

INBREEDING DEPRESSION OF PLANT QUALITY REDUCES INCIDENCE OF AN INSECT-BORNE PATHOGEN IN A WILD GOURD

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The distribution of infectious diseases within populations can be characterized in terms of variation in the susceptibility and exposure of individual hosts. In general, selfing has been associated with an increase in susceptibility to pathogens, though selfing effects on plant quality may further affect pathogen exposure due to foraging insects. We observed lower incidence of a beetle-vectored bacterium, *Erwinia tracheiphila*, in self-fertilized wild gourds (*Cucurbita pepo* ssp. *texana*) over 2 yr of field-scale epidemics in 50 : 50 mixtures of selfed and outcrossed wild gourds. Subsequent inoculation experiments revealed no significant association between inbreeding and susceptibility, suggesting differential exposure in selfed and outcrossed plants as an explanation for the observed pattern of incidence. Selfed *C. pepo* tend to be smaller and produce fewer flowers and fruits, which are attractive to the specialist beetles that vector *Erwinia*. We experimentally manipulated plant size by transplanting first-generation selfed and outcrossed seedlings to the field on three staggered dates and found that larger plants had higher incidence of *Erwinia* regardless of inbreeding. We conclude that vector selection of larger plants leads to increased exposure of outcrossed plants. Thus, disease mortality risk due to vector behavior is a potential cost to outcrossed plants.

Keywords: cucumber beetles, disease susceptibility, herbivory, inbreeding depression, mixed mating systems, vector-borne pathogen, *Cucurbita pepo*, *Erwinia tracheiphila*.

Introduction

Self-pollination and inbreeding depression may generate variability in both the susceptibility and the exposure of individuals to pathogens. Susceptibility, the occurrence of disease following challenge with a pathogen, may be affected by both direct and indirect means. The increases in homozygosity that accompany inbreeding may have effects on the many genes that determine pathogen resistance (Thompson and Burdon 1992). Furthermore, inbreeding is generally associated with a reduction in plant vigor (Charlesworth and Charlesworth 1987; Husband and Schemske 1996), which may indirectly affect susceptibility to pathogens by reducing the resources available to fight off infection. Less often considered is the potential effect that inbreeding may have on pathogen exposure through altered interactions with disease vectors (though see Ouborg et al. 2000). Many plant pathogens are vectored by foraging insects; inbreeding effects on plant quality may alter vector foraging behavior, resulting in differential rates of visitation and foraging, which may affect rates of contact with pathogens.

In plant systems, individual variation, genetic or otherwise, can result in differential visitation by foraging arthropods (pollinators and herbivores) that can act as vectors for pathogens. Individuals of *Silene latifolia* with many flowers receive more

visits from pollinators and have higher rates of the pollinator-borne smut fungus *Microbotryum violaceum* (Alexander 1987; Thrall and Jarosz 1994; Shykoff and Bucheli 1995; Ferrari et al. 2005). Power (1991) found that genetic variability in a population of oats resulted in increased aphid movement and shortened foraging bouts, resulting in reduced transmission of barley yellow dwarf virus.

The net effect of self-pollination on interactions with vectors may be either positive or negative, as it affects both host quality and resistance to herbivores (Ouborg et al. 2000; Carr and Eubanks 2002). On average, selfing results in detrimental changes in phenotype that affect the preference and performance of herbivores (Charlesworth and Charlesworth 1987; Carr and Eubanks 2002). Intuitively, selfing may reduce the quality of host plants, resulting in relatively lower exposure of inbred hosts to the herbivore vectors (Ouborg et al. 2000; Carr and Eubanks 2002). Alternatively, selfing may reduce host plant defensive capabilities and/or lengthen the time that host plants remain in the most vulnerable stages of development and thereby may result in relatively more herbivory and pathogen exposure (Carr and Eubanks 2002).

In this article, we consider the effect of self-pollination on the incidence of the beetle-transmitted pathogen *Erwinia tracheiphila* in the self-compatible, monoecious wild gourd *Cucurbita pepo* ssp. *texana*. Field observation over 4 yr revealed higher incidence of *Erwinia* in outcrossed plants. We examine this pattern through retrospective analyses of *Erwinia* incidence in experimental plots of wild gourd, field-scale experimental

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epidemics, and lab-scale inoculation trials. Through a synthesis of these data, we consider the contribution of inbreeding to variation in susceptibility and in exposure via interactions with the specialist herbivore vectors.

Material and Methods

Cucurbita-Erwinia Model System

The wild gourd *Cucurbita pepo* ssp. *texana* is an annual, monoecious, self-compatible vine native to Texas and adjacent regions and is thought to be the wild progenitor of cultivated squashes (Decker 1988; Kirkpatrick and Wilson 1988). *Cucurbita* spp. produce large yellow flowers that are pollinated primarily by squash bees (Winsor et al. 2000) but that also attract cucumber beetles (the primary herbivores) to the flowers and plants (Andersen 1987; Hesler 1998). Several volatile compounds produced by *Cucurbita* blossoms have been shown to be attractive to cucumber beetles (Andersen and Metcalf 1986; Andersen 1987; Metcalf and Lampman 1991), and different diabroticine species have been shown to discriminate between the volatile profiles of *C. pepo* (squash) varieties (Andersen and Metcalf 1987; Lampman and Metcalf 1988). The cucumber beetles feed on the leaves and other organs of both cultivated varieties of squash and wild species of *Cucurbita* throughout their native ranges (see Robinson and Decker-Walters 1997).

Cucumber beetles are the primary vector of the bacterial pathogen *Erwinia tracheiphila* (Yao et al. 1996), which infects cultivated members of the Cucurbitaceae and wild species of *Cucurbita* throughout their native ranges (see Robinson and Decker-Walters 1997). In Pennsylvania, the bacterium is transmitted via the feeding of the striped and spotted cucumber beetles (*Acalymma vittata* and *Diabrotica undecimpunctata howardi*) (Fleischer et al. 1999). *Erwinia* proliferates in the xylem and secretes a mucilaginous matrix that cuts off water supply, resulting in wilting and eventual death of the plant. Wilt symptoms typically develop 10–15 d following infection in mature plants and are nearly always fatal.

An experimental population of *C. pepo* ssp. *texana* was initiated from seeds sampled randomly from a natural population in Texas. A random sample of five progeny was used to found five maternal lines, and the remaining lines were reserved as potential pollen donors. A multiyear crossing program was used to generate plants with a range of inbreeding coefficients to study inbreeding depression in a host of traits (Stephenson et al. 2001, 2004; Hayes et al. 2004, 2005a, 2005b, 2005c). For the purposes of this study, we are concerned only with offspring from outcrossed matings ($f = 0$) and first-generation self-matings ($f = 0.5$) in each year.

Self-pollination in *C. pepo* ssp. *texana* has been associated with inbreeding depression for a range of reproductive traits: pollen performance (Stephenson et al. 2001; Hayes et al. 2005a), male and female flower production, and fruit number and weight (Stephenson et al. 2004; Hayes et al. 2005a). Inbreeding also affects interactions with natural enemies; inbred plants tend to be more susceptible to aphid-borne viral pathogens, and they sustain relatively more herbivory on new growth because of cucumber beetles (*D. undecimpunctata* and *A. vittata*) (Stephenson et al. 2004). Paradoxically, de-

spite having higher levels of foliar herbivory, inbred plants have lower incidence of *Erwinia* infection, which is vectored by cucumber beetles.

Retrospective Analyses of Erwinia Incidence

In 2002 and 2003, three 0.4-ha fields were planted with selfed and outcrossed offspring from the five maternal lines for studies of inbreeding effects on a variety of fitness measures (Stephenson et al. 2004). During these studies, the incidence of *Erwinia* was recorded in each field at monthly intervals between June and September. In both years, some plants received an insecticide spray treatment. We eliminated these individuals from our analyses. Within each field, there were equal numbers of unsprayed plants from each maternal line and breeding treatment (sample sizes are given in fig. 1).

Monthly surveys were also conducted to assess the presence of wild disease symptoms and the degree of leaf herbivory due to cucumber beetles on new growth. We used a qualitative scale ranging from 0 (no damage) to 5 (severe) (see Stephenson et al. 2004). In addition, the numbers of male and female flowers per plant were recorded at ca. 3-d intervals (because each flower lasts for only a single morning, these flower counts are an unbiased estimate of annual flower production per plant). For consistency, beetle damage and flower counts were repeated during the 2004 and 2005 field experiments by using the same methodology.

Field Epidemics

In the summers of 2004 and 2005, we conducted field experiments to examine the effects of inbreeding and genetic variability on rates and patterns of spread of *Erwinia*. In both years, we germinated selfed and outcrossed progeny from the five maternal lines in a greenhouse and transplanted them to two 0.4-ha fields at the Pennsylvania State Agricultural Experimental Station at Rock Springs, Pennsylvania, on May 18. Plants were arrayed in a 12 × 15 grid in a systematic pattern to assure that maternal lines and breeding treatments were evenly distributed across each field. Wilt epidemics began from natural reservoirs, and we surveyed fields from June 1 to September 7 at ca. 3-d intervals; the occurrence of wilt disease symptoms and mortality was recorded as well as the numbers of male and female flowers per plant. In order to make comparisons between *Erwinia* transmission in genetically similar and variable populations, we planted two additional 0.4-ha fields in 2004 with only selfed progeny of a single maternal line (one field of line I1 and one field of line J3). One of these fields (with the J3 selfed plants) was only 0.32 ha and was planted with 150 plants at the same spacing as the other fields.

The spatially explicit incidence time series from each field was analyzed using an autologistic model (Besag 1974). The response variable $y_{i,t}$ was defined as 1 if the plant in location i at time t became infected and 0 otherwise. The probability $p_{i,t}$ of the plant in location i becoming infected at time t was modeled using a general linear model with binomial error, including terms for maternal line, breeding treatment, total number of infected plants at time $t - 1$, and standardized flower number at time t . Because beetle foraging and behavior are likely to be affected by the relative quality of plants

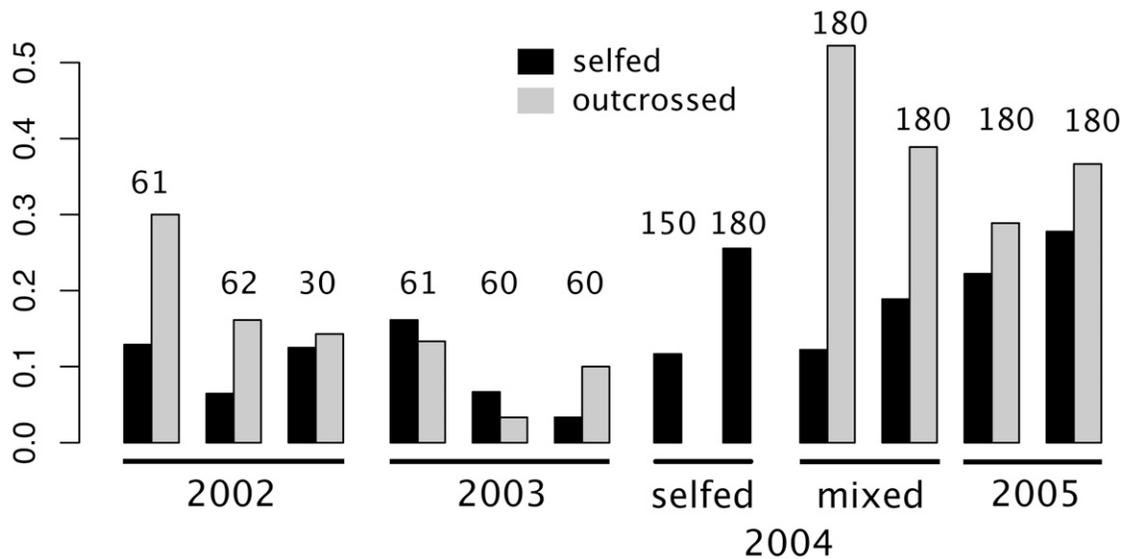


Fig. 1 Final proportion of selfed and outcrossed plants that were infected with *Erwinia tracheiphila* during growing season. Paired bars represent individual fields. In 2004, two fields had only selfed plants. The 2005 fields are the even-aged fields. Numbers indicate the number of unsprayed plants in each field.

(e.g., size, number of flowers), we centered the flower number for each sampling date on the median flower number in that field on date t ; thus, the flower number covariate is a measure of how many flowers each plant had relative to the average number of flowers. To account for spatial autocorrelation in infection, we included a term for the distance-weighted coupling to the infected plants at time $t - 1$ (Augustin et al. 1996). The coupling term is given by

$$\text{coupling}_{i,t} = \sum_{j=\text{infected}} e^{-\alpha d_{ij}},$$

where α is the distance decay parameter and d_{ij} is the distance between plant i and infected plant j . To account for differences between fields, we fitted a separate distance decay term, α , for each field.

Plant size is confounded with plant breeding (i.e., outcrossed plants typically grow faster and larger; Stephenson et al. 2004), which may affect visitation by beetles and thus exposure to *Erwinia*. To control for this effect, in 2005 we conducted a field experiment to test for the effect of plant size, independent of breeding. Each of two additional 0.4-ha fields were divided into three blocks with four rows of 15 *C. pepo* ssp. *texana*. Within each block, the position of each plant was arrayed systematically with respect to five maternal lines and two breeding treatments (as previously). Plants were randomly assigned one of three planting dates within each block. On each date (May 18, June 10, June 30), we planted 60 2-wk-old seedlings into each field. Thus, within each block, two plants from each of five maternal lines and two breeding treatments (self or outcross) were planted on each of the three dates. Following each of the first two planting dates, seedlings were sprayed at weekly intervals with Asana XL (Dupont, Wilmington, DE), a contact insecticide, to kill the herbivores and limit disease transmission. One day

after the third planting, all plants were sprayed for the final time. We refer to these as mixed-age fields, in contrast to the even-aged fields (all seedlings transplanted on a single day) planted in 2004 and 2005. Note that the even-aged fields were never sprayed with insecticide.

In order to monitor beetle numbers in these fields, we placed onto each of the four fields four wire-mesh cages that were coated with Tanglefoot (a sticky, non-water-soluble substance; Tanglefoot, Grand Rapids, MI) and baited with an unsprayed potted wild gourd plant. Throughout the period of spraying, the traps on the mixed-aged fields captured only 4.8% of the beetles that were captured in the traps on the even-aged, unsprayed fields. Moreover, an assessment of beetle damage in late June revealed very low levels of cucumber beetle herbivory in the mixed-aged fields compared with similar assessments in the even-aged fields. Thus, on release from spraying, the plants in the mixed-aged fields were nearly unexposed to herbivory or disease at three size/age treatments; the mean number of beetles captured per day in the mixed-age fields increased from 0.14 to 75.2 after the cessation of spraying (fig. 3B, inset). Following release from spraying, two plants at the center of each of the two mixed-aged fields were inoculated with *Erwinia* to initiate epidemics. Fields were surveyed at ca. 3-d intervals, and the disease status and flower production of each plant were recorded. The probability of a plant becoming infected by September 8 was analyzed using a generalized linear model with binomial error, with planting date, maternal line, and breeding used as explanatory variables.

Inoculation Trials

In 2005, we also conducted greenhouse inoculation trials to investigate differences in susceptibility to *E. tracheiphila* among maternal lines and breeding treatments when the

exposure is constant. We inoculated seedlings with bacterial culture isolated from wilted *C. pepo* ssp. *texana* in the field in 2004. The bacterial strain resembled known *E. tracheiphila* in colony and cell morphology. Previous trials had shown that the isolate remained virulent and capable of infecting seedlings. Cultures were maintained on peptone nutrient agar at 35°C and transferred to new media biweekly.

Ten self seeds and 10 outcrossed seeds from each of the five maternal families were sown in 15-cm pots in Pro-Mix (Rivière-du-Loup, Quebec) soil. To control for seedling size and development, the seedlings were inoculated with *E. tracheiphila* between the first and second node when the second true leaf had fully expanded. Seedlings varied in the number of days to germination and developmental rate, and so inoculations were conducted between May 5 and June 14, with 90% of the plants inoculated on or before June 7. The inoculum consisted of 2-d-old peptone nutrient broth cultures, diluted 1 : 100 with sterile distilled water. A 0.01-mL aliquot of inoculum was injected into the stem, just above the cotyledons, with a microliter syringe. Following inoculation, seedlings were placed in growth chambers (29°C for 16 h, 22°C for 8 h) under cool-white fluorescent lamps. Plants were scored as infected if any leaf showed unambiguous wilting in full light. We analyzed the infection outcome (binary, infected/not infected) and the time to infection to test for the effects of maternal line and breeding.

Results

Inbreeding and the Incidence of Erwinia

In 2002, 2004, and 2005, there was higher incidence of *Erwinia* infection in outcrossed plants (fig. 1). There was no clear pattern in 2003, although that year was very wet (including periods of time in which there was standing water on the fields) and the incidence of *Erwinia* was low overall (8%). In 2004, there was no difference in the total incidence of *Erwinia* in the fields containing only selfed plants and in those with mixed breeding (both selfed and outcrossed) treatments (fig. 1).

We fitted a spatially explicit, autologistic epidemic model to the incidence time series for the even-aged fields in 2004 and 2005. The odds of infection increased with the frequency of infected plants in the field but not with the proximity to infected plants (after frequency was in the model; table 1). Flower number was marginally correlated with infection risk, indicating a possible preference for beetles to visit larger plants. Outcrossed plants had a significantly higher probability of infection than did selfed plants after controlling for the frequency of infected plants and the tendency for outcrossed plants to have more flowers. There was no significant effect of maternal line (table 1).

Inoculation Trials

There was no significant effect of either maternal line ($P = 0.11$) or breeding ($P = 0.39$) on the probability of infection for inoculated seedlings (fig. 2; table A1). Conditional on becoming infected, there were marginally significant differences in the time to symptoms among maternal lines ($P = 0.07$) but no significant difference between selfed and

Table 1

Analysis of Deviance for a Stepwise Logistic Regression for Infection of *Cucurbita pepo* ssp. *texana* with *Erwinia tracheiphila* in Even-Aged Field Epidemics

Variable	Deviance	<i>P</i>
Total deviance	1782.28	
Year	1779.00	.07
Field (within year)	1774.67	.11
Frequency	1758.92	<.001
Spatial coupling	1758.31	.44
Standardized flower number	1754.85	.06
Maternal line	1748.77	.19
Breeding	1724.21	<.001

Note. Year indicates 2004 or 2005 experiments, field indicates four replicate fields, frequency indicates the number of infected plants, spatial coupling is the sum of the weighted distance to infected plants, standardized flower number is a covariate for each plant of number of flowers standardized by the median flower number on each sampling date, maternal line indicates one of five independent breeding lineages, and breeding indicates selfed ($f = 0.5$) or outcrossed ($f = 0$) progeny. Underlined values are significant explanatory variables.

outcrossed seedlings ($P = 0.9$) (fig. 2*b*; table A2). For both responses, there was no consistent trend of inbreeding on susceptibility within families (fig. 2); in fact, there was a significant interaction between breeding and family for the time to wilting ($P = 0.01$; table A2).

Plant Size and Incidence of Erwinia

Both *Diabrotica undecimpunctata* and *Acalymma vittata* are attracted to volatile constituents of *Cucurbita pepo* blossom odor. Outcrossed plants tend to produce more flowers and more blossom volatiles than do selfed plants. From 2002 to 2005, plants that blossomed early in the season (before the first full disease census, 3 wk after planting) were twice as likely to become infected as were plants that blossomed later (fig. 3A).

Because plant size is confounded with inbreeding, in 2005 we conducted an experiment that controlled for size by transplanting selfed and outcrossed seedlings at three dates. After an initial period of vegetative growth (five to seven nodes), *C. pepo* ssp. *texana* produces one flower per node, and thus the number of total flowers produced is strongly correlated with plant size (Avila-Sakar et al. 2001). The average numbers of flowers (male and female) per plant in August were 69, 58, and 24 for the early, middle, and late planting dates, respectively; thus, the planting treatment generated a 2.8-fold range in average plant sizes. (Note that within planting date, outcrossed plants produced more total flowers [$P = 0.001$], though the effect was much smaller than the planting date effect.) The proportion of plants that became infected with *Erwinia* increased with earlier planting dates (fig. 3B). The planting date effect was significant ($P < 0.001$) after controlling for maternal line and breeding (table A3). Interestingly, there was no significant effect of breeding on the proportion of plants infected in the mixed-aged fields in 2005, while in the even-aged fields planted in 2005, there was higher

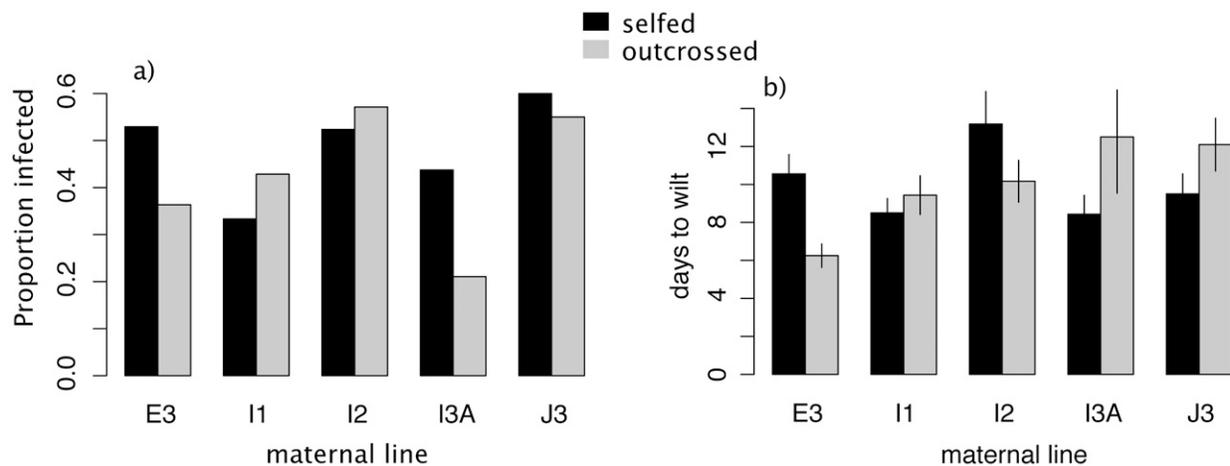


Fig. 2 a, Proportion of *Cucurbita pepo* seedlings that exhibited wilt symptoms following challenge with *Erwinia tracheiphila*. b, Mean number of days until first wilt symptoms for *C. pepo* seedlings that became infected following challenge with *E. tracheiphila*. Vertical bars indicate ± 1 SE.

incidence of *Erwinia* in outcrossed plants (fig. 1), suggesting that the effect of breeding on infection risk may be due to the increased size and flowering of outcrossed plants.

Discussion

Patterns of pathogen/parasite incidence are generated by individual differences in exposure and susceptibility to infection (Zuk and McKean 1996; Ferrari et al. 2004). One often-cited source of variation in pathogen incidence is inbreeding (Levin 1975; Lively and Howard 1994). In general, inbreeding has been hypothesized to affect susceptibility to pathogens at the population scale through the loss of heterozygosity (Lively and Howard 1994; Agrawal and Lively 2001) and at the individual level through the expression of deleterious recessive alleles (Spielman et al. 2004). Inbreeding is also known to affect foraging behavior of herbivores (Ouborg et al. 2000; Carr and Eubanks 2002) and pollinator visitation rates (Ivey and Carr 2005), which may result in additional variation in pathogen exposure. The incidence of *Erwinia* disease in outcrossed wild gourds was higher than in selfed plants in 3 of 4 yr of field observation. This result is in stark contrast to the correlation between inbreeding and parasite burden across a wide range of taxa (Charlesworth and Charlesworth 1987; Busch et al. 2004). In light of the historical patterns over 3 yr and the specific experiments conducted in 2004–2005, we can begin to address the susceptibility and exposure hypotheses for this pattern.

The simplest explanation for the observed pattern is that outcrossed plants are more susceptible to infection with *Erwinia* than are selfed plants. The evidence, however, clearly does not support this hypothesis. Among experimentally infected seedlings, there was no difference between selfed and outcrossed plants in either the probability of wilt disease or the time to the onset of disease. This lack of an apparent effect is striking, given that previous work on *Cucurbita pepo* ssp. *texana* has shown consistent advantage to outcrossing for a

variety of growth and reproductive traits (Hayes et al. 2004, 2005a, 2005c) as well as incidence of aphid-borne viral diseases in the field (Stephenson et al. 2004). The susceptibility of seedlings in the lab may not reflect that of mature plants in the field; however, we expect that differences in inherent susceptibility would be most pronounced in young plants, as the effect in adult plants would be confounded by the differences in plant size and growth rate between selfed and outcrossed individuals. The results of our field experiments indicate a role of floral visitation in *Erwinia* transmission; clearly, transmission rates for injections in the stem may not reflect transmission via a floral route. We are currently investigating the role that floral transmission may play in these patterns.

The patterns in the field and the natural history of the system suggest that differential visitation by cucumber beetles to outcrossed plants results in higher rates of exposure. Exposure of *C. pepo* ssp. *texana* to *Erwinia* is mediated by the foraging behavior of the specialist herbivores *Diabrotica undecimpunctata* and *Acalymma vittata*. Our analyses of the even-aged fields showed that infection risk indeed increases with flower number relative to the field average, suggesting a preference for larger plants. The breeding effect was significant even after controlling for the tendency of outcrossed plants to have more flowers. The distribution of beetles in the field has been shown to be strongly affected by the amount and relative composition of blossom volatiles (Andersen and Metcalf 1987; Lampman et al. 1987; Lampman and Metcalf 1988). Ferrari et al. (2006) showed that inbreeding affects the volatile profile of *C. pepo* ssp. *texana* blossoms. Selfed offspring produced, on average, fewer total volatiles per flower than did outcrossed plants. In addition to quantitative differences in the blossom odor per flower, outcrossed plants tend to produce more flowers than do inbred plants (Hayes et al. 2005a). Thus, outbred plants are likely to be more attractive to cucumber beetles, providing a potential mechanism for increased exposure to *Erwinia*.

We hypothesized that if exposure via cucumber beetles is due to selection based on plant size and flower number,

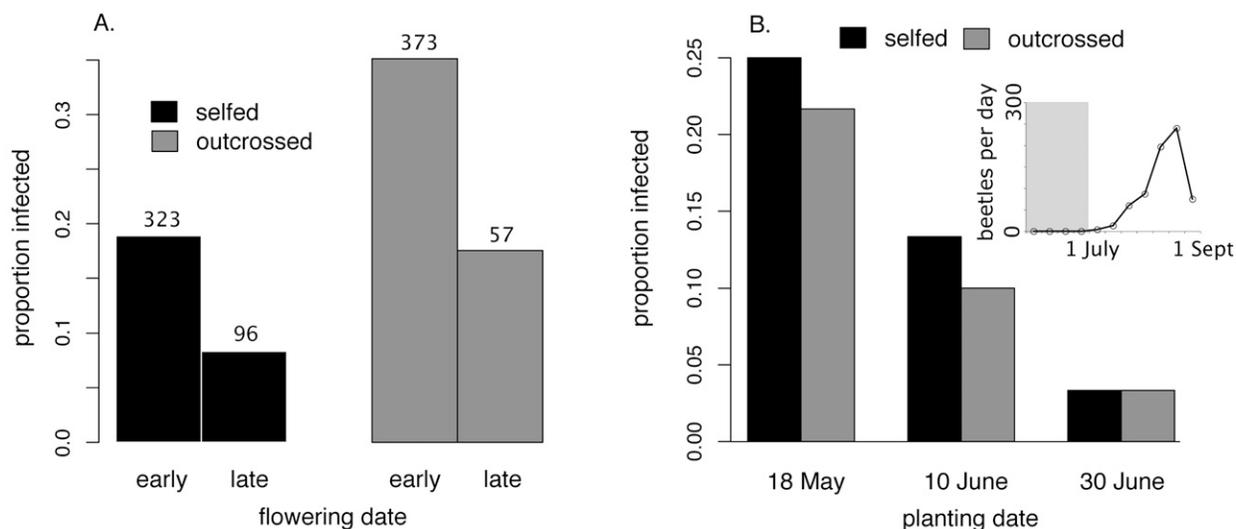


Fig. 3 A, Proportion of early- and late-blossoming *Cucurbita pepo* ssp. *texana* plants in all even-aged fields from 2002 to 2005 that were infected with *Erwinia*. Early blooming is defined as before the first full disease census, 3 wk after planting. Numbers above the bars indicate sample size. B, Proportion of selfed and outcrossed plants in mixed-age fields exhibiting symptoms of infection with *Erwinia* as a function of planting date. Note that the earlier-planted individuals are also larger. Inset gives the mean number of beetles caught per day in the mixed-age fields during insecticide spraying (shaded area) and after the cessation of spraying on July 1.

controlling the size of selfed and outcrossed plants should eliminate the breeding effect. By controlling plant size through staggered planting, we showed that, indeed, *Erwinia* incidence is strongly correlated with plant size and flowering; thus, the effect of breeding on incidence was eliminated. Strikingly, in fields of even-aged plants in the same year (2005; fig. 1), there was higher incidence of *Erwinia* in outcrossed plants. Of course, in these experiments, plant size is confounded with plant age and exposure to pesticide by the nature of the design. However, the experimental results are consistent with the anecdotal observation from our retrospective analyses that early-flowering plants, in unsprayed fields of even age, tended to have higher incidence of *Erwinia*.

The assembled field observations and experiments tell an interesting story about the role of inbreeding on host-vector-pathogen interactions and the potential for selective vectors to influence patterns of disease incidence. However, many more interesting questions have been raised by these patterns than have been answered. Most important, the behavioral link between floral characteristics and visitation by cucumber beetles has been largely inferred. A formal test of behavioral responses of striped and spotted cucumber beetles to floral volatiles of inbred and outcrossed plants is planned. Furthermore, Hayes et al. (2004) and Stephenson et al. (2004) found a positive relationship between inbreeding coefficient and measures of leaf damage by cucumber beetles to new tissue. While apparently contradictory to the conclusion that beetles are more attracted to outcrossed plants, this measure was not designed to quantify the number of beetles or rate of beetle visitation and thus may not be reflective of pathogen exposure. Moreover, these studies did not measure herbivory in the short-lived flowers. If the attraction of beetles to floral

odors leads to higher levels of floral herbivory on the flowers of outcrossed plants, then our previous measures of leaf herbivory underestimate pathogen exposure on outcrossed plants. The effect of selfing on the cucurbit-diabroticite beetle interaction is likely to be complicated, as it will be determined by both the foliar and blossom volatile cues used by beetles to locate plants and the concentration and rate of induction of cucurbitacins, bitter plant secondary compounds specific to the Cucurbitaceae that stimulate beetle feeding. This may prove to be a useful model system for studying the effects of individual variation in plant chemistry on insect behavior and multitrophic interactions.

The classical models for frequency-dependent selection by parasites rely on the escape from infection of rare genotypes because the parasite is better adapted to the common genotype (Lively and Howard 1994; Agrawal and Lively 2001). This makes an implicit assumption that exposure to parasites is proportional to genotype frequency. The introduction of a selective vector to the system casts doubt on this assumption and may result in counterintuitive patterns of pathogen incidence due to differential rates of exposure (Carr and Eubanks 2002). The collection of patterns over several years of both field observation and experiments suggests that, in the *Cucurbita-Erwinia* system, selection of outcrossed individuals by the beetle vectors results in higher rates of disease incidence and mortality. This observation is congruent with Hull-Sanders and Eubanks's (2005) application of plant defense theory to interactions between inbred plants and herbivores. They predicted, from the plant vigor hypothesis (Price 1991), that specialist herbivores should be attracted to and perform better on outbred plants. Stephenson et al. (2004) reported a higher incidence on inbred *C. pepo* ssp. *texana* of viral

diseases vectored by generalist aphids, which is also consistent with Hull-Sanders and Eubanks's (2005) prediction that generalist herbivores should be attracted to and perform better on inbred plants. These findings together suggest that Hull-Sanders and Eubanks's (2005) predictions can be extended to include susceptibility to diseases that are vectored by specialist and generalist herbivores.

The behavior of arthropod vectors can play an active role in host-pathogen dynamics (Power 1991; Real et al. 1992; McElhany et al. 1995). Vectors generate heterogeneities in exposure through selection of host characteristics independent of pathogen susceptibility (Shykoff and Bucheli 1995). This may result in counterintuitive predictions for the direction of selection if pathogen resistance and vector preference are correlated (Ouborg et al. 2000). Further, as most hosts are subject to a community of pathogens, which may be transmitted

by an equally diverse community of vectors (i.e., both specialist and generalist), the role of vector behavior in mediating pathogen-induced selection warrants further consideration.

Acknowledgments

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Appendix

Table A1

Analysis of Deviance for Stepwise Logistic Model for Probability of *Cucurbita pepo* ssp. *texana* Inoculated with *Erwinia tracheiphila* Developing Wilt Symptoms in Inoculation Trials

Variable	Deviance	<i>P</i>
Total deviance	268.84	
Maternal line	261.36	.12
Breeding	260.76	.44
Line × breeding	257.63	.54

Note. Maternal line indicates one of five independent breeding lineages, and breeding indicates selfed ($f = 0.5$) or outcrossed ($f = 0$) progeny.

Table A2

ANOVA for Linear Model Fit of Time to the Onset of Wilt Symptoms for *Cucurbita pepo* ssp. *texana* Seedlings Inoculated with *Erwinia tracheiphila* in Inoculation Trials

Source	df	MS	<i>F</i>	<i>P</i>
Maternal line	4	33.76	2.26	.07
Breeding	11	.07	.00	.95
Line × breeding	4	53.11	3.56	<u>.01</u>
Error	79	14.93		

Note. Maternal line indicates one of five independent breeding lineages, and breeding indicates selfed ($f = 0.5$) or outcrossed ($f = 0$) progeny. Underlined value is significant explanatory variable.

Table A3

Analysis of Deviance for Stepwise Logistic Model for Probability of Infection of *Cucurbita pepo* ssp. *texana* with *Erwinia tracheiphila* in Staggered Planting Experiment

Variable	Deviance	<i>P</i>
Total deviance	275.1	
Block	263.2	<u>.035</u>
Maternal line	257.5	.225
Breed	257.1	.517
Planting date	232.6	<u><.001</u>

Note. Maternal line indicates one of five independent breeding lineages, breeding indicates selfed ($f = 0.5$) or outcrossed ($f = 0$) progeny, and planting date indicates one of three planting dates (May 18, June 10, or June 30). Underlined values are significant explanatory variables.

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