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EFFECTS OF HOSTPLANT SPECIES AND ARTIFICIAL DIET ON GROWTH OF BUCKEYE (*JUNONIA COENIA*) AND PAINTED LADY (*VANESSA CARDUI*) CATERPILLARS (NYMPHALIDAE)

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ABSTRACT. We tested the effect of artificial diets and hostplant species on growth of larvae of the Buckeye (*Junonia coenia*) and the Painted Lady (*Vanessa cardui*) in the laboratory. The two hostplant species, *Plantago lanceolata* and *Plantago major* (Plantaginaceae), both contain a particular class of plant secondary compounds, iridoid glycosides. Two artificial diets were also used, one containing dried, ground *P. lanceolata* leaves and the other containing dried, ground *P. major* leaves. In the laboratory, Buckeye larvae, specialists on plants containing iridoid glycosides, grew best on leaf diets; Painted Ladies, a generalist species, showed the opposite trend, exhibiting a higher growth rate on artificial diets. Pupation and survival rates, used as indicators of larval fitness, were also affected by diet. Buckeye larvae feeding on *Plantago* leaves had higher survival rates and pupated sooner than larvae feeding on artificial diets. Painted Lady caterpillars pupated soonest on the artificial diet with *P. major* and had the lowest survival on the artificial diet with *P. lanceolata*. In a complementary field experiment conducted in an experimental garden with both caterpillar species reared on the two *Plantago* species, Painted Lady larvae grew equally well on the two hostplant species, while Buckeye larvae performed significantly better on *P. lanceolata*. The results of these experiments suggest that, for some caterpillar species, laboratory experiments and field experiments may provide different information about larval performance.

Additional key words: iridoid glycosides, *Junonia coenia*, plant-insect interactions, *Plantago*, *Vanessa cardui*.

Generalist and specialist herbivores have been predicted to have different abilities to utilize particular hostplant species, and the chemical compounds that they contain. Specifically, specialists have been predicted to be more efficient at finding, feeding on, digesting, and detoxifying their hostplant than generalists feeding on the same foods (Feeny 1976, Cates 1980, Fox & Morrow 1981, Wiklund 1982). Tests of this prediction, however, have yielded equivocal results: in some cases specialists do perform better than generalists on a particular hostplant or hostplant chemical (e.g., Blau et al. 1978, Kraft & Denno 1982, Bowers & Puttick 1988, 1989), whereas in other cases this is not so (e.g., Scriber & Feeny 1979, Futuyma & Wasserman 1981).

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To further compare the characteristics of generalist and specialist insect herbivores, we examined the performance of larvae of two nymphalid butterflies, on leaf diets and artificial diets containing ground leaves of the same hostplants. As the specialist, we used larvae of the Buckeye, *Junonia coenia* Hübner, and as the generalist, we used larvae of the Painted Lady, *Vanessa cardui* L. Larvae of *J. coenia* specialize on plants containing iridoid glycosides and have been recorded from plants in five families, Plantaginaceae, Scrophulariaceae, Acanthaceae, Verbenaceae, and Cornaceae (Bowers 1984, Scott 1986), all of which contain iridoid glycosides (Bowers 1984). Although larvae of *V. cardui* are quite general in their feeding habits, using plants in over 20 families (Scott 1986, Garrigan 1994), including the Plantaginaceae and Verbenaceae, they apparently prefer various composite species (Scott 1986). There does not appear to be any particular chemical compound(s) involved in determining this species' hostplant range.

Two common hostplant species that *J. coenia* and *V. cardui* share are narrow-leafed plantain, *Plantago lanceolata* L. and common plantain, *Plantago major* L. (Plantaginaceae) (Bowers 1984, Scott 1986). Both these plant species contain iridoid glycosides: *P. lanceolata* contains aucubin and catalpol (Duff et al. 1965, Bowers & Stamp 1992, 1993, Bowers et al. 1992) and *P. major* contains only aucubin (Duff et al. 1965, Bowers, unpubl. data).

We performed two experiments, one in the lab and one in the field, to determine how these two hostplant species affected growth, survival, and development time of *J. coenia* and *V. cardui*. We also wanted to compare the ability of these two caterpillar species to grow and survive on artificial diets, so we reared larvae on one of two different artificial diets: one containing large amounts of dried, ground leaves of *P. lanceolata*, and thus high in both aucubin and catalpol; the other containing a trace of *P. major*, thus containing small amounts of aucubin.

MATERIALS AND METHODS

Study organisms. The Buckeye is found primarily in the south and west of the U.S. (Scott 1986). Despite the toxicity of iridoids to some insect species (Bowers & Puttick 1988, 1989), iridoids function as feeding and oviposition stimulants for Buckeyes (Bowers 1984, Pereyra & Bowers 1988). In the field, Buckeyes have 1–3 broods per year (Scott 1986). The Painted Lady occurs in many of the same habitats as Buckeyes, but has a more cosmopolitan distribution around the world (Garrigan 1994). Painted Ladies are known to have mass migrations, north in the spring and again south in the late fall (Brown 1974, Tilden 1962, Scott 1986, Shields 1992). During their growing season, they typically produce 2–4 broods per year (Opler & Krizek 1984, Scott 1986).

The caterpillars for both experiments originated from well-established lab colonies. The Painted Ladies were obtained from a commercial colony in North Carolina (Carolina Biological), where they are maintained on an artificial diet that contains primarily ground leaves of mallow (*Malva* sp., Malvaceae), a commonly used hostplant (Scott 1986, Garrigan 1994). The Buckeyes came from a colony maintained at the University of Colorado, and fed on leaves of *P. lanceolata* or on an artificial diet containing ground leaves of *P. lanceolata*.

Plantago lanceolata and *Plantago major* are weedy annuals or short-lived perennials that contain iridoid glycosides in their leaves, stalks, and inflorescences (Bowers & Stamp 1992, Bowers et al. 1992). Iridoids are cyclopentanoid monoterpene-derived plant secondary chemicals that are found in members of more than 50 plant families (Jensen 1991). Iridoid content in these plantain species varies both among plants and among leaves on an individual plant (Bowers & Stamp 1992, 1993). The leaf iridoid content of *Plantago lanceolata* plants varies from 1–12% dry weight of the leaf, with new leaves having a higher concentration than mature leaves (Bowers & Stamp, 1992, Bowers et al. 1992). Aucubin concentrations of *Plantago major* range from 0.28 to 1.03% of the leaf dry weight (Bowers, unpubl. data).

A higher iridoid content of *Plantago* leaves increases its oviposition attractiveness to specialists (Klockars et al. 1993) and might therefore increase herbivory by specialists. However, higher iridoid levels have been shown to be toxic and deterrent to generalists such as the gypsy moth, *Lymantria dispar* L. (Lymantriidae) (Bowers & Puttick 1988). Thus, the iridoid content of these *Plantago* plants may have significant growth and survival implications for both the plants and their insect herbivores.

Laboratory experiment. Painted Ladies and Buckeyes were reared on each of four experimental diets. Two were leaf diets of *Plantago lanceolata* or *Plantago major*, and two were artificial diets. For the leaf diets, leaves were collected weekly from mature plants in the vicinity of the University of Colorado, Boulder campus and refrigerated in a moist bag. All leaves were washed prior to use. The artificial diets were prepared following a basic recipe developed by F. Nijhout (Duke University) (Table 1). The artificial diet was modified by adding either 0.5 g dried, powered *P. major* leaves (AD w/ Pm) or 5.1 g dried, powered *P. lanceolata* leaves (AD w/ Pl). The addition of this small amount of leaf material to the AD w/ Pm was necessary for the specialist species to eat the diet and *P. major* was chosen because its leaves have a lower overall iridoid concentration than *P. lanceolata* (*P. major* leaf iridoid content = 0.55% (±0.05 SE) dry weight, n = 15; Bowers, unpubl. data). The artificial diet with *P. lanceolata* leaves has an iridoid glycoside concentration similar to what might be found in an average plant leaf, albeit rather low (see Bowers & Stamp 1992).

TABLE 1. Components of artificial diets used to feed *J. coenia* and *V. cardui* in the laboratory experiments.

Ingredient	Amount or Volume
Cellulose (alphacel)	1.7 g
Sucrose	6.3 g
Wesson salts	2.5 g
Wheat germ	16.3 g
Cholesterol	0.3 g
Vitamin and inhibitor mix	3.6 g
Linseed oil	1.0 ml
Formalin (10%)	0.5 ml
Agar	4.1 g
Water	227.0 ml
Dried, ground *Plantago*	
If *P. major*	0.5 g
If *P. lanceolata*	5.1 g

Eighty Buckeye and 80 Painted Lady caterpillars were taken from eggs hatched on each of the diets. The caterpillars fed *ad libitum* on their experimental diets from egg hatching and throughout the experiment. As larvae molted to the third instar, 20 caterpillars of each species were weighed and reared through to pupation on each of the four diets. Each larva was kept in a separate 150 mm Ξ 50 mm petri dish, with humidity maintained by a moist piece of paper towel taped to the lid. They were reared in growth chambers set to a constant 25°C temperature and 15L:9D photoperiod. The caterpillars were weighed every second day throughout their development and until pupation. Mortality and development time were recorded.

Field experiment. The complementary field experiment was carried out in an experimental garden near the University of Colorado, Boulder. Thirty *P. lanceolata* and thirty *P. major* plants were collected in early June 1993, from the Boulder area, potted and allowed to acclimate in the greenhouse. On 2 July, they were transplanted into the experimental garden, 0.5 m apart, in single-species rows of 10 plants each. All plants were surrounded by a ring of 15 cm high aluminum edging with a band of Tanglefoot© to prevent the caterpillars from escaping and terrestrial arthropod predators from entering. In addition, to prevent bird predation and deer herbivory, each row was covered with screening held 32 cm above the plant by wooden stakes. Plants were monitored and watered daily.

On 14 July 1993, after plants had recovered from transplanting, one newly molted third instar Buckeye and one newly molted third instar Painted Lady were placed on each of these 60 plants. Larvae were weighed every other day and reared until they molted to the fifth instar.

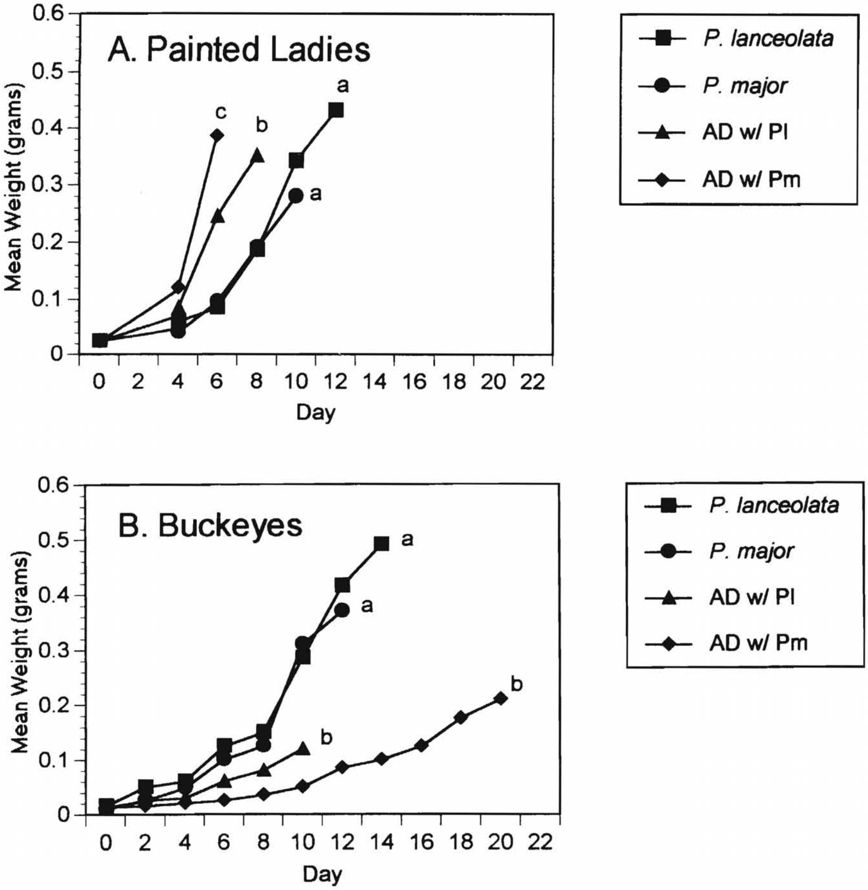

FIG. 1. Growth of the generalist, Painted Ladies (A) and the specialist, Buckeyes (B) on four diets: leaves of *Plantago lanceolata*, leaves of *P. major*, artificial diet with *P. lanceolata* leaves (AD w/ Pl), artificial diet with *P. major* leaves (AD w/ Pm). Lines followed by different letters indicate different growth rates as indicated by Sheffe's post-hoc comparisons.

After weighing, each larva was returned to the plant from which it came. Individuals noted to be missing or dead were not replaced.

RESULTS

Laboratory experiment. The generalist *V. cardui* caterpillars grew two to three times faster than the Buckeyes (Fig. 1A, B). In addition, these *V. cardui* caterpillars grew at different rates on the four diets (Fig. 1A, repeated measures ANOVA, diet factor, $F = 28.43$, $df = 3, 114$, $p < 0.001$) and grew best on the artificial diet with *P. major* (AD w/ Pm)

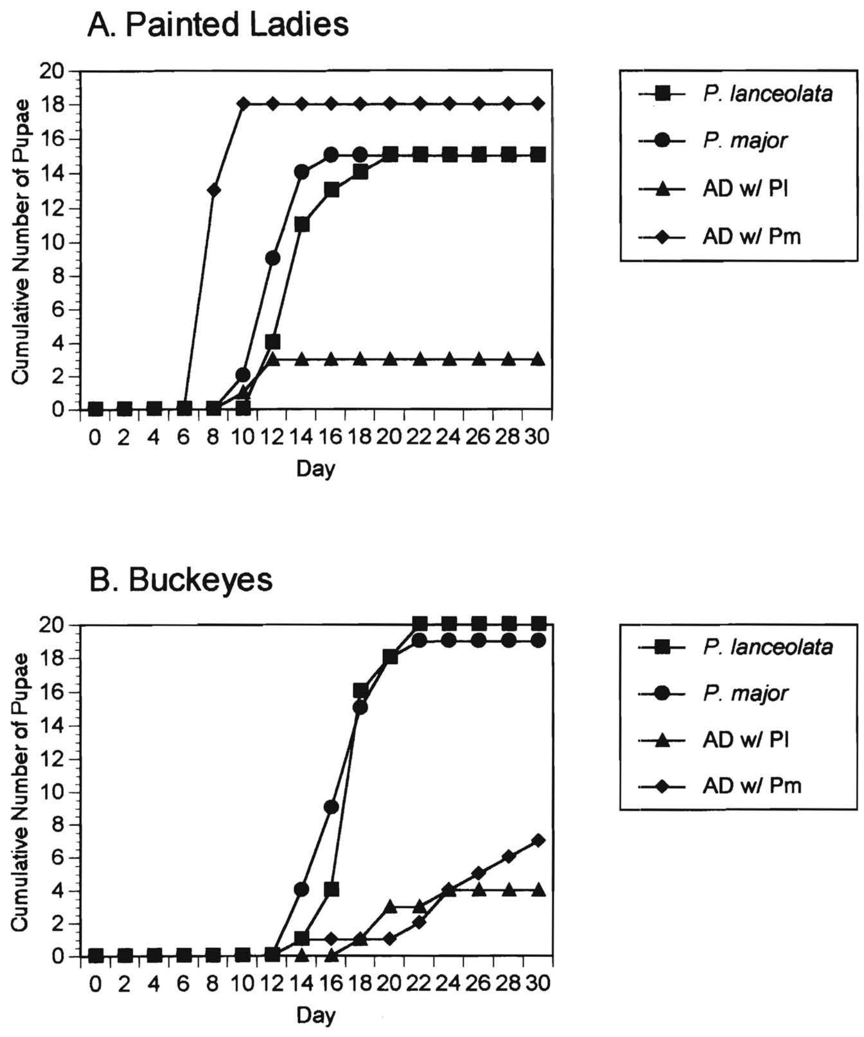

FIG. 2. Time to pupation for the generalist, Painted Ladies (A) and the specialist, Buckeyes (B) on four diets: leaves of *Plantago lanceolata*, leaves of *P. major*, artificial diet with *P. lanceolata* leaves (AD w/ Pl), artificial diet with *P. major* leaves (AD w/ Pm).

(Fig. 1A) and most poorly on the two *Plantago* leaf diets (Fig. 1A). Buckeyes also grew at different rates on the four diets (Fig. 1B, repeated measures ANOVA, diet factor, F = 24.11, df = 3, 280, $p < 0.001$), however, they grew best on the leaf diets (Fig. 1B). Sheffe's post-hoc comparisons showed that Painted Lady larvae grew similarly on both *P.*

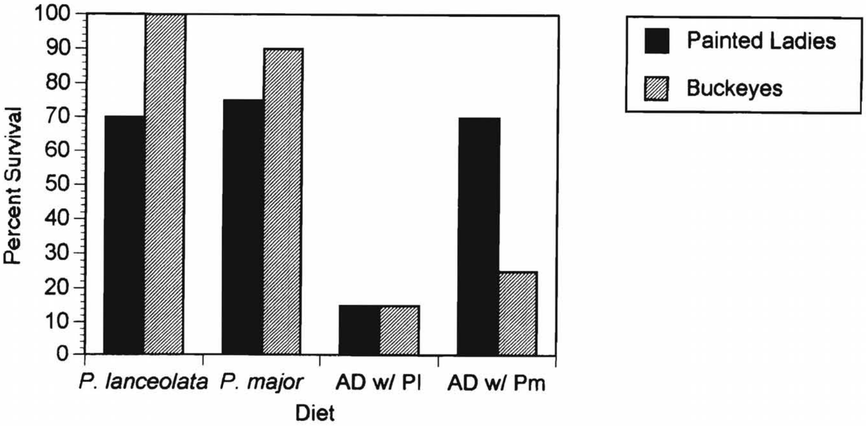

FIG. 3. Survival of Painted Ladies and Buckeyes on four different diets: leaves of *Plantago lanceolata*, leaves of *P. major*, artificial diet with *P. lanceolata* leaves (AD w/ Pl), artificial diet with *P. major* leaves (AD w/ Pm).

lanceolata and *P. major* (Fig. 1A). This was also true for Buckeye larvae (Fig. 1B).

Time to pupation also differed between the two caterpillar species (Fig. 2A, B). The Painted Ladies began to pupate after 8 days, whereas the Buckeyes did not begin to pupate until 14 days (Fig. 2A, B). The diet of the caterpillars also affected time to pupation: Painted Ladies pupated earliest on the artificial diets (Mann-Whitney *U*-test, $z = -2.16$, $p < 0.001$), whereas Buckeyes pupated earliest on the leaf diets (Mann-Whitney *U*-test, $z = -4.44$, $p = 0.003$) (Fig. 2A, B).

Survival of Buckeye larvae varied significantly among diets ($\chi^2 = 9.92$, df = 3, $p = 0.025$), but that of Painted Ladies did not ($\chi^2 = 5.94$, df = 3, $p > 0.05$) (Fig. 3). Survival of the two caterpillar species differed on two of the diets: *P. lanceolata* leaves ($\chi^2 = 7.06$, df = 1, $p > 0.01$) and AD with *P. major* (AD w/ Pm) ($\chi^2 = 8.12$, df = 1, $p < 0.005$). However, there was no significant difference in survival of the two caterpillar species on *P. major* leaves ($\chi^2 = 1.56$, df = 1, $p > 0.05$) or on the AD w/ Pl, in which both species had mortality of over 80% (Fig. 3).

Field experiment. Painted Ladies grew faster on both *P. lanceolata* and *P. major* than did Buckeyes, as shown by the difference in the log-transformed biomass at eight days for these two caterpillar species (Fig. 4, two-way ANOVA, species, $F = 16.45$, df = 1,28, $p < 0.001$). There was no effect of diet on biomass at eight days (Fig. 4, $F = 2.64$, df = 1,28, $p = 0.115$); but there was a significant interaction (Fig. 4, $F = 4.71$, df = 1,28, $p < 0.05$), indicating that the two caterpillar species responded differently to the two host plant species.

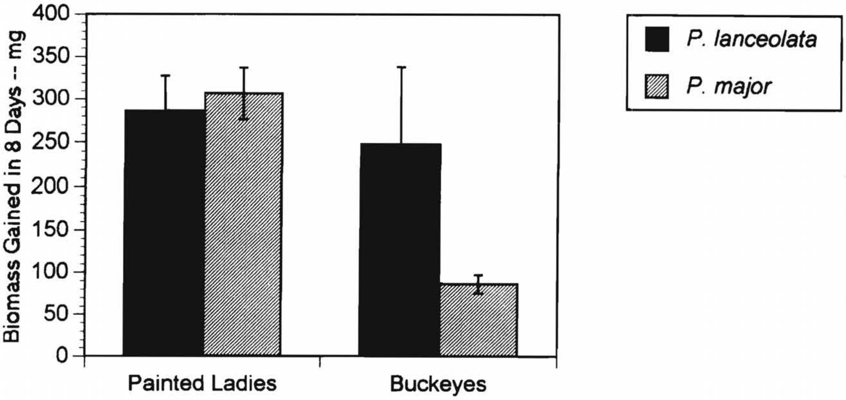

FIG. 4. Biomass gained in eight days by Painted Ladies and Buckeyes fed in the field, on either *P. lanceolata* or *P. major*. Means with standard errors are shown.

DISCUSSION

Explanations for the predominance of specialists among the Lepidoptera (and indeed herbivorous insects in general) have included host availability, predation, and hostplant chemistry (Dethier 1954, Fraenkel 1959, 1969, Ehrlich & Raven 1964, Smiley 1978, Bernays & Graham 1988, Bernays & Chapman 1994). Particularly well-studied is the idea that generalists and specialists may differ in their ability to cope with a particular set of plant chemicals: specialists may be more tolerant of or more efficient at detoxifying or tolerating these chemicals than generalists (Dethier 1954, Ehrlich & Raven 1964, Cates 1980). Our data show that experiments done in the field compared to those done in the laboratory may provide different degrees of support for this idea.

Our data from the laboratory experiments indicate that the specialist, *J. coenia*, grows better and has higher survival when fed on *P. lanceolata* or *P. major* leaf diets, and that the generalist *V. cardui* has reduced growth and fitness on these diets. One reason for this ability of *V. cardui* to perform well on artificial diets is that these larvae were from a colony maintained on artificial diet by Carolina Biological. Although our Buckeye colony was also fed artificial diets during the winter months when plant leaves are not available; they were fed leaves whenever available. Thus in the laboratory experiment, adaptation to artificial diet by *V. cardui* may be an important reason for the differences we observed between the two caterpillar species.

However, the results of the laboratory experiment also revealed that if we consider only the results for larvae reared on plant material (*P. lanceolata* or *P. major*), growth of Painted Lady larvae did not differ on the two hostplant species (Fig. 1A), nor did that of *J. coenia* (Fig. 1B). However, the data from the field experiments do not confirm these observations: the Painted lady larvae grew faster than the Buckeye larvae on *P. major*, and the two species grew at similar rates on *P. lanceolata*.

Although in the laboratory Buckeye larvae grew equally well on the two different *Plantago* species, their performance in the field was quite different on these two plants: larvae on *P. major* attained approximately one-quarter the mass of larvae on *P. lanceolata*. Reasons for the difference between the laboratory and field experiments are not known, but may be due to a variety of factors. For example, there may have been differences in hostplant quality due to differences in the sources of the plant material fed to the larvae. For the field experiment, larvae were confined to plants grown in our experimental garden, where the plants were watered as necessary, but were not fertilized in any way. Leaves fed to caterpillars in the laboratory experiment were collected from naturally occurring populations located around the University of Colorado campus, usually in lawns that were fertilized and frequently watered. Although we did not measure the iridoid glycoside, water or nitrogen content of plants used in the laboratory or field experiments, differences in these features of plants from these two different sources may have contributed to the differences noted between the laboratory and field experiments.

Another reason for differences between the laboratory and field experiments may be related to the ability of larvae in the field to choose what part of the plant on which to feed. Leaves of *P. lanceolata* vary in their iridoid glycoside content, with newer leaves being high in iridoid glycosides (up to 12% dry weight, Klockars et al. 1993) and older leaves being low (2% to unmeasurable amounts of iridoid glycosides, Klockars et al. 1993). It is likely that a similar pattern occurs in *P. major*. In the field, caterpillars may have been able to choose certain leaves over others, but in the laboratory they were forced to eat the leaves we provided. The potential to choose leaves may have allowed caterpillars in the field to attain more similar growth rates on the two *Plantago* species than would have been predicted from the laboratory experiments.

In conclusion, our data suggest that experiments designed to compare the performance of generalist and specialist insects on particular hostplant species may yield different results when they are performed in the laboratory versus in the field. Although there may be difficulties in conducting field experiments, the use of entire, intact plants growing in relatively natural conditions; the ability of insects to make choices about on what, when and where to feed; and the exposure of both plants and insects to natural fluctuations in temperature, light, humidity, and water, may provide different information about insect performance on hostplants than experiments conducted under laboratory conditions.

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