Effects of *Brassica oleracea* waxblooms on predation and attachment by *Hippodamia convergens*

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Abstract

Four mutations that reduce waxbloom in *Brassica oleracea* L. were examined for their effects on predation, mobility, and adhesion to the plant surface by the general predator *Hippodamia convergens* (Guérin-Menéville) (Coleoptera: Coccinellidae). The mutations reduce waxbloom to different degrees, but all produce a 'glossy' phenotype. Plants tested were inbred lines, near isogenic lines, or segregating F_2 populations, depending on the mutation. In an experiment on caged leaves, predation of *Plutella xylostella* L. larvae by *H. convergens* adult females was significantly greater on glossy types as compared with 'normal-wax' or wild-type counterparts. Although the trend was the same for each mutation, individual comparisons between glossy and normal-wax lines or segregants were only significant for two of them, those producing mutant alleles gl_a and gl_d. Individual *H. convergens* were observed to spend more time walking on leaf edges and less time walking on leaf surfaces of normal-wax plants than glossy plants. *Hippodamia convergens* also obtained better adhesion to the surfaces of glossy plants than to normal-wax plants when tested using a centrifugal device. Two of the mutations produced similarly strong effects on predation, behaviour, and adhesion by *H. convergens*. These two are the same previously determined by us to provide the strongest similar effects on another generalist predator, *Chrysoperla plorabunda* (Fitch). The results indicate that waxbloom variation in nature could affect herbivore populations through its effects on generalist predators.

Introduction

Plant surface waxes often form conspicuous crystalline 'waxblooms' over the underlying amorphous epicuticular wax layer (Martin & Juniper, 1970). The minute crystals ($\approx$ 1–30 μm) can easily be abraded from the surface and this interferes with attachment by insects (Stork, 1980). Through this mechanism, waxblooms can provide protection against insect herbivory or other kinds of depredation (Stork, 1980; Bodnaryk, 1992; Juniper, 1995). Waxblooms also potentially affect attachment by predatory insects and thus influence trophic interactions among insects on the plants (Eigenbrode et al., 1995, 1996, 1998, 1999).

Genetic variation in waxbloom provides an effective tool for examination of how waxbloom can influence insect herbivory and predation. In *Brassica oleracea* L., several mutations occur that re-

duce waxbloom (Macey & Barber, 1970; Eigenbrode et al., 1991b). The mutations reduce crystal densities to varying degrees and alter crystal shape. The changes in morphology are associated with differences in composition of the waxes (Macey & Barber, 1970; Eigenbrode et al., 1991a, b). In a recent study (Eigenbrode et al., 1999) we examined the effects of four such waxbloom mutations on the behaviour, effectiveness and adhesion to the plant surface by the generalist predator *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae). All the mutant types enhanced predation and adhesion by this insect feeding on *Plutella xylostella* L. larvae, but to different degrees.

Here we report the results of a set of experiments to examine the effects of the same four waxbloom mutations on walking behaviour, adhesion, and effectiveness of a coccinellid generalist predator, *Hippodamia convergens* (Guérin-Menéville). The objective

was to determine whether effects similar to those observed for *C. plorabunda* extend to a representative of the Coccinellidae, one of the most important taxa of generalist predators in natural systems and biological control.

Materials and methods

Plants. *Brassica oleracea* expressing four recessive mutations that reduce surface waxbloom, and their wild-type counterparts, were used in the experiments. In this paper, those with reduced waxbloom will be referred to as 'glossy' and the wild-types will be referred to as 'normal-wax'. These glossy mutations were described and assigned gene labels by Eigenbrode et al. (1991b). All have been associated with resistance to Lepidoptera, aphids, or both in the field (Dickson & Eckenrode, 1975, 1980; Stoner, 1990; Eigenbrode et al., 1991b). The mutant allele gl_a was tested in glossy broccoli NY 1406 (obtained from M. H. Dickson, Cornell University) and compared with the cabbage hybrid 'Round-Up'. The mutant allele gl_d was tested in the inbred cauliflower line 'Glossy Andes' (obtained from K. A. Stoner, Connecticut Agricultural Experiment Station) and compared with its parental variety 'Andes'.

Plants. Two of the glossy mutations were tested in segregating F_2 populations obtained from K. A. Stoner. The KCR4 × 'Packman' F_2 population segregates for gl_{a-1} and the Broc5 × 'Packman' F_2 population segregates for gl_c. 'Packman' is a standard broccoli variety with a normal-wax bloom. KCR4 is an inbred glossy line of kale and Broc5 is an inbred glossy line of broccoli. Glossy segregants were compared with normal-wax types in each of these populations. The KCR4 × 'Packman' F_2 included three phenotypes based on their appearance to the unaided eye and under scanning electron microscopy (Eigenbrode et al., 1999): a normal-wax, a glossy wax, and an intermediate wax phenotype. Each of these three phenotypes was tested separately. To simplify presentation in this paper, *B. oleracea* varieties, inbred lines, or segregating phenotypes will be referred to generically as wax types.

All plants were grown in the greenhouse under supplemental metal halide lighting (L18:D6) (500 μmol photosynthetically active radiation m^{-2}), in 10-cm pots with greenhouse potting soil (Sunshine Mix 1, SunGro Horticulture, Bellevue, WA).

Plants were used for experiments six to 10 weeks after germination.

Insects. *Plutella xylostella* larvae were from a colony maintained on artificial diet (Shelton et al., 1991) at the University of Idaho and derived from the 'Geneva 88' population (A. M. Shelton, Cornell University). *Hippodamia convergens* adults were obtained commercially (Nature's Control, Medford, OR). Females that had been starved for 24 h were used in all bioassays.

Predation by H. convergens *on caged leaves.* Individual leaves (5th to 7th fully expanded) on intact plants of each *B. oleracea* type were enclosed in plastic cages (20 cm × 40 cm × 10 cm) screened on two sides with 'no-see-um' netting (REI, Spokane, WA, USA). The petioles passed through foam gaskets, sealed with 'Tack'n Stick®' adhesive (American Glue Corp.). Each leaf was infested with 30 *P. xylostella* eggs, with visible developing embryos, collected from the colony on aluminum foil. The foil strip with eggs was pinned to the abaxial side of the leaf. After 48 h, the foil strip was removed and the number of eggs that had hatched was determined by counting empty chorions. Two female adult *H. convergens* were then introduced into one of the cages on each plant and the other cage served as a predator-free control. Forty-eight hours after infestation, the number of surviving *P. xylostella* was counted on each caged leaf. The proportion of *P. xylostella* surviving was calculated by dividing the number of surviving larvae by the number of eggs that had hatched on each leaf. There were 6 replicates of each of the 9 wax types × 2 predator treatments, for a total of 108 caged leaves.

The effect of wax phenotype, predator, and their interaction on proportion of *P. xylostella* surviving were examined with a two-way analysis of variance (ANOVA) on the transformed proportions (arcsin $\sqrt{x}$). For this analysis, wax phenotypes were classified as normal-wax, glossy, or intermediate. A 1-way ANOVA was also calculated, with each treatment combination (line or wax type within a population × predator absent or present) considered a class level. Planned contrasts compared the effect of adding *H. convergens* versus controls on *P. xylostella* survival within each wax type. An estimate of the reduction in *P. xylostella* survival attributable to predation on each wax type was obtained by computing Abbot's (1925) formula for effectiveness: (*P. xylostella* survival on predator-free control − *P. xylostella* survival with

H. convergens)/(Predator-free control $+$ *P. xylostella* survival with *H. convergens*) $\times$ 100.

Hippodamia convergens *behaviour.* Individual *H. convergens* adult females were observed on each *B. oleracea* wax type for 5 min. Eighteen animals were observed on the KRC4 $\times$ 'Pacman' F_2 intermediate and 24 to 32 on each of the other test entries. The beetle's movement on the plant was continuously recorded using The Observer (Noldus Information Technologies, Wageningen). The distribution of walking among leaf upper surfaces, leaf lower surfaces, and leaf edges was compared with multivariate analysis of variance (MANOVA) (Harris, 1985) with percentage of time allocated to each location as dependent variables and wax type as the independent variable. Significance of the model was assessed on the basis of Wilks' Lambda statistic. Individual ANOVAs were compared for walking on each leaf part (upper surface, lower surface, edge) if the overall MANOVA was significant. Planned contrasts compared walking in each location on glossy and normal-wax paired lines or segregants within an F_2.

Hippodamia convergens *adhesion.* Adhesive force (mN) produced by *H. convergens* adult females on the leaf surfaces of each *B. oleracea* wax type was measured with a centrifugal device (Eigenbrode et al., 1999). Twelve insects were tested on the upper leaf surface of each of the wax types. Each insect was tested on a fresh leaf segment and there were two leaf segments from each of six plants from each wax type. Analysis of raw data was performed with ANOVA.

Statistical analyses of the experiments were performed using the procedures MEANS and GLM with LSMEANS in SAS (SAS Institute, Cary, NC).

Results and discussion

Predation by H. convergens. Based on two-way ANOVA, waxbloom class (glossy, normal-wax, intermediate) (df $=$ 2, 99; F $=$ 1.57; P $=$ 0.0115) and *H. convergens* (df $=$ 1, 99; F $=$ 7.24; P $=$ 0.0084) significantly affected *P. xylostella* survival on caged leaves. The interaction between *H. convergens* and waxbloom class was not significant (with α $=$ 0.05) (df $=$ 2, 99; F $=$ 2.88; P $=$ 0.0611). Suppression of *P. xylostella* survival by *H. convergens* was consistently greater on glossy types within pairs of lines or F_2 populations (Table 1). Although the effect of glossy

wax on *H. convergens* predation of *P. xylostella* is not as great as previously reported between two cabbage genotypes differing in expression of gl_a (Eigenbrode et al., 1995), here we have shown that four different glossy mutations in *B. oleracea* can enhance predation by this insect. Together with our previous study of *C. plorabunda* (Fitch) predation on these *B. oleracea* wax types (Eigenbrode et al., 1999), this result indicates that different reduced waxbloom mutations can enhance predation by at least two active predators in different orders. The greatest increase in *H. convergens* predation occurred on plants expressing gl_a (NY 1406) or gl_d (Glossy Andes), which produce the strongest reductions in wax crystal density and wax load (μg cm^{-2}) (Eigenbrode et al., 1991b; 1999; Eigenbrode unpubl.). These two characteristics are apparently most important for affecting predator effectiveness. The trend within the KCR4 $\times$ 'Packman' F_2 towards increasing predation from normal-wax, through intermediate, to glossy types (Table 1), also is consistent with wax phenotype affecting predation. The intermediate phenotype has a crystal density intermediate between normal-wax and glossy types in the KCR4 $\times$ 'Packman' F_2 (Eigenbrode et al., 1999).

Plutella xylostella survival was similar on controls without *H. convergens,* except for 'Round-Up', on which survival was reduced. This hybrid variety matured faster than the other test entries and the leaves were tougher, apparently producing this effect. Reduced predation on this line relative to NY 1406 may have been caused by lower prey populations as well as wax phenotype.

Hippodamia convergens *behaviour.* *H. convergens* adult females spent $\geq$ 75% of the time walking during 5-min observations on all wax types. This walking was distributed differently among parts of leaves of the *B. oleracea* wax types (Table 2). The insects consistently and significantly spent more time walking on upper or lower leaf surfaces of glossy types than normal-wax types, and more time walking on leaf edges of normal-wax types than glossy types (Table 2). The proportion of time *H. convergens* spent walking on upper surfaces and edges of intermediate KCR4 $\times$ 'Packman' F_2 was similar to the proportion of time spent walking on glossy segregants in this cross. Walking on lower surfaces was similar on the intermediate and normal-wax segregants in the KCR4 $\times$ 'Packman' F_2 population.

These differences in the distribution of walking by *H. convergens* can explain the effectiveness of

Table 1. Survival (% ± SEM) of neonate *Plutella xylostella* on caged leaves of *Brassica oleracea* with different surface waxbloom with and without the predator *Hippodamia convergens*

Lines varieties or population	Wax class[a]	Predator-free control	With *H. convergens*	Reduction attributed to *H. convergens*[b]	P[c]
NY 1406	G	69.9 ± 5.7	39.9 ± 7.1	27.3	0.0175
'Round -Up'	N	32.5 ± 12.5	26.0 ± 8.2	11.1	0.5473
KCR4 ×	G	61.3 ± 12.5	40.0 ± 6.2	21.0	0.0715
'Packman' F2	I	64.8 ± 10.0	55.7 ± 10.0	7.6	0.3954
	N	61.1 ± 6.3	65.1 ± 11.8	−3.2	0.4993
'Glossy Andes'	G	47.0 ± 6.3	20.5 ± 6.3	39.3	0.0157
'Andes'	N	55.7 ± 5.1	48.8 ± 6.8	6.6	0.5703
Broc5 ×	G	51.8 ± 9.0	31.8 ± 2.0	23.9	0.1288
'Packman' F2	N	62.5 ± 6.4	48.5 ± 5.1	12.6	0.2475

ANOVA df 17, 90; F = 3.82; P = 0.0001.
[a]G = glossy, reduced waxbloom; N = normal, wild-type waxbloom; I = intermediate waxbloom.
[b]Mortality attributed to predation was estimated by Abbot's (1925) formula: (*P. xylostella* survival on predator-free control − *P. xylostella* survival with *H. convergens*) / (Predator-free control + *P. xylostella* survival with *H. convergens*) × 100.
[c]P value for planned contrast comparing *P. xylostella* survival on Control with *P. xylostella* survival with *H. convergens*.

Table 2. Percentage of time (± SEM) *Hippodamia convergens* spent walking on leaf parts of *Brassica oleracea* with different surface waxbloom

Lines varieties or population	Wax class[a]	*n*	Upper surfaces[b]	Lower surfaces[b]	Edges[b]
NY 1406	G	28	42.2 ± 3.6***	6.1 ± 1.0**	47.9 ± 3.0***
'Round -Up'	N	28	13.8 ± 3.0	0.0 ± 0.0	76.0 ± 4.5
KCR4 ×	G	24	14.2 ± 3.5***	2.3 ± 1.0*	54.2 ± 5.7*
'Packman' F2	I	18	25.6 ± 3.5***	0.9 ± 0.9*	54.3 ± 5.2**
	N	28	5.7 ± 1.9	0.8 ± 0.6	68.7 ± 4.3
'Glossy Andes'	G	30	13.8 ± 2.2**	9.4 ± 2.4**	52.1 ± 3.8*
'Andes'	N	32	4.9 ± 1.7	4.3 ± 1.6	65.5 ± 4.3
Broc5 ×	G	27	15.4 ± 2.4**	3.9 ± 1.6*	52.4 ± 4.6*
'Packman' F2	N	30	2.8 ± 1.0	0.7 ± 0.5	66.8 ± 4.4
ANOVA					
df			8, 244	8, 244	8, 244
F			23.39	6.05	4.57
P			<0.0001	<0.0001	<0.0001

MANOVA Wilk's Lambda, df = 24, 702.5 F = 10.68; P < 0.0001.
[a]G = glossy, reduced waxbloom; N = normal-wax, wild-type waxbloom; I = intermediate waxbloom.
[b]Asterisks indicate P value for planned contrast between reduced waxbloom (G or I) and normal waxbloom type (N) within each group; * =≤ 0.05, ** =≤ 0.01, *** =≤ 0.001.

Table 3. Adhesive force (mN ± SEM) produced by *Hippodamia convergens* on *Brassica oleracea* with different surface waxbloom

Lines varieties or population	Wax class[a]	mNewtons[b]
NY 1406	G	16.5 ± 3.7***
'Round -Up'	N	1.9 ± 0.1
KCR4 ×	G	17.3 ± 3.5***
'Packman' F2	I	1.9 ± 0.2
	N	1.2 ± 0.1
'Glossy Andes'	G	36.5 ± 5.3***
'Andes'	N	1.0 ± 0.1
Broc5 ×	G	13.4 ± 0.2**
'Packman' F2	N	1.0 ± 0.1
ANOVA		
df		8, 99
F		24.47
P		< 0.0001

[a]G = glossy, reduced waxbloom; N = normal, wild-type waxbloom; I = intermediate waxbloom.
[b]Asterisks indicate P value for planned contrast between reduced waxbloom (G or I) and normal waxbloom type (N) within each group; ** =≤ 0.01, *** =≤ 0.001.

this predator on the *B. oleracea* lines tested. *Plutella xylostella* tend to feed or mine on the leaf surface, especially on lower leaf surfaces (Salinas, 1984; Eigenbrode, 1990). The greater time these predators spend walking over leaf surfaces of glossy plants should increase their rates of encounter with *P. xylostella*.

Hippodamia convergens *adhesion.* Adhesive forces generated by *H. convergens* differed significantly among the *B. oleracea* wax types, ranging from nearly 10-fold to 30-fold higher on glossy versus normal-wax types within pairs of lines or F2 segregants (Table 3). Adhesive force produced by *H. convergens* on the KCR4 × 'Packman' F2 intermediate wax type was not different from normal-wax segregants in this population.

Differences in adhesion to the plant surface provide a mechanistic explanation for *H. convergens* walking more on leaf surfaces of glossy *B. oleracea*. On normal-wax plants, increased walking on the leaf edge must compensate for poor adhesion to the leaf surface. Walking and feeding on leaf edges is a strategy employed by some chrysomelid species for coping with poor adhesion to the leaf surface (Edwards &

Wanjura, 1990). Insects straddling a leaf edge can probably use muscular force to increase molecular or frictional adhesion between opposing tarsi and the leaf surface.

In this experiment, *H. convergens* adhesion to the upper leaf surface was measured. Adhesion to the lower surface may help explain behavioural differences because time walking on the lower and upper surface did not correspond. For example, *H. convergens* allocated walking time similarly to the upper surface of KCR4 × 'Packman' F2 intermediate and glossy segregants, but walking on the lower surface of intermediates was similar to that on normal-wax segregants.

Conclusions

The three experiments presented here support the hypothesis that genetic reduction in waxbloom of *B. oleracea* improves adhesion, mobility (indicated by the insects walking over more of the leaf surface), and predatory effectiveness of *H. convergens* foraging on the plants. This result is similar to that obtained with first instar *C. plorabunda* on the same set of *B. oleracea* types (Eigenbrode et al., 1999). This neuropteran predator obtained better adhesion, greater mobility, and was more effective as a predator on the glossy types. The general agreement between the results with two predators suggests reduced waxbloom may benefit other active generalist predator species. However, effects of waxbloom on behaviour of the two predator species differ in detail. In contrast to *H. convergens*, *C. plorabunda* spent more total time walking on glossy types and most of this walking was on leaf surfaces. *Chrysoperla plorabunda* spent little time walking on leaf edges and this did not differ substantially among the *B. oleracea* types (Eigenbrode et al., 1999).

Previously reported observations of *H. convergens* and *C. plorabunda* on a normal-wax and glossy cabbage (expressing gl_a) (Eigenbrode et al., 1996) also differ in detail from those we report here. In that study, differences were observed in grooming behaviour and time spent walking on glossy and normal-wax plants by both predator species. The genetic background in which the glossy gene was expressed, and the environmental conditions for plant production (L13:D11 natural lighting versus L16:D8 supplemented lighting in the present study; 25-cm diam versus 15-cm diam pots in the present study) may account for these differences. Nonetheless, predation (Eigenbrode et al.,

1995) by *H. convergens* and *C. plorabunda* on the glossy type were similar to those reported here and in Eigenbrode et al. (1999).

All the work examining this phenomenon has been done in laboratory or greenhouses and its importance in the field is uncertain. However, the results suggest that improvement in predator effectiveness for biological control might be achieved by deploying *B. oleracea* varieties with reduced waxbloom. The gene gl_a apparently provides the greatest potential benefit of this type. This strategy would have tradeoffs because glossy types may be more susceptible to attack by *Phyllotreta* flea beetles. At least this was shown to be the case for other reduced wax mutations of *B. napus* (Bodnaryk, 1992). In natural systems, intra- and interspecific variation in waxbloom (e.g., Martin & Juniper, 1970; Mulroy, 1976; Tipton & White, 1995) could affect active generalist predators and have consequences for regulation of herbivore populations.

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