Koa Looper Caterpillars (*Scotorythra paludicola*, Geometridae) Have Lower Fitness on Koa (*Acacia koa*, Fabaceae) True Leaves than on Phyllodes

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Abstract. Native plant-herbivore interactions on islands remain understudied due to the widely discussed idea that island plants have weaker defenses than their continental relatives. In Hawaii, the native moth *Scotorythra paludicola*, a specialist on the native *Acacia koa*, can undergo outbreaks that defoliate tens of thousands of acres of native koa forest, sometimes leading to massive stand mortality. Such extreme herbivory events are expected to exert strong selection pressure for defense in *A. koa*. Because mature *A. koa* trees often re-flush juvenile true leaves after defoliation, we predict that true leaves are better defended against *S. paludicola* than phyllodes, consistent with the phenomenon of induced resistance. A no-choice bioassay was conducted in the laboratory to compare *S. paludicola* development on true leaves vs. phyllodes. Consistent with our predictions, caterpillars reared on true leaves had a significantly higher mortality rate and took longer to pupate than caterpillars reared on phyllodes. Additional sources of variation in *S. paludicola* development included sex, phyllode age (young vs. mature), and host tree identity. Further research is needed to determine the mechanistic traits underlying *A. koa* resistance to *S. paludicola*, and to test whether true leaf development does in fact contribute to a reduction in *S. paludicola* performance and population stability on previously defoliated trees.

Key words: Lepidoptera, insect outbreaks, resistance, heteroblasty, induced defense

Introduction

Herbivory is a universal threat to plants, and one of the primary goals of ecology is to understand how plant traits mediate this interaction. A substantial body of research has characterized the chemical, physical, and indirect defense traits of plants, documenting extensive variation within and among species (Denno and McClure 1983, Agrawal 2011). However, very little of this research comes from island systems (Sardans et al. 2010, Peñuelas et al. 2010, Funk and Throop 2010, Barton 2013). Because native island communities often lack herbivore guilds present in continental communities (such as grazing and browsing mammals), island plants are predicted to have relatively weak defenses (Ziegler 2002, Carlquist 1970), and as a consequence, few studies have explored island plant defenses.

Hawaii is an ideal setting for investigating herbivory and the evolution of defense in native island plants because although vertebrate herbivore guilds are largely absent from the native fauna, others, most

notably insect herbivores, are diverse and abundant. One striking example of a native herbivore that is likely to exert strong selection pressure for defense on its host is the koa moth, *Scotorythra paludicola* Butler (Geometridae). Endemic to Oahu, Maui and Hawaii (Heddle 2003), *S. paludicola* is a specialist folivore on the native koa tree (*Acacia koa* A. Gray, Fabaceae). Populations of *S. paludicola* occasionally undergo massive increases, leading to outbreaks that defoliate entire koa forests (Haines et al. 2009). The largest outbreak on record is currently underway on the island of Hawaii, where approximately 70,000 acres of *A. koa* forest have been defoliated (in some areas multiple times) since the outbreak was first discovered in January 2013. Previous *S. paludicola* outbreaks have slowed tree growth and caused as much as 35% mortality of mature defoliated *A. koa* trees (Stein and Scowcroft 1984), indicating the strong fitness impact of this interaction on *A. koa*.

Considering the specificity of this interaction, the magnitude of *S. paludicola* damage, and the documented fitness consequences, it is likely that strong selection pressure has resulted in well developed defenses in *A. koa*. Although damage tolerance via survival and re-growth are expected to be important for plants that experience herbivore outbreaks (Kessler et al. 2012), we focus here on evidence for resistance traits that directly affect *S. paludicola* caterpillars and, in particular, investigate whether resistance is associated with leaf heteroblasty. Like many species of *Acacia*, *A. koa* is heteroblastic, with plants undergoing a striking shift from a juvenile stage characterized by bipinnately compound "true leaves" to a mature stage with phyllodes (Fig. 1). The transition from juvenile to mature stages typically occurs within a few years of germination (Pasquet-Kok et al. 2010), but there is considerable variation in the size of plants when they begin to develop phyllodes (Barton, personal observation), suggesting genetic and/or environmental variation in the timing of this heteroblastic shift. Anatomical and physiological differences between true leaves and phyllodes are consistent with juvenile leaves maximizing growth and phyllodes having greater drought tolerance (Pasquet-Kok et al. 2010). While considerable research has examined the ecophysiology of *A. koa* (Craven et al. 2010, Ares and Fownes 1999), nothing is known about its defenses against *S. paludicola*, and whether defense or nutritional quality differ across the heteroblastic phase change. This is an important oversight, because extensive research has shown that herbivory and defense vary significantly across plant ontogeny in general (Barton and Koricheva 2010), and observations of the *A. koa–S. paludicola* interaction suggest that leaf form may play an important role during outbreaks.

Following a 2004 *S. paludicola* outbreak on Maui, mature *A. koa* trees were observed to flush juvenile true leaves, primarily via epicormic growth from main trunks or branches, rather than from terminal shoots (Haines et al. 2009). Induced juvenilization is not commonly observed in response to herbivory, although it has been previously documented in poplar and birch (Chapin et al. 1985). Whether juvenile true leaves confer an advantage to defoliated *A. koa* via increases in growth rate (i.e., tolerance mechanisms) or via induced resistance to further damage by caterpillars (i.e., resistance mechanisms), or whether they develop simply as a consequence of anatomical and physiological constraints following defoliation, is unknown.

To investigate the suitability of *A. koa* true leaves and phyllodes for *S. paludicola* development, we performed a no-choice bioassay in which caterpillars were reared

Figure 1. *Acacia koa* displaying both phyllodes and true leaves. Photo taken by K.E. Barton, Waimea Valley, Oahu, June 2013.

on each of the two leaf types. Moreover, because resistance often changes during leaf expansion and maturation due to decreases in defensive plant secondary metabolites and nutrients, and simultaneous increases in leaf toughness (Koricheva and Barton 2012, Kursar and Coley 2003), we reared caterpillars on young as well as mature phyllodes; true leaves were only assessed at the mature stage due to the lack of availability of young true leaves. We used *S. paludicola* performance as an indirect measure of resistance. Caterpillar survival, development time to pupation, and pupal weight were compared among groups reared on true leaves, young phyllodes, and mature phyllodes. Lower survival and pupal weight, and longer development time, would reduce fitness of *S. paludicola*, reflecting a higher resistance in *A. koa*. As a specialist of *A. koa*, it is likely that *S. paludicola* performance will

be high, but that performance will vary among leaf types consistent with observations that caterpillars prefer phyllodes in the field (Haines, personal observation), and in the laboratory (B. Peck personal communication).

Materials and Methods

A no-choice bioassay was conducted in the laboratory (St John Plant Sciences Building, University of Hawaii at Manoa campus) in July–August 2013. *Scotorythra paludicola* caterpillars were obtained from eggs laid by wild-caught female moths. Approximately 40 female moths were collected from swarms in the Ocean View Estates subdivision (District of Kau, HI, N 19.142°, W 155.761) on 20 Jul 2013. Female *S. paludicola* moths insert eggs into bark crevices and patches of moss on the trunks of host trees (Zimmerman 1958), though it is unknown what specific

cues they use to recognize host plants. To obtain eggs, female moths were housed together at 23°C in a screen cage containing moss clumps, pieces of koa bark, and sprigs of koa foliage (to provide a potential oviposition cue) for 5 days. Moths were fed honey water sprayed on damp paper towels draped over the cages. Moss and bark containing eggs was removed daily after overnight exposure to moths, and placed in sealed 35-oz plastic containers without foliage until the eggs hatched (6–7 days). Caterpillars were presumably from several different broods produced by different mothers, and were randomly assigned to diet treatments.

Diets consisted of a single leaf type throughout the development of the caterpillars. Phyllodes and true leaves were collected weekly from *A. koa* in Manoa Valley, Oahu. Trees were marked so that caterpillars could be reared on foliage from the same individual tree throughout the bioassay. Ten trees were used for the bioassay, and multiple caterpillars were reared on foliage from the same tree, providing the replication necessary for testing whether *S. paludicola* performance varied significantly among *A. koa* trees due to genetic and/or environmental variation in resistance.

Caterpillars were reared singly in small plastic dishes fitted with moist filter paper. Fresh foliage and clean filter papers were provided daily, when caterpillar survival could also be noted. Upon pupation, dishes were set aside to allow pupal cases to harden completely for at least one day, and pupation date was recorded. Pupae were then weighed fresh to the nearest 0.01 mg on a Mettler Toledo microbalance. The sex of each pupa was determined by examining differences in the genitalic bumps on the ventral surface of the terminal abdominal segments, allowing us to assess whether performance differed between male and female *S. paludicola*. The total

sample size was N=134 caterpillars, but replication within leaf types varied as a consequence of foliage availability (n=43 for true leaves; n=47 for young phyllodes; n=44 for mature phyllodes).

Data were analyzed using SAS version 9.2 (Cary, North Carolina). Survival on diets composed of the three leaf types was analyzed with a Fisher's exact test. Days to pupation and pupal mass met assumptions of normality and were analyzed with mixed model ANOVA's in which the main factors of diet (3 levels: true leaves, young phyllodes, mature phyllodes), *S. paludicola* sex (2 levels), and tree identity (10 levels) were tested. Interactions between diet and sex were not significant and were removed from the final models. Tree identity was considered a random factor, the significance of which was tested by running the models with and without the random effect, and then calculating the log-likelihood ratio statistics, which can be compared to a chi-square distribution with one degree of freedom (Littell et al. 1996). The Kenward-Roger method was used to calculate degrees of freedom (Kenward and Roger 1997). Tukey-adjusted least-square mean comparisons were used to interpret significant differences among diets.

Results

Caterpillar survival varied significantly among diets (Fisher's exact test P = 0.03857), with less than half as many caterpillars surviving to pupation on true leaves (32.6%) as young (72.3%) or mature (71.1%) phyllodes. Larval development time varied significantly among caterpillars reared on the three different diets ($F_{2,73.6} = 30.38$, P < 0.0001). Development to pupation was fastest on young phyllodes (least-square mean 23.6 days ± 0.6 standard error), was intermediate on mature phyllodes (least-square mean 27.7 ± 0.6 days), and took longest on true

leaves (mean 31.5 ± 1.0 days). Least-square mean comparisons of development time revealed that all three diets were significantly different from each other (P < 0.0001).

Pupal biomass also depended significantly on leaf type ($F_{2,72.8} = 4.45$, P = 0.0151). Pupae reared on young phyllodes (least-square mean 0.07492 ± 0.00374 g) were smaller than those reared on true leaves (least-square mean 0.07912 ± 0.00501 g), which were smaller than those reared on mature phyllodes (least-square mean 0.08532 ± 0.00380 g). However, pupal weight only differed significantly between groups reared on mature vs. young phyllodes (P = 0.0040), and pupal weight did not differ significantly between true leaves and either young (P = 0.4045) or mature (P = 0.2218) phyllodes.

Significant variation among trees in their suitability as host plants was detected for pupal mass ($\chi^2 = 11.1$, $P = 0.0004$), but not for development time ($\chi^2 = 0.7$, $P = 0.2013$). Sex significantly influenced *S. paludicola* development time ($F_{1,71.7} = 19.53$, P < 0.0001) and pupal mass ($F_{1,67.3} = 86.08$, P < 0.0001), with females taking approximately five days longer to pupate and weighing nearly twice as much as male pupae.

Discussion

In this study, we provide clear evidence that *A. koa* leaves differ in their suitability for *S. paludicola* development. Caterpillars reared on true leaves suffered from higher mortality and took longer to develop than those reared on phyllodes. This suggests that juvenile *A. koa* trees that retain their true leaves are more resistant than mature trees to herbivory by this specialist herbivore, though young trees are likely less tolerant to defoliation, due to lower capacity to store resources in the trunk and roots and slower growth rates following damage (Stevens et al.,

2008, Nykänen and Koricheva 2004). An ontogenetic decline in resistance is uncommon in tropical woody plants, although it is common in boreal forest where ground-dwelling mammals exert strong selection pressure for defense in juveniles (Barton and Koricheva 2010, Swihart and Bryant 2001). Furthermore, the tendency for some *A. koa* to produce more resistant true leaves through epicormic growth following *S. paludicola* outbreaks (Haines et al. 2009) may be considered a form of induced resistance in response to these extreme defoliations. To determine whether induced resistance via true leaf development contributes to local declines in *S. paludicola* outbreaks requires further research. Field studies that quantify *S. paludicola* population dynamics on trees that vary in their development of true leaves vs. phyllodes following outbreak defoliations would be particularly enlightening.

Caterpillar development was also affected by phyllode age when reared on phyllodes. *Scotorythra paludicola* caterpillars took longer to develop on mature than on young phyllodes, but they were also ultimately larger, even after taking into account the strong sexual dimorphism in size. Although a longer larval stage poses additional risk for predation and parasitism, this cost may be outweighed by the benefit gained in pupal size, given the strong relationship between pupal size and reproductive fitness in Lepidoptera (Honek 1993).

Faster development time on young phyllodes has important implications regarding the causes of *S. paludicola* outbreaks, which are as yet undetermined (Haines et al. 2009). Caterpillars of *S. paludiciola* are known to be impacted by native and non-native predators, parasitoids, and pathogens (Haines et al. 2009), but it is unknown to what extent these top-down pressures affect population fluctuations.

Our data demonstrate that bottom-up factors are potentially important regulatory factors as well. Although much more ecological data will be required to elucidate the causes of *S. paludicola* outbreaks, they could be caused by rare interactions that optimize conditions for caterpillar survival. For example, an increased availability of young phyllodes could cause a sudden population increase, especially if it happens to be synchronized with an unrelated release from top-down regulatory factors such as parasitism or disease.

Although it is clear that *S. paludicola* caterpillars are strongly affected by *A. koa* leaf type and age, the traits underlying this resistance remain unknown. Further research is needed to investigate whether *A. koa* resistance is chemical (i.e., phenolics, alkaloids), physical (i.e., toughness, trichomes), or nutritional (i.e. nutrient content or availability), and to demonstrate how these traits differ among leaf types. The significant variation detected among trees for pupal mass (but not development time) indicates that besides the variation due to leaf type, *A. koa* trees vary in their resistance to *S. paludicola*, suggesting the possibility for further evolution by natural selection of resistance traits. Thus, the *A. koa*–*S. paludicola* interaction presents an ideal system for studying the evolution in action of island plant defenses, and herbivore offenses.

Acknowledgments

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