous butterflies. Alternately, the avoidance of non-hosts may rest on the presence of deterrents. Both hypotheses are not mutually exclusive, and a combination of these mechanisms may increase ovipositional accuracy. Often, the relative ratio of compounds interpreted as stimulatory and deterrent determines the host range of a butterfly species (Renwick and Huang, 1994; Honda and Hayashi, 1994). I predict that *P.*

glaucus rejects redbay due to the presence of deterrent compounds in the leaves.

Several studies have shown that volatiles and non-volatile compounds found in non-host foliage deter landing and oviposition (Feeny *et al.* 1989). For example, volatiles from cabbage, a non-host, inhibited *Papilio polyxenes* from landing on cellulose sponges treated with host plant extract (Feeny *et al.* 1989). Electrophysiological studies with *P. polyxenes* have shown that a single neuron in the tarsal chemoreceptors responds in a characteristic deterrency pattern to polar cabbage extracts. The action potentials generated by the deterrent neuron differ in amplitude and time period of response from those generated by the 2 neurons that respond to the stimulatory host plant extract (Roessingh *et al.* 1991). The differential coding of deterrent and stimulatory sensory input may provide the basis for the distinct behavioral responses.

The interpretation of certain compounds as deterrent may have evolved as a means of avoiding plants toxic to the larvae. The deterrent compound itself may be toxic, or reliably indicate the presence of other toxic compounds (Dethier 1980, Bernays and Cornelius 1992). Neural recognition of such compounds from the foliage of non-hosts may confer a selective advantage in habitats where hosts and non-hosts occur sympatrically. The ability of populations of *P. glaucus* from southern Florida to recognize deterrent compounds in redbay, a non-host found commonly in the same habitat as sweetbay, was tested. Northern populations of *P. glaucus*, outside the range of redbay, were also tested to see if they have the ability to recognize deterrents from a non-host they never encounter.

Materials and Methods

Extraction of redbay (Persea borbonia):

In 1995, 72.9 g of freeze-dried, milled redbay leaves collected from Highlands County, Florida were extracted once with ca. 400 ml of hexane for 1 minute, and then extracted with 400 ml of hexane for two 6 h periods in an extraction column with stopcock drain. The procedure was repeated with ethyl acetate and methanol to yield 1.1, 1.2 and 16.4 g of hexane, ethyl acetate and methanol crude extracts, respectively.

A second batch of intact redbay leaves stored at -20°C from the spring of 1995 were extracted in 1996 according to the above protocol with 1200ml of each solvent. The procedure yielded 0.3, 1.7 and 5.7 g of hexane, ethyl acetate and methanol extract, respectively. An aliquot of the methanol extract (1.9 g) was redissolved in 20 ml of methanol and 130 ml of water and extracted 3x with 50 ml of ethyl acetate. The ethyl acetate and water fractions contained 0.3 g and 1.6 g of material, respectively. The ethyl acetate fraction was dried *in vacuo*, and the aqueous extract was stored at -15°C until use in the bioassay.

General bioassay design

Each gravid female was placed separately in a round, plastic container (26 cm diam. x 9 cm) lined on the bottom with a paper towel. Redbay extracts were dissolved in their parent solvent and sprayed with a chromatographic sprayer onto both sides of tulip tree leaves. The leaf petioles were placed in a water-filled aquapic to maintain leaf turgor. Within each bioassay container, the female could contact all treatment and control leaves. The tulip tree leaves were harvested the day of their placement in the bioassay from either potted tulip trees or a tulip tree growing on the confines of Michigan State University. The leaves were rinsed with distilled water and dried before application of extracts and placement in the bioassay. Throughout the bioassay, the butterflies were fed a 20% honey solution once daily while eggs were tallied and the old leaves replaced. Bioassay containers were stacked on turntables that rotated once every six minutes and were illuminated one side with incandescent lights (see Scriber 1993). Eggs laid greater than an inch away from a substrate (the approximate distance between a female's foretarsi and curled ovipositor), or equal distances between two treatments, were scored as "container." The bioassay continued over several days, and analysis was performed on the percentage of total eggs laid by each female on each substrate over the course of the bioassay.

Bioassays with P. glaucus from Florida (1995)

First generation offspring of *P. glaucus* females collected from Florida in March 1995 were reared on sweetbay (*M. virginiana*). After eclosion, the female butterflies were stored at 18°C until they were hand-paired and placed in the bioassay 24 hours after mating.

Three bioassays were employed. The first bioassay tested oviposition preferences between three choices. The "all" treatment consisted of gram leaf equivalents of all three extracts (hexane, ethyl acetate and methanol) dissolved together in a mixture of the three solvents and sprayed onto a tulip tree leaf. A mixture of equal proportions of hexane,

ethyl acetate and methanol was sprayed onto a leaf to form the "solvent" treatment. The control category consisted of a completely untreated tulip tree leaf. Therefore, each female could choose simultaneously between the "all," "solvent" and "control" leaves.

The second bioassay design was composed of 5 choices. The hexane, ethyl acetate and methanol extracts were dissolved individually in their respective solvents and sprayed on separate tulip tree leaves. The three treatments were placed in each arena along with a "solvent" leaf (treated as above) and the "control" leaf.

The third bioassay consisted of each extract (hexane, ethyl acetate and methanol) assayed individually with a “solvent” leaf (the same solvent used to redissolve the extract) and "control" leaf for a total of 3 choices.

Bioassay with northern populations of P. glaucus *(1996)*

P. glaucus pupae from Ohio, Kentucky and Pennsylvania, reared from females collected in 1995, were removed from cold-storage (4°C) and allowed to eclose. Adult female *P. glaucus* were stored at 18°C until they were hand-paired to males (field captured *Papilio canadensis* or lab-reared *P. glaucus*).

Results from the previous studies (1995) indicated that only the methanol extract inhibited oviposition by Florida *P. glaucus*. Therefore, only the methanol extract was tested with northern populations.

In the first bioassay, 5 females were each placed in a separate bioassay arena containing an untreated tulip tree leaf, a tulip tree leaf sprayed with methanol, and a tulip tree leaf sprayed with the methanol extract dissolved in methanol. The first day of the bioassay, approximately 0.04 g of extract were sprayed onto each treatment leaf. The next 2 days the dose was reduced to 0.02 g•leaf^{-1}, and for the duration of the bioassay 0.01 g•leaf^{-1} was used. Although I cannot account for antagonistic interactions at chemosensory receptor sites, I wanted to be sure that the extract layer did not serve as an impermeable physical barrier to the perception of tulip tree chemistry. By reducing the dose, I effectively reduced thickness of the redbay extract layer on the surface of the tulip tree leaf. Reduction of the dose did not affect the results of the bioassay. Butterflies were allowed to oviposit until their death, and the lifetime percentage of eggs laid on each leaf was calculated.

The second bioassay consisted of 4 choices. The ethyl acetate extract was redissolved in ethyl acetate and sprayed onto tulip tree leaves. The aqueous fraction was removed from the freezer, thawed, and sprayed directly onto tulip tree leaves. The amounts of both extracts used was their gram yield proportional to the amount of crude methanol extract partitioned between the two solvents (1.9 g), multiplied by 0.01 (the dose of the crude methanol extract). Controls consisted of a leaf sprayed with ethyl acetate and an untreated leaf. Nine butterflies were allowed to oviposit until their death.

Statistical analysis

The percentage of eggs laid by individual females on each treatment throughout the duration of the bioassay was calculated. The percentages were arcsine transformed prior to their analysis to normalize the variances. A standard two-way analysis of variance (ANOIA) for unreplicated data was performed, with treatments and butterflies as the main effects (Sokal and Rohlf 1981). The term "unreplicated" refers to the fact that only 1 butterfly occupied each block, or bioassay arena. If treatment was found to be significant, a one-way ANOIA was performed on the arcsine transformed percentages, excluding the "container" category to maintain independence between treatments. Specific significant differences between treatments could then be characterized with the Student-Newman-Keuls test (SuperANOVA 1993).

Results

Florida populations of Papilio glaucus (1995)

In a three-choice bioassay, *P. glaucus* females avoided tulip trees sprayed with the combination of all three extracts (Figure 6). A two-way ANOIA on arcsine transformed percentages revealed a significant treatment effect ($F_3 = 19.44$, $p < 0.0001$), but not a significant butterfly effect ($F_{13} = 0.003$, $p = 1$). A one-way ANOIA confirmed the significant treatment effect ($F_2 = 19.09$, $p < 0.0001$). The mean percentage of eggs laid on "all" was significantly lower than that of "solvent" ($p < 0.01$) and "control" ($p < 0.01$). "Solvent" and "control" were not significantly different from each other.

Two-way ANOIA performed on the arcsine transformed percentages from the 5-choice oviposition trials with *P. glaucus* from Florida (Figure 7) revealed treatment ($F_5 = 4.69$, $p = 0.0014$), but not butterflies ($F_{10} = 0.11$, $p = 0.9997$), to be a significant main effect. One-way ANOIA similarly found a significant difference between treatment means ($F_4 = 3.97$, $p = 0.0072$). Significantly more eggs were laid on leaves treated with the hexane extract than with the methanol extracts ($p < 0.01$). Although not detected to be significantly different, fewer eggs were laid on methanol extract-coated leaves than "solvent" and "control" leaves.

The percentages of eggs laid did not differ between treatments or butterflies in the three choice bioassays with the hexane extract (Figure 8) according to two-way and one-way ANOIA. The butterflies tended to lay eggs equally on all three choices. A significant difference between treatments was found by two-way ANOIA ($F_3 = 5.72$, $p = 0.018$), but not one-way ANOIA, in the three choice bioassay with ethyl acetate extract (Figure 9) with Florida *P. glaucus*. Variation between treatments may be attributed to an increase in the percentage of eggs laid on leaves sprayed with ethyl acetate extract relative to the "solvent" and "control" treatments. However, due to the small sample size ($n = 4$) the results should be interpreted with caution.

Significant differences between treatments, but not butterflies, was revealed by two-way ANOIA in three-choice bioassays (Figure 10) between leaves sprayed with methanol extract, "solvent" leaves and "control"leaves ($F_2 = 10.48$, $p = 0.0084$). One-way ANOIA similarly detected a significant difference between treatments ($F_2 = 10.74$, $p = 0.014$). The butterflies laid significantly fewer eggs on leaves sprayed with methanol extracts of redbay than on "solvent" or "control" leaves ($p < 0.05$).

The percentages of eggs laid on "solvent" leaves was not significantly different from the percentages laid on "control" leaves in any of the trials.

Populations of Papilio glaucus from Ohio, Kentucky and Pennsylvania (1996)

Like the populations of *P. glaucus* from Florida, *P. glaucus* from Ohio, Kentucky and Pennsylvania deposited fewer eggs on tulip tree leaves coated with methanol extract than on untreated tulip tree leaves and tulip tree leaves sprayed with solvent (Figure 11). A two-way ANOIA revealed a significant treatment effect ($F_2 = 112$, $p < 0.0001$), but not a significant butterfly effect ($F_4 = 0.092$, $p = 0.982$). One-way ANOIA similarly revealed a significant treatment effect ($F_2 = 161$, $p < 0.0001$). A significantly lower percentage of eggs were laid on leaves coated with methanol extract than on "solvent" or "control" leaves ($p < 0.01$).

In 4-choice bioassays with the aqueous and ethyl acetate fractions of the active methanol extract (Figure 12), two-way ANOIA revealed only a significant treatment effect ($F_3 = 79.94$, $p < 0.0001$), as did the one-way ANOIA ($F_3 = 106.09$, $p < 0.0001$). Leaves sprayed with the aqueous and ethyl acetate fractions received significantly fewer eggs than the "solvent" and "control" leaves ($p < 0.01$).

Discussion

The presence of deterrents in the leaves of non-hosts is a common phenomena. A significantly larger number of compounds inhibit feeding and oviposition than stimulate those responses (Bernays and Chapman 1994). Plants that possess deterrent compounds may actually prove adequate for development when consumed by larvae whose gustatory sense has been removed. Therefore, the evolution and maintenance of deterrent compounds may enable a plant to repel unwanted herbivores (Dethier 1980). Perception of deterrent compounds that truly signal toxicity may also benefit the herbivores who avoid oviposition and consumption of poisonous foliage (Dethier 1980).

Females of the polyphagous *P. glaucus* do oviposit on and the larvae consume certain species of Lauraceae, such as sassafras and spicebush, albeit with lower resulting survivorships than with more favored host plants such as tulip tree and black cherry (Scriber *et al.* 1991). However, *P. glaucus* caterpillars fail to survive on redbay (Scriber *et al.* 1991), and adults do not oviposit on this tree (see Table 4). This suggests that deterrent compounds in redbay accurately reflect the presence of toxins, and the ability of *P. glaucus* to detect the deterrents benefits this species. These results also suggest that redbay is perceived by *P. glaucus* to be more chemically different from spicebush and sassafras than they are from each other, at least in terms of ovipositional activity. Perhaps redbay foliage contains different, more potent toxins, or the same compounds are present in higher quantities in redbay than in spicebush and sassafras.

It is interesting that northern populations of *P. glaucus* that never encounter redbay still respond to deterrents present in foliar extracts. It is possible that redbay compounds have inherent deterrent properties to *P. glaucus* and the ancestor of the *glaucus-troilus* lineages (see Figure 1). *P. palamedes* may have adapted to efficiently utilize redbay foliage during its divergence from the *glaucus* group. Alternately, *P. glaucus* may have evolved the ability to perceive and respond to compounds found in redbay. *P. borbonia* may have been among those species inhabiting the Midwestern United States that were pushed southward toward southern Florida and Texas by global cooling (Scriber 1988). The divergence of the *glaucus* and *troilus* groups may have occurred before this period of global cooling, and the detection and interpretation of redbay chemistry as deterrent may have resulted from frequent encounters by *P. glaucus* unable to detoxify the foliage. The relative reluctance of northern populations to oviposit on sassafras and spicebush may be attributed to the legacy of ovipositional inhibition by *Persea* chemistry mediated by compounds of similar or identical structure.

The detection of deterrents enables *P. glaucus* to avoid ovipositing on non-host foliage. Swallowtail butterflies normally fly away immediately after alighting on non-host leaves, in contrast to their continued sampling and drumming of host leaves (Damman and Feeny 1988, Rausher 1995). Olfactory and gustatory perception of deterrent compounds may provide butterflies with an efficient mechanism for evading non-host foliage, especially in areas where the growth forms of host and non-host trees intermingle (Feeny *et al.* 1989, Roessingh *et al.* 1991) as is the case in the Hammock habitat of central Florida. Sensitivity to strongly deterrent compounds in toxic plants may play an important in host location by polyphagous species, who are neurally wired to accept a greater number and taxonomic variety of species (Bernays and Chapman 1994).

The acceptance or rejection of a plant by ovipositing polyphagous butterfly species, such as *P. glaucus*, is probably not dictated solely by the presence or absence of deterrent chemistry (Dethier 1980). Rather, acceptance of a subset of compounds more ubiquitous among plant taxa may account for use of plants from many families. Additionally, evidence exists that the balance of cues from a variety of senses, integrated by the central nervous system, determine the acceptability of a substrate for oviposition (Miller and Strickler 1984, Ramaswamy 1994). For example, Traynier (1986) discovered that when *Pieris rapae* females are presented with 2 discs of a different shade of green and a blue disc, each bearing a different concentration of oviposition stimulant, the females invaryingly placed more eggs on one of the green discs, although the blue disc may have received the higher dose of the stimulant. The green color plus the intermediate concentration of the stimulant may have more closely approximated the "average" sensory input required to stimulate oviposition (Traynier 1986). Nevertheless, in a scenario where many sources of external and internal stimuli determine the acceptability of a host, oviposition deterrents may serve to tip the balance of sensory cues toward unacceptability, causing females to reject a potentially unsuitable host.

Table 4. Percentage of eggs laid by 5 *Papilio glaucus* females from Highlands County, Florida in a 3-choice bioassay on leaves from trees found in their habitat. Sweetbay, the primary host plant, and redbay, a non-host, are abundant, while ash is rarely encountered (Scriber, unpubl.).

Papilio glaucus ♀♀from Florida	Sweetbay *Magnolia virginiana*	Redbay *Persea borbonia*	Ash *Fraxinus trifoliata*	eggs (n)
1	74.7	1.3	24.1	79
2	70.1	9.3	20.6	107
3	57.1	0	42.9	42
4	66.7	0	33.3	12
5	57.1	0	42.9	42
Mean ± S. E.	65.4 ± 3.5	2.7 ± 1.7	32.8 ± 4.6	282

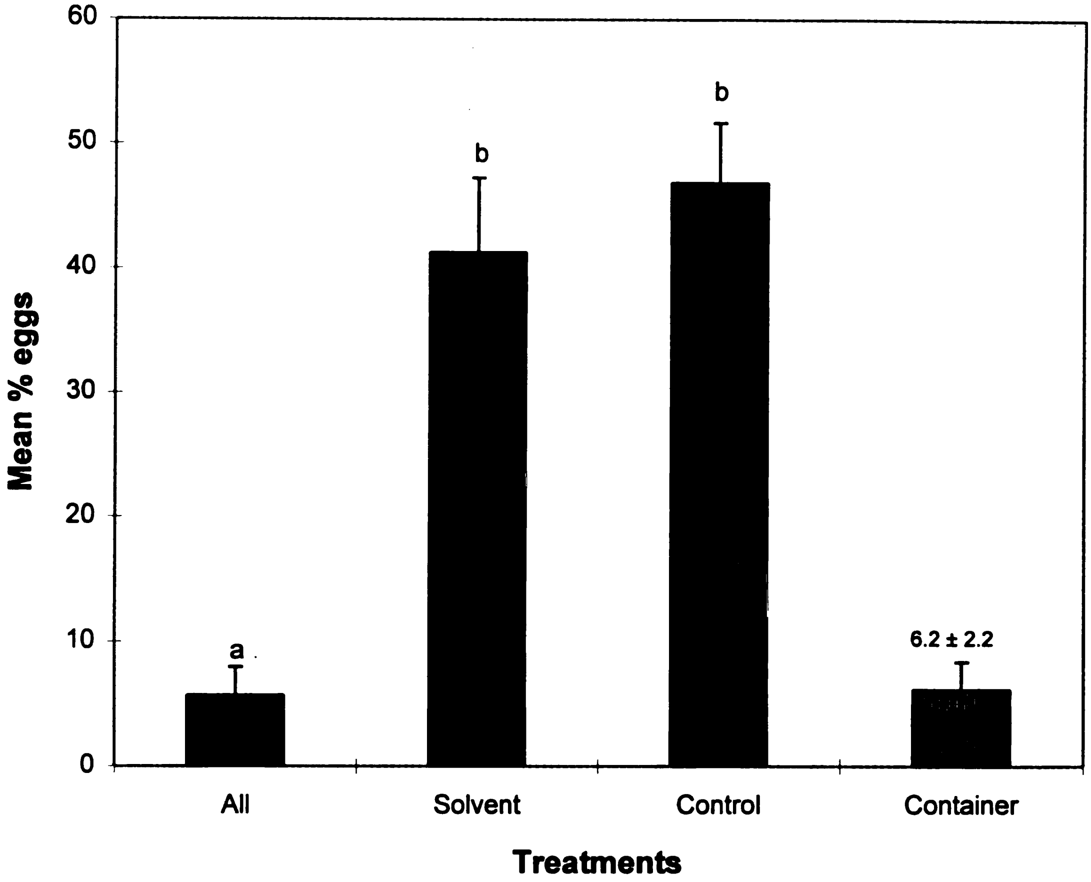

Figure 6. Percentage of eggs (mean ± S. E.) laid by *Papilio glaucus* females from Florida (n=14) on each of three choices: tulip tree leaves sprayed with a combination of the hexane, ethyl acetate and methanol extracts (all), tulip tree leaves sprayed with a 1:1:1 ratio of each solvent (solvent), and an untreated tulip tree leaf (control). Significant differences at $p < 0.01$ are shown for arcsine transformed percentages.

*Container refers to the mean percentage of eggs laid off the treatment and control substrates, and is excluded from analysis by 1-way ANOVA and the SNK test. The mean ± S. E. are shown above.

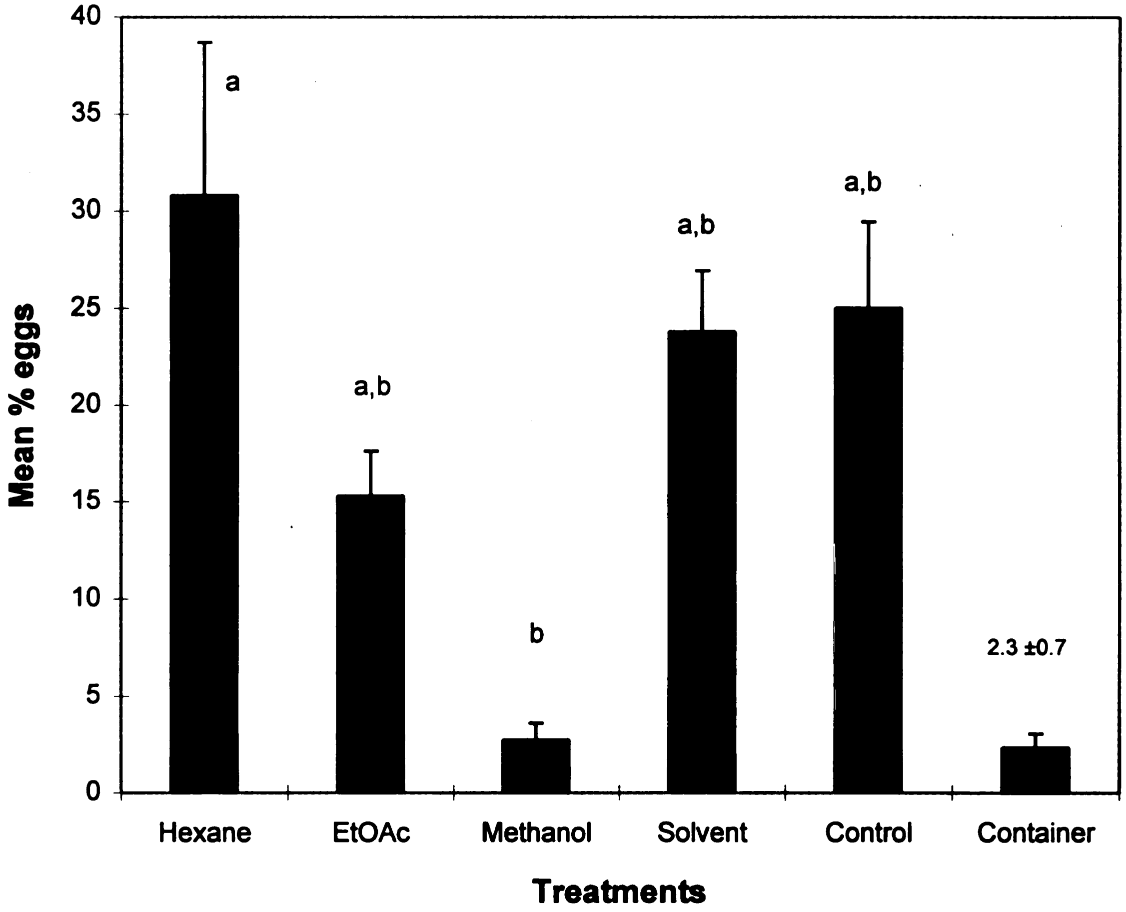

Figure 7. Percentage of eggs (mean ± S. E.) laid by *Papilio glaucus* females from Florida (n=11) on each of 5 choices: tulip trees leaves sprayed with hexane, ethyl acetate and methanol extracts, solvent or untreated. Significant differences between treatments at $p < 0.01$ for arcsine transformed percentages are shown.

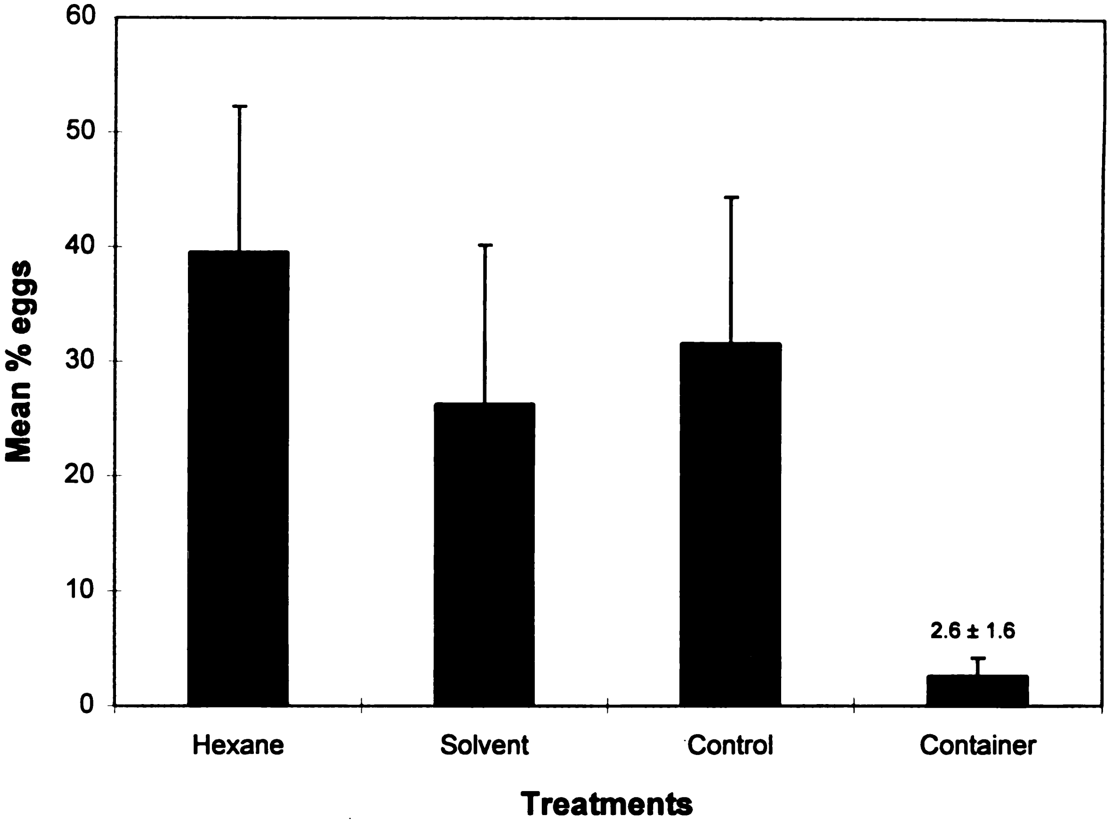

Figure 8. Percentage of eggs (mean ± S. E.) laid by *Papilio glaucus* females from Florida (n=5) on each of 3 choices: a tulip tree leaf sprayed with hexane extract (hexane), a tulip tree leaf sprayed with hexane (solvent) and an untreated tulip tree leaf (control). No siginificant differences between means were detected by 1-way and 2-way ANOVA on arcsine transformed percentages.

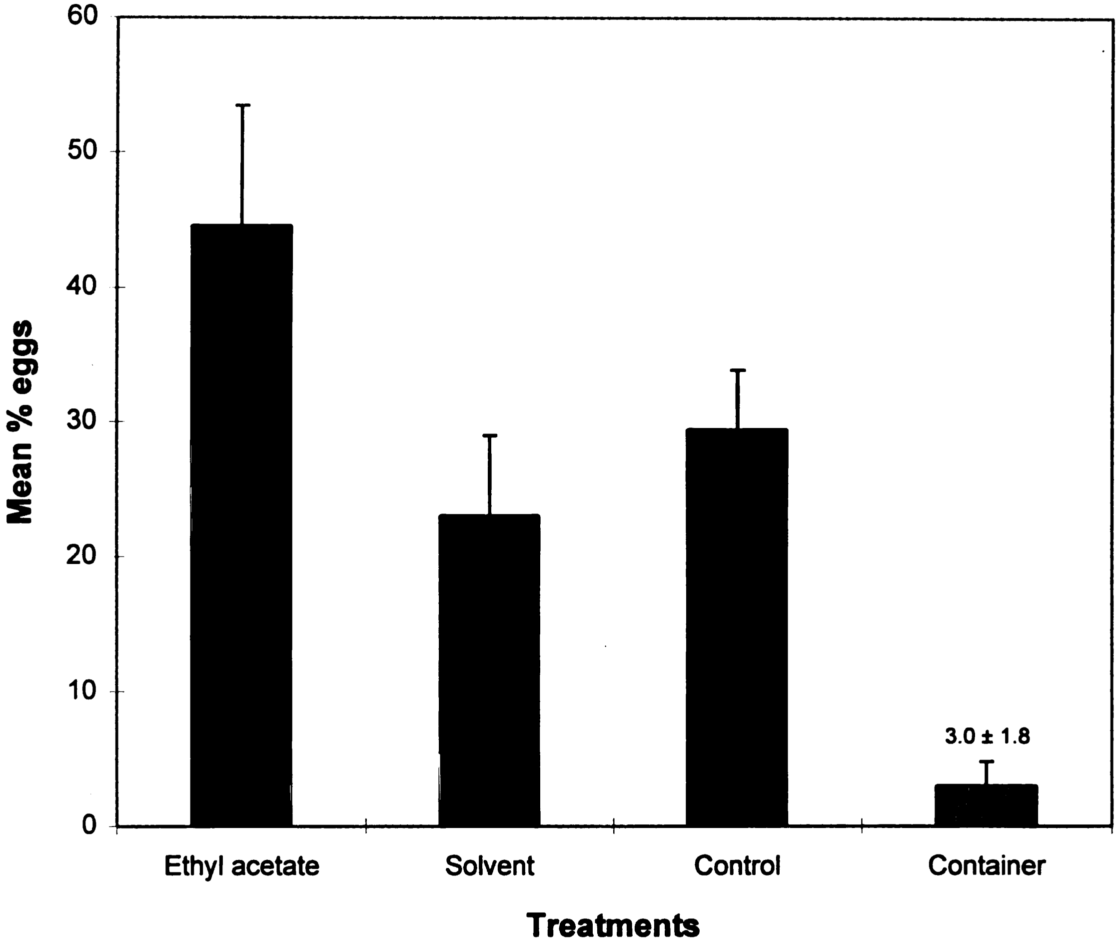

Figure 9. Percentage of eggs (mean ± S. E.) laid by *Papilio glaucus* females from Florida (n = 4) on each of 3 choices: an untreated tulip tree leaf (control), a tulip tree leaf sprayed with ethyl acetate (solvent) and a tulip tree leaf sprayed with ethyl acetate extract (ethyl acetate). No significant differences were detected by 1-way ANOVA on arcsine transformed percentages.

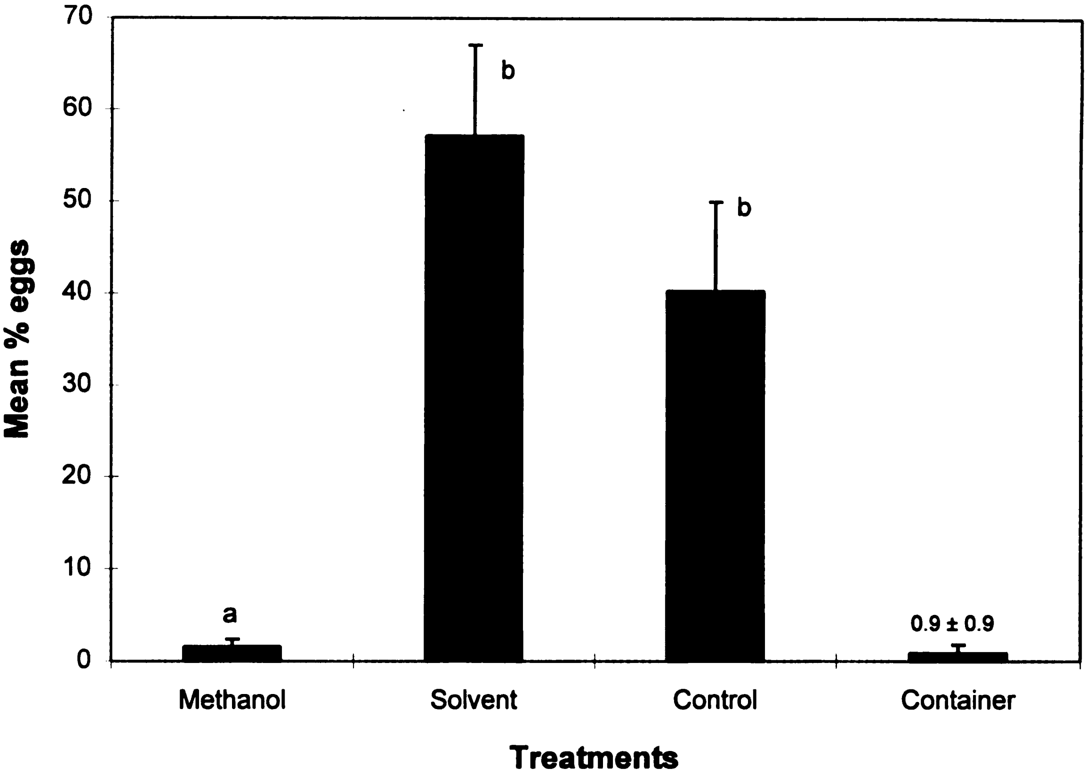

Figure 10. Percentage of eggs (mean ± S. E.) laid by *Papilio glaucus* females from Florida (n=3) on each of 3 choices: a tulip tree leaf sprayed with methanol extract, a tulip tree leaf sprayed with methanol (solvent) and an untreated tulip tree leaf (control). Significant differences between means ($p < 0.05$) for arcsine transformed percentages are indicated.

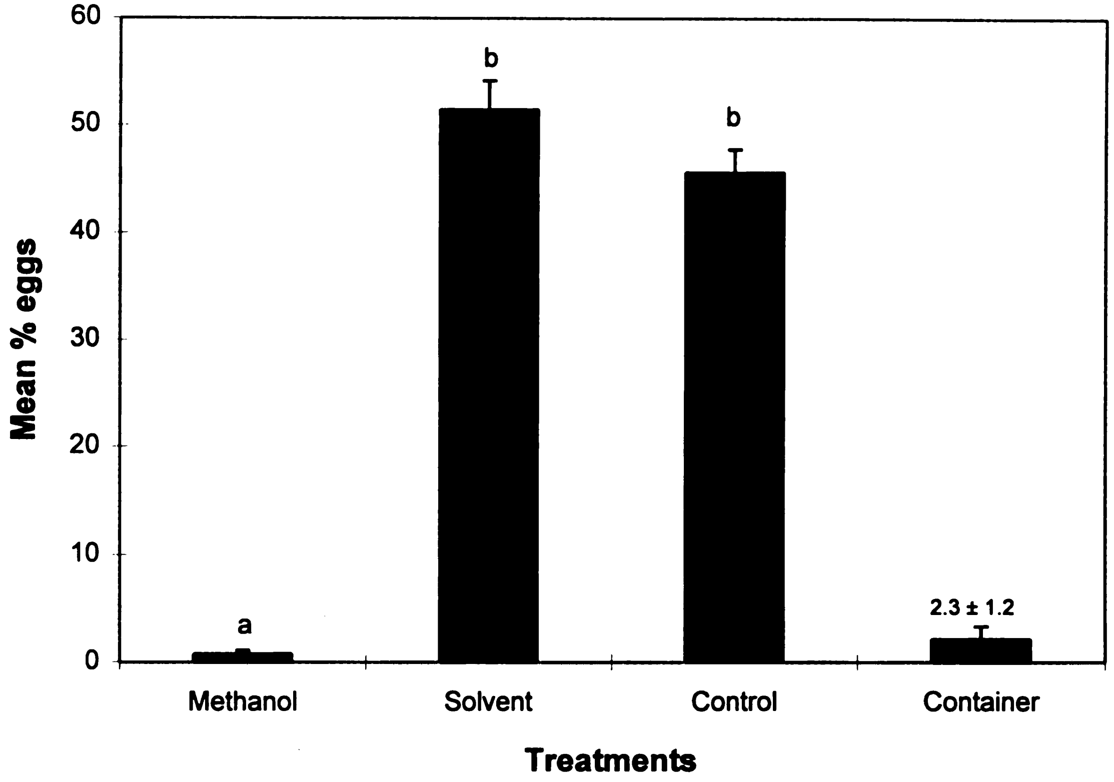

Figure 11. Percentage of eggs (mean ± S. E.) laid by *Papilio glaucus* females (n = 5) from OH, PA and KY on each of 3 choices: a tulip tree leaf sprayed with methanol extract (methanol), a tulip tree leaf sprayed with methanol (solvent) and an untreated tulip tree leaf (control). Significant differences between means ($p < 0.01$) are for arcsine transformed percentages are shown.

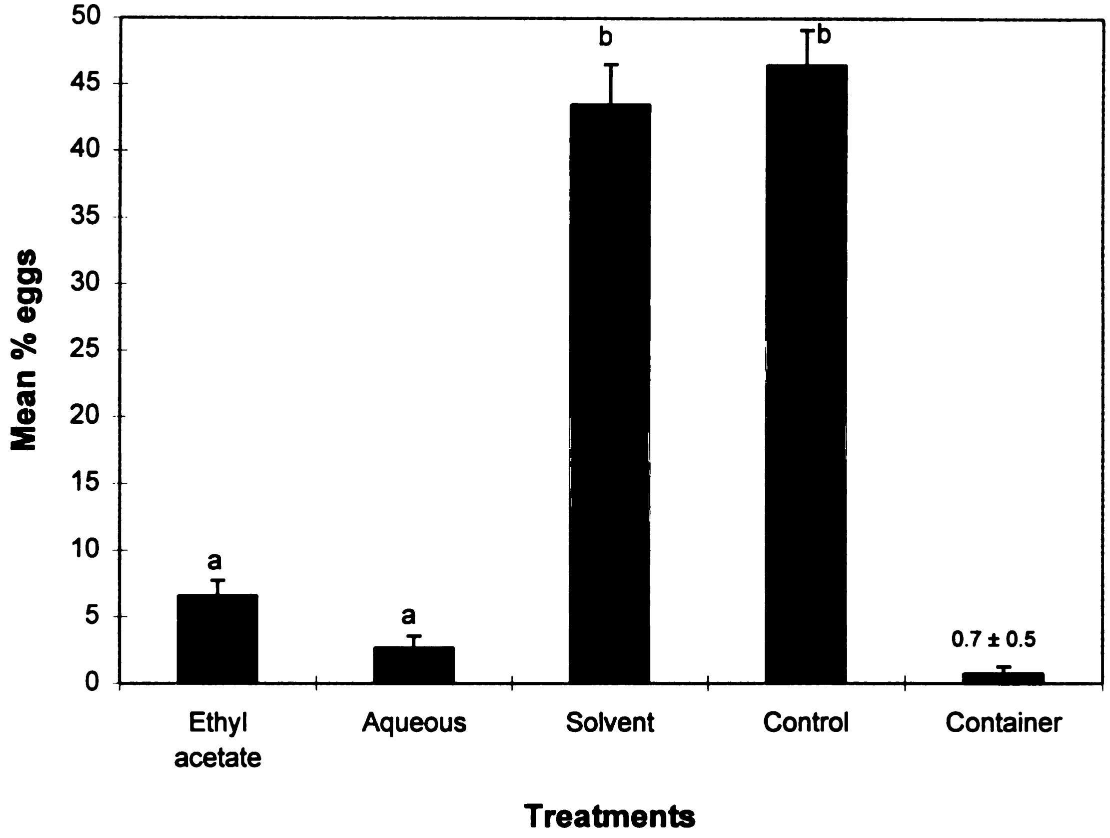

Figure 12. Percentage of eggs (mean ± S. E.) laid by *Papilio glaucus* females (n=9) from OH, PA and KY on each of 4 choices: an untreated tulip tree leaf (control), a tulip tree leaf sprayed with ethyl acetate (solvent), a tulip tree leaf sprayed with the ethyl acetate fraction (ethyl acetate), and a tulip tree leaf sprayed with the aqueous fraction (aqueous). The ethyl acetate and aqueous fractions are subdivisions of the original crude methanol extract (see text p 40). Significant differences between means ($p < 0.01$) for arcsine transformed percentages are shown.

CONCLUSION

More than 560 species of swallowtail butterflies (Lepidoptera: Papilionidae), inhabit the world, with over 200 species in the genus *Papilio*. The majority of the species inhabit tropical zones, and decline in number over the temperate and neartic latitudes (Scriber 1995). However, it is thought that the origin of the Papilionidae is in eastern (Hancock 1983) or western North America (Scriber *et al* 1991). The evolutionary history that has shaped the geographic distribution and speciation of these butterflies remains an uncertainty, although clues from paleontology, biogeography and botanical affiliation have generated hypothesis concerning many swallowtail species.

The study of the chemical mechanisms governing host plant acceptance can provide clues to the taxonomic relationships between swallowtail butterfly species as well as provide explanations of how current patterns of host use may have diverged from the ancestral. The chemical facilitation hypothesis, generated by Dethier (1941) and Jermy (1976, 1984) and expanded by Feeny (1991) states that the probability of colonization of a novel host plant increases with increasing biochemical similarity of the new hosts to the current hosts. Specifically, if a plant contains compounds that a butterfly already recognizes as a oviposition or feeding stimulants, lacks chemical deterrents and can be detoxified by the larvae, the plant has increased likelihood of becoming a host. I have chosen to focus on the role that oviposition stimulants and deterrents play in host acceptance by polyphagous (*Papilio glaucus*) and oligophagous (*Papilio troilus* and *Papilio palamedes*) species of swallowtail butterflies.

My research has demonstrated a chemical basis for host acceptance by *P. troilus* and *P. palamedes*. In an all-filter paper bioassay, filter paper triangles coated with spicebush extracts received more eggs by *P. troilus* than did the untreated control. Additionally, *P. troilus* and *P. palamedes* laid more eggs on non-host leaf surfaces sprayed with the respective host plant extracts than on the untreated leaves.

Although chemical cues are responsible for maintaining ovipositional fidelity on the correct host species, other cues play a major role in eliciting oviposition. Relative to the filter paper, extracts on non-host leaf surfaces received a greater percentage of eggs. The relative unacceptability of the filter paper substrate manifest itself in the large number of eggs laid off the triangles, scattered randomly about the oviposition arena when no leaves were present. In contrast, in the all-leaf bioassays, the vast majority of oviposition was confined to the tulip tree leaf surfaces treated with host plant extract. Although color plays a large role in orienting and arousing oviposition, sensory inputs other than color may have been responsible in the recognition of the leaves as suitable host substrate in my bioassays, since the green of the methanol extract was not enough to stimulate significant oviposition. The relative role of texture, odor, and the reflectance of the host surface in eliciting oviposition remains to be assessed for these *Papilio* species.

The results of my experiments decisively show that polar fractions of non-host plant extract (*Persea borbonia*) misted onto host leaves deter oviposition by populations of *P. glaucus* from southern Florida, Ohio, Kentucky and Pennsylvania. The detection of deterrents by the adult female is advantageous when such compounds accurately reflect the toxicity of a plant to her progeny, as is the case here. Only populations of *P. glaucus* from Florida encounter *P. borbonia*, which grows alongside their host plant, *Magnolia virginiana*, in dense swamp habitat. Sensitivity to contact deterrents in redbay may prevent *P. glaucus* from making fatal oviposition mistakes in areas of dense growth of both tree species.

Populations of *P. glaucus* from Ohio, Kentucky and Pennsylvania are deterred from oviposition by extracts of a host plant that they never encounter. Gene flow could in part explain that phenomenon, but if the rejection of redbay were a recent adaptation to life in central and southern Florida, a gradient of deterrency decreasing in northern populations would be expected. An alternate explanation may be that redbay compounds have properties that activate an intrinsic deterrent response by *P. glaucus*. For example, certain compounds can generate characteristic irregular or "deterrent" temporal patterns of neural action potentials due to their interaction with the receptor cell membrane (Schoonhoven *et al.* 1992). *P. palamedes* may have adapted to interpret redbay compounds as oviposition stimulants, facilitated by historical use of lauraceous hosts by *troilus* group species. On the other hand, *P. glaucus* may have evolved to recognize redbay chemistry as deterrent. *P. borbonia* may have been among those species inhabiting the midwestern United States that were pushed southward toward southern Florida and Texas by global cooling. After the divergence of the *troilus* and *glaucus* groups, frequent and unsuccessful colonization attempts of redbay may have facilitated the evolution of its rejection by *P. glaucus*. The legacy of ovipositional inhibition by compounds in redbay may explain a relative reluctance of northern populations to oviposit on the predominant lauraceous plants (sassafras and spicebush) compared to more favored host plants such as tulip tree and white ash (see Table 1, chapter 1). The elucidation of the deterrent present in redbay foliage may likely explain the relative preference of *P. glaucus* for Magnoliaceae relative to Lauraceae and perhaps even Rutaceae.

Deterrent compounds tend to have high activity even when isolated (Honda and Hayashi 1995), which may make their identification easier than that for oviposition stimulants, which have high activity only when mixed together in synergy (Ohsugi *et al* 1985, Honda 1986, Feeny *et al* 1988, Nishida and Fukami 1989, Honda 1990, Sachdev-Gupta *et al* 1993, Nishida 1995). Elucidation of the compound(s) in redbay deterrent to *P. glaucus* is worthy of future study.

My research has demonstrated that host selection by *P. troilus*, *P. palamedes* and *P. glaucus* is mediated, in part, by detection of chemical stimulants and deterrents in host and non-hosts foliage. Biotic and abiotic factors determine the evolution of the direction and range of gustatory receptor sensitivity and its neural interpretation. Current behavioral responses to ecologically relevant compounds provide the background against which past and future host affiliations can be extrapolated.

APPENDICES

APPENDIX 1

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1996-7

Title of thesis or dissertation (or other research projects):

PHYTOCHEMICAL BASIS FOR HOST PLANT SELECTION BY GENERALIST AND SPECIALIST SWALLOWTAIL BUTTERFLIES

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)
Cheryl Frankfater

Date September 19, 1996

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original:	Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies:	Included as Appendix 1 in copies of thesis or dissertation. Museum(s) files. Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

		Number of:							
Species or other taxon	Label data for specimens collected or used and deposited	Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio glaucus (yellow)	Powell Co., Kentucky caught 5/18/96					1			
Papilio glaucus	Lawrence Co., Ohio caught 5/21/96						1		
Papilio glaucus (dark)	Lawrence Co., Ohio caught 5/22/96					1			
Papilio troilus	Oldham Co., Kentucky caught 7/3/95					1			
Papilio troilus	Lawrence Co., Ohio caught 8/2/96						1		
Papilio palamedes	Tampa, Florida caught Dec. 1993					1			
Papilio palamedes	Highlands Co., Florida caught 3/21/96						1		
	*all specimens collected by Mark D. Deering								

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Cheryl Frankfater

Date 9/19/96

Voucher No. 1996-7

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

LIST OF REFERENCES

LITERATURE CITED

Ayers, M. P. and J. M. Scriber. 1994. Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera: Papilionidae). Ecological Monographs 64: 465-482.

Berenbaum, M. R. 1995. Chemistry and oligophagy in the Papilionidae. *In*: J. M. Scriber, Y. Tsubaki and R. C. Lederhouse (eds.). Swallowtail butterflies: their ecology and evolutionary biology. Walsworth Publishing Co., Missouri. pp. 27-38.

Bernays, E. A. and M. Cornelius. 1992. Relationship between deterrence and toxicity of plant secondary compounds for the alfalfa weevil *Hypera brunneipennis*. Entomologia Expermentalis et Applicata 64: 289-292.

Bernays, E. A. and R. F. Chapman. 1994. Behavior: the process of host-plant selection. pp 95-165. *In* Miller, T. A. and H. S. van Emden (eds). Host-plant selection by phytophagous insects. Chapman & Hall, New York. Chapter 4.

Blaney, W. M. and M. S. J. Simmonds. 1990. A behavioural and electrophysiological study of the role of tarsal chemoreceptors in feeding by adults of *Spodoptera*, *Heliothis virescens* and *Helicoverpa armigera*. Journal of Insect Physiology 36: 743-756.

Bossart, J. L. 1993. Differential selection and adaptation in different host environments: genotypic and phenotypic variation in host use traits in the tiger swallowtail butterfly, *Papilio glaucus* L. Dissertation. Michigan State University, East Lansing, Michigan, USA.

Bossart, J. L. and J. M. Scriber. 1995. Genetic variation in oviposition preference in tiger swallowtail butterflies: interspecific, interpopulation and interindividual comparisons. *In*: J. M. Scriber, Y. Tsubaki and R. C. Lederhouse (eds.). Swallowtail butterflies: their ecology and evolutionary biology. Walsworth Publishing Co., Missouri. pp. 183-193.

Damman, H. and P. Feeny. 1988. Mechanisms and consequences of selective oviposition by the zebra swallowtail butterfly. Animal Behavior 36:563-573.

Dethier, V. G. 1941. Chemical factors determining the choice of food plants by *Papilio* larvae. American Naturalist 75: 61-73.

Dethier, V. G. 1980. Evolution of receptor sensitivity to secondary plant substances with special references to deterrents. The American Naturalist 115: 45-66.

Feeny, P. 1991. Chemical constraints on the evolution of swallowtail butterflies. *In*: P. Price, T. M. Lewinsohn, G. W. Fernandes and W. W. Benson (eds.). Plant-animal interactions: evolutionary ecology in tropical and temperate regions. John Wiley & Sons, Inc. pp. 315-340. Chapter 15.

Feeny, P., K. Sachdev-Gupta, L. Rosenberry and M. Carter. 1988. Luteolin 7-*0*-(6"-malonyl)-β-D-glucoside and *trans*-chlorogenic acid: oviposition stimulants for the black swallowtail butterfly. Phytochemistry 27: 3439-3448.

Feeny, P., E. Städler, I. Ahman and M. Carter. 1989. Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). Journal of Insect Behavior 2: 803-827.

Gossard, T. W. and R. E. Jones. 1977. The effects of age and weather on egg-laying in *Pieris rapae* L. Journal of Applied Ecology 14: 65-71.

Grant, G. C. and D. Langevin. 1994. Oviposition responses of four *Choristoneura* (Lepidoptera:Tortricidae) species to chemical and physical stimuli associated with host and nonhost foliage. Environmental Entomology 23: 447-456.

Grossmueller, D. W. and R. C. Lederhouse. 1985. Oviposition site selection: an aid to rapid growth and development in the tiger swallowtail butterfly, *Papilio glaucus*. Oecologia 66:68-73.

Grossmueller, D. W. and R. C. Lederhouse. 1987. The role of nectar source distribution in habitat use and oviposition by the tiger swallowtail butterfly. Journal of the Lepidopterists' Society 41: 159-164.

Hagen, R. H., R. C. Lederhouse, J. Bossart and J. M. Scriber. 1991. *Papilio canadensis* and *P. glaucus* (Papilionidae) are distinct species. Journal of the Lepidopterists' Society 45: 245-258.

Hancock, D. L. 1983. Classification of the Papilionidae (Lepidoptera): a phylogenetic approach. Smithersia 2: 1-48.

Honda, K. 1986. Flavone glycosides as ovipostion stimulants in a papilionid butterfly, *Papilio protenor*. Journal of Chemical Ecology 12: 1999-2010.

Honda, K. 1990. Identification of host-plant chemicals stimulating oviposition by swallowtail butterfly, *Papilio protenor*. Journal of Chemical Ecology 16: 325-337.

Honda, K. and N. Hayashi. 1995. A flavonoid glucoside, phellamurin, regulates differential oviposition on a rutaceous plant, *Phellodendron amurense*, by two sympatric swallowtail species, *Papilio protenor* and *P. xuthus*: the front line of a coevolutionary arms race? Journal of Chemical Ecology 21: 1531-1539.

Ilse, D. 1937. New observations on responses to colours in egg-laying butterflies. Nature 140: 544-545.

Jermy, T. 1976. Insect–host-plant relationship—Coevolution or sequential evolution? *In*: T. Jermy (Ed.). The host-plant in relation to insect behavior and reproduction. Plenum, New York. pp. 109-113.

Jermy, T. 1984. Evolution of insect/host plant relationships. American Naturalist 124: 609-630.

Kolb, G. and C. Scherer. 1982. Experiments on wavelenth specific behavior of *Pieris brassicae* L. during drumming and egg-laying. Journal of Comparative Physiology 149: 325-332.

Lederhouse, R. C., M. P. Ayers, J. K. Nitao and J. M. Scriber. 1992. Differential use of lauraceous hosts by swallowtail butterflies, *Papilio troilus* and *Papilio palamedes* (Papilionidae). Oikos 63: 244-252.

Masumoto, T., S. Nomakuchi and K. Sawada. 1993. Host plant conspicuousness and the distribution of eggs and larvae in the butterfly, *Anthocharis scolymus* (Lepidoptera: Pieridae). Researches in Population Ecology 35: 241-250.

Miller, J. R. and K. L. Strickler. 1984. Finding and accepting host plants. *In*: W. J. Bell and R. T. Cardé (eds.). Chemical ecology of insects. Sinauer Associates, Inc., Sunderland, Massachusetts. pp. 127-157. Chapter 6.

Murphy, D. D. 1983. Nectar sources as constraints on the distribution of egg masses by the checkerspot butterfly, *Euphydryas chalcedona* (Lepidoptera: Nymphalidae). Environmental Entomology 12: 463-466.

Myers, J. H. 1985. Effect of physiological condition of the host plant on the ovipositional choice of the cabbage white butterfly, *Pieris rapae*. Journal of Animal Ecology 54:193-204.

Nishida, R. 1995. Oviposition stimulants of swallowtail butterflies. *In*: J. M. Scriber, Y. Tsubaki and R. C. Lederhouse (eds.). Swallowtail butterflies: their ecology and evolutionary biology. Walsworth Publishing Co., Missouri. pp.17-26.

Nishida, R. and H. Fukami. 1989. Oviposition stimulants of an Aristolochiaceae-feeding swallowtail butterfly, *Atrophaneura alcinous*. Journal of Chemical Ecology 15: 2565-2575.

Nishida, R., T. Ohsugi, H. Fukami and S. Nakajima. 1990. Oviposition deterrent of a Rutaceae-feeding swallowtail butterfly, *Papilio xuthus*, from a non-host rutaceous plant, *Orixa japonica*. Agric. Biol. Chem. 54: 1256-70.

Nitao, J. K., M. P. Ayers, R. C. Lederhouse and J. M. Scriber. 1991. Larval adaptation to lauraceous hosts: geographic divergence in the spicebush swallowtail butterfly. Ecology. 72: 1428-1435.

Nylin, S. 1988. Host plant specialization and seasonality in a polyphagous butterfly, *Polygonia c-album* (Nymphalidae). Oikos 53: 381-386.

Ohsaki, N. and Y. Sato. 1994. Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. Ecology 75:59-68.

Ohsugi, T., R. Nishida and H. Fukami. 1985. Oviposition stimulant of *Papilio xuthus*, a *Citrus*-feeding swallowtail butterfly. Agric. Biol. Chem. 49: 1897-1900.

Oyeyele, S. O. and M. P. Zalucki. 1990. Cardiac glycosides and oviposition by *Danaus plexippus* on *Asclepias fruticosa* in south-east Queensland (Australia), with notes on the effect of plant nitrogen content. Ecological Entomology 15:177-185.

Papaj, D. and M. D. Rausher. 1987. Components of conspecific host-discrimination behavior in the butterfly, *Battus philenor*. Ecology 68: 245-53.

Ramaswamy, S. B. 1994. Physiological basis of feeding and oviposition behavior in moths. *In*: T. N. Ananthakrishnan (ed). Functional dynamics of phytophagous insects. Science Publishers, Lebanon, N. H. pp. 55-78.

Rausher, M. D. 1978. Search image for leaf shape in a butterfly. Science 200:1071-1073.

Rausher, M. D. 1995. Behavioral ecology of oviposition in the pipevine swallowtail, *Battus philenor*. *In*: J. M. Scriber, Y. Tsubaki and R. C. Lederhouse (eds.). Swallowtail butterflies: their ecology and evolutionary biology. Walsworth Publishing Co., Missouri. pp. 53-62.

Rausher, M. D. and D. R. Papaj. 1983. Demographic consequences of discrimination among conspecific host plants by *Battus philenor* butterflies. Ecology 64:1402-1410.

Renwick, J. A. A. and F. S. Chew. 1994. Oviposition behavior in lepidoptera. Annual Review of Entomology 39:377-400.

Renwick, J. A. A. and Huang. 1994. Interacting chemical stimuli mediating oviposition by Lepidoptera. *In*: T. N. Ananthakrishnan (ed). Functional dynamics of phytophagous insects. Science Publishers, Lebanon, N. H. pp 79-94.

Robertson, H. G. 1987. Oviposition site selection in *Cactoblastis cactorum* (Lepidoptera): constraints and compromises. Oecologia 73: 601- 608.

Roessingh, P., E. Städler, R. Schöni and P. Feeny. 1991. Tarsal contact chemoreceptors of the black swallowtail butterfly *Papilio polyxenes*: responses to phytochemicals from host- and non-host plants. Physiological Entomology 16: 485-495.

Sachdev-Gupta, K., P. Feeny and M. Carter. 1993. Contact oviposition stimulants for the pipevine swallowtail butterfly, *Battus philenor*, from an *Aristolochia* host plant: synergism between inositols, aristolochic acids and a monogalactosyl diglyceride. Chemoecology 4: 19-28.

Saxena, K. N. and S. Goyal. 1978. Host-plant relations of the citrus butterfly *Papilio demoleus* L.: orientational and ovipositional responses. Entomologica Experimentalis et Applicata 24: 1-10.

Schoonhoven, L. M., W. M. Blaney and M. S. J. Simmonds. 1992. Sensory coding of feeding deterrents in phytophagous insects. *In*: E. Bernays (ed.). Insect-plant interactions. Volume IV. pp. 59-79. Chapter 3.

Scriber, J. M. 1984. Larval foodplant utilization by the world Papilionidae (Lep.): latitudinal gradients reappraised. Tokurana (Acta Rhaloceralogica) 2: 1-50.

Scriber, J. M. 1986. Origins of the regional feeding abilities in the tiger swallowtail butterfly: ecological monophagy and the *Papilio glaucus australis* subspecies in Florida. Oecologia 71:94-103.

Scriber, J. M. 1988. Tale of the tiger: beringial biogeography, binomial classification, and breakfast choices in the *Papilio glaucus* complex of butterflies. In: K. C. Spencer (ed.). Chemical mediation of coevolution. Academic Press, New York. pp. 240-301.

Scriber, J. M. 1993. Absence of behavioral induction in oviposition preference of *Papilio glaucus* (Lepidoptera: Papilionidae). The Great Lakes Entomologist 26:81-95.

Scriber, J. M. 1994. Climatic legacies and sex chromosomes: Latitudinal patterns of voltinism, diapause, size, and host-plant selection in two species of swallowtail butterflies at their hybrid zone. *In*: H. V. Danks (ed.). Insect Life-cycle Polymorphism. Kluwer Academic Publishers, the Netherlands. pp. 133-171

Scriber, J. M. 1995. Overview of swallowtail butterflies: taxonomic and distributional latitude. *In*: J. M. Scriber, Y. Tsubaki and R. C. Lederhouse (eds.). Swallowtail butterflies: their ecology and evolutionary biology. Walsworth Publishing Co., Missouri. pp 3-8. Chapter 1.

Scriber, J. M. 1996. Tiger tales: natural history of native North American swallowtails. American Entomologist 42: 19-32.

Scriber, J. M. and R. C. Lederhouse. 1992. The thermal environment as a resource dictating geographic patterns of feeding specialization of insect herbivores. *In*: M. R. Hunter, T. Ohgushi and P. W. Price (eds.). Effects of resource distribution on animal-plant interactions. Academic Press, San Diego, CA. pp. 429-466.

Scriber, J. M., R. C. Lederhouse, and R. C. Hagan. 1991a. Foodplants and evolution within the *Papilio glaucus* and *P. troilus* species groups (Lepidoptera: Papilionidae). *In*: P. W. Price, T. M. Lewinsohn, G. W. Fernandes, and W. W. Benson (eds.). Plant-animal interactions: evolutionary ecology in tropical and temperate regions. Wiley and Sons, Inc. pp. 341-373.

Scriber, J. M., B. L. Giebink and D. Snider. 1991b. Reciprocal latitudinal clines in oviposition behavior of *Papilio glaucus* and *P. canadensis* across the Great Lakes hybrid zone: possible sex-linkage of oviposition preferences. Oecologia 87: 360-368.

Scriber, J. M. and A. Redman. 1993. Interspecific competition as a cause of hostplant shifts in Michigan Lepidoptera. Bulletin of the Entomological Society of America 74: 432-433.

Shorey, H. H. 1964. The biology of *Trichoplusia ni* (Lepidoptera: Noctuidae). III. Response to the oviposition substrate. Annals of the Entomological Society of America 57: 165-170.

Singer, M. C., D. Ng, and C. D. Thomas. 1988. Heritability of oviposition preference and its relationship to offspring performance within a single insect population. Evolution 42: 977-985.

Smiley, J. T. 1978. Plant chemistry and the evolution of host specificity: new evidence from *Heliconius* and *Passiflora*. Science 201: 745-747.

Smiley, J. T. 1985. Are chemical barriers necessary for evolution of butterfly-plant associations? Oecologia 65: 580-583.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. W. H. Freeman & Co. San Fransisco. 859 pp.

Städler, E. 1974. Host plant stimuli affecting oviposition of the eastern spruce budworm. Entomologica Experimentalis et Applicata 17: 176-188.

Strong, D. R. 1988. Special feature: insect host range (series of articles). Ecology 69: 886-915.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomologica Experimentalis et Applicata 47: 3-14.

Thompson, J. N. and O. Pellmyr. 1991. Evolution of oviposition behavior and host preference in lepidoptera. Annual Review of Entomology 36:65-89.

Traynier, R. M. M. 1984. Associative learning in the ovipositional behaviour of the cabbage butterfly, *Pieris rapae*. Physiological entomology 9: 465-472.

Traynier, R. M. M. 1986. Visual learning in assays of sinigrin solution as an oviposition releaser for the cabbage butterfly, *Pieris rapae*. Entomologica Experimentalis et Applicata 40: 25-33.

Underwood, D. L. A. 1994. Intraspecific variability in host plant quality and ovipositional preferences in *Eucheira socialis* (Lepidoptera: Pieridae). Ecological Entomology 19: 245-256.

van Loon, J. J. A., T. C. Everaarts and R. C. Smallegange. 1992. Associative learning in host-finding by female Pieris brassicae butterflies: relearning preferences. *In*: S. B. J. Menken, J. H. Visser and P. Harrewijn (eds). Proc. 8th Int. Symp. Insect-Plant Relationships. Dordrecht: Kluwer Acad. Publ.

Watanabe, M. 1995. Population dynamics of Papilio xuthus larvae in relation to the life history of the host tree. *In*: J. M. Scriber, Y. Tsubaki and R. C. Lederhouse (eds.). Swallowtail butterflies: their ecology and evolutionary biology. Walsworth Publishing Co., Missouri. pp. 101-106.

Wiklund, C. 1984. Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. Oecologia 63: 23-29.

Williams, E. H. 1981. Thermal influences on oviposition in the montane butterfly *Euphydryas gillettii*. Oecologia 50:342-346.

Williams, K. S. 1983. The coevolution of *Euphydryas chalcedona* butterflies and their larval host plants. Oecologia 56: 336-340.

Wolfson, J. L. 1980. Oviposition response of *Pieris rapae* to environmentally induced variation in *Brassica nigra*. Entomologica Experimentalis et Applicata 27:223-232

Zangerl, A. R. and M. R. Berenbaum. 1992. Oviposition patterns and hostplant suitability: parsnip webworms and wild parnsip. The American Midland Naturalist 128: 292-298.