Induced Plant Resistance and Susceptibility to Late-Season Herbivores of Wild Radish

ANURAG A. AGRAWAL AND MARGARET F. SHERRIFFS

Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada

ABSTRACT Wild radish is an annual plant that exhibits broad spectrum induced resistance to herbivores. In two experiments, we placed potted plants [control, manually clipped, and damaged by *Pieris rapae* (L.) larvae] in the field and assayed for oviposition by *P. rapae* (a specialist herbivore), damage by flea beetles (a specialist herbivore), and damage by rabbits (a generalist herbivore). Induced responses attracted oviposition by *P. rapae* and increased damage by flea beetles, while having a minimal effect on rabbit herbivory. Plant families had different levels of resistance to herbivory by rabbits and to oviposition by *P. rapae*, but not to herbivory by flea beetles. Manual clipping was a poor inducer of plant responses. Induced responses in wild radish can be a double-edged sword, increasing herbivory by some herbivores under certain conditions, while reducing herbivory by other herbivores.

KEY WORDS *Raphanus raphanistrum*, induced defense, plant-insect interactions, generalist versus specialist, trade-offs

MANY PLANTS ACTIVELY respond to herbivore damage by producing chemicals or structures that reduce future attack (Karban and Baldwin 1997). Such induced responses that increase plant resistance are thought to be adaptive in that they can increase the fitness of plants in environments with herbivory, but may reduce fitness in the absence of herbivory (Agrawal 1998). One benefit of induced responses over constitutive strategies is that the plant-defense strategy is not on all of the time, and thus potential host-finding cues for specialist herbivores are not present when plants are not induced (Agrawal and Karban 1999). For example, damage-induced responses in *Cucurbita moschata* (Duchesne) deterred feeding by one herbivore [*Epilachna tredecimnotata* (Latreille)], while they attracted another species [*Acalymma vittata* (F.)] (Carroll and Hoffman 1980). In this case, the inducible strategy may protect the plant against one attacker, but also prevents the plant from being devoured by the other.

Plants in the family Brassicaceae produce a group of secondary metabolites known as mustard oil glycosides, or glucosinolates, which play a role in plant defense (Chew 1988, Louda and Mole 1992). Within the family, constitutive levels of glucosinolates vary between species and individuals, and in addition, many brassicaceous plants produce higher levels of indole glucosinolates after herbivory (Koritsas et al. 1991, Bodnaryk 1992, Griffiths et al. 1994, Baur et al. 1998, Agrawal et al. 1999a). Glucosinolates have been found to deter herbivory by generalist herbivores and attract specialist herbivores (Huang and Renwick 1994, Giamoustaris and Mithen 1995). Because glucosinolates play this dual role, it might benefit bras-

sicaceous plants to produce high levels of glucosinolates only after herbivory has occurred, thereby reducing attraction of specialist herbivores when they are not under attack.

We have examined the induced responses of wild radish plants [*Raphanus raphanistrum* (L.) and *R. sativus* (L.)] to place the evolutionary ecology of induced resistance in a community context (Agrawal 1998, 1999a, 1999b, 2000; Agrawal et al. 1999a, 1999b). In this system, plants are attacked by many specialist and generalist herbivores and respond by increasing concentrations of indole glucosinolates and densities of setose trichomes. During the natural phenology of wild radish, induced resistance has been reported against seven species of herbivores in three different feeding guilds, reducing their preference or performance, yet other herbivores seem unaffected by the induced plant responses (Agrawal 2000).

In this study we sought to examine the consequences of induced responses to herbivory for late-season herbivores of wild radish, several of which are specialist feeders. Specifically we asked the following question: What are the consequences of caterpillar damage (removal of one leaf) and manual clipping (removal of one leaf) for oviposition by *Pieris rapae* (L.) (a specialist herbivore), damage by flea beetles (a specialist herbivore), and damage by rabbits (a generalist herbivore)?

Materials and Methods

Raphanus raphanistrum (Brassicaceae) is a cosmopolitan, self-incompatible, annual plant found in disturbed sites. In northern California, where this study

was conducted, wild radish plants germinate in the late winter (December–February), flower through spring (February–May), and produce indehiscent seeds by early summer (May–July). Generalist and specialist folivores of wild radish include various caterpillars, aphids, beetles, and mollusks. The common herbivores of wild radish that are negatively affected by induced resistance include earwigs, grasshoppers, aphids, and several species of lepidopteran larvae, including *P. rapae* (Agrawal 1998, 1999a, 1999b).

In June and July 1996, we planted seeds of 13 maternal families of wild radish (full- or half-sibs) in greenhouse soil in 800-ml pots. Family structure was used to minimize genetic variance and maximize ability to measure treatment effects. We grew two cohorts of ≈80 plants, separated by 1 wk, that were each randomly divided into three treatment groups: (1) unmanipulated controls, (2) damaged by a single *P. rapae* larva confined to a clip-cage (one full leaf removed per plant), and (3) one full leaf clipped with a scissors at the petiole. Treatments were imposed when the plants had two to three true leaves. Clip-cages were made from ventilated tops of 5-cm petri dishes attached to either side of a hair clip. Several other experiments in this system have showed that empty clip-cages attached to leaves do not induce responses or affect seed set (Agrawal 1999a, 1999b; Lehtilä and Strauss 1999). The clip-cages were moved twice daily to allow the caterpillar to consume the entire leaf; the larvae completed their feeding within 2 d. The clipping treatment was employed because unpublished data suggested that clipping did not induce resistance (for examples of this in field experiments with wild radish see also Agrawal 1998, 1999a) or induction of glucosinolates (Agrawal et al. 1999c). Therefore, clipping could be used as a leaf-damage control, whereby caterpillar-treated and clipped plants had equal leaf tissue removed, but only the former with induced plant responses. Many other systems show minimal or reduced induced responses to manual clipping damage (Haukioja and Neuvonen 1985, Hartley and Lawton 1987, Mattson and Palmer 1988, Baldwin 1990, Lin et al. 1990, Turlings et al. 1990, Bodnaryk 1992, Korth and Dixon 1997). Induced plant responses are thought to be minimized by clipping damage because of the absence of herbivore saliva, the greatly reduced area of actual leaf tissue that is damaged, and rapid nature of the removal.

The day after the treatments were imposed, the plants were set out in a field ≈50 m from a cabbage patch at the University of California—Student Experimental Farm in Davis, CA. The field had a large population of morning glories, *Convolvulus arvensis* (L.), at which the specialist herbivore, *P. rapae*, frequently takes nectar. Another specialist herbivore, the flea beetle (*Phylotreta* spp.), was present. Generalist pests in the area included thrips, rabbits, and grasshoppers. The plants were randomly arranged in two staggered rows. The rows were 2 m apart, and the plants were 2 m apart within the rows. The plants were set in plastic dishes and given 1 cm of water from below each morning to prevent desiccation. After 3 d,

P. rapae eggs that had been deposited on the plants were counted and herbivore damage was recorded. We only observed significant damage by rabbits and flea beetles, which have very distinct types of feeding. Rabbit feeding is typically characterized by the consumption of large chunks of leave, whereas flea beetle feeding is characterized by clusters of many small pits. Percent leaf area removed was calculated by visual estimation (i.e., each leaf of a plant that has five leaves = 20%; if 20% of one leaf is damaged we recorded 4% total leaf damage). After the initial assay, the eggs were removed from each plant using fine tweezers, and plants were left in the field for an additional 3 d. The number of newly laid eggs was counted, and the cumulative herbivory by rabbits and flea beetles was recorded. This experiment was repeated with the second cohort of plants.

The percentage data were arcsine square-root transformed for analysis. We analyzed the effects of treatment (control, clipped, induced), plant family, and trial on the number of eggs, amount of flea beetle damage, and rabbit damage per plant (on 2 d each = six response variables) using a multivariate analysis of variance (MANOVA). We employed the MANOVA approach because the egg laying and herbivory are potentially not independent response variables. Interaction terms were not significant, and were thus not included in the model. Following a significant MANOVA result, we used univariate analyses to examine effects on the different response variables. In addition, because we had the a priori hypothesis that clipped plants would not show induced responses, we examined the pair-wise differences between treatments using Fisher's least significant difference (LSD) test.

Results and Discussion

MANOVA revealed significant main effects of induction treatments, plant family, and trial (Table 1). Induced responses attracted oviposition by specialist *P. rapae* and increased damage by specialist flea beetles, but only on the first census date (Fig. 1). Manual clipping was a poor inducer of wild radish, and did not increase plant susceptibility to *P. rapae* oviposition or flea beetle damage (Fig. 1). However, on the second sampling date, clipped plants received significantly less damage from rabbits than controls. In field experiments that paralleled the natural phenology of wild radish, induced plants received less natural herbivory and produced more flowers and seeds than controls (Agrawal 1998, 1999a). There is also strong evidence for induced resistance to several herbivores from different feeding guilds that were not encountered in this field experiment (Agrawal 1999a, 2000). However, in the current experiment we found evidence for induced susceptibility of plants, with induced plants receiving more *P. rapae* eggs and damage by flea beetles than control plants. These experiments were conducted at a time when specialist herbivores abound due to the cultivation of brassicaceous agricultural

Table 1. MANOVA and univariate analyses for the effects of induction treatments, plant family, and experimental trial on the number of *P. rapae* eggs, percent leaf damage by flea beetles, and percent leaf damage by rabbits on wild radish plants
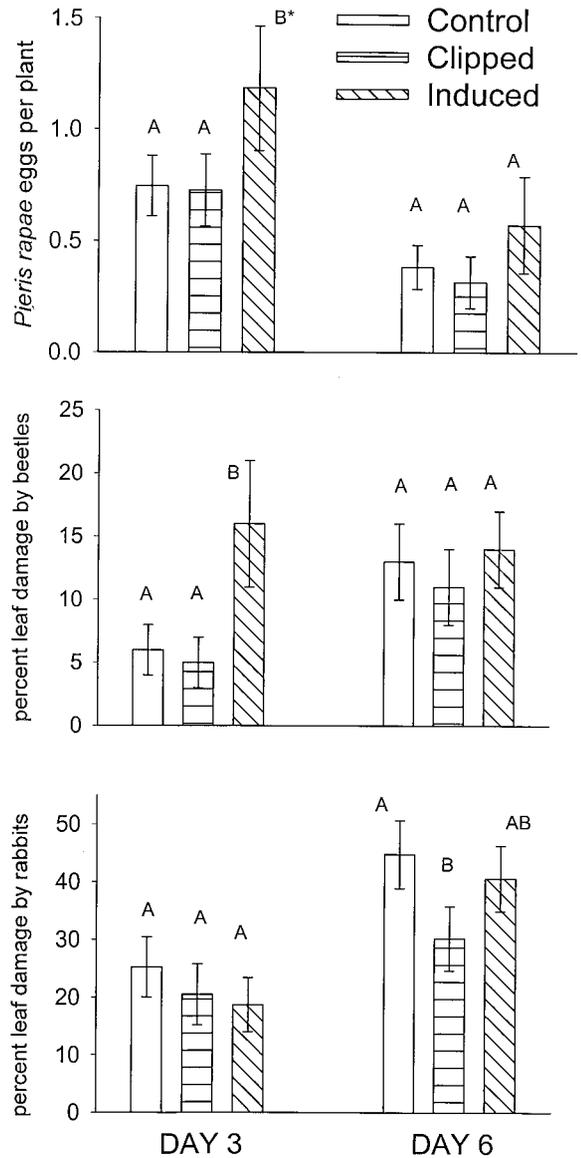
	F	df	P
Induced responses (MANOVA)	1.881	12,256	0.037
Univariate analyses:			
Pieris rapae eggs–day 3	1.668	2,133	0.193
Pieris rapae eggs–day 6	0.507	2,133	0.603
Flea beetle damage–day 3	2.997	2,133	0.053
Flea beetle damage–day 6	0.443	2,133	0.643
Rabbit damage–day 3	0.658	2,133	0.520
Rabbit damage–day 6	3.105	2,133	0.048
Plant family (MANOVA)	1.306	108,740	0.027
Univariate analyses:			
Pieris rapae eggs–day 3	1.064	18,133	0.395
Pieris rapae eggs–day 6	1.712	18,133	0.044
Flea beetle damage–day 3	1.001	18,133	0.463
Flea beetle damage–day 6	1.162	18,133	0.302
Rabbit damage–day 3	1.763	18,133	0.036
Rabbit damage–day 6	2.191	18,133	0.006
Trial (MANOVA)	3.839	6,128	0.001
Univariate analyses:			
Pieris rapae eggs–day 3	0.696	1,133	0.406
Pieris rapae eggs–day 6	1.285	1,133	0.259
Flea beetle damage–day 3	3.290	1,133	0.072
Flea beetle damage–day 6	2.667	1,133	0.105
Rabbit damage–day 3	0.607	1,133	0.437
Rabbit damage–day 6	15.453	1,133	<0.001

Percentage data were arcsine square-root transformed before the analyses.

Fig. 1. Effects of clipping and caterpillar damage (induced) on the number of *P. rapae* eggs, percent leaf damage by flea beetles, and percent leaf damage by rabbits on wild radish plants. Error bars are mean ± SE. Raw data are presented, although percentage data were arcsine square-root transformed for analyses. Different letters represent statistically different values (*P* < 0.05, Fisher's LSD, except B* where *P* = 0.1).

crops, while many wild brassicaceous species are senescing.

The finding that different life stages of herbivores are differentially affected by plant traits may be common. In previous studies we have found strong evidence of induced resistance in wild radish to *P. rapae* larvae (Agrawal 2000), yet here adults were attracted to induced plants. It has been well documented that *P. rapae* adults are attracted to indole glucosinolates (Huang and Renwick 1994), the same class of glucosinolates that are induced by herbivory in many brassicaceous plants, including wild radish (Koritsas et al. 1991, Bodnaryk 1992, Doughty et al. 1995, Agrawal et al. 1999a). It is not known, however, if induced indole glucosinolates are the active component of induced resistance to larvae. In an analogous system, larvae of the specialist *Junonia coenia* (Hübner) have high mortality on genotypes of *Plantago lanceolata* (L.) that contain high levels of iridoid glycosides (Adler et al. 1995), yet adults of *J. coenia* are attracted to high levels of iridoid glycosides (Bowers 1992). Divergent effects of plant traits on different life stages have also been reported in tri-trophic interactions where adult parasitoids are attracted to particular compounds, but the same suites of traits have negative effects on parasitoid larvae (Thaler 1999, Havill and Raffa 2000). Because it appears that adults are often attracted to putative defenses, the net consequences of the negative effects on larvae depend on the relative difficulty of host-finding compared with coping with toxic hosts.

One of the hypotheses put forth to explain the evolution of inducible defenses is that expression of inducible defense avoids creating a persistent target for herbivores that are use these 'defenses' as host-finding cues (Agrawal and Karban 1999, Agrawal et al. 1999c). In addition to *P. rapae,* specialist flea beetles (Feeny et al. 1970, Giamoustaris and Mithen 1995), diamondback moths (Reed et al. 1989, Riggin-Bucci and Gould 1996, Verkerk and Wright 1996), and root flies (Griffiths et al. 1994, Baur et al. 1996a, 1996b, 1998) are also attracted to glucosinolates, although
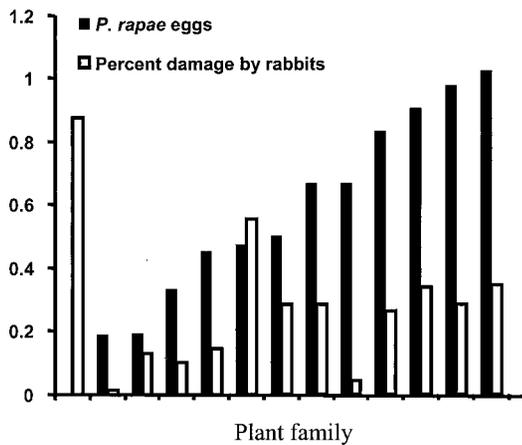
Fig. 2. Variation in the number of *P. rapae* oviposited and percent leaf herbivory by rabbits on 13 families of wild radish. Data for each family is the average of several individual plants censused on two dates. There is no correlation between the two types attackers (Bartlett chi-square = 0.200, df = 1, P = 0.655).

only a few of these experiments have documented induced susceptibility to these herbivores. The most puzzling results have been reported for flea beetles, in which induced resistance has been reported under some conditions (Palaniswamy and Lamb 1993, Agrawal 1998), whereas induced susceptibility resulted under other conditions (Vaughn and Hoy 1993, this study).

In addition to levels of plant resistance and susceptibility being affected by environmental conditions (i.e., previous herbivory), we demonstrated that families of wild radish plants also vary in their resistance to oviposition by *P. rapae* and herbivory by rabbits (Table 1; Fig. 2). Family level variation was striking, spanning an order of magnitude in attack by *P. rapae* and rabbits. There was no correlation between these two attackers (Fig. 2). Although there was no overall effect of induction on rabbits, the family level variation is intriguing, and perhaps suggests that rabbit herbivory has selected for constitutive resistance over induced resistance. We did not find evidence that induced resistance varied across families (i.e., a genotype-by-environment interaction indicating genetic variation in induction). In other experiments we found evidence of genetic variation in wild radish for induced resistance to aphids (Agrawal 1999a), and such genetic variation has been reported for several other plant-herbivore systems (Agrawal 1999b). Thus, plant genotype, environment, and their interaction have the potential to influence generalist and specialist herbivores of wild radish.

Acknowledgments

References Cited

Adler, L. S., J. Schmitt, and M. D. Bowers. 1995. Genetic variation in defensive chemistry in *Plantago lanceolata* (Plantaginaceae) and its effect on the specialist herbivore *Junonia coenia* (Nymphalidae). Oecologia (Berl.) 101: 75–85.

Agrawal, A. A. 1998. Induced responses to herbivory and increased plant performance. Science 279: 1201–1202.

Agrawal, A. A. 1999a. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology 80: 1713–1723.

Agrawal, A. A. 1999b. Induced plant defense: Evolution of induction and adaptive phenotypic plasticity, pp. 251–268. *In* A. A. Agrawal, S. Tuzun, and E. Bent [eds.], Inducible plant defenses against pathogens and herbivores: Biochemistry, ecology, and agriculture. American Phytopathological Society Press, St. Paul, MN.

Agrawal, A. A. 2000. Specificity of induced resistance in wild radish: Causes and consequences for two specialist and two generalist caterpillars. Oikos 89: 493–500.

Agrawal, A. A., P. M. Gorski, and D. W. Tallamy. 1999a. Polymorphism in plant defense against herbivory: Constitutive and induced resistance is *Cucumis sativus.* J. Chem. Ecol. 25: 2285–2304.

Agrawal, A. A., and R. Karban. 1999. Why induced defenses may be favored over constitutive strategies in plants, pp. 45–61. *In* R. Tollrian and C. D. Harvell [eds.], The ecology and evolution of inducible defenses. Princeton University Press, Princeton, NJ.

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999b. Transgenerational induction of defenses. in animals and plants. Nature (Lond.) 401: 60–63.

Agrawal, A. A., S. Y. Strauss, and M. J. Stout. 1999c. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. Evolution 53: 1093–1104.

Baldwin, I. T. 1990. Herbivory simulations in ecological research. Trends Ecol. Evol. 5: 91–93.

Baur, R., A.N.E. Birch, R. J. Hopkins, D. W. Griffiths, M.S.J. Simmonds, and E. Stadler. 1996a. Oviposition and chemosensory stimulation of the root flies *Delia radicum* and *D. floralis* in response to plants and leaf surface extracts from resistant and susceptible *Brassica* genotypes. Entomol. Exp. Appl. 78: 61–75.

Baur, R., V. Kostal, B. Patrian, and E. Staedler. 1996b. Preference for plants damaged by conspecific larvae in ovipositing cabbage root flies: Influence of stimuli from leaf surface and roots. Entomol. Exp. Appl. 81: 353–364.

Baur, R., E. Staedler, K. Monde, and M. Takasugi. 1998. Phytoalexins from *Brassica* (Cruciferae) as oviposition stimulants the cabbage root fly, *Delia radicum.* Chemoecology 8: 163–168.

Bodnaryk, R. P. 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry 31: 2671–2677.

Bowers, M. D. 1992. Iridoid glycosides, pp. 297–325. *In* G. A. Rosenthal and M. R. Berenbaum [eds.], Herbivores: their interactions with secondary plant metabolites, 2nd ed.,

vol. I: The chemical participants. Academic, San Diego, CA.

Carroll, C. R., and C. A. Hoffman. 1980. Chemical feeding deterrent mobilized in response to insect herbivory and counteradaptation by *Epilachna tredecimnotata.* Science 209: 414–416.

Chew, F. S. 1988. Searching for defensive chemistry in the Cruciferae, do glucosinolates always control interactions of Cruciferae with their potential herbivores and symbionts? No! pp. 81–112. *In* K. C. Spencer [ed.], Chemical mediation of coevolution. Academic, San Diego, CA.

Doughty, K. J., G. A. Kiddle, B. J. Pye, R. M. Wallsgrove, and J. A. Pickett. 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38: 347–350.

Feeny, P., K. L. Paauwe, and N. J. Demong. 1970. Flea beetles and mustard oils: host plant specificity of *Phyllotreta cruciferae* and *P. striolata* adults (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 63: 832–841.

Giamoustaris, A., and R. Mithen. 1995. The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. oleifera) on its interaction with specialist and generalist pests. Ann. Appl. Biol. 126: 347–363.

Griffiths, D. W., A.N.E. Birch, and W. H. Macfarlane-Smith. 1994. Induced changes in the indole glucosinolate content of oilseed and forage rape (*Brassica napus*) plants in response to either turnip root fly (*Delia floralis*) larval feeding or artificial root damage. J. Sci. Food Agric. 65: 171–178.

Hartley, S. E., and J. H. Lawton. 1987. Effects of different types of damage on the chemistry of birch foliage and the responses of birch feeding insects. Oecologia (Berl.) 74: 432–437.

Haukioja, E., and S. Neuvonen. 1985. Induced long-term resistance of birch foliage against defoliators: defensive or incidental? Ecology 66: 1303–1308.

Havill, N., and K. Raffa. 2000. Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. Ecol. Entomol. 25: 171–179.

Huang, X., and J.A.A. Renwick. 1994. Relative activities of glucosinolates as oviposition stimulants for *Pieris rapae* and *P. napi* oleracea. J. Chem. Ecol. 20: 1025–1037.

Karban, R., and I. T. Baldwin. 1997. Induced responses to herbivory. University of Chicago Press, Chicago, IL.

Koritsas, V. M., J. A. Lewis, and G. R. Fenwick. 1991. Glucosinolate responses of oilseed rape, mustard and kale to mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala*). Ann. Appl. Biol. 118: 209–222.

Korth, K. L., and R. A. Dixon. 1997. Evidence for chewing insect-specific molecular events distinct from a general wound response in leaves. Plant Physiol. 115: 1299–1305.

Lehtilä, K., and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum.* Ecology 80: 116–124.

Lin, H., M. Kogan, and D. Fischer. 1990. Induced resistance in soybean to the Mexican bean beetle (Coleoptera: Coccinellidae): comparisons of inducing factors. Environ. Entomol. 19: 1852–1857.

Louda, S., and S. Mole. 1992. Glucosinolates chemistry and ecology, pp. 123–164. *In* G. A. Rosenthal and M. R. Berenbaum [eds.], Herbivores: their interactions with secondary plant metabolites, 2nd ed., vol. I: The chemical participants. Academic, San Diego, CA.

Mattson, W. J., and S. R. Palmer. 1988. Changes in levels of foliar minerals and phenolics in trembling aspen *Populus tremuloides* in response to artificial defoliation, pp. 157–170. *In* W. J. Mattson, J. Levieux, and C. Bernard-Dagan [eds.], Mechanisms of woody plant defenses against insects: search for pattern. Springer, Berlin.

Palaniswamy, P., and R. J. Lamb. 1993. Wound-induced antixenotic resistance to flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), in crucifers. Can. Entomol. 125: 903–912.

Reed, D. W., K. A. Pivnick, and E. W. Underhill. 1989. Identification of chemical oviposition stimulants for the diamondback moth, *Plutella xylostella,* present in three species of Brassicaceae. Entomol. Exp. Appl. 53: 277–286.

Riggin-Bucci, T. M., and F. Gould. 1996. Effects of surfactants, *Bacillus thuringiensis* formulations, and plant damage on oviposition by diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 89: 891–897.

Thaler, J. S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature (Lond.) 399: 686–688.

Turlings, T.C.J., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250: 1251–1253.

Vaughn, T. T., and C. W. Hoy. 1993. Effects of leaf age, injury, morphology, and cultivars on feeding behavior of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Environ. Entomol. 22: 418–424.

Verkerk, R.H.J., and D. J. Wright. 1996. Multitrophic interactions and management of the diamondback moth: a review. Bull. Entomol. Res. 86: 205–216.