Epicuticular wax on pea plants decreases instantaneous search rate of *Hippodamia convergens* larvae and reduces attachment to leaf surfaces

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Abstract—Crop cultivar can affect the ability of natural enemies to control pest populations. Peas, *Pisum sativum* L. (Fabaceae), with a reduced epicuticular wax bloom have reduced pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), populations in the field than peas with a normal-wax bloom. In this paper we use the functional response to examine predation by *Hippodamia convergens* Guérin de Méneville (Coleoptera: Coccinellidae) larvae foraging on pea plants with a normal- and a reduced-wax bloom. We found that *Hippodamia convergens* shows a Type II functional response on both phenotypes of peas. *Hippodamia convergens* consumed significantly more pea aphids on reduced-wax plants than on normal-wax plants. The instantaneous search rate, *a*, was higher for predators on reduced-wax plants, but the handling time, T_h, was similar for predators on both wax phenotypes. In addition, we tested the ability of *H. convergens* larvae to attach to the surface of normal-wax and reduced-wax pea leaves. We found that *H. convergens* larvae attach more strongly to reduced-wax peas than to normal-wax peas. These results suggest that predation of pea aphid by *H. convergens* is enhanced on reduced-wax peas due to increased ability of predators to attach to these plants, and as a result, search for and find aphids.

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Résumé—Le type de cultivar peut affecter la capacité des ennemis naturels à lutter contre les populations d'insectes nuisibles. Les pois, *Pisum sativum* L. (Fabaceae), qui ont une production de cire épicuticulaire réduite sont moins parasités par les populations du puceron du pois, *Acyrthosiphon pisum* (Harris) (Hemiptera : Aphididae), en nature que les pois avec une couche cireuse normale. Nous avons examiné la réponse fonctionnelle de la prédation exercée par les larves d'*Hippodamia convergens* Guérin de Méneville (Coleoptera : Coccinellidae) se nourrissant sur des plants de pois à production normale ou réduite de cire. *Hippodamia convergens* a une réponse fonctionnelle de Type II sur les deux phénotypes de pois, mais consomme significativement plus de pucerons du pois sur les plants moins cireux. La vitesse instantanée de recherche, *a*, est plus grande chez les prédateurs qui sont sur les plants moins cireux, mais la durée de manipulation, T_h, est semblable sur les deux types de plants. Nous avons également examiné la capacité des larves d'*H. convergens* de s'agripper à la surface de feuilles normales et de feuilles à couche de cire réduite. Les larves d'*H. convergens* se fixent plus solidement sur ces dernières que sur les feuilles normales. Ces résultats indiquent que la prédation exercée sur les pucerons du pois par *H. convergens* est favorisée sur les plants à

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couche de cire réduite parce que les prédateurs ont plus de facilité à s'y agripper et peuvent donc plus aisément chercher et trouver les pucerons.

[Traduit par la Rédaction]

Introduction

Crop characteristics unquestionably have the potential to influence the ability of biological control agents to regulate insect populations in the field (Cortesero *et al.* 2000). Manipulation of crop phenotype has the potential to provide food sources for biological control agents (Limburg and Rosenheim 2001), provide refugia from desiccation (domatia) (Walter 1996; Agrawal and Karban 1997), or increase the attractiveness of the crop to searching natural enemies (Turlings *et al.* 1991; Röse *et al.* 1997; Kessler and Baldwin 2001). In addition, plant morphological features such as plant size, architecture (Kareiva and Perry 1989; Kareiva and Sahakian 1990; Frazer and McGregor 1994; Coll *et al.* 1997), and smaller scale features such as trichomes (Obrycki and Tauber 1984) can affect a predator's effectiveness.

One morphological feature that has the potential to impact predator foraging success is plant epicuticular waxes. Epicuticular waxes cover the primary aerial surfaces of all higher terrestrial plants (Jenks and Ashworth 1999). Epicuticular waxes frequently occur as a thin amorphous layer, but some plant species have distinctly crystalline epicuticular waxes forming a wax "bloom" on the plant's surface. Some plant species are polymorphic for wax bloom, with individuals exhibiting either amorphous epicuticular waxes (bloomless) or a prominent bloom (Jenks and Ashworth 1999). The maintenance of epicuticular wax polymorphisms suggests diverse and variable ecological roles for epicuticular wax blooms. Postulated reasons for epicuticular wax polymorphism include protection from variable drought in grasses (Daly 1964) and protection from variable UV in *Dudlya brittonii* Johansen (Crassulaceae) (Mulroy 1979).

Epicuticular wax variation also influences susceptibility to herbivores. Reduced epicuticular wax bloom in crops that typically have a heavy wax bloom can produce either increased or decreased resistance to insect herbivory (Eigenbrode and Espeli 1995). Pea plants, *Pisum sativum* L. (Fabaceae), with the *wel* mutation, which reduces EW, support lower pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), populations in the field than plants with normal wax levels (White and Eigenbrode 2000*b*). This difference has been attributed to the greater impact of coccinellid adults and larvae attacking aphids on the reduced-waxy-bloom plants in controlled laboratory cage experiments (Eigenbrode *et al.* 1998; White and Eigenbrode 2000*a*). In the field, excluding naturally occurring predators (mostly coccinellids) with cages reduces the differences in aphid populations on the pea isolines, suggesting enhanced predation is important for full expression of apparent resistance in reduced-wax peas (Rutledge *et al*. 2003). Growth and fecundity assays both in the field and the greenhouse show pea aphids do equally well on plants with normal- and reduced-wax levels (Rutledge *et al*. 2003).

The dominant aphid predator in the Palouse region of northern Idaho where we have conducted our fieldwork is the coccinellid, *Hippodamia convergens* Guérin de Méneville (Coleoptera: Coccinellidae). This species has been the focus of our laboratory and field experiments on the effects of waxy bloom on pea–aphid predation (Eigenbrode *et al.* 1998; White and Eigenbrode 2000*a*, 2000*b*). Observations of individual *H. convergens* larvae reveal that they walk less on peas with waxy blooms than on reduced-waxy-bloom peas (White and Eigenbrode 2000*a*); however, determining by direct observation how epicuticular waxes affect their foraging proved intractable because continuous observations during extended periods (>12 h) were required. To understand how epicuticular waxes affect foraging behavior of individual coccinellids, we conducted functional response experiments with the larvae. The functional response approach has proven useful in understanding how plant morphology impacts other predators (Carter *et al.* 1984; Treacy *et al.* 1987; deClerq *et al.* 2000) and allowed us to distinguish between the effects of waxy bloom on *H. convergens* searching capacity and ability to capture and subdue prey. Based on our functional response result, we conducted an additional experiment to measure the ability of *H. convergens* larvae to attach to the leaf surface of peas with reduced- and normal-wax phenotypes.

Materials and methods

Peas

Two sister lines of peas differing in epicuticular waxes were developed from accession PI W6-15368 (Marx 406). As described in Eigenbrode *et al.* (1998), the lines differ in expression of the wax mutation *wel* (Marx 1969). One of the lines is homozygous for *Wel* and has a normal-wax bloom; the other line is homozygous for *wel* and has a reduced-wax bloom. The mutation *wel* reduces wax expression over the entire plant surface by approximately 80%, and changes wax composition and structure (Eigenbrode *et al.* 1998). Epicuticular waxes on normal-wax plants have a crystalline structure, whereas epicuticular waxes on reduced-wax plants are amorphous. Both lines are homozygous for the recessive mutation *tl* (acacia leaf), which expands tendrils to leaflets.

Peas were grown in 10-cm^2 pots and were potted in a 2:1 ratio of commercial potting mix (Sunshine Mix 1, SunGro Horticulture, Bellevue, Washington) and sand. Plants were grown in a greenhouse at 25°C and 16L:8D and bottom-watered once a week. Seedlings at the 3- to 4-node stage were used in the experiment. Occasionally, it was necessary to use older seedlings trimmed to 4 nodes.

Insects

Acrythosiphon pisum

Aphids were from a virus-free clonal colony started from a field-collected individual. Aphids used for feeding ladybugs were reared on fava beans, *Vicia faba* L. (Fabaceae). Aphids used as prey in the experiments were reared on peas (variety Columbia) so that they would settle more readily onto the pea plants. Aphids in the second through the penultimate instar were used in the experiments to avoid reproduction of aphids during the 24 h of the experiment.

Hippodamia convergens

Adult *H. convergens* were obtained from Nature's Control (Medford, Oregon). The sex of adults were determined and were placed in 28-mL plastic cups as mating pairs. They were fed a honey–yeast mixture supplemented with 10–20 *A. pisum* daily. Cups were checked daily for eggs, which were removed to another container. When eggs hatched individual larvae were placed into 28-mL plastic cups and fed 10–20 *A. pisum* daily. Molting was noted during the daily feeding, and instar and date of molting were marked on the cup. We used fourth instar larvae the day after molting in all experiments. *Hippodamia convergens* were reared in an incubator at 24°C and 16L:8D at 60% RH.

Functional response experiment

Hippodamia convergens were starved for 18 h before use in experiments to equalize motivational state. To ensure that only active, healthy larvae were used in the experiment, larvae were tested with an aphid before use. Larvae that did not attempt to capture an offered aphid within 30 s were discarded. Only active larvae contribute to aphid mortality in the field, so this was considered a reasonable condition for including larvae in our bioassay.

Aphids were collected from their colony and placed on peas using a paintbrush. Aphids were distributed approximately equally over the surface of the plants and given 2 h to redistribute themselves and to settle onto the plants before predators were introduced. Pea plants to be used in the experiment were grown within plastic funnels, the small opening of which was placed over the seedling soon after germination. The funnels were made from 2-L plastic beverage bottles with bottoms cut off. Their interior surfaces were coated with fluon (Anti-Ant™, Farnam Companies Inc, Phoenix, Arizona) to provide an arena from which nonflying insects could not escape. Each predator was introduced by letting it slide down the inside of the funnel to the bottom of the plant.

After 24 h, predators were removed and peas were destructively sampled. We assumed missing aphids were eaten. Replicates were discarded if first-instar aphids were present, indicating that reproduction had taken place over night. Replicates were also discarded if the predator had escaped, or appeared unhealthy, at the end of the experiment. Predators were deemed unhealthy if they did not respond when prodded by soft forceps.

At least 12 replicates of the experiment were conducted at aphid densities of 2, 4, 8, 16, 32, 64, and 128 aphids. Owing to the large number of trials, replicates were accumulated over time. Experiments were conducted in a greenhouse where conditions were kept as consistent as possible between days (24°C, 45% RH, and 16L:8D).

Because we did not replace aphids as they were eaten, the prey density decreased with each prey item removed. Therefore, Holling's (1966) disk equation was not the appropriate model for the data. This problem was addressed by Rogers (1972) and his random predator equation

$$N_e = N_o\{1 - \exp[a(T_h N_e - T)]\}$$

where N_e is the number of prey eaten, N_o is the number of prey originally present, a is the instantaneous search rate, T_h is handling time, and T is the length of the experiment. We followed the methods of Juliano (1993) to fit our data to the random predator equation in PROC NLIN (SAS Institute Inc 2002). Differences in the overall predator efficacy (aphids killed) and the parameters T_h and a between the two pea isolines were calculated using a dummy variable technique (Bates and Watts 1988) in PROC NLIN (SAS Institute Inc 2002). T_h and a were estimated after fitting Rogers' equation to the data and were not measured independently.

Attachment of larval *Hippodamia convergens* to pea leaves

Attachment measurement device

A centrifugal device was used to measure the ability of larval *H. convergens* to attach to pea leaves. A full description of the device is found in Eigenbrode and Kabalo (1999). A weighed insect is placed on the surface of a turntable and covered with a clear plastic canopy. The turntable is rotated at increasing speed until the insect is detached from the surface by centrifugal force. Detachment is observed with the aid of a

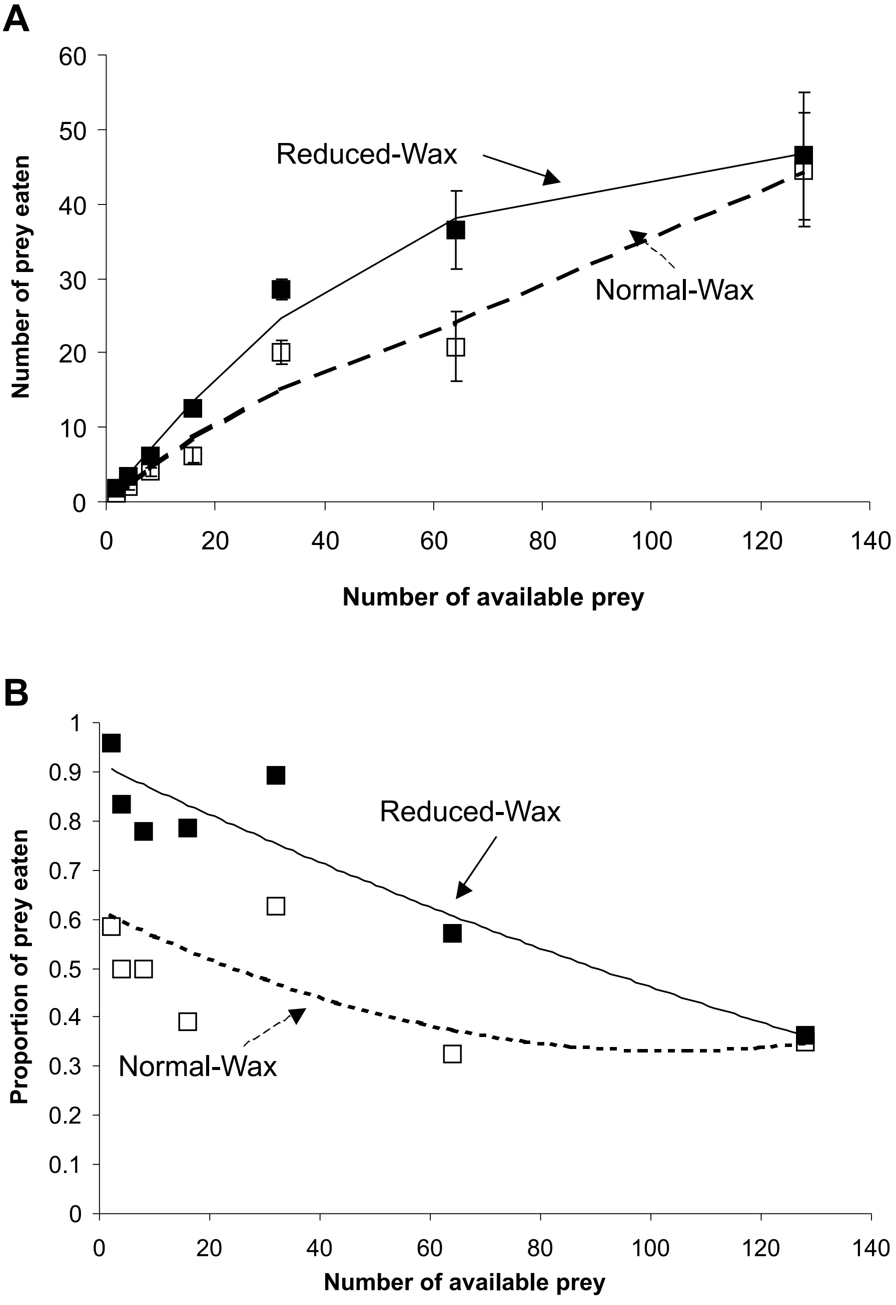

FIGURE 1. Functional response of *Hippodamia convergens* larvae to *Acrythosiphon pisum* on reduced wax and normal wax peas. (A) Number of prey available *versus* number of prey eaten. (B) Number of prey available *versus* proportion of prey eaten. Observed data are presented as means ± SE (A), and lines show predicted values derived from regressions that fit individual points to Rogers' modified Type II functional response (n = 12–16 replicates/aphid density).

synchronized strobe. The turntable revolutions per second, f (Hertz), required to detach the insect and the radius, r (metres), of the insect location on the turntable were used to calculate the effective velocity, v (metres per second). Together with the individual insect mass, m (grams), this gave the attachment force, F (milliNewtons) according to the following equation:

$$F = m\frac{v^2}{r} = m\frac{(2\pi rf)^2}{r} = 4\pi^2 mrf^2$$

Experimental design

For these measurements pea leaflets were taken from plants grown in the greenhouse and attached to the surface of the turntable using plastic clay. A new leaflet was used for each measurement. Each *H. convergens* larva was used on the day after it molted to fourth-instar (the same age used in the functional response experiments), weighed, and tested three times to obtain an average attachment force for that individual. Twenty replicates each were conducted for both the adaxial and abaxial surfaces of pea leaflets of reduced-wax and normal-wax peas.

The average attachment force for each individual was used in the analyses. A factorial analysis of plant type, leaf side, and their interaction was conducted in SAS (PROC ANOVA; SAS Institute Inc 2002).

Results

Individual predators displayed a Type II functional response on both of the pea phenotypes (Figs. 1A, 1B) as shown by the continuously decreasing proportion of prey eaten with increasing number of prey available (Fig. 1B). Individual predators were more efficient (removed more aphids) on the reduced-wax peas than on the normal-wax peas ($F_{2,161} = 12.1924$, $P < 0.0001$). The instantaneous search rate, a, was higher for predators on reduced-wax peas than for predators on normal-wax peas. Handling time, T_h, was not significantly different on the two pea types (Table 1).

Hippodamia convergens larvae generated greater attachment force to reduced-wax leaves, 0.182 (±SE 0.0255) μN, than to normal-wax leaves, 0.109 (±SE 0.0171) μN (Table 2). There was no difference between the abaxial and adaxial leaf surfaces, and no interaction between plant line and leaf surface (Table 2).

Discussion

Predators more effectively removed aphids on reduced-wax plants than on normal-wax plants as shown by the functional response experiments. Based on the fitted Rogers' random search model, predator searching efficiency was higher on reduced-wax plants than on normal-wax plants, whereas T_h was not significantly different on the two plant types. These results suggest that waxy bloom reduces the mobility of predators exploring the plant for prey but has little effect on the time required for predators to subdue, consume, and process prey. Consistent with this interpretation, previous data show that on normal-wax plants, larvae spend less time walking and more time in potentially unproductive behaviors such as grooming, scrambling (moving their legs without generating forward motion), and falling from the plant (White and Eigenbrode 2000*b*). The pattern of decreased searching efficiency and decreased mobility on normal-wax plants is associated, probably causally, with reduced attachment by *H. convergens* larvae to normal-wax plants on the surfaces of leaflets (Table 2).

Table 1. Random predator equation parameters for *Hippodamia convergens* larvae foraging on reduced wax and normal wax peas.

	Reduced wax*	Normal wax*	$F_{2,161}$	P
Instantaneous search rate a (h^{-1})	0.114±0.016	0.033±0.004	22.93	<0.0001
Handling time T_h (h)	0.375±0.068	0.235±0.011	1.18	0.2782

Note: Values are given as the mean ± SE. SE are the asymptotic standard errors estimated from fitting the data to Rogers' random predator equation (PROC NLIN, SAS 2002).

Table 2. Analysis of the attachment force generated by *Hippodamia convergens* to pea leaves.

Source	Anova SS	Mean square	F_1	P
Wax	10.6580	10.6580	11.07	0.0014
Side	0.0005	0.0005	0.00	0.9819
Wax × side	0.8820	0.8820	0.92	0.8820

Note: Wax refers to the two plant lines and side to the abaxial and adaxial surfaces of the leaves.

The advantage for predators on the reduced-wax plants over those on normal-wax plants dissipated at the highest density of aphids (128/plant and 42.6/plant node). At this density, the distance between aphids on the plant is apparently small enough that predator mobility and resulting a become inconsequential for predator effectiveness. An aphid density of 42/plant node is unusually high for dry peas in our area. In the field over the last 6 years of intensive sampling, aphid densities on peas not treated with insecticide have rarely exceeded 20 aphids/node (SD Eigenbrode, personal observation). Thus, greater searching ability is a factor that can lead to improved effectiveness of larval coccinellids on reduced-wax peas under typical field conditions.

Pea aphids drop from plants when threatened and this behavior can contribute to aphid mortality if aphids that drop fail to regain a plant (Roitberg and Myers 1979). This source of aphid mortality probably did not substantially influence our estimates of *H. convergens* functional response parameters. First, the fluon-coated funnels surrounding our plants ensured that aphids falling from plants landed within approximately 1 cm of the plant stem and prevented their climbing the funnel, away from the plant. Second, greenhouse temperatures were moderate and soil in the pots was moist, so aphid mortality on the soil was probably minimal; we found few dead aphids on the soil. Third, although pea aphids in the presence of predators tend to drop more from reduced-wax peas than from normal-wax peas (CE Rutledge, unpublished data), if this was a substantial source of aphid mortality in our bioassay, the T_h estimate on reduced-wax plants should have been relatively reduced, but this was not observed.

These results are consistent with, and provide a potential mechanism for, the observed lower populations of pea aphids on reduced-wax peas in the field (White and Eigenbrode 2000*a*) and for greenhouse experiments in which pea aphid populations were reduced by *H. convergens* larvae on reduced-wax peas but not on normal-wax peas (White and Eigenbrode 2000*b*). A reduced-waxy bloom is an example of a morphological change that can improve the crop habitat to favor predators (Cortesero *et al.* 2000).

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