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Potential for host shifting in *Papilio palamedes* following invasion of laurel wilt disease

Adam D. Chupp · Loretta L. Battaglia

Abstract In the southeastern US, laurel wilt disease (LWD) is causing widespread mortality of species in the Lauraceae. The principal target, *Persea borbonia*, is the primary larval host of *Papilio palamedes*, which is known to feed on other Lauraceae species. Among these potential hosts, the exotic *Cinnamomum camphora* is the only species that has shown resistance to LWD. We hypothesized that oviposition preference for *C. camphora* and *P. borbonia* would correspond to larval performances on these species and that the relative host suitability of *C. camphora* would indicate an opportunity for host-switching. We used laboratory experiments and field observations to compare performance and preference of *P. palamedes* between *C. camphora* and *P. borbonia* foliage. Our results indicate moderate survivorship on *C. camphora* compared to *P. borbonia* and no differences in first and fourth instar growth rates between treatments. Fourth instars consumed relatively less of *C. camphora* foliage compared to that of *P. borbonia*, but metabolic efficiency did not differ between treatments. Rearing on the foliage of *P. borbonia* stump sprouts from LWD-infected trees resulted in significantly higher growth rates and metabolic efficiency as first and fourth instars, respectively. In the field and laboratory,

we found no oviposition preference for *C. camphora*. While females laid eggs on *C. camphora* during laboratory trials, the same number of eggs was also laid on inanimate objects. We conclude that *C. camphora* is suitable for larval development but host-switching to this species by *P. palamedes* will be primarily constrained by the ecological factors that govern oviposition behaviors.

Keywords *Cinnamomum camphora* · Host shifting · Laurel wilt disease · *Papilio palamedes* · *Persea borbonia* · Preference-performance relationship

Introduction

Species invasions and disease outbreaks can dramatically alter the relative abundances of native and exotic species and facilitate the formation of novel species associations (Agosta et al. 2010). Under these circumstances, new relationships between exotic and native species may form on the basis of compatibility and thus do not represent the outcome of a long history of coevolution; the term "ecological fitting" has been used to explain such novel associations (Janzen 1980, 1985; Agosta 2006; Agosta and Clemens 2008). By forming relationships with native species in the wake of disturbance, exotic species can maintain an interactive system (i.e. herbivory, pollination, and dispersal) when they have functions that are similar to

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that of a native species (Zamora 2000). Such functional equivalence may provide resiliency to disturbance. On the other hand, associations with exotic species may alter ecological processes and degrade the function of native systems (Simberloff and Von Holle 1999). In either scenario, the results will have important implications for conservation programs. The initial steps of forecasting novel species associations and disseminating these predictions should be a major goal for theoretical conservation biologists.

For insect herbivores, which provide an important link between primary producers and higher trophic levels, predicting shifts in host plant associations may have broad implications for community dynamics and ecosystem processes (Sih et al. 1985). Such forecasts are complicated by the complex set of biological and ecological factors that can shape host selection at multiple insect life stages (see Pearse and Altermatt 2013; Pearse et al. 2013). Larvae of many insect herbivores, such as those of the Lepidopterans, are relatively sedentary. Therefore, host plant selection is made by ovipositing adult females who should optimize fitness by selecting host plants that maximize the performance of their larval offspring (Jaenike 1978). However, interactions with exotic host plants may often result in suboptimal relationships between oviposition preference and larval performance (Karowe 1990, Larsson and Ekbom 1995; Schlaepfer et al. 2005; Gillespie and Wratten 2011). For example, female common coppers (*Lycaena salustius*) will recognize and oviposit on exotic buckwheat (*Fagopyrum esculentum*) despite poor larval performance on this species. The morphological and chemical similarities between *F. esculentum* and the ancestral (native) hosts of *L. salustius* are apparently driving the behavior of ovipositing females (Gillespie and Wratten 2011). Although analyses of oviposition behaviors suggest ecological fitting between *L. salustius* and *F. esculentum*, the novel host plant may be an evolutionary trap if the physiological performance of larvae remains low (Schlaepfer et al. 2005). Predicting novel host colonization requires an understanding of both the preference and performance of an insect herbivore on a potential host. By considering herbivore and host species traits and their evolutionary histories we can identify where good ecological fits may occur (Pearse et al. 2013). The availability and occupancy of potential hosts may be of utmost importance (Forister and Wilson 2013), especially

in situations where exotic species invasions and disease outbreaks are drastically altering the relative abundances of host species.

In North America, several well documented cases of forest disease and insect outbreaks, e.g., chestnut blight (Anagnostakis 1987), hemlock woolly adelgid (Orwig and Foster 1998), and emerald ash borer (Haack et al. 2002), have resulted in dramatic declines in the dominance of native species. However, the greatest reductions in species richness are expected to occur among groups of insect herbivores that specialize on the impacted native species (Gandhi and Herms 2010). Despite this expectation, estimates of these effects on insect herbivores are rare (but see Work and McCullough 2000; Scriber 2004; Wagner 2007). To more completely understand the impacts of widespread forest disturbances, we need thorough documentation of herbivores and their associations before they become permanently altered (Gandhi and Herms 2010).

Throughout the Coastal Plain of the southeastern US, laurel wilt disease (LWD) has decimated populations of species in the Lauraceae family (USDA Forest Service 2013). Although this newly discovered exotic fungal pathogen, *Raffaelea lauricola*, and its beetle vector, *Xyleborus glabratus* (Coleoptera: Curculionidae), have caused mortality in several Lauraceae species, most incidences of LWD are observed on redbay, *Persea borbonia* L. (Laurales: Lauraceae) (Fraedrich et al. 2008). In *P. borbonia* populations, mortality is >95 % for trees above 2.5 cm diameter at breast height (DBH). The symptoms progress rapidly as the fungal pathogen apparently blocks water transport in the xylem, resulting in canopy wilting and death within weeks to a few months (Fraedrich et al. 2008; Mayfield 2008; pers. obs. A Chupp). Although stump sprouting appears to be a common response in some populations of *P. borbonia*, re-infection of sprouts via *X. glabratus* and/or spreading of *R. lauricola* through the root system is evident (Spiegel and Leege 2013). More recent studies also suggest that sprouting responses may do little to sustain populations of *P. borbonia.* (Evans et al. 2013)

With the persistence of *P. borbonia* in question, there is concern for the herbivores which obtain resources from this species. Across the Gulf and Atlantic Coastal Plain, *P. borbonia* is a common subcanopy species in many forested habitats and appears to play an important role as a food source for many native species (Brooks 1962; Goodrum 1977; Landers

et al. 1979; Brendemuehl 1990; Van Deelen 1991, Leege 2006). In the literature, *P. borbonia* is perhaps best known as the primary larval host of the palamedes swallowtail butterfly, *Papilio palamedes* Drury (Lepidoptera: Papilionidae). This long-tongued pollinator is abundant on the southeastern Coastal Plain and has a distribution which roughly mirrors that of *P. borbonia*. Laboratory observations suggest that female oviposition preferences and larval performance are highest on *P. borbonia* when compared to other species of Lauraceae (Brooks 1962; Scriber et al. 1991; Lederhouse et al. 1992). However, there are also accounts of *P. palamedes* using other species within the Lauraceae, including the exotic camphor tree, *Cinnamomum camphora* L. (Laurales: Lauraceae). In laboratory experiments, 15 % of *P. palamedes* larvae survived when reared on *C. camphora* (Lederhouse et al. 1992). However, with such a small number surviving to adulthood (n = 3), conclusions drawn from other measures of larval performance (i.e. lifetime larval growth rate, larval duration, and pupal mass) were insignificant. A year earlier, the same authors reported survivorship on *C. camphora* to be 50 % (Scriber et al. 1991), however, larvae were reared for only 12 days, which is 1/3 of the total larval development time on *C. camphora* (Lederhouse et al. 1992). The studies by Scriber et al. (1991) and Lederhouse et al. (1992) provide minimal data regarding the performance of *P. palamedes* on *C. camphora*. In addition, we found no studies of adult female oviposition preferences for *C. camphora*. Altogether, it is unclear to what degree *P. palamedes* may use *C. camphora*.

Following the widespread mortality of *P. borbonia*, we submit several reasons for why *C. camphora* may be the best alternative host for *P. palamedes*. First, *C. camphora* has a close phylogenetic relationship with *P. borbonia* and shares morphological and chemical characteristics (Chanderbali et al. 2001, A. Chupp pers. obs). Such similarities have been useful for predicting trait matching (i.e. ecological fitting) between plants and herbivores and subsequent colonization of novel plant species (as reviewed by Pearse et al. 2013). Second, *C. camphora* has a distribution which overlaps that of *P. borbonia* and is expanding throughout the Atlantic and Gulf Coastal Plains; this sub-canopy tree is listed as a naturalized invasive species in eight states of the southeastern US, as well as California, Hawaii and the territory of Puerto Rico (USDA, NRCS 2013). Third, observations of LWD in *C. camphora* suggest a resistance to the disease and/or its beetle vector. In Florida and Georgia, infected individuals showed minimal stem die-off and in no case did complete canopy wilting occur (Smith et al. 2009). Results from single point inoculation trials in the field and laboratory failed to produce LWD symptoms in *C. camphora* despite systemic colonization by *R. lauricola*; multiple point inoculations caused only localized branch dieback (Fraedrich et al. in press). These trials provide the most recent evidence that *C. camphora* is more resistant to LWD than native Lauraceae species. Finally, successful development on *C. camphora* has been reported for several species of Papilionidae from North America [*Papilio glaucus* L. (Lepidoptera: Papilionidae) and *Papilio troilus* L. (Lepidoptera: Papilionidae)], Tasmania [*Graphium macleayanus moggana* Leach (Lepidoptera: Papilionidae)] and mainland Australia [*Papilio aegeus* Donovan (Lepidoptera: Papilionidae)] (Morris 1989; Scriber et al. 2006, 2007, 2008a, b). These observations suggest a pattern of compatibility between *C. camphora* and closely related *Papilio* spp.

The objective of this study was to test the suitability of *C. camphora* as a host for *P. palamedes*. However, like all Lepidopterans, *P. palamedes* has a complex life history and both larvae and adults have distinct interactions with host plants. We measure adult oviposition preference and larval performance on both *C. camphora* and *P. borbonia*. We hypothesized that adult females would display some preference for ovipositing on *C. camphora* and that larval performance would correspond with this preference. We provide empirical data on the nature of these interactions prior to widespread disturbance by LWD. Finally, we discuss the ecological factors that may also govern the potential colonization of *C. camphora*.

Methods

Larval survival, growth, and metabolic efficiency

Papilio palamedes eggs were obtained from adult females collected in Jackson County, Mississippi on the Grand Bay National Estuarine Research Reserve (GBNERR). Captured females were kept in clear plastic containers (35 × 20 × 13 cm) and placed in a VWR® signature diurnal growth chamber (Sheldon Manufacturing Inc.). Twigs of *P. borbonia*, which

were collected from GBNERR, were also placed in the containers. Aquapics were used to maintain turgor pressure within stems and leaves. The growth chamber was maintained at 30 and 22 °C during 16-h day and 8-h night cycles, respectively. Humidity was kept between 60 and 80 % using a Hunter® 3.4 L humidifier (Model # 33119).

Eggs from 11 different females were kept in separate petri dishes in the growth chamber until hatching. Randomly selected larvae were assigned to one of three host-plant treatments: (1) foliage of *C. camphora* (2) foliage of healthy *P. borbonia* or (3) foliage of *P. borbonia* stump sprouts produced after LWD-induced canopy death (hereafter *P. borbonia* LWD). Although larvae were randomly selected, we systematically placed larvae from each family (i.e. egg-laying female) into all three treatments. Healthy foliage of *C. camphora* and *P. borbonia* were collected from trees on GBNERR. Foliage of *P. borbonia* LWD was collected two kilometers north of GBNERR where impacts from LWD have been recently observed (A. Chupp pers. obs.). In the laboratory, larvae were reared individually in clear plastic containers (35 × 20 × 13 cm). Containers were lined with moist paper towels to maintain saturated humidity and fluorescent grow lights (L:D 16:8) maintained temperatures between 27 °C (lights on) and 23 °C (lights off). Larvae were presented with foliage that was refreshed daily. The duration of each developmental stage was closely monitored and we recorded the date and time of larval mortality, molting, pre-pupation, pupation, and adult emergence. We measured the initial mass of larvae in the following stages: neonate (i.e. first instar), second instar, fourth instar, and pupa. Mass was measured to the nearest 0.1 mg and the time of each weighing to the nearest minute. The sex of each individual was determined only for emerged adults; the sex of larvae that died prior to this stage is unknown.

Growth rates (RGR) of each first, second, and fourth instar larva were calculated as follows:

$$\text{RGR} = \left[\ln\left(M_f\right) - \ln\left(M_i\right)\right]/T_{f-I},$$

where M_i is initial mass, M_f is final mass, and T_{f-I} is time elapsed in days. For calculations of first instar RGR, final weight (M_f) was multiplied by 0.9 to correct for overestimation of RGR due to initial gut filling (Lederhouse et al. 1992). Larvae were weighed immediately after molting. Lifetime larval growth rates were calculated using the following equation:

$$\text{Lifetime RGR} = \left[\ln\left(DM_p\right) - \ln\left(DM_i\right)\right]/T_{ld},$$

where DM_p is pupal dry mass, DM_i is the dry neonate mass, and T_{ld} is larval duration in days (does not include the prepupal stage). Dry pupal mass (DM_p) was calculated as $0.219 \cdot M_p$ for males and $0.250 \cdot M_p$ for females, where M_p is pupal fresh mass. Dry neonate mass (DM_i) was calculated as $0.125 \cdot M_i$, where M_i is the fresh neonate mass. Conversions to dry mass avoided underestimation of lifetime RGR due to lower water content in pupae relative to larvae (Lederhouse et al. 1992). We considered estimates of dry female pupal mass (i.e. body size) as a surrogate for fecundity. Across a range of insect orders, female body size is commonly the primary constraint on insect fecundity (as reviewed by Honek 1993).

Indices of metabolic efficiency were calculated for each fourth instar larva. Larvae were weighed immediately after molting into the fourth instar and then closely monitored for the next 7 days. During this time we weighed the fresh mass of all leaves that were presented to larvae. At the end of this period, we collected and dried (50 °C) all uneaten leaf material and frass. The fresh weight of larvae was also recorded at this time. Larval fresh weight was multiplied by a constant (0.125) to approximate dry weight (Lederhouse et al. 1992, Ayres and Scriber 1994). To determine the initial dry weight of fresh leaves presented to larvae, we used a regression equation developed from the fresh and dry weights of leaves collected separately throughout the experimental period (n = 40 per treatment) (Levesque et al. 2002). We then calculated total food ingested (dry weight), total frass (dry weight), consumption rate (CR), approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI). Calculation of these indices followed that of Scriber and Lederhouse (1983):

$$\text{CR} = \text{mg food ingested d}^{-1}$$
$$\text{AD} = \left[\left(\text{mg food ingested} - \text{mg frass}\right)/\right.$$
$$\left.\text{mg food ingested}\right] \cdot 100$$
$$\text{ECD} = \left[\text{mg larval biomass gained}/\right.$$
$$\left.\left(\text{mg food ingested} - \text{mg frass}\right)\right] \cdot 100$$
$$\text{ECI} = \left(\text{mg biomass gained}/\text{mg food ingested}\right) \cdot 100$$

Adult oviposition preferences

Papilio palamedes females were captured in Jackson County, Mississippi on the GBNERR. To understand the relative preferences of adult females, we conducted both choice and no-choice experiments. In no-choice experiments, females were allowed to oviposit in clear plastic containers (35 × 20 × 13 cm) lined with paper towels. Females were presented with either foliage of healthy *P. borbonia* or *C. camphora.* Aquapics helped to maintain turgor pressure in stems and leaves. We also tested oviposition preferences using synthetic foliage. In preliminary observations, females often laid eggs on inanimate objects (e.g. container surface) and trials with synthetic foliage were conducted to see if these behaviors could be stimulated by non-living plant material. This foliage was presented to a smaller subset of females and was similar in appearance to the living foliage of *P. borbonia* and *C. camphora*. In choice experiments, females were placed in wooden framed rearing cages (40 × 32 × 55 cm) lined with no-see-um/mosquito netting. The larger space provided by rearing cages allowed for the physical separation of *P. borbonia* and *C. camphora* foliage in opposite corners of the enclosure. Synthetic foliage was not presented during these trials. Live stems were placed in water-filled Erlenmeyer flasks (125 mL) to maintain turgor pressure. The height and girth of presented foliage was consistent between host-plant types.

In both choice and no-choice experiments, enclosures were placed in a VWR® signature diurnal growth chamber (Sheldon Manufacturing Inc.). The growth chamber was maintained at 30 and 22 °C during 16-h day and 8-h night cycles, respectively. Humidity was kept between 60 and 80 % using a Hunter® 3.4 L humidifier (Model # 33119). In both experiment types, each female was observed for 72 h. After this period, the foliage was removed and the total number of eggs laid on each host plant was recorded. We also recorded the number of eggs laid on inanimate objects (i.e. the enclosure, flasks, or aquapics). To corroborate results from laboratory experiments, we recorded observations of oviposition and larval development in the field during extensive periods of live female capture. These observations were also documented during collection of host plant material in the field. We documented the size class of each tree upon which eggs were laid. All individuals <1.5 m in height were included in size

class 1. Individuals ≥1.5 m in height were then classed by DBH as follows: size class two contained individuals <7 cm DBH and size class three contained individuals ≥7 cm DBH.

Statistical analyses

At each stage of larval development (1st–4th instar, pre-pupa, and pupa), we analyzed differences in mortality among treatments (i.e. host plant type) using contingency table analysis with Pearson's Chi squared test. For all measures of larval performance and metabolic efficiency, we used one-way ANOVA and Tukey's post hoc analysis to test for significant differences among treatments; separate analyses of female pupal weight were also conducted. We used a randomized block model (one-way ANOVA) to test for a family effect on the growth rate of larvae during the first instar. Square-root transformations were applied to any data that were not normally distributed and/or violated the assumption of variance homogeneity.

To analyze female oviposition preferences during choice experiments, we used a one-sample t test to determine if the proportion of eggs laid on *P. borbonia* or *C. camphora* significantly departed from 50 %. In no-choice trials, we used a one-way ANOVA to test for significant differences in the number of eggs laid by females on the different host plants. In the statistical analyses for both choice and no-choice experiments, the number of eggs laid on inanimate objects was discarded. We applied square-root transformations to all data that were not normally distributed. All statistical procedures were conducted using the SAS 9.2 software package (SAS Institute 2007).

Results

Larval survival, growth, and metabolic efficiency

We reared a total of 72 *P. palamedes* larvae (n = 23–25 per treatment). There were no significant differences in survivorship between larvae reared on the foliage of healthy *P. borbonia* and those reared on *P. borbonia* LWD (Fig. 1). Survivorship was reduced for individuals reared on the foliage of *C. camphora*; significant declines occurred prior to the fourth instar ($\chi^2 = 7.38$, $P < 0.010$). On both *P. borbonia* treatments, no individuals died after the second instar
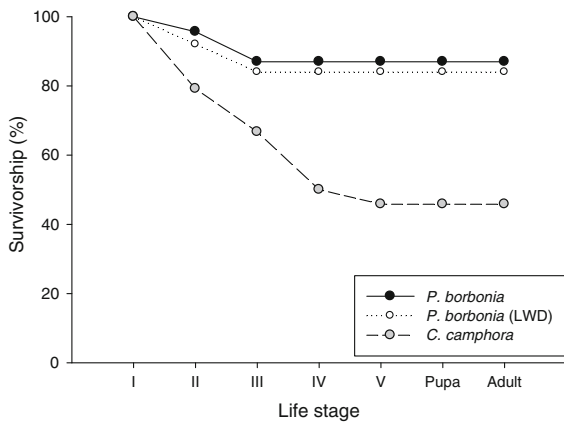
Fig. 1 Survivorship of *P. palamedes* on three host plant treatments. Points represent the percentage of individuals that were alive at the beginning of each life stage

whereas one individual died on *C. camphora* after the third instar (Fig. 1).

Growth rates of larvae in the first instar were significantly higher for those reared on *P. borbonia* LWD ($F_{2,61} = 8.50$, $P = 0.0006$). First instar growth

rates of larvae reared on healthy *P. borbonia* and *C. camphora* did not differ (Fig. 2). The randomized block model indicated no effect of family on growth rates. During the second-third instar period, growth rates were significantly lower for larvae reared on *C. camphora* when compared to both *P. borbonia* treatments ($F_{2,50} = 27.16$, $P < 0.0001$), which did not differ from each other (Fig. 2). This same pattern was observed for lifetime larval growth rates where the *C. camphora* treatment produced significantly lower growth rates than both *P. borbonia* treatments ($F_{2,49} = 24.97$, $P < 0.0001$) which again did not significantly differ from one another. No significant differences among treatments were observed for larval growth rates during the fourth instar (Fig. 2). Total larval duration (excludes pre-pupal and pupal stages) was significantly longer for larvae reared on *C. camphora* ($F_{2,29} = 22.77$, $P < 0.0001$) and there was no significant difference between *P. borbonia* treatments. There was also a marginally significant effect of treatment on pupal mass ($F_{2,49} = 3.59$, $P = 0.0350$); *C. camphora* produced lighter pupae

Fig. 2 Measures of *P. palamedes* larval performance on three host plant treatments. Samples sizes for first instar growth rate were from left to right: 19, 22, 23. Sample sizes for second-third instar growth rate were: 12, 20, 21. Sample sizes for all other measures were: 11, 20, 21. *Bars* represent the mean ± standard error
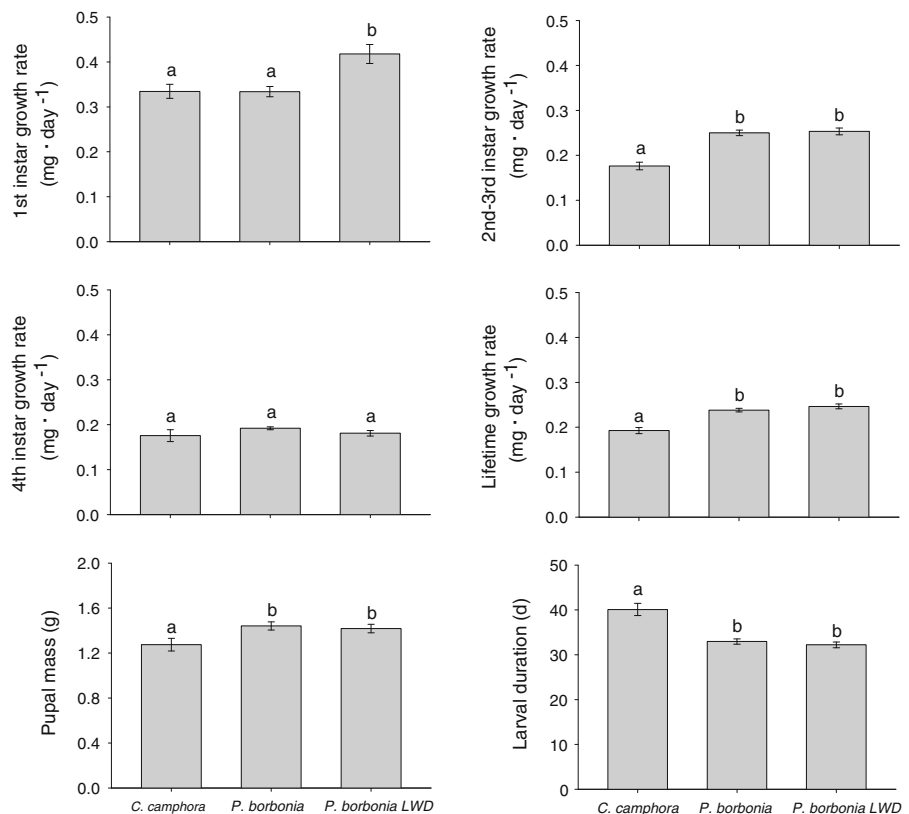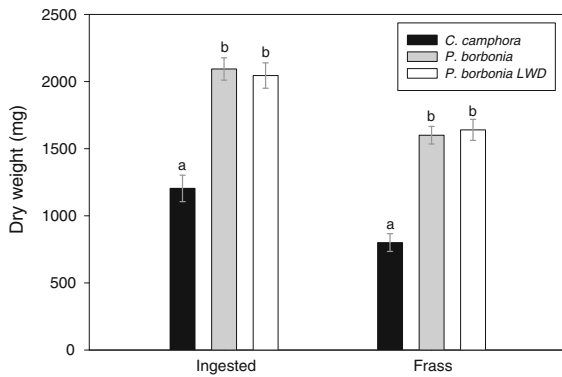
Fig. 3 Amount of plant material ingested and excreted (frass) from larvae during a 7-day period of the fourth instar when reared on three host plant treatments. *Bars* represent the mean ± standard error
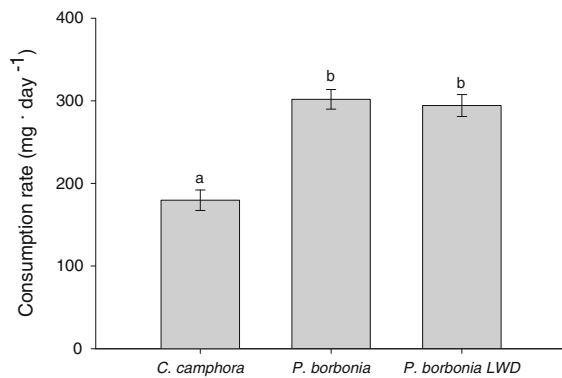

Fig. 4 Consumption rate of larvae during a 7-day period of the fourth instar when reared on three host plant treatments. *Bars* represent the mean ± standard error

Fig. 5 Metabolic indices of larvae during a 7-day period of the fourth instar when reared on three host plant treatments. *AD* Approximate digestibility, *ECD* efficiency of conversion of digested food, *ECI* efficiency of conversion of ingested food. *Bars* represent the mean ± standard error

on *P. borbonia* and *P. borbonia LWD* were non-significant (Figs. 3, 4). Among all three host-plant treatments, there were significant differences in approximate digestibility (AD). The AD of *C. camphora* was significantly higher than that of *P. borbonia* which was greater than that of *P. borbonia LWD* ($F_{2,45} = 15.91$, $P < 0.0001$, Fig. 5). The efficiency of conversion of digested plant material (ECD) was significantly higher for larvae reared on *P. borbonia LWD* and there was no difference between *P. borbonia* and *C. camphora* ($F_{2,45} = 11.12$, $P = 0.0001$). Finally, the efficiency of conversion of ingested material to biomass (ECI) was significantly higher for larvae reared on *C. camphora* when compared to larvae reared on healthy *P. borbonia*. All other differences were non-significant (Fig. 5).

Adult oviposition preferences

To collect adult females, we spent approximately 350 h in the field. We accumulated additional hours of observation while collecting fresh foliage of *P. borbonia* and *C. camphora*. Although these collections were made daily, we did not quantify the total observation time. During these collection periods, we witnessed egg laying by eight females. While we observed five *P. palamedes* females ovipositing on *P. borbonia* (two on size class 1, two on size class 2, and one on size class 3), no females of this species were seen laying eggs on *C. camphora*. However, we did observe 3 *P. troilus* females ovipositing on *C.*

when compared to both *P. borbonia* treatments which again did not differ from each other (Fig. 2). The same pattern was observed for female pupal mass (surrogate for fecundity) but there were greater differences between *C. camphora* and both *P. borbonia* treatments ($F_{2,22} = 8.53$, $P = 0.002$). Analysis of emerged adults revealed that females represented 55, 50, and 52 % of individuals in *C. camphora*, *P. borbonia*, and *P. borbonia LWD* treatments, respectively.

During the fourth instar, more detailed measures of metabolic efficiency were made on a total of 48 larvae. Consumption rate and the mass of plant material ingested and excreted (frass) showed the same trend across treatments. All three measures were significantly lower for those larvae reared on *C. camphora* ($P < 0.0001$) while differences between larvae reared
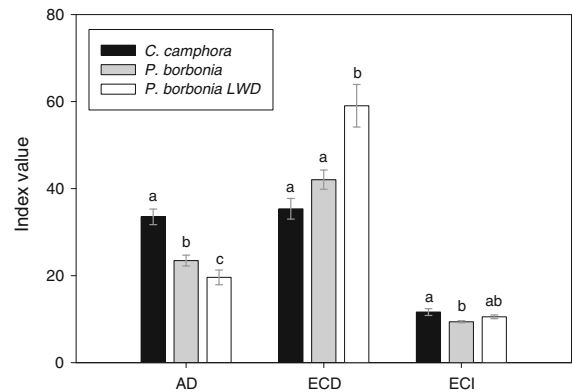
camphora (one on size class 1 and two on size class 3). Oviposition events were verified by checking foliage for eggs.

In no-choice experiments, we used a total of 46 live-captured female *P. palamedes*; sample sizes for *P. borbonia*, *C. camphora*, and synthetic plant foliage were 20, 18, and 8, respectively. Eleven females oviposited a total of 451 eggs on *P. borbonia*; this includes one female who laid 222 eggs and was discarded from the statistical analyses. Twenty-four eggs were oviposited on inanimate objects during no-choice trials with *P. borbonia*. In no-choice experiments with *C. camphora*, two females laid a total of 22 eggs on host plant material and another 55 eggs on inanimate objects. In trials with synthetic foliage, there were no eggs laid on the foliage or on inanimate objects. The total number of eggs laid by each female was square-root transformed to better approximate a normal distribution and all eggs laid on inanimate objects were omitted from statistical analyses. Significantly more eggs per female were laid on *P. borbonia* when compared to both *C. camphora* and synthetic foliage and there was no difference between *C. camphora* and synthetic foliage ($F_{2,43} = 7.99$, $P = 0.0011$).

In choice experiments, we used a total of 19 live-captured females. Six females laid a total of 154 eggs on *P. borbonia*. Two of those 6 females also laid 1 egg each on *C. camphora*. One of those two females also laid 3 eggs on inanimate objects that were later omitted during statistical analyses. There were no females that only oviposited on *C. camphora* or inanimate objects. The proportions of eggs laid on *P. borbonia* and *C. camphora* were square-root transformed to more approximate assumptions of normality and homogeneity of variance. A significantly greater proportion of eggs was laid on *P. borbonia* than would be expected due to chance (i.e. 50 %) ($t = 13.38$, $P < 0.0001$).

Discussion

While several factors may govern the colonization of novel host plants by herbivorous insects, understanding the relationship between adult oviposition preferences and larval performance is an essential part of forecasting novel host colonizations and the consequences of these host shifts (Keeler and Chew 2008;

Forister and Wilson 2013). Here, using a native insect herbivore, *P. palamedes*, and an exotic host plant, *C. camphora*, we documented moderate levels of larval performance despite no oviposition preferences for this species in both choice and no-choice trials. Reports of this type (i.e. no preference and moderate/high performance) have rarely been documented in Lepidopteran species (but see Karowe 1990). On the contrary, observations of high preference and low performance on novel host plants are quite common, a trend that is more frequently observed in generalist species that are relatively less discriminating (e.g. Gripenberg et al. 2010; Harvey et al. 2010; Nakajima et al. 2013). In those circumstances, the host plant may function as an evolutionary trap when low performance leads to a reduction in the realized fitness of the native insect (Keeler and Chew 2008). However, selective processes can increase larval performance over time and lead to the successful colonization of a novel host (e.g. Thompson 1988). Because our results indicate that *P. palamedes* has no preference for ovipositing on *C. camphora*, we argue that the evolution of larval performance on this species has been minimal. Instead, we suggest that the physiology of *P. palamedes* larvae is largely pre-adapted to *C. camphora* foliage whereby the physical and chemical properties of leaf tissues are suitable for complete development. That is to say, based on larval performance, these two species make a relatively good fit despite no history of association and evolution of host-specific physiological adaptations.

Of the total number of eggs laid on *P. borbonia* and *C. camphora* ($n = 629$) during choice and no-choice trials, only 3.8 % were placed on the foliage of *C. camphora*. While 18 females laid eggs on *P. borbonia*, only four females oviposited on *C. camphora*. However, in situations where egg laying on *C. camphora* was observed, we conclude that no preference was actually given to this species because an equal or greater number of eggs was laid on inanimate objects. This conclusion is also supported by field observations where we found no evidence of female oviposition or larvae on *C. camphora*. Our laboratory results agree with studies that have shown that perfect discrimination of optimal hosts by egg laying herbivores is improbable (Rausher 1985). While captive butterflies may develop behaviors that are not consistent with wild individuals (Lewis and Thomas 2001), the females used in our study were caught locally and kept in oviposition cages for no more than 72 h. It is

also interesting to note that in the 8 females we tested on synthetic foliage, no eggs were laid on the foliage or on inanimate objects. We suggest that while *C. camphora* foliage is sub-optimal for oviposition, it may stimulate a less discriminating egg laying behavior in some cases (i.e. no-choice trials where a small number of eggs were laid on both *C. camphora* and inanimate objects). In choice trials, we agree that ovipositing females may have difficulty discriminating between preferred and sub-optimal surfaces, thereby leading to "mistakes" when similar cues are being produced by different species (Fox and Lalonde 1993). In a natural setting, such behaviors can promote the colonization of novel host plants, especially when larvae perform well on these species (e.g. Thompson 1988). While the results of our choice trials suggest that these mistakes occur roughly 3 % of the time, it is unclear how often they would occur in nature where numerous biological and ecological factors may alter the perceived suitability of egg-laying substrates (Forister and Wilson 2013).

For larvae, the suitability of host plants is largely determined by the chemical constituents of plant tissues. Specifically, larvae are sensitive to the types and quantities of secondary compounds and the availability of nutrients (Slansky 1992). Adult insect herbivores may use these chemical signatures as "fingerprints" by which the most suitable larval host plants are recognized. For *Papilio palamedes* and other specialists, their senses are finely tuned as they target one species within a community of closely related species that may have very similar fingerprints (Feeny 1976). Our results suggest an optimal relationship between oviposition preferences and larval performance whereby *P. palamedes* prefers to oviposit on the species which yields the highest larval performance. This relationship is well documented, especially in specialist insect herbivores (as reviewed by Gripenberg et al. 2010). While the ability to discriminate only the most suitable host may be an advantage, it is commonly reported that specialization in general is disadvantageous to coping with disturbance (e.g. McKinney 1997; Hobbs 2000; Colles et al. 2009; Clavel et al. 2011). Disturbances that cause widespread decline and possible extinction of a primary host species may be extremely threatening. Although adult phytophagous insects exhibit behavioral flexibility during host selection, which can facilitate the selection of the most abundant host (Cunningham

et al. 2001; West and Cunningham 2002), specialist insect herbivores may be less capable of such adaptations. Plasticity of oviposition behavior and subsequent novel host colonization will be required for a specialist herbivore like *P. palamedes* to persist in the wake of LWD.

During the course of this study, we recorded the first observations of LWD at the GBNERR in coastal Mississippi; mortality of *P. borbonia* was patchy (n = 40–50) and the presence of LWD was not yet pervasive. If the effects of LWD are similar to those on the Atlantic Coastal Plain, then 75–97 % of *P. borbonia* trees will be dead within 2–4 years (Spiegel and Leege 2013). Here, we conclude that subsequent stump sprout production and persistence will dictate the consequences of LWD for *P. palamedes*. Our field observations indicated that female *P. palamedes* will readily oviposit on redbay of the smallest size class (height <1.5 m), including sprouts. Laboratory results revealed that larval performance on the foliage of stump sprouts from infected *P. borbonia* was comparable to those reared on the foliage of healthy *P. borbonia*. In addition, fourth instar larvae feeding on sprout foliage converted digested leaf material into biomass more efficiently than in other treatments. These results suggest that the physical and/or chemical properties of foliage from *P. borbonia* sprouts could lead to enhanced performance on this species. We also observed that sprouts from experimentally cut trees in the field were heavily colonized by herbivores compared to mature canopy foliage. While it has been frequently documented that seedlings and sprouts are better defended than mature plants, a recent meta-analysis indicated that ontogenetic patterns of secondary defense compounds are inconsistent and vary with a range of biotic factors including types of herbivores, defense traits, and plant life forms (Barton and Koricheva 2010 and citations therein). We suspect that *P. borbonia* sprouts are less defended and are therefore a more efficient nutrient source for developing larvae. Regardless, the relative availability of *P. borbonia* and *C. camphora* will be regulated by the persistence of these vegetative sprouts and will therefore be an important component of predicting novel host plant colonization in *P. palamedes* (Forister and Wilson 2013; Pearse et al. 2013).

While foreign host plants can provide new opportunities for native insect herbivores (e.g. Siemann et al. 2006; Harvey et al. 2010), the colonization of

these exotic species is more likely to occur in areas where the invader has been present for a longer period of time (Siemann et al. 2006). Following their initial establishment, novel host plants go through a period of "naturalization" where they accumulate herbivores; the equilibration of these herbivore communities may take centuries (Strong 1974). For *C. camphora*, which was introduced to Florida circa 1875 (Langeland and Craddock Burks 1998), it is unclear if populations throughout the southeastern US have completed this process of naturalization.

In the eight southeastern states where it occurs, *Cinnamomum camphora* is considered an exotic invasive species (USDA, NRCS 2013). In the coastal counties of Mississippi, Alabama, and Florida, it grows well in disturbed areas (e.g., along roadsides and power line right-of-ways) and is also present in forested habitats where larger individuals reach the sub-canopy layer (pers. obs. A. Chupp). Comparisons of multiple plant databases indicate that its distribution is still expanding. For example, in Florida, *C. camphora* is naturalized in 27 counties (USDA, NRCS 2013) but has been vouchered in 36 counties (Wunderlin and Hansen 2013). For these reasons, we argue that availability of *C. camphora* is sufficient to support herbivore populations across much of the southeastern Coastal Plain. While quite common in this region, this invader has not garnered the same level of attention as several other exotic tree species (e.g. Chinese tallow) (Renne et al. 2002; Rogers and Siemann 2004; Battaglia et al. 2009). For such a widespread species that is continuing to invade new areas, there has been surprisingly little research focused on the ecology of *C. camphora* in its introduced range.

Here, we report field observations of *C. camphora* colonization by *P. troilus*. We witnessed oviposition by females and observed later instars on *C. camphora* foliage. Complete development of *P. troilus* on *C. camphora* was also reported in New Orleans, LA (Linda Auld pers. comm.). Interestingly, phylogentic and biogeographic analyses of Lauraceae suggest that the primary host of *P. troilus* [spicebush, *Lindera benzoin* L. (Laurales: Lauraceae)] is more closely related to *C. camphora* than the primary host of *P. palamedes* (*P. borbonia*) (Chanderbali et al. 2001), corroborating the observed pattern that *P. troilus* appears to have greater compatibility with *C. camphora* than *P. palamedes*.

During field observations of *C. camphora*, we also confirmed the leaf rolling behavior of *P. troilus* larvae, a characteristic not exhibited by *P. palamedes*. In Lepidopterans, this behavior has been shown to reduce the risk of predation from carnivorous insects (Damman 1987). However, it has also been suggested that birds can cue in on leaf rolls, actually increasing predation rates in species that make larger and more conspicuous leaf rolls (Murakami 1999). The leaf rolling behavior has also been shown to increase the quality of leaf tissue. For example, the rolling of Japanese lilac [*Syringa reticulate* Blume (Lamiales: Oleaceae)] leaves by the larvae of the Holly tortix moth [*Rhopobota naevana* Hubner (Lepidoptera: Tortricidae)] increased leaf nitrogen content by 20 % and reduced leaf toughness and total phenolic content by 21 and 55 %, respectively. The mechanisms that underlie these changes are not understood, although other symbiotic organisms are apparently benefitting from them (Fukui et al. 2002). While the occupancy of *C. camphora* by *P. troilus* may preclude oviposition by female *P. palamedes* and/or provide competition for larvae, leaf rolls could also provide opportunities for improviing larval performance. In any case, this leaf rolling behavior may explain much about the current and potential future interactions between *C. camphora*, *P. troilus*, and *P. palamedes*. Ultimately, the suitability of *C. camphora* for colonization by *P. palamedes* may be largely influenced by the occupancy of this novel host by *P. troilus*.

Although *C. camphora* was relatively unsuitable for oviposition by female *P. palamedes*, we also showed that larvae are physiologically capable of using this exotic host and in many cases individuals were very well adapted. While it is unclear how predation and other interspecific interactions would affect larval performance in a natural setting, our results suggest that *C. camphora* will not function as an evolutionary trap for *P. palamedes*. Instead, the future colonization of *C. camphora* by *P. palamedes* will depend largely on the factors influencing adult female oviposition preferences. Among these factors, we argue that host availability and occupancy will be most important following the impacts of LWD. The colonization of *C. camphora* could save *P. palamedes* from the heightened risk of extinction following the decline of its native primary and alternative hosts.

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