Attachment to Plant Surface Waxes by an Insect Predator¹

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SYNOPSIS. Insects foraging on plant surfaces must attach to the layer of lipophilic materials known as epicuticular waxes (EW) that cover these surfaces. In this paper, we briefly review the evidence that variation in EW morphology can influence the ecology of herbivorous insects directly, by affecting their attachment to plant surfaces, and indirectly by affecting attachment by actively foraging predatory insects to plant surfaces. We then present new data examining how EW micromorphology and chemical composition of Brassica oleracea influence attachment by the predatory beetle, Hippodamia convergens (Coccinellidae). Bioassays with genotypes of B. oleracea differing in wax characteristics, and with EW extracts from these plants applied to glass, show that wax crystals disrupt attachment. In addition, bioassays show that attachment by H. convergens differs among EW extracts prepared to have smooth surfaces without crystals. The differences in attachment under these conditions are evidently due to the chemical composition of the waxes. Bioassays with two pure wax constituents show that wax composition can significantly affect attachment by H. convergens.

The study opens the way for using a similar approach to understand attachment by insects to waxy plant surfaces.

CONTEXT OF THE STUDY: ATTACHMENT TO PLANT SURFACE WAXES BY INSECTS

Both insect herbivores and predators that attack them must attach effectively to plant surfaces (Southwood, 1986; Eigenbrode, 1996), which implies that they must attach to the waxy materials that cover these surfaces. The cuticle of the primary organs of higher plants consists of a polymeric cutin matrix and cuticular waxes soluble in organic solvents such as hexane or dichloromethane. The waxes are complex mixtures of very-long-chain aliphatics including primary alcohols (n-alkan-1-ols), aldehydes, fatty acids (n-alkanoic acids) and alkyl esters, all of which occur predominantly with even-numbered chain lengths, and hydrocarbons, secondary alcohols and ketones with predominance of odd-numbered chain lengths (Walton, 1990). Small proportions of these materials may have branched carbon chains. Cuticular waxes from certain species and organs also contain relatively large proportions of triterpenoids, including oleaneans and uranes, and phytosterols (Walton, 1990). Although these patterns are robust, the majority of plant species has yet to be examined and our current information is based on an overrepresentation of temperate species (Riederer and Markstädtler, 1996).

A portion of plant cuticular waxes is located outside the cuticular matrix and, hence, is exposed on the immediate surface of the plant. These “epicuticular waxes” (EW) generally form a thin, continuous film but can also be decorated with protruding microscopic crystals occurring as filaments, rods, platelets, tubes, and complex dendritic structures (Jeffree, 1986; Barthlott et al., 1998). Grossly, such crystals give the plant surface a whitish (“glaucous”) appearance or waxy bloom.

Comparative chemical and micromorphological investigations have shown that special wax constituents form the EW crystals on diverse plant surfaces (Jeffree et al., 1975; Jetter and Riederer, 1994, 1999; Meusel et al., 2000) and the composition of these crystals sometimes appears to differ from the underlying intracuticular waxes. This has been determined by very brief extraction (Silva Fernandes et al., 1964) or mechanically stripping the EW from the cuticle (Baker et al., 1983; Jetter et al., 2000; Jetter and Schäffer, 2001).

Although it is clear that the bulk wax mixture is important for waterproofing (Schönherr, 1976), the necessity of EW for this function of the cuticles is not certain (Baur, 1998). Meanwhile, EW may have other ecological functions including shielding of UV-B (Mulroy, 1979; Day et al., 1992), protection against pathogen invasion (Blakeman, 1973; Carver and Thomas, 1990; Nielsen et al., 2000), and influencing insect behavior by functioning as allelochemicals (Eigenbrode and Espelie, 1995; Spencer, 1996; Udayagiri and Mason, 1997; Cervantes et al., 2002).

In addition to their demonstrable allelochemical effects on insects, EW also can influence insect adhesion or attachment to the plant surfaces, as was first demonstrated by Kernan von Marilau (1898), Haberlandt (1909) and Knoll (1914). The ecological implications of this phenomenon have been documented in four contexts: (i) EW crystals in pitchers of the carnivorous genera Nepenthes and Darlingtonia generate slippery surfaces, which are pivotal in prey capture (Juniper, 1995); (ii) EW crystals on stems of the paleotropical ant-plant genus Macaranga generate slippery surfaces that protect mutualistic partner ants against generalist competitors (Fedele et al., 1997); (iii) EW crystals on floral stems of certain plants prevent ants from removing pollen (Harley, 1991); (iv) differences in adhesion
or attachment to EW crystals mediate interactions involving herbivorous insects and their insect natural enemies. This last aspect is the context of the present report, and will be reviewed in more detail.

**EW effects on attachment by herbivorous insects**

Crop species with typically glaucous appearance, caused by EW crystals, sometimes produce so-called “glossy” mutations in which the waxy bloom is greatly reduced (Eigenbrode and Espelie, 1995). The glossy forms are often more susceptible to certain insect pests (Tsumuki et al., 1987; Stoner, 1990; Bodnaryk, 1992) and this has been shown in some cases to be related to an increased ability of these insects to attach or adhere to plants with reduced waxy blooms. A glossy variety of *Brassica oleracea* provided better adhesion for the chrysomelid beetle, *Phaedon cochleariae*, than a variety with typical waxy bloom (Stork, 1980a). In the field, damage by flea beetles, *Phyllotreta cruciferae*, was greater on *Brassica* spp. with reduced waxy bloom (Stoner, 1990; Eigenbrode et al., 2000). Bodnaryk (1992) showed that waxy bloom was the responsible factor in *B. napus* by removing the wax crystals mechanically, rendering the plants more susceptible to beetle damage. For similar reasons, reduced waxy bloom peas, *Pisum sativum*, are more susceptible to damage by the pea leaf weevil *Sitona lineata* (unpublished data, SDE).

Similar effects have been documented in natural systems. Juvenile leaves of some *Eucalyptus* spp. possess a waxy bloom that prevents attachment and feeding by beetles (Edwards, 1982). The distribution and abundance of leaf feeding beetle species on *Eucalyptus* spp. is related to the waxy bloom on the trees and the attachment abilities of the individual beetle species (Edwards and Wanjura, 1991).

**EW effects on attachment by predatory insects**

Contrary to expectations based on the foregoing examples, agricultural crop varieties with reduced waxy blooms typically are relatively resistant to insects as compared with normal waxy bloom varieties (75% of the reported occurrences) (Eigenbrode and Espelie, 1995). This has been reported for soybeans (Baker et al., 1985), onions (Molenaar, 1984), sorghum (Starke and Weibel, 1981), wheat (Lowe et al., 1985) and *Brassica* crops (e.g., Way and Murdie, 1965; Dickson and Eckenrode, 1980; Stoner, 1990).

Evidence is accumulating that at least a partial explanation for the apparent resistance of reduced waxy bloom crop varieties is enhanced action by insect predators on these plants, as first suggested by Way and Murdie (1965). Three taxonomically diverse species of generalist predators (*Chrysoperla plorabunda, Orius insidiosus*, and *Hipposandma convergens*) more effectively reduce populations of the diamondback moth, *Plutella xylostella*, on a glossy cabbage (Eigenbrode et al., 1995). A similar pattern occurs for *H. convergens* attacking aphids on glossy peas (Eigenbrode et al., 1998; White and Eigenbrode, 2000). In the field, glossy *Brassica napus* has lower densities of aphids but higher densities of the aphid predator *H. convergens* than normal EW *B. napus* (Eigenbrode et al., 2000).

EW blooms in general appear to interfere with predator mobility (von Arzet, 1973; Shah, 1982; Grevstad and Klepetka, 1992). Observations on glossy and glaucous plants show that the predators are more active, cover more of the plant surface, and fall less from the reduced EW *Brassica* and *Pisum* (Eigenbrode et al., 1996, 1998; White and Eigenbrode, 2000). These behavioral differences are related to substantially greater attachment forces achievable by predators on glossy leaf surfaces than on glaucous ones (Eigenbrode et al., 1998). Indeed, differences in attachment force generated by two predator species to *B. oleracea* differing in EW bloom are correlated with effectiveness of these predators at taking prey on the plants (Eigenbrode and Kabalo, 1999; Eigenbrode et al., 1999).

Although it is clear that EW blooms interfere with attachment by predatory and phytophagous insects, the EW properties responsible for the effects are not yet understood. It is not known to what extent the morphology of plant wax crystals, their specific composition, the density of wax crystals, and the composition of underlying waxy layers of the cuticle influence insect attachment. These potential factors are confounded in EW mutants because composition and crystal morphology and density are all changed to some degree by the mutations (Macey, 1970; Macey and Barber, 1970; von Netting and Wettstein-Knowles, 1972; Holloway et al., 1977; Eigenbrode et al., 1991a; b; Eigenbrode, 1998). In addition, the work thus far has focused on plants that differ obviously in waxy bloom, but has left unexamined the effects on insect attachment of amorphous waxes that differ only in composition, despite the fact that such variation is widespread in nature (Walton, 1990).

**Experiments: Attachment to Plant Waxes by a Predatory Insect, *Hippodamia convergens***

To develop a better understanding of how plant EW affect insect attachment, we conducted experiments with *H. convergens*, a predator known to forage on diverse plants, including grasses, forbs, and perennial shrubs (Beers et al., 1993; Hoffmann and Frodsham, 1993). We first examined our previously reported data (Eigenbrode and Kabalo, 1999) on attachment by *H. convergens* to eight *Brassica oleracea* genotypes differing in waxy bloom. We then measured attachment by *H. convergens* to these plants with EW altered mechanically and to EW extracted from the plants for *in vitro* tests.

**Study 1: attachment to B. oleracea leaf surfaces with varying amounts and shapes of epicuticular wax crystals**

**Materials and methods.** We used four mutations affecting EW in different genetic backgrounds of *Brassica oleracea*. The mutants and wildtype counterparts

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The text above is a natural representation of the document as if you were reading it naturally. The key points include:

- The interaction between herbivorous and predatory insects and the role of waxy blooms.
- The influence of reduced waxy blooms on insect attachment and feeding behavior.
- The role of EW in affecting attachment forces and predator mobility.
- Experiments conducted with *Hippodamia convergens* to study attachment to plant waxes.
- The role of waxy bloom in affecting attachment by predatory insects.

These points are integrated into the natural text to provide a coherent understanding of the document's content.
provide a range of EW phenotypes as shown by SEM (Fig. 1A, C–F). With their normal counterparts, these produce 9 EW types referred to in this paper as Brassica types. The mutant allele gla was tested in glossy cabbage NY1406 (obtained from M. H. Dickson, Cornell University) and compared with its normal waxy bloom counterpart “Round-Up” cabbage. The mutant allele gla was tested in the inbred cauliflower line “Glossy Andes” (obtained from K. A. Stoner, Connecticut Agricultural Experiment Station) and compared with its normal waxy bloom counterpart “Andes.” The two other glossy mutations were compared to glaucous phenotypes within segregating F2 populations (obtained from K. A. Stoner). KCR4 × “Packman” F2 population segregates for gla and the Broc5 × “Packman” F2 population segregates for gla. The KCR4 × “Packman” F2 populations include an intermediate EW phenotype (Eigenbrode et al., 1999), which was not included in our experiments. Plants were grown in the greenhouse under supplemental metal halide lighting (L18:D6) (500 μmol photosynthetically active radiation/m²), 18°C and 25°C (night,
day), and average approx. ambient RH of 60%, in 10-cm pots with greenhouse potting soil (Sunshine Mix 1, SunGro Horticulture, Bellevue, WA). Six to 10 weeks after germination, leaves from all eight *B. oleracea* types were harvested for adhesion experiments.

Field-collected *Hippodamia convergens* of undetermined age were maintained for several weeks on a diet of pea aphids (*Acyrthosiphon pisum*). Only female insects that had been provided water but no prey for 24 hours were used in the experiments.

Lateral attachment forces by *H. convergens* were measured with a centrifugal device as described by Eigenbrode et al. (1999) and Federle et al. (2000). Leaf pieces (or wax mixtures deposited on glass, see below) were fastened to a horizontal aluminum turntable (30 cm diam). An individual insect was placed on the test material within a clear plastic canopy to eliminate effects of air resistance. The preparation was then attached to the turntable, which was accelerated gradually until the insect was separated from the leaf surface by centrifugal force. To aid observation of the insect during the test, a stroboscopic light directed at the insect was triggered to flash on each rotation of the turntable. The turntable revolutions per second $f$ [Hz] required to detach the insect and the radius $r$ [m] of the insect location on the turntable were used to calculate the effective velocity $v$ [m sec$^{-1}$]. Together with the individual insect mass $m$ [g] this gave the attachment force $F$ [milli-Newton] according to:

$$F = \frac{m v^2}{r} = m \left(\frac{2\pi f r^2}{r}\right) = 4\pi^2 m r f^2$$

Typical forces were more conveniently expressed in $\mu$N. Attachment by each insect was measured 2 to 3 times and the average of these values was used as a single observation. Ten to 12 insects were tested on the upper leaf surface of each of the *Brassica* wax types. Each insect was tested on a fresh leaf piece and there were two leaf pieces from each of six plants from each *Brassica* type.

Following a similar protocol, at least 20 insects were tested on leaf pieces of each of the same *Brassica* types mechanically polished by gently wiping them with a lint-free cloth. This procedure eliminated the waxy bloom and removed most of the EW crystals or incorporated them into the wax surface, or both, as revealed by scanning electron microscopy (SEM) (e.g., in Fig. 1B). The gross appearances of the glossy phenotypes were not visibly affected by this polishing.

For statistical analysis, attachment forces to plant surfaces were transformed [$\ln(x)$] and compared with analysis of variance (ANOVA). Planned contrasts compared attachment to each wild-type and its respective reduced wax counterpart; Pearson’s correlation coefficient was calculated between *H. convergens* attachment and the previously reported (Eigenbrode et al., 1991b) mean EW crystal density (crystals per $\mu$m$^2$) for the eight *Brassica* types.

**Results.** While glaucous types had surfaces with dense networks of rods and filaments (Fig. 1A), the EW of glossy phenotypes had less dense vestures of filaments, rods, angular and irregular plates, polygons and globules (Fig. 1C–F). Attachment by *H. convergens* to untreated leaf surfaces of the *B. oleracea* genotypes spanned a 30-fold range (Fig. 2A), differing significantly among the types ($P \leq 0.0001$). With one exception (Broc 5 × Packman F$_2$ Normal vs. Broc 5 × Packman F$_2$ Glossy), the reduced waxy bloom line in each pair allowed significantly greater attachment force by *H. convergens*. Crystal counts on the untreated plant surfaces and attachment by *H. convergens* were negatively correlated ($r = -0.930$, $P = 0.0001$). Mechanical polishing did not alter attachment to the glossy types from the KCR4 × Packman F$_2$ and NY1406, as expected from their unchanged surface appearance. The low adhesion force on Broc 5 × Packman F$_2$ Glossy and the high adhesion force on Glossy Andes were both brought closer to the average for all types by polishing.

**Study 2: attachment by *H. convergens* to reconstituted *Brassica* wax extracts**

In order to investigate the effect of different EW compositions and morphologies, the influence of epidermis topography and other *in situ* parameters had to be eliminated. To this end, leaf cuticular waxes of the eight *Brassica* types were extracted, analyzed, and reconstituted on glass to measure attachment force by *H. convergens*. We prepared the waxes for testing either so as to allow semi-natural crystals to form, which should influence attachment as on intact leaf surfaces, or as amorphous films to eliminate the complex effects of crystal morphology and allow assessment of chemical composition alone on attachment by this insect.

**Materials and methods.** Previous SEM studies had shown nearly identical EW crystals on both leaf sides for all eight *Brassica* types (unpublished data, RJ). Hence, it could be expected that the qualitative wax compositions would be similar on both sides and a total leaf extract could be used to simulate adaxial cuticles. Wax extracts of each *Brassica* line were prepared by immersing leaves for 10 sec in $n$-hexane at room temperature. Extracts were concentrated to dryness under a stream of nitrogen, weighed, taken up in $n$-hexane and applied to glass coverslips. Wax coverage on the intact plants ranged 10-fold from ca. 2 to 20 $\mu$g/cm$^2$. For the present experiments, we did not test the effects of wax coverage, but used a standard superabundant deposit of 100 $\mu$g/cm$^2$ for all the wax mixtures.

Wax extract solutions were standardized to 2.42 mg/ml and 200 $\mu$l of this solution was dispensed onto a 4.84-cm$^2$ glass cover slip to deposit a 100 $\mu$g/cm$^2$ coating. The solvent was allowed to evaporate slowly to produce a bed of crystals. Two methods were employed to generate amorphous surfaces. One method was to melt the crystals of waxes deposited onto glass from solvent by warming the cover slip to approxi-
Attachment forces generated by *H. convergens* to *B. oleracea* wax extracts depended on how they were prepared for bioassay, being greatest for wax extracts presented as amorphous films on glass (mean $= 0.061 \pm 0.004$ mN), lower for waxes prepared with molded smooth surfaces ($0.018 \pm 0.002$ mN), and lowest for waxes applied to glass and allowed to crystallize ($0.012 \pm 0.002$ mN). Attachment forces to EW preparations were greater than the average attachment force to intact plant surfaces ($0.009 \pm 0.004$ mN). The pattern of relative attachment to EW extracts prepared as crystals was similar to that to intact plant surfaces (*cf.* Fig. 2A and C). As for intact plant surfaces, the effect of plant type was significant and attachment was greater to crystalline EW extracts from the glossy type in each pair, except those from...
the Broc-5 × F2 population (cf., Fig. 2A and C). On EW extracts prepared with smooth surfaces the effect of plant type was significant, but attachment tended to be greater to normal-type waxes, significantly so for two comparisons (Fig. 2D and E). In addition, the general pattern of attachment to waxes applied as smooth films tended to resemble those to plant surfaces mechanically polished to reduce EW crystals (Fig. 2B, D, and E).

Fifty-four different compounds were identified in EW mixtures from the Brassica types (data not shown) and these were grouped into homologous series representing eight classes of aliphatics (Table 1). Secondary alcohols and ketones were found to carry the functional group mainly on the central carbon atom (15-isomers). The EW extracts from glossy and glaucous Brassica types were similar qualitatively, but differed quantitatively. The glaucous Brassica types had wax coverages between 12 and 19 µg cm⁻² (Table 1). Three of these normal-wax types (Andes, Broc5 × Packman F2, and Round-Up) had similar composition, containing 3–5% primary alcohols, 10–14% secondary alcohols, 37–42% alkanes, 24–27% ketones, 6–9% esters and 1% aldehydes. Normal-wax line KCR4 × Packman F2 wax differed from the others in its lower concentration of secondary alcohols (4%) and ketones (9%), and correspondingly higher concentration of alkanes (65%) and esters (11%). The normal-wax types had similar chain-length distributions within major compound classes (data not shown). Predominant homologs were C26 for the unbranched alcohols, C27 for the branched alcohols, and C29 for the secondary alcohols, ketones, ketols and alkanes, respectively.

A group of three glossy Brassica types (Glossy Andes, KCR4 × Packman F2, glossy, and NY1406 glossy) had drastically decreased wax coverages ranging from 2 to 10 µg cm⁻² (Table 1), relative to their normal-wax counterparts. In KCR4 × Packman F2 glossy and NY1406 the overall drop in wax loads was due to reduced amounts of alkanes, secondary alcohols, ketones and ketols, with resulting dominance of primary alcohols, fatty acids and alkyl esters. These two EW types also were characterized by shifts towards smaller chain lengths (C27 more abundant than C29 alkane, secondary alcohol and ketone, respectively, data not shown). In Glossy Andes the drop in overall coverages was due to reduced amounts of all aliphatic compound classes, with slightly increased portions of alkanes and primary alcohols, and decreased percentages of esters, secondary alcohols, ketones and ketols. Broc5 × Packman F2 glossy showed a unique increase in the wax load as compared with Broc5 × Packman F2 normal-wax plants. The overall increase was based on higher levels of ketones and alkanes, over-compensating for the reduced amounts of primary and secondary alcohols.

None of the correlations examined between the relative composition for each compound class in EW extracts and attachment by H. convergens were significant. There was a marginally significant negative correlation between attachment and the relative proportion of primary alcohols (r = −0.691, P = 0.057). Study 3: attachment by H. convergens to pure wax constituents

To overcome the difficulty in interpreting attachment to complex mixtures of wax components, we used similar methods to examine H. convergens attachment to pure C22 primary alcohol and C22 fatty acid. Although not predominant homologues in natural Brassica waxes, these compounds represent two important classes of EW components in many natural waxes.

Materials and methods. Standard C22 n-alkanoic acid and C22 primary alcohol were obtained commercially (Sigma-Aldrich, Deisenhofen, Germany) and dissolved in chloroform (app. 10 mg ml⁻¹). Carefully pre-rinsed 2 × 2 cm² glass slides were mounted on aluminum spacers and placed on a heating block (35°C). Up to 1 ml of the standard solution was placed onto the glass and evaporated under a gentle stream of N2 within approx. 1 min, forming an homogenous bed of crystals. SEM confirmed that loads of 1–2.5 mg cm⁻² could guarantee continuous coverage of the glass substratum with platelet-shaped crystals. Individ-

<table>
<thead>
<tr>
<th>Comparison wax bloom</th>
<th>Andes vs. Andes G</th>
<th>Broc5 × Packman F2</th>
<th>KCR4 × Packman F2</th>
<th>Round-Up vs NY1406</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Reduced</td>
<td>Normal</td>
<td>Reduced</td>
</tr>
<tr>
<td>Alkanes</td>
<td>39</td>
<td>44</td>
<td>42</td>
<td>57</td>
</tr>
<tr>
<td>sec. Alcohols</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Ketones</td>
<td>27</td>
<td>8</td>
<td>24</td>
<td>30</td>
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<td>Ketols</td>
<td>3</td>
<td>tr</td>
<td>2</td>
<td>tr</td>
</tr>
<tr>
<td>prim. Alcohols</td>
<td>5</td>
<td>14</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Alkanoic acids</td>
<td>tr</td>
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<td>tr</td>
<td>1</td>
</tr>
<tr>
<td>Alkyl esters</td>
<td>7</td>
<td>5</td>
<td>9</td>
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<td>9</td>
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<td>tr</td>
</tr>
<tr>
<td>Not identified</td>
<td>4</td>
<td>10</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Coverage [µg cm⁻²]</td>
<td>12.0</td>
<td>2.3</td>
<td>12.7</td>
<td>20.2</td>
</tr>
<tr>
<td>S.D. [µg cm⁻²]</td>
<td>10.7</td>
<td>1.1</td>
<td>7.6</td>
<td>9.6</td>
</tr>
</tbody>
</table>
TABLE 2. Attachment force [μN] by H. convergens to artificial surfaces, reconstituted in vitro as continuous layers of representative was constituents.

<table>
<thead>
<tr>
<th></th>
<th>Crystallized on glass</th>
<th>Molded, with tarsal claws intact</th>
<th>Molded, with claws removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;22&lt;/sub&gt; primary alcohol</td>
<td>5.9 ± 0.3</td>
<td>14.3 ± 0.8</td>
<td>15.3 ± 0.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;22&lt;/sub&gt; n-alkanoic acid</td>
<td>4.9 ± 0.3</td>
<td>12.2 ± 0.9</td>
<td>11.4 ± 1.1</td>
</tr>
<tr>
<td>P value for t-test</td>
<td>0.012</td>
<td>0.12</td>
<td>0.006</td>
</tr>
</tbody>
</table>

usual crystal preparations were transformed into amorphous surfaces using the molding procedure described above.

Attachment to these materials by H. convergens was measured as described above. In addition, to isolate the effects of attachment to pure compounds using tarsal adhesion from attachment using pretarsal claws as anchors, an experiment was conducted using insects with the pretarsal claws removed surgically. Tarsal claws were removed under a microscope using fine scissors. After the surgery, the animals were fed aphids ad libitum and allowed to recover for 48 hr. Those exhibiting normal activity levels after 48 hours were used in the attachment bioassay.

Students t-test was used to compare Hippodamia convergens attachment to C<sub>22</sub> primary alcohol vs. C<sub>22</sub> n-alkanoic acid in each of the three experiments. Attachment data were log transformed before the analysis.

Results. Attachment to C<sub>22</sub> primary alcohol tended to be greater than attachment to C<sub>22</sub> n-alkanoic acid regardless of the method of preparation and whether the tarsal claws were removed from the insects before testing (Table 2). Attachment to both of these compounds was approximately 3-fold greater when they were presented as molded solids than as beds of crystalline deposits of the extracted EW. Crystalline deposits of the extracted EW types reproduce the relative attachment potential of intact plants. These crystalline deposits allow lower attachment forces by H. convergens than EW deposited as smooth films on glass or as solids with smooth, molded surfaces. Finally, two pure wax components crystallized on glass allowed lower attachment forces than the same compounds tested as smooth solids.

We also show that waxes differing in chemical composition but prepared with identical amorphous surfaces allow significantly different attachment by H. convergens. Brassica EW extracts as smooth films on glass or as molded solids yielded qualitatively similar patterns of attachment (Fig. 2D and E) characterized by greater attachment to extracts from wildtype (normal) waxes than to extracts from mutants. A similar but weaker pattern appears to be emerging on intact leaf surfaces wiped to remove EW crystallites. This procedure does not eliminate crystals (Fig. 1), but may reduce their influence on attachment, allowing stronger influence of chemical composition on H. convergens attachment. Although our result shows that chemical composition of waxes influences H. convergens attachment, the effects of specific EW components or component classes remain to be determined. Correlation analysis suggests that an increased proportion of primary alcohols, as occurs in EW from reduced EW Glossy Andes, NY1406, and in reduced waxy bloom KCR4 × Packman F<sub>2</sub> individuals, contributes to lower attachment by H. convergens to extracts of these waxes.

Discussion. Here we have reviewed the published evidence that reductions in the amount of epicuticular wax crystals allow insects to achieve greater forces of attachment to plants, thereby affecting their trophic interactions. The published work has not attempted a systematic experimental approach to deciphering what EW attributes influence insect attachment. We present definitive experimental evidence that crystallization of EW is a principal factor determining attachment by H. convergens. Specifically, attachment by H. convergens to leaves from eight Brassica types is correlated with the density of wax crystals on these leaves. Polishing B. oleracea with prominent waxy blooms to remove crystallites increases attachment by this insect, but polishing has little effect on B. oleracea with genetically reduced waxy blooms. Crystalline deposits of the extracted EW from eight B. oleracea types reproduce the relative attachment potential of intact plants. These crystalline deposits allow lower attachment forces by H. convergens than EW deposited as smooth films on glass or as solids with smooth, molded surfaces. Finally, two pure wax components crystallized on glass allowed lower attachment forces than the same compounds tested as smooth solids.
agent). Our result suggests that the wetting agent employed by *H. convergens* needs to be characterized as part of continued efforts to understand the basis of its attachment to EW.

**Conclusion**

Using a new approach, we have demonstrated the effect of wax crystals on plant surfaces in reducing attachment forces obtainable by an insect. This confirms the importance of wax crystals for influencing the ecology of insects that must adhere to plants to feed or forage for prey. We additionally show that composition of natural waxes prepared as smooth surfaces influences insect attachment. Thus, plant epicuticular waxes have the dual potential for influencing insect ecology through their allelochemical activity and through their influence on insect attachment to plant surfaces.

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