



Plant resistance attenuates the consumptive and non-consumptive impacts of predators on prey

Ian Kaplan and Jennifer S. Thaler

I. Kaplan (ikaplan@purdue.edu), Dept of Entomology, Purdue Univ., Smith Hall, West Lafayette, IN 47907, USA. – J. S. Thaler and I.K. Depts of Entomology and Ecology and Evolutionary Biology, Cornell Univ., Comstock Hall, Ithaca, NY 14853, USA.

Plant resistance and predation have strong independent and interacting effects on herbivore survival, behavior, and patterns of herbivory. Historically, research has emphasized variation in the consumption of herbivores by enemies. Recent work, however, demonstrates that predators also elicit important changes in the traits of their prey, but we do not know how this is influenced by plant quality. In this study, we quantify how the consumptive and non-consumptive effects of predators vary along a gradient of plant resistance using tomato plants (*Solanum lycopersicum*), tobacco hornworms (*Manduca sexta*), and predaceous stinkbugs (*Podisus maculiventris*). We manipulated resource quality using three tomato lines that vary in the expression of the jasmonate pathway, a phytohormonal pathway that is central in mediating resistance to insects. Resistant plants had higher levels of defensive proteins and glandular trichomes than low resistance plants. The consumptive and non-consumptive effects of predators were quantified on the three tomato lines by comparing the impact of 'lethal' predators that both kill and scare prey with 'risk' predators whose mouthparts were surgically impaired to prevent killing. Across several field experiments, the total cascading effect of predators on plant damage was 80.4% lower on jasmonate-overexpressing (highly resistant) plants compared to that on wild-type or jasmonate-insensitive (low resistance) plants. This dramatic attenuation of predator effects was due to a 66% reduction in consumption on high resistance plants, and also because of a 65% decline in non-consumptive effects. Numerous studies in natural and agricultural habitats have documented that predator effects tend to be weaker on well-defended plants; our results provide novel mechanistic insight into this pattern by demonstrating that plant resistance substantially weakens both the consumptive and non-consumptive impacts of predators.

A central pursuit in the study of tri-trophic interactions is understanding how plant traits affect the interplay between predators and herbivores (Price et al. 1980, Hare 2002). Variation in plant quality, for example, has well-documented effects on the ability of natural enemies to suppress herbivore populations and indirectly benefit plants (Campbell and Duffey 1979, Boethel and Eikenbary 1986, Forkner and Hunter 2000, Denno et al. 2002). Although numerous mechanisms may underlie tri-trophic interactions, none of which are mutually exclusive, some of the more widely-cited mechanisms include: (1) delayed herbivore development on resistant plants increasing exposure time to enemy attack (i.e. the slow-growth–high-mortality hypothesis; Clancy and Price 1987, Benrey and Denno 1997, Kaplan et al. 2007); (2) cascading effects via secondary chemistry whereby herbivores sequester plant toxins for their own defense (Campbell and Duffey 1979, Barbosa et al. 1991, Thaler 2002); (3) locally-induced plant resistance increasing within-plant movement of herbivores, thereby increasing apparency to visually-oriented predators (Bergelson and Lawton 1988, Kaitaniemi et al. 2004); (4) attraction of natural enemies through herbivore-induced plant cues

(Turlings et al. 1990, Thaler 1999, Kessler and Baldwin 2001, Rasmann et al. 2005); and (5) structural plant traits (e.g. trichomes) that impact predator and parasitoid foraging (Marquis and Whelan 1996, Yang 2000, Styrsky et al. 2006).

In spite of the large number of potential mechanistic pathways that can mediate tri-trophic interactions, all of the above-cited mechanisms emphasize how plants alter the ability of natural enemies to locate and kill their prey. Recent studies, however, demonstrate that killing (i.e. the consumptive effect of predators) is only one component of how predators impact herbivores and plants. A substantial portion of the net predator effect can be attributed to non-consumptive effects that occur when predator presence alone alters the behavior, physiology, or life-history of the surviving prey (Lima and Dill 1990, Peacor and Werner 2001, Werner and Peacor 2003, Schmitz et al. 2004). These so-called 'fear' or 'intimidation' effects were found to be as important as killing on prey demography, and the magnitude of non-consumptive effects are, in fact, often stronger than consumptive effects when considered at the level of the basal resources (Preisser et al. 2005).

While much is known about the consequences of plant resistance traits for consumptive predator effects, remarkably little is known about the impact of plants on non-consumptive effects, and even less about how plants affect the relative importance of consumptive vs non-consumptive pathways. We hypothesized that plant resistance will play an important role in modulating non-consumptive effects based on theoretical predictions of optimal foraging strategies. Under predation risk, organisms are predicted to respond less to predators when their physiological state or resource quality is poor (Houston et al. 1993, Werner and Anholt 1993). In support of this theoretical expectation, several studies demonstrate that behavioral responses to predators tend to be stronger when organisms are fed ad libitum compared with individuals that are deprived of food (Horat and Semlitsch 1994, Anholt and Werner 1998, Hazlett 2003, Fraker 2008). In other words, organisms fed a low quality diet may be in such poor physiological condition that they simply take more risks than they would otherwise because the threat of food deprivation (and its associated effects on survival, fecundity, etc.) outweighs the probability of being killed by a predator.

We explored the complex mechanisms by which plants affect the magnitude of consumptive and non-consumptive effects in a predator–prey interaction by experimentally manipulating plant resistance and predation risk in a model tri-trophic system. Resource quality was varied using three genetically modified lines of tomato *Solanum lycopersicum* to both increase and decrease plant resistance to herbivores: (1) jasmonate-insensitive (non-inducible, highest quality), (2) wild-type (jasmonate-induced resistance intact, intermediate quality), and (3) jasmonate-overexpressor (constitutively-induced, lowest quality). The jasmonate response is a near-universal biochemical pathway induced by herbivory that confers resistance to a wide range of herbivores (Thaler et al. 2001, Kessler et al. 2004). The two mutant plants, jasmonate-insensitive (*jai-1*; Li et al. 2004) and jasmonate-overexpressor (*prosystemin*; McGurl et al. 1994), are in the same genetic background as our wild-type (Castlemart). These lines are at opposite ends of the spectrum in their quality for leaf-chewing insects. The jasmonate-insensitive plants do not induce protease inhibitors and other secondary chemicals in response to herbivory and thus represent a high quality resource for consumers (Howe et al. 1996, Li et al. 2002). In contrast, the overexpressing plants constitutively express the jasmonate pathway and are therefore a poor quality resource.

The tobacco hornworm *Manduca sexta* (Lepidoptera: Sphingidae), was used as the focal herbivore in this study. Largely a specialist on solanaceous plants, hornworms are voracious consumers of tomato leaves. Despite their well-known adaptations to chemical resistance traits in the Solanaceae (Wink and Theile 2002), *Manduca* larvae experience high mortality and feed poorly on jasmonate-overexpressing tomato lines, and have low mortality and rapid growth on jasmonate-insensitive plants (Orozco-Cardenas et al. 1993, Howe et al. 1996). In addition to their susceptibility to plant resistance traits, hornworms are heavily attacked by natural enemies and possess a suite of anti-predator behaviors ranging from reduced movement and feeding (Bernays 1997, Griffin and Thaler 2006, Thaler and Griffin 2008) to rapid

striking, thrashing and regurgitation during close physical encounters (Walters et al. 2001).

One of the primary enemies of hornworms in natural and agricultural habitats is the predaceous stinkbug, *Podisus maculiventris* (Hemiptera: Pentatomidae). These omnivorous insects stalk their prey through stealthy movements and can best be described as sit-and-pursue predators. Importantly, hornworms feed less in the presence of *Podisus* and our prior work in this system has demonstrated that such non-consumptive effects contribute to $\approx 54\%$ of the total predator effect on leaf damage (Griffin and Thaler 2006, Thaler and Griffin 2008). Moreover, both the predator and herbivore are native to the eastern United States, thus allowing for coevolutionary dynamics to shape current ecological patterns of stinkbug–hornworm interactions.

The specific goals of this study were to use the aforementioned tri-trophic system consisting of tomatoes, hornworms, and stinkbugs to: (1) quantify the impact of plant resistance on caterpillar predation and cascading effects of predators on leaf damage; (2) assess the relative impact of consumptive versus non-consumptive predator effects and, more notably, determine how the strength of these two distinct trophic pathways varies along a gradient of plant resistance; and (3) tease apart the (plant) physiological and (insect) behavioral mechanisms underlying such patterns.

Material and methods

Insects and plants

Manduca caterpillars were obtained as eggs from the North Carolina State University insectary and reared on tomato before use. *Podisus* adults were collected using pheromone traps in Ithaca, New York and maintained in colony on caterpillars and mealworms. We used second to third instar hornworm larvae and adult stinkbugs in all experiments.

Field experiments

To determine how plant resistance affects the magnitude of consumptive and non-consumptive predator effects on herbivore performance, survival, and patterns of herbivory, we experimentally manipulated plant jasmonate expression and predator exposure to hornworm larvae. Plant resistance was altered using the three tomato lines described above: (1) jasmonate-insensitive (non-inducible), (2) wild-type (inducible), and (3) jasmonate-overexpressor (constitutively-induced). Although these plant-types differ greatly in their physical resistance traits (e.g. trichomes) and secondary chemical profiles, the three lines are virtually indistinguishable based on general indices of growth and outward appearance (e.g. size, structure, phenology; Kaplan and Thaler unpubl.).

The consumptive and non-consumptive components of stinkbug predators were assessed using the following three treatments: (1) predator-free control; (2) lethal predators that could both kill and scare (consumptive + non-consumptive); and (3) risk predators that had their stylets impaired and could only scare but not kill (non-consumptive). Predaceous stinkbugs have piercing-sucking mouthparts

which they use to impale their caterpillar prey. In prior work we have found that by removing the last 1 mm segment of this stylet using surgical scissors, stinkbugs are no longer able to penetrate the cuticle of soft-bodied insects such as caterpillars (Griffin and Thaler 2006, Thaler and Griffin 2008). However, risk predators are still able to plant-feed and consume dead caterpillars, and typically survive for 3–4 weeks in this altered state. The behavior of risk predators does not differ from that of lethal predators without their stylets altered (Griffin and Thaler 2006, Thaler and Griffin 2008). Thus, risk predators provide the cues (visual, olfactory, vibrational) that are indicative of predation risk, but without a corresponding change in herbivore abundance.

The three plant-types and three predator treatments were fully-crossed in a factorial design resulting in nine treatment combinations of tomato jasmonate lines and predator effects (replication 10–16 per treatment in 2006 and 10–15 in 2008). The experimental arenas consisted of 1-m³ cages constructed of a pvc frame and covered with translucent spun-polyester sleeves. These cages were tied closed on the top and buried into the soil on the bottom so they excluded most other organisms from entering. Each cage contained two plants of the same jasmonate type at the three-leaf stage transplanted from the greenhouse and six hornworm caterpillars were added to all cages (three caterpillars per plant) at the beginning of the experiment. Cohorts of six caterpillars were weighed prior to use and initial weights were approximately standardized. Each cage, the unit of replication, was assigned to one of the nine predator-plant treatment combinations in a randomized block design. A single stinkbug that was starved for 24 h was added to cages assigned to predator addition.

After 72 h, cages were harvested. Caterpillar and stinkbug survival were recorded and the surviving caterpillars were weighed. We used an acetate grid to visually assess the quantity of leaf tissue (mm²) consumed by hornworms. We also removed undamaged leaflets from the first fully-expanded leaf in control cages to confirm that the three jasmonate lines differed in their physical and chemical resistance traits. Three hole punches (2 mm diameter) were removed from leaflets and trichomes were counted on the adaxial surface of each leaf disc using a dissecting microscope. The three within-leaflet counts were averaged to provide a single estimate of trichome density. Tomato leaves are covered with a thick layer of both non-glandular trichomes that physically impede insect foraging and glandular trichomes that exude toxins (Duffey 1986). Leaflets that were not used to measure trichomes were immediately placed on dry ice and stored at –80°C until secondary chemical analysis. Trypsin protease inhibitor activity and polyphenol oxidase activity, two jasmonate regulated proteins with known anti-herbivore properties (Wang and Constabel 2004, Zavala et al. 2004, Bhonwong et al. 2009), were subsequently quantified in leaf tissue using the protocol described in Viswanathan and Thaler (2004).

Two trials of this experiment were conducted in 2006 (one in June, the other in July) and three trials were conducted in 2008 (all in August). The only difference in experimental design and protocol between the two years is that trichomes were not counted on plants in 2006.

One-way ANOVA was used to compare protease inhibitor and polyphenol oxidase activity, and trichomes across the three plant-types, with trial as a blocking effect (Proc GLM; all statistical analyses were performed using SAS, ver. 9.2). Two-way ANOVA was used to assess the impact of our treatments on caterpillar performance (weight), survival, and leaf damage, with plant-type and predator manipulation as main effects and trial as a blocking effect (Proc GLM). Two additional analyses were conducted to determine whether: (1) caterpillar predation was affected by plant-type, and (2) caterpillar survival in the risk predator treatment differed from that of the control (given that risk predators are surgically altered to prevent killing, this comparison is necessary to confirm that our manipulation was successful). To address the first question we paired the control and lethal predator treatment for each plant-type within spatial blocks of our experiment and for each pairing we subtracted the number of caterpillars remaining in predator cages from that of the control. This provides a measure of predation pressure on caterpillars as plant resistance varies from low to high. The effect of plant-type on predation rate was then tested using one-way ANOVA (Proc GLM). To address the second question of the efficacy of our risk manipulation, we re-analyzed the above-described two-way ANOVA on caterpillar survival, but we removed the lethal predator treatment from the dataset such that our analysis specifically compares the control and risk predator treatments. Leaf damage data were square-root transformed to meet assumptions of normality and homogeneity of variances.

Behavioral assay

To assess the singular and interactive effects of plant resistance and predation risk on caterpillar foraging behavior, we observed caterpillars using a combination of different plant-types and predator presence, similar to the above-described field experiment. Specifically, we recorded hornworm behavior on the three jasmonate lines in the presence and absence of lethal stinkbug predators, resulting in six different treatment combinations ($n = 12$ – 13 observation periods per treatment).

Observational arenas were constructed with cylindrical plastic tubes (25 height \times 22 cm \varnothing) placed over a single tomato plant at the three-leaf stage. The bottom lip of the tube was buried in soil to prevent predators from escaping and the top was covered with mesh to allow ventilation. Continuous observations were conducted in a laboratory setting and observational periods lasted for 45 min. New individual plants, caterpillars, and predators were used for each assay. Caterpillars were reared on artificial diet and transferred to one of the three plant resistance lines 48 h before observations. A single caterpillar was placed on the terminal leaflet of leaf three and, in cages assigned to predator addition, one adult stinkbug was released. The predators were exposed to tomato plants and hornworm larvae and then starved for 24 h prior to use in observations. Six cages were observed simultaneously by two observers and therefore all six treatment combinations were utilized in each observation session. This eliminates potential bias in the date or time during which the observations were made.

Caterpillar behaviors were placed into one of the following four categories: resting (remaining motionless), eating (chewing leaf tissue), moving (changing position within the plant), and trichome shaving (moving mandibles back-and-forth over the leaf surface, but without causing defoliation). These activities constitute the vast majority (>95%) of the time budget for hornworm larvae. We also noted the location and general behavior of stinkbugs in each enclosure. If a caterpillar was killed by a stinkbug during the assay then this observation was excluded from our dataset.

The effects of stinkbug presence and plant jasmonate-type on caterpillar behavior were assessed using two-way MANOVA, followed by univariate ANOVAs for each of the four behaviors observed (Proc GLM). Data were log transformed to meet assumptions of normality and homogeneity of variances.

Results

Field experiments

The three tomato jasmonate lines successfully manipulated both chemical and physical plant traits associated with resistance to herbivorous insects, with the expression of such traits increasing in response to elevated jasmonate levels. Polyphenol oxidase activity was 63% higher in jasmonate-overexpressing plants compared to the wild-type, whereas activity was 46% lower in jasmonate-insensitive plants (plant-type effect: $F_{2,80} = 18.07$, $p < 0.0001$; polyphenol oxidase activity, mean $\Delta OD \text{ g}^{-1} \text{ min}^{-1} \pm SE$: jasmonate-insensitive = 0.19 ± 0.04 , wild-type = 0.35 ± 0.03 , jasmonate-overexpressor = 0.57 ± 0.07). Protease inhibitor activity was almost twice as high in overexpressing versus insensitive plants (plant-type effect: $F_{2,76} = 16.72$, $p < 0.0001$; protease inhibitor activity [% trypsin inhibition], mean $\pm SE$: jasmonate-insensitive = 29.41 ± 3.57 , wild-type = 48.45 ± 3.59 , jasmonate-overexpressor = $54.41 \pm$

4.30). Last, the number of trichomes were similar between wild-type and jasmonate-overexpressing lines, but jasmonate-insensitive plants possessed 87% fewer trichomes than the wild-type (plant-type effect: $F_{2,42} = 48.95$, $p < 0.0001$; trichome density [no. mm^{-2}], mean $\pm SE$: jasmonate-insensitive = 0.63 ± 0.13 , wild-type = 5.00 ± 0.54 , jasmonate-overexpressor = 5.46 ± 0.36).

The performance of caterpillars developing on the three tomato lines increased in response to decreasing jasmonate expression and thus was negatively correlated with plant resistance traits. More specifically, caterpillar final weights were ~20% higher on jasmonate-insensitive plants compared to wild-type and jasmonate-overexpressors (plant-type effect: $F_{2,227} = 3.55$, $p = 0.0302$; *Manduca* weight [mg], mean $\pm SE$: jasmonate-insensitive = 41.91 ± 2.95 , wild-type = 35.29 ± 1.96 , jasmonate-overexpressor = 35.32 ± 2.80). These results combined with those reported below on survival and leaf consumption demonstrate that increased jasmonate levels strongly reduce plant quality for *Manduca*. The presence of predaceous stinkbugs, however, did not affect caterpillar weight (predator effect: $F_{2,227} = 0.44$, $p = 0.6466$; plant-type \times predator: $F_{4,227} = 1.18$, $p = 0.3215$). Trial number (i.e., temporal block) was highly significant ($p < 0.0001$) for this and all subsequent response variables, but there were no treatment \times trial interactions.

Caterpillar survival was affected by tomato line ($F_{2,241} = 3.97$, $p = 0.0201$), predator presence ($F_{2,241} = 46.57$, $p < 0.0001$), and the interaction between plant-type and predator ($F_{4,241} = 4.53$, $p = 0.0015$). These patterns were consistent across both years of the study with similar treatment effects in 2006 (Fig. 1A) and 2008 (Fig. 1B). Although survival was slightly lower when exposed to risk predators (control vs risk treatment: $F_{1,140} = 8.75$, $p = 0.0036$; *Manduca* survival [no. caterpillars cage^{-1}], mean $\pm SE$: control = 4.53 ± 0.14 , risk treatment = 3.99 ± 0.18), this decline represents only a small fraction of the number of caterpillars consumed by lethal predators. Moreover, minor reductions in caterpillar abundance can not explain the large cascading

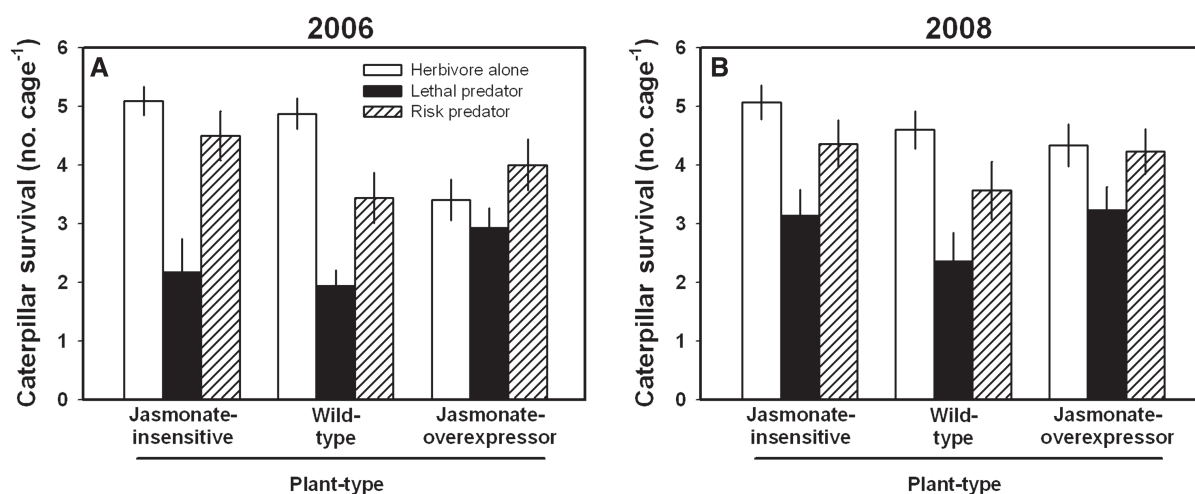


Figure 1. The impact of lethal and risk predators on caterpillar survival (mean \pm SE) on each of three plant-types that vary in resistance in a field experiment conducted during (A) 2006 and (B) 2008. Lethal predators can both kill and scare prey (i.e. consumptive + non-consumptive) and thus represent the total predator effect, whereas risk predators can only scare prey and thereby isolate the non-consumptive component of the interaction. Jasmonate-overexpressing plants are highly resistant to insect herbivory, while jasmonate-insensitive plants express low levels of resistance and the wild-type is intermediate between the two mutant lines.

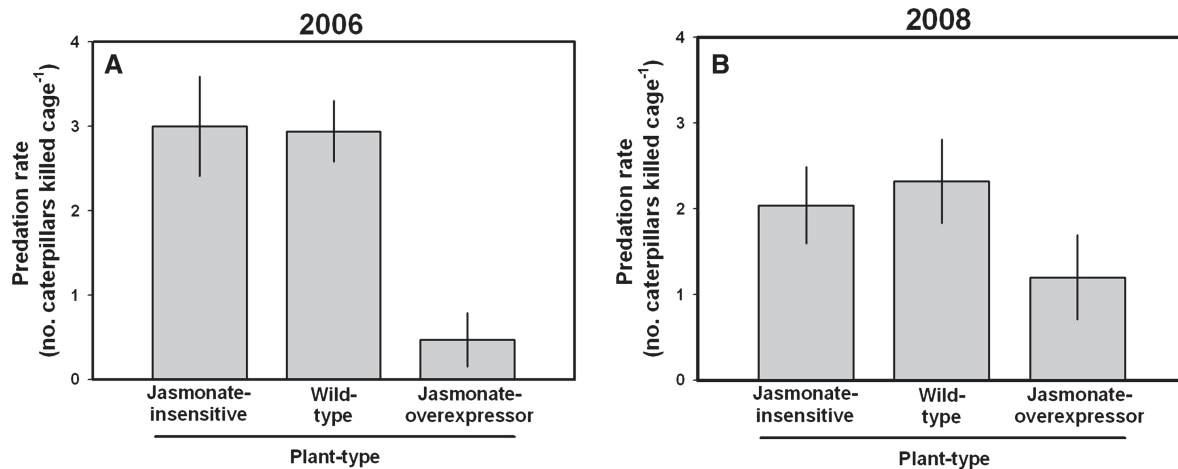


Figure 2. Predation rates (mean \pm SE) for stinkbug predators on hornworm caterpillars on each of three plant-types that vary in resistance in a field experiment conducted during (A) 2006 and (B) 2008. Jasmonate-overexpressing plants are highly resistant to insect herbivory, while jasmonate-insensitive plants express low levels of resistance and the wild-type is intermediate between the two mutant lines.

effects on herbivory that occurred in the predation risk treatment (see leaf damage results below). Finally, stinkbug presence is known to induce feeding cessation in *Manduca* (Griffin and Thaler 2006, Thaler and Griffin 2008) and thus a portion of predation risk effects may in fact entail prey starving to death, in which case caterpillar mortality is part of the non-consumptive component of stinkbugs.

The number of caterpillars killed by predators in the lethal treatment varied across plant lines (plant-type effect: $F_{2,76} = 10.79$, $p < 0.0001$), with less predation occurring on high resistance plants. In 2006, predation rate was 84% lower on jasmonate-overexpressing plants compared with the wild-type (Fig. 2A), whereas predation was 48% less in 2008 (Fig. 2B).

Caterpillar leaf consumption declined in response to increasing jasmonate (plant-type effect: $F_{2,204} = 4.16$, $p = 0.0169$) and also declined in response to lethal and risk predators (predator effect: $F_{2,204} = 18.46$, $p < 0.0001$), although

the interaction between plant-type and predator was only marginally significant ($F_{4,204} = 2.07$, $p = 0.0865$). Across all plant-types in 2006, leaf damage was reduced from 295 mm² in the predator-free control to 198 mm² in the presence of lethal predators, a 33% reduction in herbivory, and 218 mm² in the predation risk treatment, a 26% reduction in herbivory (Fig. 3A). As a result, non-consumptive predator effects contributed to 79% of the total predator effect. Patterns in 2008 were very similar with lethal and risk predators reducing leaf damage by 30% and 27%, respectively, and thus 89% of the total predator effect could be attributed to the non-consumptive pathway (Fig. 3B).

Behavioral assay

Both plant-type and predator presence affected the overall behavior of *Manduca* larvae, although there was no interaction between plant and predator (MANOVA: plant-type

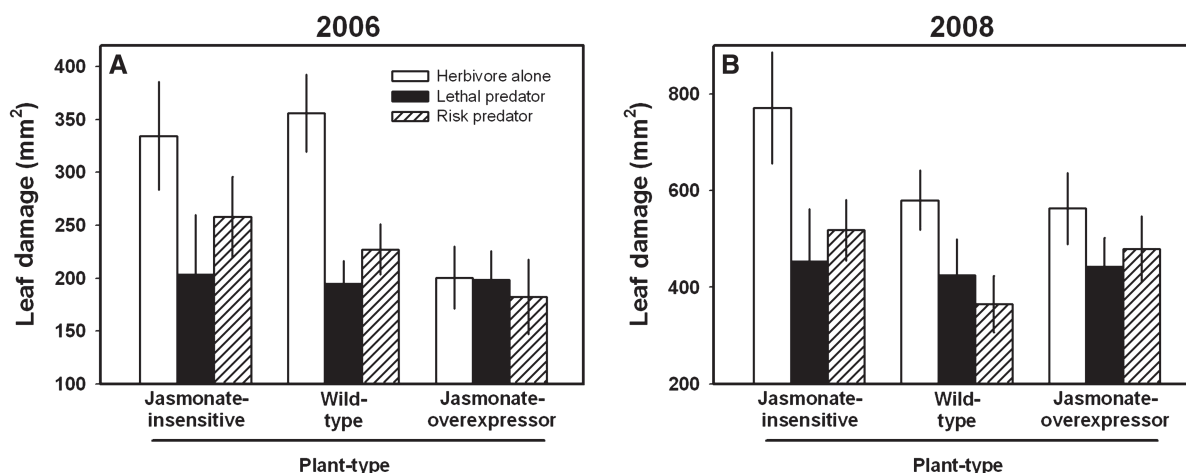


Figure 3. The impact of lethal and risk predators on caterpillar defoliation (mean \pm SE) on each of three plant-types that vary in resistance in a field experiment conducted during (A) 2006 and (B) 2008. Lethal predators can both kill and scare prey (i.e. consumptive + non-consumptive) and thus represent the total predator effect, whereas risk predators can only scare prey and thereby isolate the non-consumptive component of the interaction. Jasmonate-overexpressing plants are highly resistant to insect herbivory, while jasmonate-insensitive plants express low levels of resistance and the wild-type is intermediate between the two mutant lines.

effect, $F_{8,134} = 2.18$, $p = 0.0332$; predator effect, $F_{4,67} = 3.11$, $p = 0.0208$; plant \times predator, $F_{8,134} = 1.65$, $p = 0.1176$). More specifically, the amount of time spent eating was not affected by predators (predator effect $F_{1,70} = 1.39$, $p = 0.2428$; plant \times predator: $F_{2,70} = 0.34$, $p = 0.7125$), but the main effect of plant-type was marginally significant as a result of caterpillars spending 34% more time eating on low resistance plants ($F_{2,70} = 2.70$, $p = 0.0743$; mean time (seconds) \pm SE: jasmonate-insensitive = 995.72 ± 117.29 , wild-type = 700.50 ± 64.22 , jasmonate-overexpressor = 782.72 ± 89.20). Similarly, trichome shaving was influenced by plant-type, but not predation risk (plant-type effect: $F_{2,70} = 4.44$, $p = 0.0152$; predator effect: $F_{1,70} = 0.73$, $p = 0.3962$; plant \times predator: $F_{2,70} = 0.03$, $p = 0.9724$). In this case, caterpillars spent 37% less time trichome shaving on low resistance plants (mean time (seconds) \pm SE: jasmonate-insensitive = 662.96 ± 90.54 , wild-type = 1097.38 ± 114.74 , jasmonate-overexpressor = 1007.12 ± 113.22), which correlates well with plant-type differences in trichome density (see results for plant resistance traits reported above).

Resting and moving were opposite from eating and trichome shaving in that these behaviors were more strongly affected by predation risk than plant-type. For example, caterpillars spent 46% more time resting in the presence of predators (plant-type effect: $F_{2,70} = 0.26$, $p = 0.7733$; predator effect $F_{1,70} = 4.64$, $p = 0.0346$; plant \times predator: $F_{2,70} = 0.46$, $p = 0.6317$; mean time (seconds) \pm SE: predator-free = 667.20 ± 81.57 , predation risk = 974.54 ± 115.66), and also 44% less time moving under predation risk (plant-type effect: $F_{2,70} = 0.30$, $p = 0.7448$; predator effect $F_{1,70} = 7.51$, $p = 0.0078$; plant \times predator: $F_{2,70} = 2.70$, $p = 0.0744$; mean time (seconds) \pm SE: predator-free = 170.41 ± 40.52 , predation risk = 95.05 ± 33.14).

Discussion

Dozens of studies have now conclusively demonstrated that non-consumptive effects are central to understanding the cumulative impact of predators on their prey and indirectly on plants (Lima and Dill 1990, Peacor and Werner 2001, Schmitz et al. 2004, Preisser et al. 2005, Griffin and Thaler 2006). The challenge is to reveal the ecological factors that mediate the relative importance of killing versus fear. In food chains linking plants with phytophagous insects and their predators, plant resistance provides an important source of variation in enemy impact (Price et al. 1980, Hare 2002), yet virtually nothing is known about the consequences of plant heterogeneity for non-consumptive predator effects.

Our multi-year field experiment and behavioral observations suggest that elevated plant resistance elicits a concomitant decrease in the total effect of stinkbug predators on caterpillars and plants, with comparable declines in the magnitude of both killing and scaring. Predation rates, for example, were 66% lower on jasmonate-overexpressing plants, an effect that occurred consistently across both years of our study (Fig. 2). Thus the strength of the consumptive component of stinkbugs on hornworms weakens as plant resistance increases. Bartlett (2008) also found that *Podisus* consumes

fewer prey on herbivore-resistant versus susceptible soybean plants, although other studies investigating the interaction between plant resistance and stinkbug predation have provided mixed results (Cloutier and Jean 1998, Bell et al. 2003, Kaplan et al. 2007). This outcome largely mirrors broader patterns in the literature. While many studies have documented negative effects of plant defenses on predators and parasitoids, a similar number have found either a positive effect or no interaction altogether (Price et al. 1980, Lill and Marquis 2001, Hare 2002).

Because the jasmonate cascade regulates a suite of resistance traits, our plant-types differ in several respects making it difficult to isolate the precise mechanism underlying differential predation. Trichomes are known to impede enemy foraging on tomato (Kennedy 2003), but trichome expression on our plants mostly differed between jasmonate-insensitive (0.63 mm^{-2}) and wild-type (5.00 mm^{-2}), with a comparatively smaller increase on jasmonate-overexpressors (5.46 mm^{-2}). In contrast, consumption was similar in magnitude on jasmonate-insensitive and wild-type plants and primarily differed on the jasmonate-overexpressor (Fig. 2). Therefore, it seems unlikely that trichomes mediated this interaction.

Secondary chemistry is a more plausible mechanism, although phytochemicals have diverse effects on the development and behavior of herbivores, some of which cannot explain our results. For instance, caterpillars grow slower (Results) and increase intra-plant movement on high resistance tomato plants (Kaplan and Thaler unpubl.), both of which are predicted to increase rather than decrease predation. This allows for two remaining possibilities: cascading effects of direct defenses, or indirect defenses (i.e. volatiles). Tomato secondary chemicals are known to negatively affect the performance of *Podisus* feeding on *Manduca* (Traugott and Stamp 1997). Interestingly, *Podisus* were also far less likely to attack *Manduca* larvae when reared on tomatine or chlorogenic acid (Traugott and Stamp 1996). Of the predators that did consume prey, the duration of time spent feeding (and thus handling time) increased dramatically (~ 2.3 h feeding on control caterpillars vs ~ 5.4 h feeding on allelochemical-fed caterpillars).

In addition to direct defense, the jasmonate cascade mediates indirect defenses and thus volatile profiles likely differed between the three plant-types (Thaler et al. 2002). Predaceous stinkbugs are attracted to herbivore-induced plant volatiles and may use them as a cue to locate prey (Weissbecker et al. 1999, 2000). Jasmonate-deficient tomato plants, however, induce substantially weaker volatile responses to herbivory compared with the wild-type (Thaler et al. 2002). If differential volatile induction among our plant-types explained variable enemy impact then we would expect far more killing to have occurred on the wild-type than the jasmonate-insensitive line, which was not the case.

Based on this overall assessment of potential mechanisms, it seems likely that reduced herbivore quality from feeding on toxic plants deters stinkbug predators and explains the drastic reduction in killing that occurs on the jasmonate-overexpressing tomato line. This outcome is also consistent with the strength of transgenic manipulations of systemin-mediated resistance to herbivores on tomato (Orozco-Cardenas et al. 1993, McGurl et al. 1994).

Plant resistance and non-consumptive predator effects

While we are unaware of other studies testing the consequences of variation in plant resistance for non-consumptive predator effects, Danner and Joern (2003) explored potential interactions between plant nutritional quality and spider predation risk on grasshopper growth. The statistical interactions between predators and plant quality in this study were non-significant, but fertilizing grasses and forbs elevated herbivore performance in spite of the growth penalties that occurred when spiders were present. Surprisingly, we found that stinkbugs had no impact on hornworm growth, even though larvae consumed far less leaf tissue in the risk treatment.

The overall strength of the non-consumptive predator effect was large, accounting for 79–89% of the total predator effect on leaf damage, and decreased in magnitude with increasing plant resistance, especially between the wild-type and jasmonate-overexpressor (Fig. 3). Bernays (1997) demonstrated that caterpillars, including *Manduca*, are far less susceptible to predation when resting, and thus reducing activity levels in the presence of enemies is considered to be an adaptive response that has shaped foraging strategies in herbivorous insects (Stamp and Casey 1992). Conversely, feeding is a risky activity that greatly increases the likelihood of being detected and killed by a predator, which makes it rather surprising that caterpillars did not modify their feeding time in response to predators in our behavioral assay. Clearly *Manduca* larvae eat less when stinkbugs are present as demonstrated by the dramatic reductions in herbivory in the risk treatment of our experimental field trials (Fig. 3). The short-term observations may simply have been on too small of a time-scale to detect a response in caterpillar feeding behavior.

What is especially striking from our data is that variation in killing across the three plant-types almost perfectly mirrors that of non-consumptive effects. In both cases comparatively strong treatment effects occurred on jasmonate-insensitive plants and the wild-type with a precipitous decline on jasmonate-overexpressors (compare Fig. 2 and 3). This begs the question, are killing and fear mechanistically linked? Two scenarios could drive this type of pattern. First, if non-consumptive effects necessitated an attack to occur and thus were primarily elicited by close-distance encounters between predator and prey. Pea aphids, for example, remove their stylet from the host-plant and drop to the ground when physically harassed by predators (Nelson and Rosenheim 2006). In this case, ecological factors that reduced consumption would likely diminish non-consumptive effects as well because anti-predator responses are only induced when attack is imminent. However, we consider this to be an unlikely mechanism for stinkbug-hornworm interactions. In the majority of our field cages, predators were not found on plants, seeming to prefer the soil and sides of cages, and the same is true of our behavioral assays. In less than half of the total observational periods were stinkbugs foraging on tomato plants and this low rate of foliar-foraging was unaffected by plant-type. Moreover, when predators were found foraging on plants they were rarely observed harassing caterpillars. Responses to predation risk in this system do not appear to be affected by the spatial proximity of predator and

prey; stinkbugs can scare herbivores even when the two insects do not co-occur on the same plant.

A second, and more compelling, reason for the association between killing and fear is that caterpillars are able to gauge the actual level of risk (as opposed to perceived risk) in their environment and respond accordingly. In other words, the perceived risk is identical across the three plant-types because a single stinkbug was added to all cages, but the actual risk of attack is dramatically lower on high resistance plants because predators kill far less. It is certainly plausible that stinkbugs emit different chemical and auditory signals while in hunting mode that caterpillars exploit as predation risk cues. Another possibility is that herbivores developing on jasmonate-overexpressors cross some physiological threshold whereby the stress of food deprivation prevents caterpillars from engaging in anti-predator behaviors. *Manduca* larvae already consume less food on the overexpressor and the ingested leaf material is also less digestible compared with other plant-types (Kaplan and Thaler unpubl.). This suggests that caterpillars may be 'up against the wall' on high resistance plants and reducing food intake further would be tantamount to suicide, in which case maintaining herbivory levels in spite of predators can be viewed as an adaptive strategy of balancing risks with physiological constraints.

Conclusions

Early work on interactions between plant resistance and biological control in agriculture emphasized the potential incompatibility of these two processes (Bergman and Tingey 1979, Campbell and Duffey 1979, Boethel and Eikenbary 1986), although the relationship is not always negative (Price et al. 1980, Hare 2002). Our study demonstrates that understanding the impact of plants on predator-prey dynamics necessitates an explicit consideration of non-consumptive effects, in addition to the more traditional emphasis on consumption. Based on the total cascading effect of stinkbugs on plants in our system it would be tempting to conclude that variation in killing across plant-types drives this ecological pattern. While this is partly true, behavioral responses of prey appear to explain as much or more of the attenuation in enemy effects on resistant host-plants.

Acknowledgements – We thank Liana Nice, Andrew Tuccillo, Monica Kersch Becker, Jessica Nix, Guilherme Becker, Ordomb Huot, Stephanie Ann Thornton and Andrew Mudge for their assistance with field experiments, and Jessica Nix for help with behavioral assays. Gaylord Desurmont graciously provided us with stinkbugs from pheromone traps. Anurag Agrawal and Monica Kersch Becker offered valuable comments on later stages of this manuscript. Appropriate permits were obtained for the field release of transgenic plants. This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, Grant no. 2006-35302-17431.

References

- Anholt, B. R. and Werner, E. E. 1998. Predictable changes in predation mortality as a consequence of changes in food availability and predation risk. – *Evol. Ecol.* 12: 729–738.

- Barbosa, P. et al. 1991. Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. – Ecology 72: 1567–1575.
- Bartlett, R. 2008. Negative interactions between chemical resistance and predators affect fitness in soybeans. – Ecol. Entomol. 33: 673–678.
- Bell, H. A. et al. 2003. Impact of genetically modified potato expressing plant-derived insect resistance genes on the predatory bug *Podisus maculiventris* (Heteroptera: Pentatomidae). – Biocontrol Sci. Technol. 13: 729–741.
- Benrey, B. and Denno, R. F. 1997. The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. – Ecology 78: 987–999.
- Bergelson, J. M. and Lawton, J. H. 1988. Does foliage damage influence predation on the insect herbivores of birch? – Ecology 69: 434–445.
- Bergman, J. M. and Tingey, W. M. 1979. Aspects of interactions between plant genotypes and biological control. – Bull. Entomol. Soc. Am. 25: 275–279.
- Bernays, E. A. 1997. Feeding by lepidopteran larvae is dangerous. – Ecol. Entomol. 22: 121–123.
- Bhonwong, A. et al. 2009. Defensive role of tomato polyphenol oxidases against cotton bollworm (*Helicoverpa armigera*) and beet armyworm (*Spodoptera exigua*). – J. Chem. Ecol. 35: 28–38.
- Boethel, D. J. and Eikenbary, R. D. 1986. Interactions of host plant resistance and parasitoids and predators of insects. – Halsted Press.
- Campbell, B. C. and Duffey, S. S. 1979. Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. – Science 205: 700–702.
- Clancy, K. M. and Price, P. W. 1987. Rapid herbivore growth enhances enemy attack – sublethal plant defenses remain a paradox. – Ecology 68: 733–737.
- Cloutier, C. and Jean, C. 1998. Synergism between natural enemies and biopesticides: a test case using the stinkbug *Perillus bioculatus* (Hemiptera: Pentatomidae) and *Bacillus thuringiensis tenebrionis* against Colorado potato beetle (Coleoptera: Chrysomelidae). – J. Econ. Entomol. 91: 1096–1108.
- Danner, B. J. and Joern, A. 2003. Resource-mediated impact of spider predation risk on performance in the grasshopper *Aeneotettix deorum* (Orthoptera: Acrididae). – Oecologia 137: 352–359.
- Denno, R. F. et al. 2002. Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. – Ecology 83: 1443–1458.
- Duffey, S. S. 1986. Plant glandular trichomes: their partial role in defense against insects. – In: Juniper, B. E. and Southwood, T. R. C. (eds), Insects and the plant surface. Edward Arnold, pp. 151–172.
- Forkner, R. E. and Hunter, M. D. 2000. What goes up must come down? Nutrient addition and predation pressure on oak herbivores. – Ecology 81: 1588–1600.
- Fraker, M. E. 2008. The effect of hunger on the strength and duration of the antipredator behavioral response of green frog (*Rana clamitans*) tadpoles. – Behav. Ecol. Sociobiol. 62: 1201–1205.
- Griffin, C. A. M. and Thaler, J. S. 2006. Insect predators affect plant resistance via density- and trait-mediated indirect interactions. – Ecol. Lett. 9: 335–343.
- Hare, J. D. 2002. Plant genetic variation in tritrophic interactions. – In: Tschantke, T. and Hawkins, B. A. (eds), Multitrophic level interactions. Cambridge Univ. Press, pp. 8–43.
- Hazlett, B. A. 2003. The effects of starvation on crayfish responses to alarm odor. – Ethology 109: 587–592.
- Horat, P. and Semlitsch, R. D. 1994. Effects of predation risk and hunger on the behavior of two species of tadpoles. – Behav. Ecol. Sociobiol. 34: 393–401.
- Houston, A. I. et al. 1993. General results concerning the trade-off between gaining energy and avoiding predation. – Philos. Trans. R. Soc. Lond. B 341: 375–397.
- Howe, G. A. et al. 1996. An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. – Plant Cell 8: 2067–2077.
- Kaitaniemi, P. et al. 2004. Movement and disappearance of mountain birch defoliators are influenced by the interactive effects of plant architecture and induced resistance. – Ecol. Entomol. 29: 437–446.
- Kaplan, I. et al. 2007. Leafhopper-induced plant resistance enhances predation risk in a phytophagous beetle. – Oecologia 152: 665–675.
- Kennedy, G. G. 2003. Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. – Annu. Rev. Entomol. 48: 51–72.
- Kessler, A. and Baldwin, I. T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. – Science 291: 2141–2144.
- Kessler, A. et al. 2004. Silencing the jasmonate cascade: induced plant defenses and insect populations. – Science 305: 665–668.
- Li, L. et al. 2002. Distinct roles for jasmonate synthesis and action in the systematic wound response to tomato. – Proc. Natl Acad. Sci. USA 99: 6416–6421.
- Li, L. et al. 2004. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. – Plant Cell 16: 126–143.
- Lill, J. T. and Marquis, R. J. 2001. The effects of leaf quality on herbivore performance and attack from natural enemies. – Oecologia 126: 418–428.
- Lima, S. L. and Dill, L. M. 1990. Behavioral decisions made under the risk of predation – a review and prospectus. – Can. J. Zool. 68: 619–640.
- Marquis, R. J. and Whelan, C. 1996. Plant morphology, and recruitment of the third trophic level: subtle and little-recognized defenses? – Oikos 75: 330–334.
- McGurl, B. et al. 1994. Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. – Proc. Natl Acad. Sci. USA 91: 9799–9802.
- Nelson, E. H. and Rosenheim, J. A. 2006. Encounters between aphids and their predators: the relative frequencies of disturbance and consumption. – Entomol. Exp. Appl. 118: 211–219.
- Orozco-Cardenas, M. et al. 1993. Expression of an antisense prosystemin gene in tomato plants reduces resistance toward *Manduca sexta* larvae. – Proc. Natl Acad. Sci. USA 90: 8273–8276.
- Peacor, S. D. and Werner, E. E. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. – Proc. Natl Acad. Sci. USA 98: 3904–3908.
- Preisser, E. L. et al. 2005. Scared to death? The effects of intimidation and consumption in predator–prey interactions. – Ecology 86: 501–509.
- Price, P. W. et al. 1980. Interactions among 3 trophic levels – influence of plants on interactions between insect herbivores and natural enemies. – Annu. Rev. Ecol. Syst. 11: 41–65.
- Rasmann, S. et al. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. – Nature 434: 732–737.
- Schmitz, O. J. et al. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. – Ecol. Lett. 7: 153–163.
- Stamp, N. E. and Casey, T. M. 1992. Caterpillars: ecological and evolutionary constraints on foraging. – Chapman and Hall.
- Styrsky, J. D. et al. 2006. Plant trichomes indirectly enhance tritrophic interactions involving a generalist predator, the red imported fire ant. – Biol. Control 36: 375–384.
- Thaler, J. S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. – Nature 399: 686–688.

- Thaler, J. S. 2002. Effect of jasmonate-induced plant responses on the natural enemies of herbivores. – *J. Anim. Ecol.* 71: 141–150.
- Thaler, J. S. and Griffin, C. A. M. 2008. Relative importance of consumptive and non-consumptive effects of predators on prey and plant damage: the influence of herbivore ontogeny. – *Entomol. Exp. Appl.* 128: 34–40.
- Thaler, J. S. et al. 2001. Jasmonate-mediated induced plant resistance affects a community of herbivores. – *Ecol. Entomol.* 26: 312–324.
- Thaler, J. S. et al. 2002. Jasmonate-deficient plants have reduced direct and indirect defences against herbivores. – *Ecol. Lett.* 5: 764–774.
- Turlings, T. C. J. et al. 1990. Exploitation of herbivore-induced plant odors by host seeking parasitic wasps. – *Science* 250: 1251–1253.
- Traugott, M. S. and Stamp, N. E. 1996. Effects of chlorogenic acid- and tomatine-fed caterpillars on the behavior of an insect predator. – *J. Insect Behav.* 9: 461–476.
- Traugott, M. S. and Stamp, N. E. 1997. Effects of chlorogenic acid- and tomatine-fed caterpillars on performance of an insect predator. – *Oecologia* 109: 265–272.
- Viswanathan, D. V. and Thaler, J. S. 2004. Plant vascular architecture and within-plant spatial patterns in resource quality following herbivory. – *J. Chem. Ecol.* 30: 531–543.
- Walters, E. T. et al. 2001. Defensive responses of larval *Manduca sexta* and their sensitization by noxious stimuli in the laboratory and field. – *J. Exp. Biol.* 204: 457–469.
- Wang, J. H. and Constabel, C. P. 2004. Polyphenol oxidase overexpression in transgenic *Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). – *Planta* 220: 87–96.
- Weissbecker, B. et al. 1999. Electroantennogram responses of a predator, *Perillus bioculatus*, and its prey, *Leptinotarsa decemlineata*, to plant volatiles. – *J. Chem. Ecol.* 25: 2313–2325.
- Weissbecker, B. et al. 2000. Identification of volatile potato sesquiterpenoids and their olfactory detection by the two-spotted stinkbug *Perillus bioculatus*. – *J. Chem. Ecol.* 26: 1433–1445.
- Werner, E. E. and Anholt, B. R. 1993. Ecological consequences of the tradeoff between growth and mortality rates mediated by foraging activity. – *Am. Nat.* 142: 242–272.
- Werner, E. E. and Peacor, S. D. 2003. A review of trait-mediated indirect interactions in ecological communities. – *Ecology* 84: 1083–1100.
- Wink, M. and Theile, V. 2002. Alkaloid tolerance in *Manduca sexta* and phylogenetically related sphingids (Lepidoptera: Sphingidae). – *Chemoecology* 12: 29–46.
- Yang, L. H. 2000. Effects of body size and plant structure on the movement ability of a predaceous stinkbug, *Podisus maculiventris* (Heteroptera: Pentatomidae). – *Oecologia* 125: 85–90.
- Zavala, J. A. et al. 2004. Manipulation of endogenous trypsin proteinase inhibitor production in *Nicotiana attenuata* demonstrates their function as antiherbivore defenses. – *Plant Physiol.* 134: 1181–1190.