

PLANT DEFENSE THEORY PROVIDES INSIGHT INTO INTERACTIONS INVOLVING INBRED PLANTS AND INSECT HERBIVORES

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Abstract. Inbreeding in the form of self-fertilization is widespread among plants and typically results in broad, detrimental changes in plant morphology and physiology. Phenotypic changes associated with inbreeding are likely to alter interactions between inbred plants and other organisms, but few studies have investigated this potential. We found that inbreeding in the entire-leaf morning glory, *Ipomoea hederacea* var. *integriscula*, altered this plant's ability to resist and tolerate attack by insect herbivores. The effects of inbreeding on plant defense, however, varied among insect species, and plant defense theory helped explain this variation. If the effects of inbreeding on plant phenotype are analogous to those of environmental stresses, then the plant vigor hypothesis predicts specialist herbivores will perform better on outbred plants, and the plant stress hypothesis predicts that generalist herbivores will perform better on inbred plants. We conducted a series of greenhouse experiments in which we reared two species of specialist tortoise beetles, a generalist moth species, and a generalist aphid species on inbred and outbred morning glories to test these hypotheses. We found that specialist tortoise beetles performed significantly better when reared on outbred plants and that aphid populations grew significantly faster on inbred plants as predicted by the plant vigor and plant stress hypotheses, respectively. Beet armyworm caterpillars, however, performed better on outbred plants, not inbred plants as predicted. These results suggest that plant defense theories may be useful for predicting the effects of inbreeding on plant–herbivore interactions, but differences in herbivore feeding habit (leaf chewing vs. phloem feeding) may also help explain variation in the effects of plant inbreeding on insect herbivores.

Key words: *Aphis gossypii*; *Charidotella bicolor*; *Deloyala guttata*; herbivory; inbreeding; *Ipomoea hederacea*; plant defense; plant stress; plant vigor; self-fertilization; *Spodoptera exigua*.

INTRODUCTION

More than half of all flowering plants retain the capacity to self-pollinate (Barrett and Eckert 1990), and it is estimated that half of all higher plant species self-pollinate 20–40% of the time (Vogler and Kalisz 2001). Progeny produced as a result of self-pollination usually incur a fitness cost referred to as inbreeding depression (see Husband and Schemske [1996] for a recent review), and it is important to understand the magnitude of inbreeding depression to predict the effect of self-fertilization on plant population dynamics and the evolutionary dynamics of plant sexual systems (Ashman 2002). Almost all studies of inbreeding depression, however, have been conducted in the greenhouse (review by Charlesworth and Charlesworth [1987]). Recent studies suggest that the effects of inbreeding depression may be more severe under field conditions than in the greenhouse and that greenhouse studies may not provide accurate estimates of inbreeding depression (Ramsey and Vaughton 1998, Keller and Waller 2002).

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Dudash (1990), for example, reported that inbreeding depression as measured by survival and flower production was more severe in the field in rose-pink *Sabatia* plants, *Sabatia angularis* (L.) Pursh. (Gentianaceae). Likewise, Heschel and Paige (1995) compared the responses of 10 populations of scarlet gilia, *Ipomopsis aggregata* (Pursh) V. Grant (Polemoniaceae), to stress and found that smaller, more inbred populations were more susceptible to environmental stress than larger, outbred populations. These studies suggest that environmental stresses may exacerbate the effects of inbreeding.

One environmental stress common to all plants, but relatively understudied in inbred plants, is exposure to natural enemies. Resistance or tolerance are two strategies plants may use to defend themselves against natural enemies such as herbivores, parasites, and pathogens. Plants can reduce the amount of damage they experience (resistance) through chemical or mechanical means (Strauss et al. [2002] for review), or they can tolerate damage by increasing growth to maintain overall photosynthetic area or by replacing damaged parts (Mauricio et al. 1997). Inbreeding may alter the capacity of a plant to resist or tolerate damage. A study of the *Silene-Microbotryum* pathosystem found that some genotypes of inbred white campion, *Silene alba*

TABLE 1. Hypotheses: predictions and tests.

Hypotheses	Type of herbivore used in test	Predictions		
		Highest preference	Highest performance	Highest resistance
Plant vigor	specialists			
	golden tortoise beetles mottled tortoise beetles	outbred plants outbred plants	outbred plants outbred plants	inbred plants inbred plants
Plant stress	generalists			
	beet armyworms cotton aphids	inbred plants inbred plants	inbred plants inbred plants	outbred plants outbred plants

Notes: The plant vigor hypothesis predicts that specialist herbivores like golden and mottled tortoise beetles will prefer and perform better when reared on outbred plants than inbred plants. Conversely, this hypothesis predicts that inbred plants will have higher resistance to specialist herbivores and consequently suffer less damage. The plant stress hypothesis predicts the opposite effects of inbreeding level on generalist herbivores: generalist herbivores such as beet armyworm caterpillars and cotton aphids will prefer and perform better when reared on inbred plants than outbred plants and outbred plants will suffer less damage from generalist herbivores as a consequence.

(Mill.) Krause (Caryophyllaceae), had increased resistance to the anther-smut fungus *Microbotryum violaceum* (Persoon: Persoon) G. Deml & Oberwinkler (Basidiomycetes), whereas other inbred genotypes experienced a decrease in resistance (Ouborg et al. 2000). Carr and Eubanks (2002) found that inbred *Mimulus guttatus* Greene (Scrophulariaceae) were both less tolerant and less resistant when attacked by the spittlebug *Philaenus spumarius* (L.) (Cercopidae). However, Ivey et al. (2004) found that there was considerable variation within and among *M. guttatus* populations in the effects of inbreeding on tolerance to spittlebugs. Likewise, Hayes et al. (2004) found that damage of *Cucurbita pepo* ssp. *texana* (Cucurbitaceae) caused by *Diabrotica* beetles increased linearly with increasing levels of inbreeding depression. Thus, it appears that inbreeding can affect interactions between plants and natural enemies, but that the effects may vary within and among systems.

The mechanisms behind these interactions are poorly understood. We can speculate that inbreeding affects host plant quality. Spittlebugs had an increased biomass at pupation when fed inbred monkey flowers (Carr and Eubanks 2002), implying that inbreeding increased plant quality. *Diabrotica* beetles were found to preferentially attack *C. pepo* inbred seedlings (Hayes et al. 2004), and it is known that *Diabrotica* beetles can discriminate between plants based on cucurbitacin content (Tallamy and Krischik 1989), implying that inbreeding may affect cucurbitacin concentrations. However, more work is needed to determine how inbreeding affects mechanical or biochemical resistance mechanisms to increase host-plant quality.

One way to predict the effects of self-pollination on tolerance and resistance against natural enemies may be to relate inbreeding depression to environmental stress. The phenotypic effects of inbreeding, including reduced growth, reduced biomass, and reduced reproductive success of species in many plant families have been extensively documented (Charlesworth and Charlesworth 1987, Husband and Schemske 1996). The phenotypic effects of inbreeding are similar to the phe-

notypic effects caused by environmental stresses. For example, drought and water stress produce complex responses in plants and can significantly reduce plant biomass and survivorship (Chaves et al. 2003). Likewise, the phenotypic effect of environmental stresses, such as drought, alters the resistance and tolerance of plants to insect herbivores (White 1969, Koricheva et al. 1998). Fortunately, several plant defense theories have been developed to help predict the response of stressed plants to insect herbivores (Price 1991, Stamp 2003), and it may be possible to use these theories to predict the outcome of interactions between inbred plants and insect herbivores.

The plant vigor hypothesis predicts that healthy, rapidly growing or vigorous plants have relatively high nutritional value and relatively high levels of defense (Price 1991, Karhu and Neuvonen 1998). Specialist herbivores are more greatly affected by host-plant quality than plant defenses because they have supposedly evolved the counter-adaptations necessary to overcome plant defenses (Price 1991, Schowalter et al. 1999, Fritz et al. 2003). Consequently, specialist herbivores should prefer and perform better when reared on healthy, rapidly growing, vigorous plants. Alternatively, the plant stress hypothesis states that generalist herbivores are affected by plant defenses more than by plant quality. Therefore, they should prefer and perform better when reared on relatively stressed plants that will be poorly defended when compared to non-stressed plants (White 1969, Schowalter et al. 1999). The plant vigor and plant stress hypotheses are not mutually exclusive, so it is essential to expose inbred plants to both specialist and generalist herbivores when assessing the vulnerability of inbred plants.

Four predictions can be made for interactions between inbred plants and insect herbivores using the plant vigor and the plant stress defense hypotheses (Table 1). Under the plant vigor hypothesis, outbred plants are equated to vigorous plants (Hayes et al. 2004) with a relatively high nutritional value and inbred plants are equated to less vigorous plants with a low nutritional value (Sorensen and Campbell 1997). Thus, it is pre-

dicted that: (1) specialist herbivores will be attracted to and perform better on outbred plants; and (2) inbred plants will have relatively high resistance to specialist herbivores (Table 1). Consequently, outbred plants will suffer greater relative damage and fitness loss due to specialist herbivore attack. Under the plant stress hypothesis, inbred plants are equated to stressed plants with altered host-plant quality (Hayes et al. 2004) and compromised defenses. It is predicted that: (3) generalist herbivores are affected by plant defenses more than plant quality; therefore, generalist herbivores will prefer and perform better when reared on inbred (stressed) plants; and (4) outbred plants have a relatively high resistance to generalist herbivores and consequently inbred plants will suffer relatively greater damage and fitness loss due to generalist herbivore attack (Table 1).

MATERIALS AND METHODS

Study system

Inbreeding is common in the entire-leaf morning glory, *Ipomoea hederacea* var. *integriuscula*, although its mating system varies widely. Outcrossing rates vary from almost complete selfing (90%, $t = 0.1$) to almost complete outcrossing (5%, $t = 0.95$), with a species mean $t \approx 0.6$ (Hull-Sanders 2004). A broad range of insects attack *I. hederacea* plants in the field, including chrysomelid and curculionid beetles, beet and southern armyworm caterpillars, cotton aphids, leaf hoppers, and grasshoppers (H. M. Hull-Sanders, *personal observation*). Two specialist herbivores collected from field populations of *I. hederacea*, the mottled tortoise beetle, *Deloyala guttata* (Oliv.), and the golden tortoise beetle, *Charidotella bicolor* (F.) (formerly known as *Metriona bicolor*) (Coleoptera: Chrysomelidae: Cassidinae), were kept in colony and used for plant vigor experiments (Table 1). Although the exact defense mechanisms in this plant are not known, other *Ipomoea* species have been found to have constitutive defenses such as polyphenol oxidases localized in the latex of laticifers (Schadel and Walter 1981). Despite these high levels of defense, specialist herbivores have been shown to avoid latex-bearing secretory canals and consume large amounts of leaf tissue (Dussourd and Denno 1991). Specialist herbivores can act as an agent of selection in *Ipomoea* (Simms and Rausher 1989), thus implying a high level of coevolution that may be highly sensitive to alterations in plant quality. Specialist beetles obtained from the field were raised in growth chambers with a photoperiod of 16L:8D cycle at $27 \pm 2^\circ\text{C}$ and 90% relative humidity. Generalist beet armyworm caterpillars and cotton aphids were used to test the plant stress hypothesis (Table 1). Beet armyworm caterpillars, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), were obtained from either a colony maintained at Auburn University, Auburn, Alabama, USA, or from the USDA rearing facility in Stoneville, Mississippi,

USA. Generalist cotton aphids, *Aphis gossypii* Glover (Homoptera: Aphididae), experiments provided a contrast in feeding mechanisms to caterpillars (i.e., leaf-chewing vs. phloem-sap sucking). Cotton aphids were obtained from a colony maintained at Auburn University.

Inbred/outbred seed

In August 2000, *I. hederacea* seeds were collected from populations infesting cotton at the Auburn University EV Smith Research Center, Macon County, Alabama, USA. *I. hederacea* seeds were sown in 20 cm diameter pots with ProMix growing medium (Premier Horticulture, Dorval, Quebec, Canada). Eighteen plants were selected to generate 18 family lines. Flowers were allowed to self-pollinate without manipulation to produce inbred seed. Outbred seeds were produced by emasculating the anthers of closed flowers the afternoon before they opened by using a pair of sharp-tipped forceps to slit open flowers from the side and removing all the anthers. The anthers do not dehisce before 22:00 hours the night before opening and can be removed without the possibility of pollination (S. Chang, *personal communication*). Flowers were allowed to develop overnight and pollen was removed from undisturbed flowers from other plants and placed onto the stigma of the emasculated flower. Seeds developed within six weeks and were harvested upon maturation.

Inbred and outbred *I. hederacea* plants were grown in a greenhouse in 20 cm diameter pots using ProMix potting soil. Individual plants were covered with plastic 2-L cages with mesh sides to reduce the risk of attack by nonexperimental insects in experiments involving beetles and beet armyworms. For all experiments except cotton aphid experiments, plants were harvested after 45 days after the addition of herbivores by cutting the plant at soil level. Number of leaves, reproductive effort (flowers + buds), and total plant length (soil to apical meristem) were recorded. Soil was removed from the root system by soaking the plants in water and agitating by hand until only the roots remained. The leaves and stems were then placed in foil packets, dried at 45°C for seven days, and weighed to determine aboveground biomass (AGB) (leaves + stems) and belowground biomass (BGB) to the nearest 0.01 g.

Plant vigor hypothesis

Between December 2000 and August 2002, six blocks of 20 plants each were arranged in a 2×2 completely randomized block design with two levels of pollination treatment (outcrossed and selfed) and two levels of insect treatment (with and without insects). Combinations of five first-instar larvae of both beetle species were placed on treatment plants after five true leaves had emerged and were allowed to feed until pupation (~ 7 days). Population densities of early instar beetles on young plants are unknown. However,

this density is within the range observed for whole plants in the field (H. M. Hull-Sanders, *personal observation*). The fresh mass of pupae was measured using an electrobalance to the nearest 0.01 mg.

Choice experiments were conducted to determine if inbreeding affected the feeding preference of adult beetles. Outcrossed and self-fertilized plants were randomly paired and transplanted 20 cm apart into cages (75 × 30 × 33 cm) constructed of fine mesh mosquito netting (mesh size = 0.5 mm). Plants were allowed to grow undisturbed and remained covered until both plants had at least five leaves. An individual adult female beetle was released into each cage. The experiment was replicated 42 times with golden tortoise beetles and 22 times with mottled tortoise beetles. Specialist beetles were allowed to feed for seven days. After seven days, the insects and plants were collected, and the number of leaves damaged on each plant were recorded.

Plant stress hypothesis

Between August 2001 and August 2002, 11 blocks of 36 inbred and outbred *I. hederacea* plants were arranged in a 2 × 2 completely randomized block design in the greenhouse as described above. One beet armyworm caterpillar was placed on each treatment plant after five true leaves had emerged. Beet armyworm caterpillars were allowed to feed until pupation (~10 days).

In the laboratory, generalist beet armyworm performance experiments were conducted in a 4 × 2 completely randomized block design experiment with four maternal family genotypes and two types of pollination treatment (outcrossed and self-fertilized) with 12 replications. Larvae were fed fresh leaves daily from greenhouse plants until pupation. The number of days until pupation and fresh mass at pupation were recorded.

Generalist cotton aphid population growth experiments were conducted to determine if additional generalist herbivores responded differently to plant inbreeding. Between February and September 2003, four blocks of 32 plants each were arranged in an 8 × 2 completely randomized block design for eight replications, with eight maternal family genotypes and two levels of pollination (outcrossed and selfed) all receiving aphids. Four cotton aphids were placed on each treated plant. Aphid population growth was monitored by counting the number of cotton aphids on each plant once per week for four weeks.

To determine the effects of generalist cotton aphids on inbred and outbred plants, an additional experiment of four blocks of 32 plants each were arranged with two levels of pollination (outcrossed and selfed) and two levels of damage (damaged plants receiving four initial aphids, undamaged plants receiving none). Aphid population growth was monitored as described above. After four weeks, all plants were harvested.

Data analysis

Data were analyzed using SAS 8.2 software (SAS Institute 2001). Beetle performance data (mass at pupation) were analyzed by analysis of variance (ANOVA), and preference data were analyzed by chi-square contingency table analysis. To examine inbreeding effects on tolerance to beetle feeding and allocation patterns of plant biomass, data were transformed as needed to conform to the assumptions of normality and variance. We used multivariate analysis of variance (MANOVA) to test (1) dry leaf mass and dry stem mass and (2) dry AGB and dry BGB. The full model used block, pollination treatment (outcrossed and selfed), insect treatment (with and without beetles), and the interaction between pollination and insect treatment as fixed effects. To determine which univariate responses might be driving the results of a significant MANOVA, we used ANOVA to examine the insect and pollination effects on leaf mass, stem mass, AGB, BGB, and reproductive effort.

Generalist beet armyworm performance data (mass at pupation and days to pupation) were analyzed by ANOVA using SAS (version 8.2; SAS Institute 2001) Proc GLM with plant family as a random effect and pollination as a fixed effect (Delwiche and Slaughter 1995). To examine inbreeding effects on plant tolerance to beet armyworm caterpillar feeding, data were transformed as needed and MANOVA was used to test dry leaf mass and dry stem mass. The full model used block, pollination treatment (outcrossed and selfed), insect treatment (with and without armyworms), and the interaction between pollination and insect treatment as fixed effects. To determine which univariate responses might be driving the results of a significant MANOVA, we used ANOVA to examine the insect and pollination effects on height, number of leaves, leaf mass, stem mass, AGB, and reproductive effort.

Preliminary analyses did not reveal any significant effect of plant family for aphid population growth nor plant growth, therefore nonsignificant random effects were pooled into the error term. Generalist cotton aphid performance data (population growth) were analyzed for week 4 by ANOVA using SAS (version 8.2; SAS Institute 2001) Proc GLM with block as a random effect and pollination treatment as a fixed effect (Delwiche and Slaughter 1995). To examine inbreeding effects on tolerance to cotton aphid feeding, data were transformed as needed and MANOVA was used to test dry AGB and dry BGB. To determine which univariate responses might be driving the results of a significant MANOVA, ANOVA was used. The full model included a block effect (random), the two pollination and insect treatment effects (fixed) plus the interactions for height, number of leaves, AGB, BGB, and reproductive effort.

RESULTS

Plant vigor hypothesis

Larvae of both species of specialist tortoise beetles performed better when fed outbred plants, and female

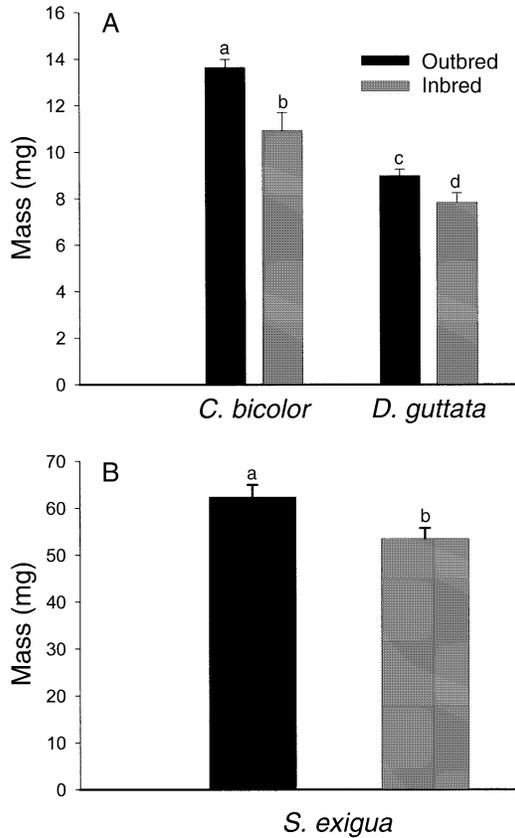


FIG. 1. Pupal mass (means + 1 SE) of (A) golden tortoise beetles, *Charidotella bicolor*, and mottled tortoise beetles, *Deloyala guttata*, and (B) beet armyworms, *Spodoptera exigua*, when fed leaves of outbred and inbred entire-leaf morning glory, *Ipomoea hederacea*. Letters above bars indicate significant differences ($P < 0.05$) based on Fisher's least significant differences (LSD; SAS Institute 2001). The study was conducted in greenhouses at Auburn University, Auburn, Alabama, USA.

golden tortoise beetles preferred to feed on outbred plants. Golden tortoise beetles and mottled tortoise beetles developed significantly larger pupae when reared on outbred plants (Fig. 1A). Golden tortoise beetle larvae reared on outbred plants were 12.76% larger at pupation than larvae reared on inbred plants (ANOVA, $F_{1,80} = 5.434$, $P = 0.022$), and mottled tortoise beetle larvae reared on outbred plants were 12.89% larger at pupation than larvae reared on inbred plants (ANOVA, $F_{1,42} = 5.144$, $P = 0.029$). In choice experiments, adult golden tortoise beetles fed on outbred plants 69% of the time ($\chi^2 = 7.00$, $df = 2$, $P = 0.03$), whereas adult mottled tortoise beetles fed on inbred and outbred plants nearly equally ($\chi^2 = 2.82$, $df = 2$, $P = 0.24$).

The MANOVA comparing treatment effects on biomass of plants fed on by tortoise beetles revealed significant effects of inbreeding (pollination effect, Wilks' lambda = 19.11, $P < 0.0001$), insect treatment (insect treatment effect, Wilks' lambda = 3.92, $P = 0.026$), and the interaction between pollination and insect treat-

ments (pollination \times insect effect, Wilks' lambda = 3.15, $P = 0.051$; Appendix A). Therefore, univariate responses to treatment effects were examined to determine which responses might be driving these results. Inbreeding significantly reduced the height, number of leaves, reproductive effort (flowers + buds), leaf and stem mass, AGB, and BGB (Appendix A). However, only stem mass showed a significant interaction with insect treatment. Outbred plants attacked by golden and mottled tortoise beetles were 24.9% smaller than outbred control plants, whereas inbred plants attacked by golden and mottled tortoise beetles were 15.04% larger than inbred control plants (Fig. 2A). BGB showed a significant insect treatment effect, but the interaction was only marginally significant. Outbred plants at-

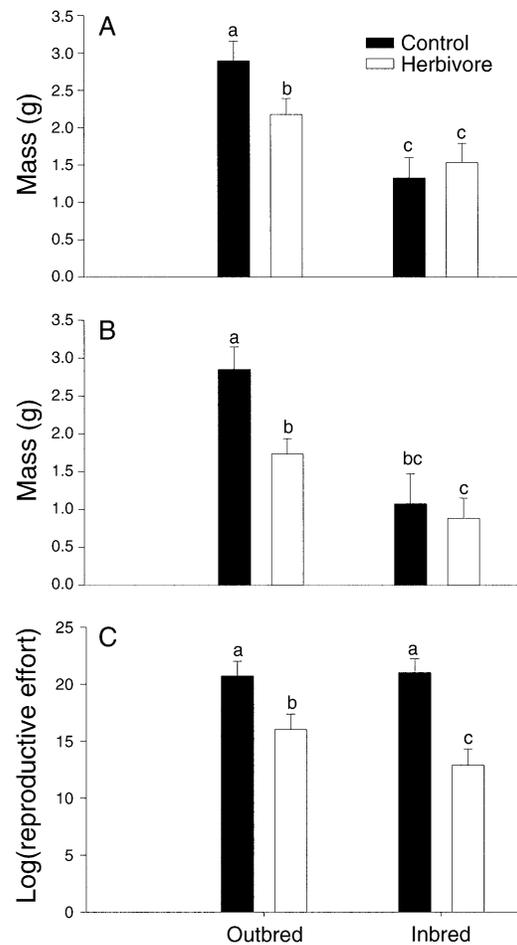


FIG. 2. Differences in entire-leaf morning glory, *Ipomoea hederacea*, performance (means + 1 SE) when attacked by herbivores: (A) aboveground biomass (AGB); (B) belowground biomass (BGB) of *Ipomoea hederacea* attacked by golden tortoise beetles, *Charidotella bicolor*, and mottled tortoise beetles, *Deloyala guttata*; and (C) reproductive effort (the sum of the number of flowers and buds) of plants attacked by beet armyworms, *Spodoptera exigua*. Letters above bars indicate significant differences ($P < 0.05$) based on Fisher's least significant difference (LSD; SAS Institute 2001).

tacked by tortoise beetles were 39.2% smaller than control outbred plants, and inbred plants attacked by golden and mottled tortoise beetles were 17.9% smaller than control inbred plants (Fig. 2B).

Plant stress hypothesis

Generalist beet armyworm caterpillars were 15.77% larger at pupation when reared on outbred plants (ANOVA, $F_{1,84} = 4.68$, $P = 0.033$; Fig. 1B). Plant family genotype did not affect beet armyworm mass at pupation. There was, however, a significant genotype \times pollination effect (ANOVA, $F_{3,84} = 6.67$, $P < 0.001$) with beet armyworm pupae consistently larger when fed outbred plants than inbred plants. On average beet armyworms needed two fewer days to reach pupation (ANOVA, $F_{1,84} = 9.61$, $P = 0.003$), and plant family genotype did not affect beet armyworm growth, and there was no genotype \times pollination effect.

The MANOVA comparing treatment effects on leaf and stem mass of plants attacked by beet armyworm caterpillars revealed significant effects of inbreeding (pollination effect, Wilks' lambda = 0.950, $P = 0.0004$) and insect treatment (insect treatment effect, Wilks' lambda = 0.903, $P < 0.0001$), but there was no interaction between pollination and insect treatments (pollination \times insect effect, Wilks' lambda = 0.999, $P = 0.8561$; Appendix B). Inbreeding significantly reduced height and stem mass (Appendix B). Outbred plants were 9.3% taller, and this resulted in 13.4% greater stem mass. Herbivory by beet armyworm caterpillars significantly affected plants regardless of plant breeding systems. Beet armyworms significantly reduced height, number of leaves, number of reproductive structures, and AGB (Appendix B). However, only reproductive effort showed a significant pollination \times insect treatment interaction (ANOVA, $F_{1,285} = 3.148$, $P = 0.002$; Appendix 2). Outbred plants had a 23.5% reduction in reproductive effort, whereas inbred plants had a 42.2% reduction when attacked by beet armyworm caterpillars (Fig. 2C).

After four weeks, generalist cotton aphid populations were significantly smaller on outbred plants than on inbred plants (ANOVA, $F_{1,84} = 3.84$, $P = 0.053$). There were 30% fewer cotton aphids on outbred plants (227.58 ± 34.66 aphids, mean ± 1 SE) than on inbred plants (324.16 ± 35.04 aphids).

The MANOVA comparing treatment effects on biomass of plants attacked by cotton aphids did not reveal any significant effects of inbreeding, insect treatment, or their interaction. There was a significant effect of pollination treatment on the number of leaves and reproductive effort (Appendix C). However, inbreeding significantly increased the number of leaves and reproductive effort. Outbred plants had 14% fewer leaves and 14% fewer reproductive structures than inbred plants. In addition, plants with cotton aphids had 24.48% greater biomass than plants without aphids (ANOVA, $F_{1,146} = 10.16$, $P = 0.002$).

DISCUSSION

Using the plant vigor hypothesis (Price 1991), we predicted that specialist herbivores would prefer and perform better on vigorous, outbred plants and that inbred plants would be more resistant to specialist herbivores and suffer less damage as a consequence (Table 1) (Schowalter et al. 1999, Fritz et al. 2003). Specialist tortoise beetles responded to outbred plants as predicted. Specialist golden and mottled tortoise beetles were significantly larger at pupation when fed outbred plants. In addition, adult golden tortoise beetles preferred to feed on outbred plants. Tortoise beetles were significantly smaller when reared on inbred plants and pupal mass strongly correlates with adult mass and subsequent fecundity in these beetles (Rausher 1983). Therefore, self-pollination increased resistance to specialist tortoise beetles in our study.

Using the plant stress hypothesis (White 1969, Schowalter et al. 1999, Thompson et al. 2001), we predicted that generalist herbivores would perform better on stressed, inbred plants and that outbred plants would be more resistant to generalist herbivores and suffer less damage than inbred plants (Table 1). Beet armyworm caterpillars did not respond to inbred plants as predicted by the plant stress hypothesis. We found that beet armyworm caterpillars performed better when reared on outbred plants. Development time was significantly reduced and pupal mass was significantly increased when beet armyworms were fed outbred plants. These results were similar to those of a study in which armyworms had relatively high performance on healthy, nonstressed plants and lower performance on drought-stressed plants (Showler and Moran 2003). Cotton aphids, on the other hand, did respond to inbred plants as predicted by the plant stress hypothesis. After four weeks, aphid populations were higher on inbred plants than on outbred plants. Aphid population growth is often related to host-plant quality (Ebert and Cartwright 1997, McVean and Dixon 2001) and has often been found to increase on stressed plants (McVean and Dixon 2001). Thus, increases in cotton aphid populations indicate that the effect on this herbivore of inbreeding in entire-leaf morning glories is similar to abiotic plant stresses.

Theoretical models predict that a selfing allele within an outcrossing population will have a 50% transmission advantage over an outcrossing allele (Fisher 1941) and that the selfing advantage is reduced only when the selfed offspring suffer a fitness cost associated with inbreeding depression (Charlesworth and Charlesworth 1987). Many plant species, however, that maintain mixed mating systems exhibit inbreeding depression that is apparently strong enough to outweigh the gene-transmission advantage (Husband and Schemske 1996). Reproductive assurance has been offered as the most likely explanation for the persistence of self-fertilization in these cases (Kalisz and Vogler 2003, Kalisz

et al. 2004). Self-fertilization, however, may provide an opportunity for the selection of traits other than those associated with plant mating systems (Crow and Kimura 1970). Ivey et al. (2004) found that inbreeding could change tolerance and resistance to herbivores, but that this effect varied among full-sib maternal families, suggesting that genetic variation for the effect of inbreeding on resistance and tolerance exists (Ouborg et al. 2000). In this study, inbreeding increased resistance to the most common specialist herbivores (tortoise beetles) as well as resistance to an opportunistic generalist herbivore (beet armyworms). If the most common herbivores reduce the fitness of outcrossed progeny, yet have little or no effect on the fitness of inbred progeny, then we can expect the frequency of selfing plants to persist in populations with high densities of these herbivores (Ashman 2002, Carr and Eubanks 2002). In these situations, herbivory could select for self-pollination and variation in herbivory could help maintain mixed mating systems (Steets and Ashman 2004).

Inbreeding is expected to change plant resistance to herbivores (Kennedy and Barbour 1992, Ashman 2002), and some studies have shown that inbreeding can change plant interactions with natural enemies (Ouborg et al. 2000, Carr and Eubanks 2002, Ivey et al. 2004). However, in this study we have shown that inbreeding not only affects the resistance of plants to herbivory, but that this effect can vary depending on the type of herbivore. Inbred entire-leaf morning glory plants were more resistant to two leaf-chewing herbivores, but less resistant to one piercing-sucking herbivore. These results are consistent with the meta-analysis by Koricheva et al. (1998) that concluded that piercing-sucking insects performed better on stressed plants, but contrast with the meta-analysis by Huberty and Denno (2004) that concluded that sap-feeders are adversely affected by continuous plant stress. However, our results are also consistent with the study by Carr and Eubanks (2002) in which inbred *Mimulus guttatus* were less resistant to xylem-feeding herbivores. These results may reflect how these herbivores avoid plant defenses. Plants of the genus *Ipomoea* are chemically defended by latex exudates that can reduce herbivore feeding (Data et al. 1996). Both golden and mottled tortoise beetles avoid the latex defenses of *Ipomoea* by feeding between the leaf veins (Dussourd and Denno 1991). Generalist herbivores such as armyworms feed on the entire leaf and often have difficulty avoiding latex secretory canals (Dussourd and Denno 1994). Inbreeding may alter the digestibility of the plants and this alteration may result in an increase in resistance to herbivores. Because aphids pierce the plant tissue and siphon nutrients directly from the phloem, aphids may be able to avoid latex ducts and other plant defenses that could be influenced by inbreeding. The chemical composition of the plant and how inbreeding changes resistance to herbivores remain to be studied.

This study marks the first attempt to use existing plant defense theory to predict interactions between multiple herbivores and inbred plants. We found that inbreeding can change the resistance of plants to herbivory and that this effect can vary significantly among different species and feeding modes of herbivores. Inbreeding may reduce the fitness of plants; however, it may increase the resistance to some of the most common detrimental herbivores. Thus, increased herbivore resistance may be an overlooked factor promoting the maintenance of mixed mating systems in plant populations.

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LITERATURE CITED

- Ashman, T.-L. 2002. The role of herbivores in the evolution of separate sexes from hermaphroditism. *Ecology* **83**:1175–1184.
- Barrett, S. C. H., and C. G. Eckert. 1990. Variation and evolution of mating systems in seed plants. Pages 229–254 in S. Kawano, editor. *Biological approaches and evolutionary trends in plants*. Academic Press, San Diego, California, USA.
- Carr, D. E., and M. D. Eubanks. 2002. Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). *Evolution* **56**:22–30.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**:237–268.
- Chaves, M. M., J. P. Maroco, and J. S. Pereira. 2003. Understanding plant responses to drought—from genes to the whole plant. *Functional Plant Biology* **30**:239–264.
- Crow, J. F., and M. Kimura. 1970. *An introduction to population genetics theory*. Burgess, Minneapolis, Minnesota, USA.
- Data, E. S., S. F. Nottingham, and S. J. Kays. 1996. Effect of sweetpotato latex on sweetpotato weevil (Coleoptera: Curculionidae) feeding and oviposition. *Journal of Economic Entomology* **89**:544–549.
- Delwiche, L. D., and S. J. Slaughter. 1995. *The little SAS book: a primer*. SAS Institute, Cary, North Carolina, USA.
- Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* **44**:1129–1139.
- Dussourd, D. E., and R. F. Denno. 1991. Deactivation of plant defense: correspondence between insect behavior and secretory canal architecture. *Ecology* **72**:1383–1396.
- Dussourd, D. E., and R. F. Denno. 1994. Host range of generalist caterpillars: trenching permits feeding on plants with secretory canals. *Ecology* **75**:69–78.
- Ebert, T. A., and B. Cartwright. 1997. Biology and ecology of *Aphis gossypii* Glover (Homoptera: Aphididae). *Southwestern Entomologist* **22**:116–153.

- Fisher, R. A. 1941. Average excess and average effect of gene substitution. *Annals of Eugenics* **11**:53–63.
- Fritz, R. S., B. A. Crabb, and C. G. Hochwender. 2003. Preference and performance of a gall-inducing sawfly: plant vigor, sex, gall traits and phenology. *Oikos* **102**:601–613.
- Hayes, C. N., J. A. Winsor, and A. G. Stephenson. 2004. Inbreeding influences herbivory in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *Oecologia* **140**:601–608.
- Heschel, M. S., and K. N. Paige. 1995. Inbreeding depression, environmental stress, and population size variation in scarlet gilia (*Ipomopsis aggregate*). *Conservation Biology* **9**:126–133.
- Huberty, A. F., and R. F. Denno. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* **85**:1383–1398.
- Hull-Sanders, H. M. 2004. Levels of inbreeding depression and selfing rate of *Ipomoea hederacea* var. *integriuscula* (Convolvulaceae). Dissertation. Auburn University, Auburn, Alabama, USA.
- Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**:54–70.
- Ivey, C. T., D. E. Carr, and M. D. Eubanks. 2004. Effects of inbreeding in *Mimulus guttatus* on tolerance to herbivory in natural environments. *Ecology* **85**:567–574.
- Kalisz, S., and D. W. Vogler. 2003. Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* **84**:2928–2942.
- Kalisz, S., D. W. Vogler, and K. M. Hanley. 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* **430**:884–887.
- Karhu, K. J., and S. Neuvonen. 1998. Wood ants and a geometrid defoliator of birch: predation outweighs beneficial effects through the host plant. *Oecologia* **113**:509–516.
- Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* **17**:230–241.
- Kennedy, G. G., and J. D. Barbour. 1992. Resistance variation in natural and managed systems. Pages 13–41 in R. S. Fritz and E. L. Simms, editors. *Plant resistance to herbivores and pathogens: ecology, evolution, and genetics*. Chicago University Press, Chicago, Illinois, USA.
- Koricheva, J., S. Larsson, and E. Haukioja. 1998. Insect performance on experimentally stressed woody plants: a meta-analysis. *Annual Review of Entomology* **43**:195–216.
- Mauricio, R., M. D. Rausher, and D. S. Burdick. 1997. Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology* **78**:1301–1311.
- McVean, R. I. K., and A. F. G. Dixon. 2001. The effect of plant drought-stress on populations of the pea aphid *Acyrtosiphon pisum*. *Ecological Entomology* **26**:440–443.
- Ouborg, N. J., A. Biere, and C. L. Mudde. 2000. Inbreeding effects on resistance and transmission-related traits in the *Silene-Microbotryum* pathosystem. *Ecology* **81**:520–531.
- Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* **62**:244–251.
- Ramsey, M., and G. Vaughton. 1998. Effect of environment on the magnitude of inbreeding depression in seed germination in a partially self-fertile perennial herb (*Blandfordia grandiflora*, Liliaceae). *International Journal of Plant Sciences* **159**:98–104.
- Rausher, M. 1983. Tradeoffs in performance on different hosts: evidence from within- and between-site variation in the beetle *Deloyala guttata*. *Evolution* **38**:582–595.
- SAS Institute. 2001. SAS. Version 8.2. SAS Institute, Cary, North Carolina, USA.
- Schadel, W. E., and W. M. Walter, Jr. 1981. Localization of phenols and polyphenol oxidase in 'Jewel' sweet potatoes (*Ipomoea batatas* 'Jewel'). *Canadian Journal of Botany* **59**:1961–1967.
- Schowalter, T. D., D. C. Lightfoot, and W. G. Whitford. 1999. Diversity of arthropod responses to host-plant water stress in a desert ecosystem in southern New Mexico. *American Midland Naturalist* **142**:281–290.
- Showler, A. T., and P. J. Moran. 2003. Effects of drought stressed cotton, *Gossypium hirsutum* L., on beet armyworm, *Spodoptera exigua* (Hubner), oviposition and larval feeding preferences and growth. *Journal of Chemical Ecology* **29**:1997–2010.
- Simms, E. L., and M. D. Rausher. 1989. Evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. *Evolution* **43**:573–585.
- Sorensen, F. C., and R. K. Campbell. 1997. Near neighbor pollination and plant vigor in coastal Douglas-fir. *Forest Genetics* **4**:149–157.
- Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology* **79**:23–55.
- Steets, J. A., and T.-L. Ashman. 2004. Herbivory alters the expression of a mixed-mating system. *American Journal of Botany* **91**:1046–1051.
- Strauss, S. Y., J. A. Rudgers, J. A. Lau, and K. G. Irwin. 2002. Direct and ecological costs to herbivory. *Trends in Ecology and Evolution* **17**:278–285.
- Tallamy, D. W., and V. A. Krischik. 1989. Variation and function of cucurbitacins in *Cucurbita*: an examination of current hypotheses. *American Naturalist* **133**:766–786.
- Thompson, V. P., A. B. Nicotra, and M. J. Steinbauer. 2001. Influence of previous frost damage on tree growth and insect herbivory of *Eucalyptus globulus globulus*. *Austral Ecology* **26**:489–499.
- Vogler, D. W., and S. Kalisz. 2001. Sex among the flowers: the distribution of plant mating systems. *Evolution* **55**:202–204.
- White, T. C. R. 1969. An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology* **50**:905–909.

APPENDIX A

A table presenting variance components and treatment effects for plants attacked by tortoise beetles is available in ESA's Electronic Data Archive: *Ecological Archives* E086-048-A1.

APPENDIX B

A table presenting variance components and treatment effects for plants attacked by beet armyworms is available in ESA's Electronic Data Archive: *Ecological Archives* E086-048-A2.

APPENDIX C

A table presenting variance components and treatment effects for plants attacked by cotton aphids is available in ESA's Electronic Data Archive: *Ecological Archives* E086-048-A3.