Plant genotype and induced responses affect resistance to herbivores on evening primrose (*Oenothera biennis*)

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Abstract. 1. Although both genotype and induced responses affect a plant's resistance to herbivores, little is known about their relative and interactive effects. This study examined how plant genotype of a native plant (*Oenothera biennis*) and induced plant responses to herbivory affect resistance to, and interactions among, several herbivores.

2. In a field experiment, genetic and environmental variation among habitats led to variation in the amount of early season damage and plant quality. The pattern of variation in early season infestation by spittlebugs (*Philaenus spumarius*, a piercing–sucking herbivore) negatively correlated with oviposition preference by a later feeding specialist weevil (*Tyloderma foveolatum*, a leaf-chewer).

3. To determine if plant genotype and induced responses to herbivory might be responsible for these field patterns, we performed no-choice and choice bioassays using four genotypes of *O. biennis* that varied in resistance. Plants were induced by either spittlebugs or weevils and assays measured the responses of the same specialist weevil as well as a generalist caterpillar (*Spodoptera exigua*).

4. Resistance to adult weevils was largely unaffected by plant genotype, while they experienced induced resistance following damage by conspecific weevils in no-choice assays. Caterpillars were more strongly affected by plant genotype than induced responses in both no-choice and choice assays, but they also fed less and experienced higher mortality on plants previously damaged by weevils. In contrast to the pattern suggested by the field experiment, spittlebugs did consistently induce resistance against either weevils or caterpillars in the bioassay experiment.

5. These results support recent findings that show herbivore species can compete via induced plant responses. Additionally, a quantitative review of the literature demonstrates that plant genotype tends to be more important than interspecific competition among herbivores (plant-mediated or otherwise) in affecting herbivore preference and performance.

Key words. Bottom-up effect, community genetics, genotype by environment, indirect effect, multi-trophic interaction, phenotypic plasticity, plant defence, plant–insect interactions, relative importance.

Introduction

Plants frequently display genetic variation within and between populations for traits that influence the preference and performance of insects on their hosts (i.e. resistance traits) (Zangerl & Berenbaum, 1990; Schappert & Shore, 1999; Elle & Hare, 2000; Agrawal *et al.*, 2002; Johnson & Agrawal, 2005). Resistance traits may also be phenotypically plastic, changing in response to abiotic or biotic environmental cues. Herbivory is particularly important in this regard, as plants frequently respond to insect damage by inducing resistance (lowering plant quality) or susceptibility (increasing plant quality) (Karban & Baldwin, 1997).

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Disentangling the relative importance of the genetic and environmental (e.g. herbivory) effects on resistance traits, plus the interaction between these factors (i.e. genetic variation for induced responses), is critical if we are to understand the ecological mechanisms controlling the abundance and distribution of insect herbivores. The objective of this study was to determine the relative importance of genotypic variation in host plant quality compared with competition among insect herbivores mediated by induced plant responses.

Induced resistance to herbivory provides a mechanism by which insect species can indirectly compete (Harrison & Karban, 1986; Hunter, 1992). Competitive interactions mediated by plants may be particularly important for insect species that share a host plant, yet are separated in either space or time (Hunter, 1992). Early evidence for plant-mediated interspecific competition came from studies of herbivory on oaks (*Quercus* spp.), where damage to leaves caused local induction of condensed tannins and was associated with reduced preference and survivorship of leaf-mining insects (West, 1985; Faeth, 1986). Later, a comprehensive review of 193 pairwise interactions between herbivorous species found that interspecific competition occurred in 76% of species interactions (Denno *et al.*, 1995); a large percentage of these competitive interactions appeared to be mediated by induced resistance. Recent studies suggest that plant-mediated competition among insects may, in fact, be a very common mechanism of competition (Agrawal, 1999; Denno *et al.*, 2000; González-Megías & Gómez, 2003; Kessler *et al.*, 2004; Van Zandt & Agrawal, 2004a; Viswanathan *et al.*, 2005).

The combined effects of plant genotype and herbivore-induced responses can lead to interactive or independent effects on herbivores. An interaction between plant genotype and induction occurs when there is genetic variation in induced resistance (Agrawal *et al.*, 2002), so that the effects of induced responses on interspecific interactions vary between plant genotypes. For example, Moran and Whitham (1990) studied plant-mediated competition between two aphid species (a leaf-feeder and root-feeder) that co-occur on lamb's quarters (*Chenopodium album*), which is polymorphic for constitutive resistance to the leaf-feeding aphid. Interactive effects occurred because the leaf-feeding aphid reduced populations of the root-feeding aphid via induced responses by 91% on susceptible plants, whereas there was no such effect on resistant plants. In contrast, plant genotype and induced responses may independently influence insect resistance when there is no genetic variation for induced resistance. For example, in common milkweed (*Asclepias syriaca*) plant genotype and root herbivory by long-horned beetle larvae (*Tetraopes tetraophthalmus*) independently affected the number of leaf mines initiated by a host-specific fly (*Liriomyza asclepiadis*) (Agrawal, 2004). Despite several studies having examined the independent and joint effects of plant genotype and insect competition (Fritz *et al.*, 1986; Karban, 1987, 1989; Mopper *et al.*, 1990; Cronin & Abrahamson, 1999, 2001), very little is known about the relative importance of these factors.

A previous field experiment showed genetic variation in common evening primrose (*Oenothera biennis*) for resistance to early season herbivory (Johnson & Agrawal, 2005). These results are extended in this study by identifying the early season herbivores and examining whether the earliest abundant herbivore negatively correlates with a later season host-specific weevil. Based on the patterns from the field, a bioassay experiment was performed to gain a mechanistic understanding of these interactions. Specifically, the following questions were examined: (1) does early season herbivory induce systemic plant responses that negatively affect later season herbivores (i.e. both conspecifics and heterospecifics)? (2) What is the relative importance of plant genotype compared with induced responses in affecting resistance to herbivores? And last (3) is there genetic variation in induced responses, indicating that plant genotype mediates competitive interactions? The results addressing these questions are combined with a comprehensive literature review, in which it is examined whether plant genotype or interspecific competition (plant-mediated or otherwise) has a stronger effect on herbivores.

Materials and methods

Study site and system

Experiments were conducted in southern Ontario, Canada, at the Koffler Scientific Reserve at Jokers Hill (Jokers Hill). Jokers Hill is a 350-ha field station owned by the University of Toronto (www.zoo.utoronto.ca/jokershill), located 50 km north of Toronto, Ontario (44 °03'N, 79 °29'W, 300 m a.s.l.).

Evening primrose (*Oenothera biennis* L., Onagraceae) is a biennial, native to North America that forms a rosette before bolting into a 0.5–2.5 m flowering stalk. It commonly occurs in open habitats at Jokers Hill, particularly in old fields, mowed lawns, and recently disturbed soil. *Oenothera biennis* primarily self-pollinates to produce clonal seeds, which is a function of its permanent translocative heterozygote genetic system (Cleland, 1972). This genetic behaviour provides a powerful ecological tool because numerous replicates of single clonal genotypes can be grown from seed.

Oenothera biennis plays host to a wide diversity of specialist and generalist insect herbivores (Johnson *et al.*, 2006). In the field experiment (see below), the two most abundant early season herbivores on *O. biennis* at Joker's Hill were the meadow spittlebug (*Philaenus spumarius*, Cercopidae) and a weevil (*Tyloderma foveolatum*, Curculionidae) (Fig. 1a). The spittlebug is a generalist xylem feeding herbivore that secretes a frothy mass of 'spittle' (Ahmed, 1950). The weevil *T. foveolatum* feeds almost exclusively on plants in the Onagraceae and is most commonly associated with *O. biennis* (Wibmer, 1981). Adult weevils feed on the leaves and drill holes into the stem where they lay single eggs that are covered with a
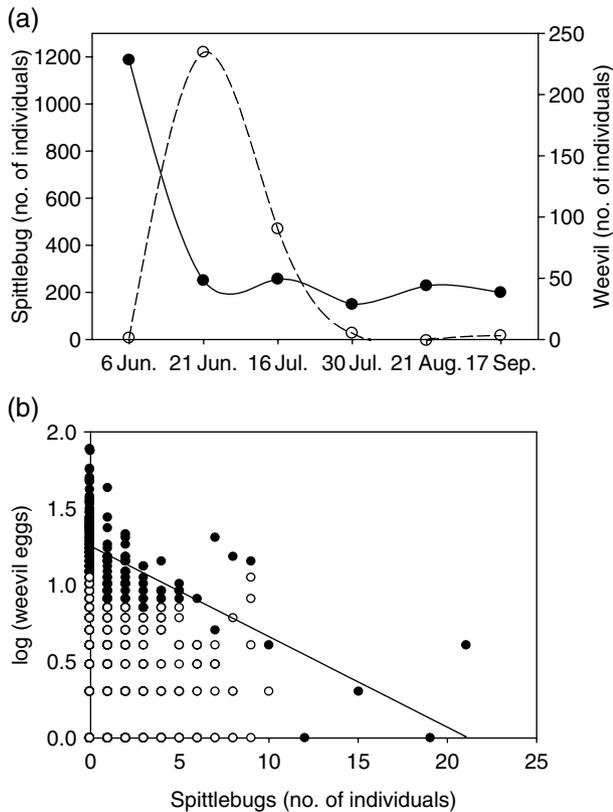
Fig. 1. The (a) phenology of the common early season herbivores and (b) the relationship between their abundances. (a) The meadow spittlebug (*Philaenus spumarius*; solid line) and the host-specific weevil (*Tyloderma foveolatum*; dashed line) were the most common early season herbivores in the field experiment. (b) To assess whether spittlebugs may negatively affect the oviposition preference of weevils, the upper 25th percentile of the total number of eggs laid per plant (●) was regressed against the number of spittlebugs per plant on 6 June ((○) represent the lower 75th percentile data). Spittlebugs and eggs were counted on each of 929 plants.

patchwork of plant fibres. Upon hatching, the larvae chew their way to the pith where they continue to feed and pupate, before emerging from the stem in late summer as adults (M. Johnson, pers. obs.). The beet armyworm caterpillar (*Spodoptera exigua*, Noctuidae) is a leaf-chewing herbivore, but unlike the weevil it has a broad host range and is common across much of North America (Berdegué *et al.*, 1998). *Spodoptera exigua* was used in this experiment to provide a standardised assay of plant resistance to a generalist herbivore.

Field experiment

Detailed methods of the field experiment were previously published (Johnson & Agrawal, 2005), so only a brief summary is provided. Seeds from 14 clonal families (hereafter genotypes) of *O. biennis* were collected between 2001 and 2002 from Jokers Hill, Toronto (50 km south) and

Mississauga (50 km south-west). Seeds were germinated in late March 2002 and plants were transplanted into five common gardens (habitats) at Jokers Hill during the third week of May. The five habitats represented a productivity gradient (M. Johnson, unpublished data) and encompass the range of environments in which *O. biennis* is locally found. Habitats were divided into four equal-sized contiguous spatial blocks (hereafter microhabitats), into which all genotypes were fully randomised. Within the microhabitats, plants were planted in a grid with 1-m spacing between the rows and columns, which should have effectively prevented light and root competition between plants. In total, the experiment consisted of 64–67 replicate plants per genotype for a total of 926 plants. Naturally colonising *P. spumarius* (spittlebugs) and *T. foveolatum* (weevils) were surveyed by counting the number of individuals on plants, as well as the number of eggs laid by weevils in the stems or petioles.

Statistical analyses

The effects of genotype, environment (habitat and microhabitat), and genotype-by-environment interactions were assessed on the number of spittlebugs and weevil eggs. The effects of both plant genotype and environment were considered because both factors can lead to heterogeneity in herbivory and plant quality, and may in turn affect the insects that colonise plants. The analyses presented here differ from previous work in this system (Johnson & Agrawal, 2005), in that the present study focuses on resistance and interactions among specific herbivore species, whereas earlier work presented descriptive measures of the community as a whole.

Analyses on the number of spittlebugs and weevil eggs were performed using restricted maximum likelihood with Proc Mixed of SAS v. 8.02 (SAS Institute, Cary, North Carolina; Littell *et al.* 1996). Genotype broad-sense breeding values were estimated with best linear unbiased prediction (BLUPs, Littell *et al.*, 1996). BLUP estimates are similar to genotype means, but are less biased by environmental effects (Shaw *et al.*, 1995).

The correlation between spittlebug abundance in early June (their date of peak abundance) and the number of weevil eggs counted in mid-July was examined with quantile regression analysis (Thomson *et al.*, 1996; Cade *et al.*, 1999). Quantile regression tests whether one variable (spittlebug abundance) imposes an upper limit on the values of a second variable (weevil eggs). This statistical method is particularly effective at identifying biological interactions where one species limits the maximum abundance of another species, even though conventional correlation methods may show no relationship (Thomson *et al.*, 1996). Quantile regression was performed by regressing the upper 25th percentile of the data for the number of weevil eggs against the number of spittlebugs. The data were log-transformed to linearise the data and meet the assumptions of homogeneity of variance.

The effects of plant genotype on the presence/absence of adult weevils on plants were assessed using logistic regression in PROC GENMOD of SAS v. 8.02 (Allison, 1999). All plants from one habitat (the disturbed site) were excluded because adult weevils were completely absent from that habitat, resulting in complete separation of the data (Allison, 1999).

Bioassay experiments

Four genotypes (E, B, L, and N) were used from the field experiment that captured the range in resistance to a suite of early season chewing herbivores, of which *T. foveolatum* was the most abundant (Fig. 2). These genotypes all originated from Jokers Hill and were collected from sites less than 2.5 km apart. To reduce maternal effects, seeds from each genotype were grown for one complete generation in a common garden. In mid-May, seeds were germinated at room temperature on moistened filter paper and exposed to natural sunlight. One week later, 130 seedlings from each of the four genotypes were individually planted into 250 ml pots containing soaked Pro Mix General Purpose Soil BX (Premier Horticulture, Dorval, Quebec, Canada). All pots were put into an outdoor meshed cage that excluded insects, but gave exposure to ambient weather conditions. After 6 weeks, all plants were covered with spun-polyester bags and randomly assigned plants to one of three treatments: (1) undamaged control, (2) three nymph spittlebugs (mostly fourth or fifth instar), or (3) one weevil. Although spittlebugs and weevils differ in their feeding style, the herbivory treatment was standardised according to
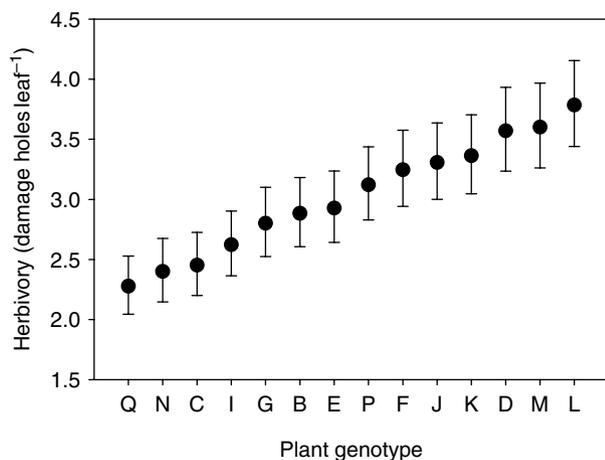

Fig. 2. Variation among plant genotypes in the amount of early season herbivory. Herbivory was measured as the number of discrete damage holes caused per leaf by leaf-chewing herbivores. Although *Tyloderma foveolatum* was the most prominent herbivore, damage was also caused by a congeneric weevil (*T. nigrum* Casey), and two Chrysomelid beetles (*Altica* sp., *Graphops pubescens* Melsheimer). Damage by each species was indistinguishable. Each point represents the best-linear unbiased predictor for a genotype ($\pm$ 1 SE) and genotypes are ordered according to increasing damage.

herbivore dry biomass; three late-instar spittlebugs are approximately equal in biomass to a single weevil (M. Johnson, unpublished data). These herbivores were selected because they were the most abundant early season herbivores on *O. biennis*, and therefore have the greatest potential to induce early season plant responses that can affect the community of herbivores that subsequently colonise plants. Spittlebugs experienced very low mortality. Weevils were allowed to remove approximately 15% of the leaf area on plants, which reflects natural levels of damage. On a few plants, individual weevils either died or were not consuming leaf tissue and these individuals were substituted with new weevils. Damage levels were typically reached after 2 days, at which time all herbivores and bags were removed; weevils were removed from plants after 1 day if 15% of leaf tissue had already been consumed.

Ten days following the cessation of herbivory, one to four newly expanded undamaged leaves (depending on availability) were removed from each plant using a clean razor blade and placed on moistened filter paper. Two of the leaves were used in no-choice assays, while the other two were used for choice assays. On the one hand, no-choice assays provide an absolute measure of the constitutive and induced resistance of plants. On the other hand, choice assays reveal how diversity in plant quality may alter the feeding choices by insects (Behmer *et al.*, 2002). Induced response assays were performed using the beet armyworm caterpillar (*S. exigua*) and adult weevils (*T. foveolatum*). Adult weevils continue to feed naturally in the field for several weeks, making them potentially susceptible to induced responses caused by conspecifics, as well as spittlebugs. The beet armyworm was employed to provide a measure of resistance against generalist leaf-chewing herbivores. To maintain independence among replicates, the leaves from a single plant were only used in a single replicate for a given assay type (i.e. choice, no-choice) and assay species (weevil, caterpillar).

All bioassays were performed in 100-mm Petri dishes lined with moistened filter paper and sealed with Parafilm. In weevil assays, a single adult weevil was placed at the centre of each Petri dish. In no-choice assays, one leaf was placed in the centre of each Petri dish (total $n = 213$ leaves, 14–24 replicates per genotype–damage treatment combination), on top of which the weevil was placed. In choice assays, weevils were presented with three leaves from a single genotype, where each leaf was of a different damage treatment (i.e. undamaged control, spittlebug, and weevil). Equal-sized leaves were placed at the periphery of a dish, equidistant from the centre (total $n = 363$ leaves, 63–117 replicate dishes per genotype). Weevils were removed after 3 days; there was no mortality. The leaves were pressed until dry and the amount of herbivory was measured as the number of discrete damage holes of a consistent size. The number of holes was well correlated with leaf area consumed ($r = 0.95$, $P < 0.001$, $n = 39$).

In caterpillar assays, freshly hatched neonate caterpillars were used following the methods used in the weevil no-choice and choice assays, but caterpillars were removed

after 1 week and mortality assessed. The number of replicate dishes in no-choice assays ranged from 12 to 21 per genotype–damage treatment combination (total $n = 194$ leaves) and 66–123 per genotype in the choice assay (total $n = 376$ leaves).

Statistical analyses

The effects of plant genotype and damage treatment on the amount of herbivory were assessed using PROC GLM of SAS v. 8.02. Plant genotype was treated as a fixed effect because genotypes were chosen to represent the range of herbivore resistance in the field experiment. For no-choice assays, the following model was used: herbivory = $mean_{overall}$ + genotype + damage + genotype × damage + error. A split-plot design was used for choice assays using the model: herbivory = $mean_{overall}$ + genotype + whole-plot error + damage + genotype × damage + dish(genotype) + split-plot error, where 'dish' represents the random effect of Petri dish. The significance of genotype was tested with respect to the whole-plot error, while the split-plot error was used for all other effects in the model. When statistically significant, leaf area was included as a covariate in analyses, which was calculated by measuring leaf length and applying the equation: area = $0.63 \times length^{1.72}$, which accurately converts to leaf area ($r = 0.98$, $P < 0.001$, $n = 30$). Data were transformed with log- or square-root transformations when necessary to improve normality and homogeneity of variance. Figures report the back-transformed data in the original units. For the mortality data, the data were fit to a binomial distribution using generalized linear models in PROC GENMOD of SAS (Allison, 1999). The model for this analysis was: N_{alive}/N_{total} = genotype + damage + genotype × damage. Likelihood-ratio tests were used to test the significance of each effect in the model (Allison, 1999).

Results

Field experiment

Plant genotypes varied in the amount of early season herbivory by 66% from the least resistant to the most resistant genotype ($\chi^2_1 = 4.9$, $P = 0.01$; Fig. 2; Appendix C,

Johnson & Agrawal, 2005). The abundance of spittlebugs and weevils peaked in the first and third weeks of June respectively (Fig. 1a). Spittlebug abundance varied between plant genotypes by as much as 130%, but this effect was only marginally significant (Table 1), and variation between and within habitats strongly affected their abundance (Table 1). The number of weevil eggs varied significantly between plant genotypes (Table 1) by 2.3-fold, while there was no effect of genotype on the occurrence of adults ($\chi^2_{13} = 13.3$, $P = 0.42$), although there was an effect of habitat on adults ($\chi^2_3 = 134.16$, $P < 0.001$). Consistent with spittlebugs negatively affecting the preference of weevils, spittlebugs appear to have imposed an upper limit on the number of eggs that weevils laid on plants. The upper 25th percentile of the number of eggs negatively correlated with the number of spittlebugs on plants early in the season [log_e(weevil eggs) = $1.25 - 0.06$(spittlebugs), $R^2 = 0.42$, $P < 0.001$; Fig. 1b]. In contrast, spittlebug abundance in early June (i.e. during their peak abundance period) did not correlate with the presence of adult weevils in mid-June, when adult weevils were most abundant (logistic regression: $\chi^2_1 = 1.08$, $P = 0.30$).

Bioassay experiments

Weevil, no-choice. Weevils were negatively affected by previous damage by conspecific weevils, evident from the 40% reduction in herbivory compared with controls (Fig. 3b). Induced responses were more important than genetic variation in resistance (Fig. 3a), as the damage treatment accounted for 4% of the total variation while plant genotype was not significant and accounted for < 1% of the variation (Table 2). There was no interaction between plant genotype and damage. Spittlebug feeding caused leaves to become wavy and hard textured at the site of damage, but there was no detectable systemic induction effect on weevils ($t_{Tukey–Kramer} = -0.09$, $P > 0.95$).

Weevil, choice. Consistent with the induced resistance observed in the no-choice assay, weevil herbivory was 29% lower on leaves from plants previously damaged by weevils, but this reduction was not significant ($t_{Tukey–Kramer} = -1.51$, $P = 0.29$). Surprisingly, weevil herbivory was 70% greater

Table 1. The effects of plant genotype (G), habitat (H), and microhabitat (M) on spittlebug abundance and the number of weevil eggs. Habitat was a fixed effect and statistical significance was determined using an *F*-statistic. All other effects were random and their significance was calculated using the χ^2 statistic from log-likelihood ratio tests. Square brackets indicate nested effects. Effects in bold are significant at $P < 0.05$.

Source	Spittlebug abundance		Weevil eggs	
	F/χ^2	P	F/χ^2	P
Habitat	20.99	**<0.001**	88.2	**<0.001**
Microhabitat[H]	38.6	**<0.001**	36.9	**<0.001**
Genotype	2.1	0.07	17.7	**<0.001**
G × H	0.7	0.2	8.1	**0.002**
G × M[H]	0.7	0.2	0	–

on plants damaged by spittlebugs compared with controls ($t_{\text{Tukey–Kramer}} = 3.12$, $P = 0.006$), indicative of induced susceptibility. Genetic differences between plants had little effect on herbivory by weevils, although there was a weak interaction between plant genotype and previous damage (Table 2), caused by some genotypes exhibiting stronger induced susceptibility than others.

Caterpillar, no-choice. Both plant genotype and previous damage affected the amount of herbivory by generalist caterpillars (Table 3). Plant genotype accounted for 27% of the variation in consumption, with mean herbivory varying eight-fold between genotypes (Fig. 4a). The relative difference in herbivory between genotypes closely reflected their rank-order of resistance in the field experiment. Weevils induced resistance to caterpillars, causing a 45% reduction in consumption compared with controls (Fig. 4b), yet the damage treatment explained just 3% of the total variation. Herbivory was 9% lower on spittlebug-damaged plants compared with controls, but this effect was not significant. Plant genotype did not interact with the damage treatment to affect caterpillar herbivory.

The effects of plant genotype and damage on caterpillar mortality mirrored the results for herbivory. Caterpillar mortality varied substantially between genotypes ($\chi^2_3 = 58.6$, $P < 0.001$; Fig. 5a) and survivorship was lowest on plants previously damaged by weevils ($\chi^2_2 = 6.77$, $P = 0.03$, Fig. 5b). As with herbivory, plant genotype and the damage treatment did not interact ($\chi^2_6 = 6.83$, $P = 0.34$).

Caterpillar, choice. Plant genotype again had the strongest effect on herbivory by caterpillars, explaining 13% of the total variation (Table 3). The genotypes varied seven-fold in the amount of herbivory by caterpillars and their rank-order in resistance was nearly identical to that observed in the no-choice assay. Leaves from weevil-damaged plants received 36–74% less herbivory among plant genotypes than control plants, but herbivory levels were generally low and this effect was not significant (Table 3). There was a weak interaction between plant genotype and damage, whereby spittlebug damage resulted in a 59% reduction in herbivory by caterpillars on a single genotype (L).

Discussion

The principal finding of this paper is that both plant genotype and induced responses independently influenced resistance to herbivores. In the field, plant genotypes varied in the amount of herbivory they received by early season herbivores (Fig. 2). This variation in resistance corresponded to the performance of a generalist leaf chewing caterpillar in a bioassay experiment, where constitutive variation in resistance among genotypes was more important than induced resistance caused by weevils (Table 2; Fig. 4). In contrast, the specialist weevil tended to be most strongly affected by induced resistance in response to damage by conspecifics. Genetic variation in resistance had little to no effect on herbivory by adults (Fig. 3), but did affect oviposition preference (Table 1).
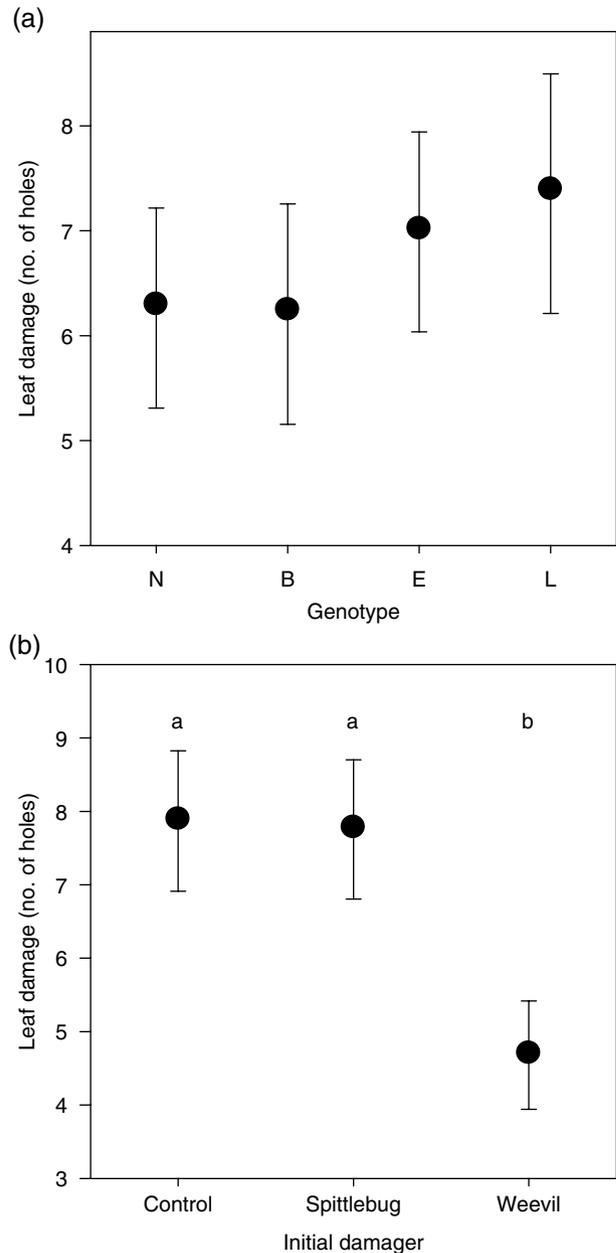
Fig. 3. The amount of herbivory among (a) genotypes and (b) damage treatments by the specialist weevil *Tyloderma foveolatum.* Herbivory was measured as the total number of holes of a consistent size per leaf; the least-squares means ($\pm$ 1 SE) are reported. Means with different lower-case letters are significantly different at $P < 0.05$, according to *post hoc* Tukey–Kramer tests.

Induced responses and plant-mediated competition

The field results show that a combination of genetic and environmental effects lead to variation in the amount of early season damage and plant quality. This heterogeneity has the potential to affect the large suite of insects that subsequently colonise *O. biennis.* In the field, the degree of

infestation by spittlebugs was negatively correlated with the number of eggs laid by weevils on plants (Fig. 1b), yet there was no consistent negative effect of spittlebug feeding on resistance to weevils in the bioassay experiment. This lack of correspondence between oviposition preference and herbivory by adult weevils is not entirely surprising, as the preference and performance of insects are often poorly correlated (Thompson, 1988), and spittlebugs may have affected plant quality in different ways between the two experiments. Spittlebugs also had little effect on caterpillar performance (Table 2; Figs 4 and 5). The specialist weevil on the other hand, negatively affected both conspecific weevils (Fig. 3) and caterpillars (Fig. 4) that fed on newly expanding undamaged leaves from damaged plants. From the bioassay experiment, the contrasting effects of spittlebugs (a xylem-feeder) and weevils (a leaf-chewer) support competition theory that proposes that negative interactions between insect species in the same feeding guild should be stronger than competition among insects of different feeding guilds (Denno *et al.*, 1995).

In spite of earlier arguments to the contrary (Strong *et al.*, 1984), interspecific competition is now recognised as an important factor controlling insect populations and communities (Denno *et al.*, 1995). This paradigm shift was in part motivated by the recognition that insect damage can induce changes that reduce plant quality (Haukioja & Niemelä, 1979) and mediate interactions within and between insect species (Haukioja, 1980; West, 1985; Faeth, 1986). These plant-mediated interactions can have important consequences for multiple species of co-occurring insects (Agrawal, 1999; Kessler & Baldwin, 2001; Thaler *et al.*, 2001; Viswanathan *et al.*, 2005), and may influence the diversity and structure of insect communities (Kessler *et al.*, 2004; Van Zandt & Agrawal, 2004a). The results from this paper support the hypothesis that induced responses act as a mechanism for intra- and interspecific competition among insects (Figs 3, 4 and 5). Furthermore, these results demonstrate specificity in the elicitation of responses by different herbivores but no specificity in the effect of these responses (Van Zandt & Agrawal, 2004b; Viswanathan *et al.*, 2005). In particular, weevils caused strong and relatively consistent effects of induced responses while spittlebugs had inconsistent to no effect on resistance to herbivores, yet they induced visible morphological changes in the plant.

Genetic variation and induced responses

This study found no evidence of genetic variation in induced responses. Plant genotype and the initial damage treatment never interacted to affect resistance to the second herbivore in no-choice assays, and therefore plant genotype did not mediate interspecific interactions among insects (Tables 2 and 3). In choice assays, the weak interactions and induced susceptibility caused by spittlebug damage provide evidence that diversity in plant quality may alter the feeding choices by insects (Behmer *et al.*, 2002;

Rodriguez-Saona & Thaler, 2005), but the overwhelming evidence from the combination of choice and no-choice assays is that these factors act independently. This may not be the case for all species interactions on *O. biennis* in the field, and further research that looks at a wider array of species is warranted.

To compare the findings of this paper with that of other studies, a review was carried out of all studies that report adequate empirical data on the effects of plant genotype and interspecific competition (regardless of mechanism) among insects in natural systems (Table 4). In these studies, plant genotype significantly affected the preference and/or performance of a focal herbivore in two-thirds of pair-wise interactions (64%), while interspecific competition occurred in less than half of the interactions (41%). Of the 12 unique competitive interactions reported, genetic variation in resistance mediated interactions among herbivores in a minority of studies (25%). In the three pairwise interactions where plant genotype mediated species interactions, initial herbivory either had a large effect on plant morphology (Mopper *et al.*, 1990; Cronin & Abrahamson, 1999), or genetic variation in resistance strongly affected the competitor itself (Moran & Whitham, 1990), which reduced its density and ameliorated the competitor's effects on the focal herbivore. The likelihood that genetic variation will mediate competitive interactions will depend on several factors, including the magnitude of variation in constitutive resistance and induced responses, the types of induced responses (e.g. chemical, morphological, etc.), the covariance between constitutive and induced resistance, and the offensive traits and sensitivity of the herbivores to resistance (Karban & Agrawal, 2002). Theoretical and empirical research in this area is greatly needed.

When the results from the present study are fit into the body of literature accumulating on this topic (Table 4), it is clear that plant genotype tends to have a stronger influence than insect competition on the preference and performance of insect herbivores. Plant genotype explained more variation [mean = $20.7 \pm 5.7\%$ (SE)] than both the main effect of interspecific competition (mean = $3.4\% \pm 1.0$) as well as the interaction between plant genotype and competition (mean = $4.2\% \pm 1.3$). The difference in the amount of variation explained by the main effects of plant genotype and competition is significant ($F_{1,12} = 8.47$, $P = 0.01$). Feeding mode of the competing insect did not influence the amount of variation explained by the focal herbivore's response to plant genotype ($F_{2,19} = 0.14$, $P = 0.87$), but it did affect the amount of variation explained by the focal herbivore's response to competition ($F_{2,19} = 4.16$, $P = 0.03$). Competition with leaf chewing (mean = 3.7 ± 1.3) and endophytic competitors (mean = 5.5 ± 2.8) explained more variation than competition with piercing–sucking herbivores (mean = 0.7 ± 0.8). The feeding mode of the focal herbivores had no effect on the amount of variation explained by either plant genotype ($F_{4,17} = 1.87$, $P = 0.16$) or competition ($F_{4,17} = 1.84$, $P = 0.17$). Thus, it appears that the feeding mode of the competitor, and not the focal herbivore, is most important in affecting interspecific competition.

Table 2. The effects of plant genotype and damage treatment on the amount of herbivory by the weevil *Tyloderma foveolatum* in choice and no-choice assays. In the analyses of choice assays, dish nested within genotype was included as a blocking factor and leaf length was used as a covariate. Square brackets indicate nested effects and effects in bold are significant at $P < 0.05$.

Source	d.f.	SS	F	P
No-choice assay				
Genotype (G)	3	1.68	0.28	0.84
Treatment (T)	2	17.96	4.48	**0.01**
G × T	6	14.91	1.24	0.29
Error	200	401.41		
Choice assay				
Genotype	3	4.44	1.28	0.28
Whole-plot error	134	154.11		
Treatment	2	16.47	10.88	**<0.001**
G × T	6	8.98	1.98	0.07
Leaf length	1	5.78	7.64	**0.01**
Dish[G]	117	140.02	1.58	**0.002**
Split-plot error	216	163.59		

Table 3. The effects of plant genotype and damage treatment on the amount of herbivory by *Spodoptera exigua* neonate caterpillars in choice and no-choice assays. In the analyses of choice assays, dish nested within genotype was included as a blocking factor and leaf length was used as a covariate. Square brackets indicate nested effects and effects in bold are significant at $P < 0.05$.

Source	d.f.	SS	F	P
No-choice assay				
Genotype (G)	3	58.82	24.05	**<0.001**
Treatment (T)	2	5.46	3.35	**0.04**
G × T	6	4.18	0.85	0.53
Error	181	147.58		
Choice assay				
Genotype	3	28.01	16.77	**<0.001**
Whole-plot error	162	90.21		
Treatment	2	1.17	1.17	0.31
G × T	6	6.2	2.08	0.06
Leaf length	1	2.18	4.39	**0.04**
Dish[G]	121	68.69	1.14	0.2
Split-plot error	235	116.97		

Unfortunately, the mechanism of competition was rarely identified in the studies reviewed, so the generality of these results maybe sensitive to the type of competition among insects (see Denno *et al.*, 1995). Nonetheless, these patterns are likely reflective of natural systems, given that the majority of the studies reviewed were field-based.

Community genetics

Recent attention has been paid to the community-level importance of intraspecific genetic variation (Agrawal, 2003). Although community genetics research clearly shows that intraspecific genetic variation can play a prominent role in structuring arthropod communities (Whitham *et al.*, 2003), there have been few attempts to assess the relative importance of plant genotype vs. those factors (such as the abiotic environment, predation, mutualism, interspecific resource diversity, and competition) traditionally thought most important in affecting populations and communities of insects (e.g. Fritz *et al.*, 1986; Karban, 1987; Fritz & Price, 1988; Karban, 1989). This study, combined with a quantitative review of the literature, show that intraspecific genetic variation tends to be more important than interspecific competition in affecting resistance to individual insects. These results contribute to ongoing community genetics research on interactions between *O. biennis* and its arthropod fauna (Johnson & Agrawal, 2005; Johnson *et al.*, 2006), which highlight the importance of genetic variation in affecting the ecology of species interactions. More studies are needed to gain a comprehensive understanding of the relative and interactive role that intraspecific genetic variation plays in affecting insect populations and communities.
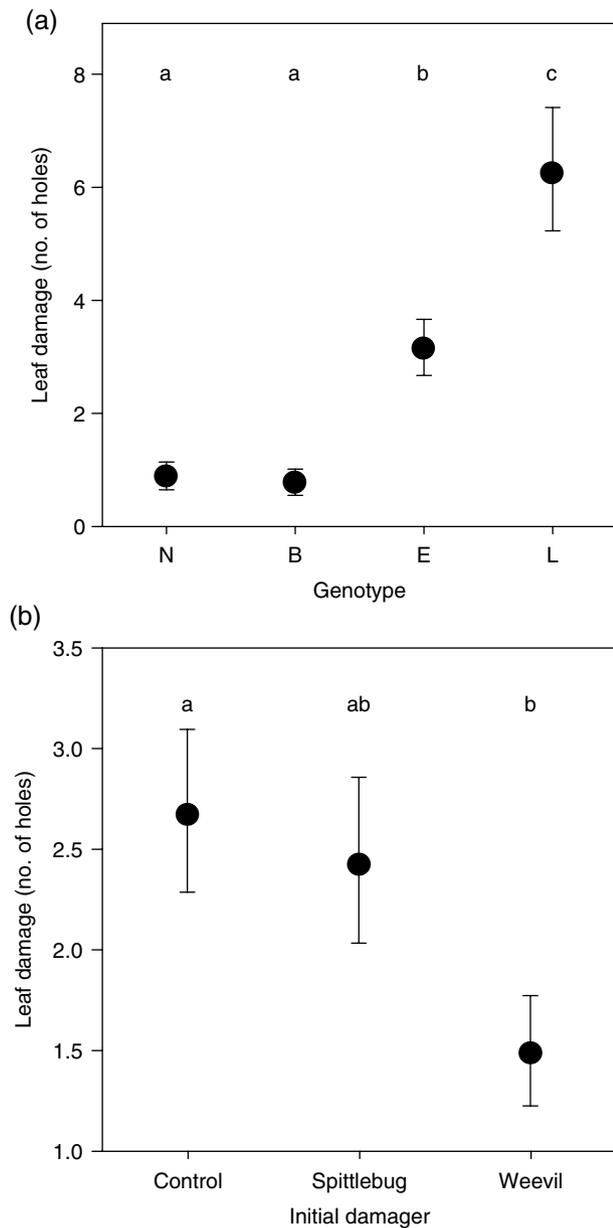
Fig. 4. The amount of herbivory among (a) genotypes and (b) damage treatments by the generalist caterpillar *Spodoptera exigua*. Herbivory was measured as the total number of holes of a consistent size per leaf; the least-squares means ($\pm$ 1 SE) are reported. Means with different lower-case letters are significantly different at $P < 0.05$, according to *post hoc* Tukey–Kramer tests.
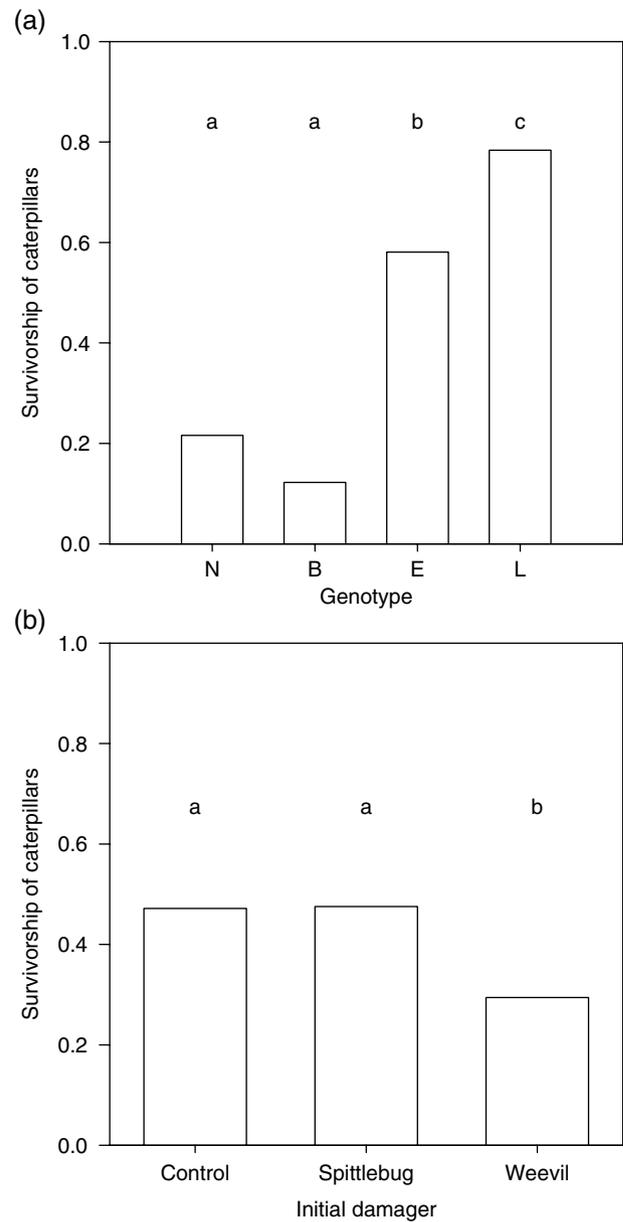

Fig. 5. The proportion of *Spodoptera exigua* caterpillars surviving on leaves from different (a) genotypes and (b) damage treatments in the no-choice assay. Different lower-case letters above the means indicate the means that are significantly different at $P < 0.05$, according to *post hoc* Tukey–Kramer tests.

Acknowledgements

Table 4. A review of studies that simultaneously investigated the effects of plant genotype and interspecific competition among insect herbivores. The following is reported for each study: the acting competitor species, the focal herbivore species, their respective feeding modes (type and location of feeding), the measured response variable for the focal herbivore, the amount of variation explained by plant genotype (G), competition (C), the interaction (I) between plant genotype and competition, and the mechanism (Mech.) of competition. The amount of variation explained was determined by dividing the sums-of-squares reported for the effect by the total sums-of-squares. Significant effects at $P < 0.05$ are in bold. The mechanism of competition was not identified in most studies. Several studies reported multiple response variables for each focal herbivore, and although these different variables typically gave qualitatively similar results, we chose the response variable that was most similar to variables reported in other studies.

Study	Competitor	Feeding mode	Focal species	Feeding mode	Response	G	C	I	Mech.
Agrawal (2004)	*Tetraopes tetraophthalmus*	C, L	*Liriomyza asclepiadis*	M, L	Abundance	3.9	**11.4**	4.1	I
Cronin & Abrahamson (2001)	*Philaenus spumarius*	P, X	*Eurosta solidaginis*	En-G, S	Ovipunctures	**46.6**	**0.6**	17.1	I?
Cronin & Abrahamson (2001)	*Trirhabda* sp.	C, L	*E. solidaginis*	En-G, S	Ovipunctures	**71.3**	4.4	8.3	U
Cronin & Abrahamson (2001)	*P. spumarius*	P, X	*E. solidaginis*	En-G, S	Gall size	**55.4**	1.7	11.8	I
Cronin & Abrahamson (2001)	*Trirhabda* sp.	C, L	*E. solidaginis*	En-G, S	Gall size	**41.2**	1.9	21.2	U
Cronin & Abrahamson (1999)	*P. spumarius*	P, X	*E. solidaginis*	En-G, S	Ovipunctures	1.8	**2.4**	**3.8**	I?
Mopper *et al.* (1990)	*Dioryctria albovitella*	C, L	*Neodiprion edulicolis*	En-G, S	Various			Yes[a]	U
Moran & Whitham (1990)	*Hayhurstia atriplicis*	P, Ph	*Pemphigus betae*	P, Ph	Abundance			Yes[a]	I
Karban (1987) Year 1	*P. spumarius*	P, X	*A. secticornis*	Ce, L	Abundance	**21.8**	0.5	0.7	U
Karban (1987) Year 1	*Platyptilia williamsii*	C, L	*A. secticornis*	Ce, L	Abundance	**21.8**	1.5	0.2	U
Karban (1987) Year 2	*P. spumarius*	P, X	*A. secticornis*	Ce, L	Abundance	**16.3**	0.6	0.8	U
Karban (1987) Year 2	*P. williamsii*	C, L	*A. secticornis*	Ce, L	Abundance	**16.3**	<0.1	0.3	U
Karban (1989) Year 1	*P. williamsii*	C, L	*P. spumarius*	P, X	Mass (male)	0.5	1	2.9	U
Karban (1989) Year 2	*P. williamsii*	C, L	*P. spumarius*	P, X	Mass (female)	11.4	1.6	**12.6**	U
Karban (1989) Year 3	*P. williamsii*	C, L	*P. spumarius*	P, X	Persistence	1.6	**8.4**	2.3	U
Karban (1989) Year 1	*P. spumarius*	P, X	*P. williamsii*	C, L	Persistence	<0.1	0.9	NS	U
Karban (1989) Year 2	*P. spumarius*	P, X	*P. williamsii*	C, L	Persistence	0.6	<0.1	NS	U
Karban (1989) Year 3	*P. spumarius*	P, X	*P. williamsii*	C, L	Persistence	3.3	0.3	NS	U
Fritz *et al.* (1986)	*Euura lasiolepis*	En-G, S	*Pontania* sp. nov.	En-G, L	Abundance	**18.9**	**9.4**	10.6	I?
Fritz *et al.* (1986)	*E. lasiolepis*	En-G, S	*P.* sp. nov.	En-G, L	Abundance	**19.9**	0.2	6.7	I?
Fritz *et al.* (1986)	*E. lasiolepis*	En-G, S	*Phyllocolpa* sp.	Ex-G, L	Abundance	**23.6**	**6.9**	4.7	I?
McGuire & Johnson (this study)	*P. spumarius*	P, X	*T. foveolatum*	C, L	Herbivory	1.9	<0.01	2.1	I
McGuire & Johnson (this study)	*P. spumarius*	P, X	*Spodoptera exigua*	C, L	Herbivory	**26.5**	0.1	1.4	I
McGuire & Johnson (this study)	*Tyloderma foveolatum*	C, L	*S. exigua*	C, L	Herbivory	**32.7**	**3.4**	1.3	I

[a]Design not fully factorial or relevant statistics not reported; NS, interaction not significant and statistics not reported. Feeding mode: C, free-roaming chewer; Ce, cell content feeder; En-G, endophytic galler; Ex-G, exophytic galler; M, miner; P, piercing–sucking. Feeding location: L, leaf; Ph, phloem; S, stem; X, xylem. Mechanism of competition: I, induced response; I?, probably induced response; U, unknown.

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