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Effect of epicuticular wax on adhesion of psyllids to glaucous juvenile and glossy adult leaves of *Eucalyptus globulus* Labillardière

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Abstract In the heteroblastic plant *Eucalyptus globulus*, the glaucous juvenile leaves are coated in epicuticular wax that is relatively absent on the glossy adult leaves. The adhesion of three psyllid species (*Ctenarytaina eucalypti* Maskell, *C. spatulata* Taylor and *Glycaspis brimblecombei* Moore) to glossy adult leaves, adult leaves to which the juvenile leaf wax was added, glaucous ('waxy') juvenile leaves, and 'de-waxed' juvenile leaves was compared. Wax reduced adhesion of all three species but *C. spatulata* and *G. brimblecombei* were most affected. Unlike the other two species, *C. eucalypti* could consistently climb the adaxial and abaxial surfaces of vertically inclined 'waxy' juvenile leaves. *Ctenarytaina spatulata* and *G. brimblecombei* were able to climb the 'waxy' juvenile leaves only by clinging to protruding veins of abaxial leaf surfaces. Psyllid adhesion to adult leaves was reduced when juvenile leaf wax was transferred to glossy adult leaves. These results are consistent with previous findings that juvenile leaves are resistant to *C. spatulata* and *G. brimblecombei* but are susceptible to *C. eucalypti*. Observations under light microscopy suggested that the tarsi of *C. eucalypti* are better adapted for adhering to the epicuticular wax-coated surfaces than those of the other two species. *Ctenarytaina eucalypti* used all legs to adhere to flat surfaces while the other species used only the front two pairs of legs. All three psyllid species had adhesive pulvilli on their basal tarsomeres; but *C. eucalypti* had eight and 11 times more total pulvillar area per unit of body weight than did *C. spatulata*, and four and five times more total pulvillar area than did *G. brimblecombei*, for males and females, respectively. Based on the 'foot prints' of the psyllids on soot-coated glass, *C. eucalypti* caused less exfoliation than the other two species when walking.

Key words *Ctenarytaina eucalypti*, *Ctenarytaina spatulata*, *Glycaspis brimblecombei*, leaf waxes, pulvilli.

INTRODUCTION

Plant epicuticular waxes affect insects both physically and chemically (Eigenbrode & Espelie 1995; Eigenbrode 1996). Waxes alter the slipperiness of plant surfaces, which affects adhesion of herbivores (Stork 1980b; Edwards 1982), predators (Eigenbrode *et al.* 1996, 1999), ants (Federle *et al.* 1997), and trapped insects to plant surfaces (Juniper & Burras 1962). Although epicuticular waxes affect resistance to several Homoptera (Thompson 1963; Lowe *et al.* 1985; Åhman 1990; Blua *et al.* 1995), little is known about the mechanisms of this resistance (Powell *et al.* 1999). To our knowledge the effects of epicuticular waxes on adhesion of psyllids have not been investigated.

Glaucousness is common in *Eucalyptus* and is often due to the presence of light-scattering epicuticular waxes (Barber 1955; Penfold & Willis 1961; Wirthensohn & Sedgley 1996). Despite considerable information on the structure (Hallam & Chambers 1970), development (Hallam 1970; Wirthensohn & Sedgley 1996) and abiotic affects of *Eucalyptus* epicuticular waxes (Cameron 1970; Banks & Whitecross 1971; Thomas & Barber 1974; Potts & Jackson 1986), little is known about their effect on insects. To our knowledge the only prior work on the effects of *Eucalyptus* waxes on insects (Edwards & Wanjura 1990; Edwards 1982) indicated that waxes reduced adhesion of chrysomelid beetles.

Heteroblasty describes plants with juvenile foliage that is morphologically different from the adult foliage (Day 1998). In California the heterblastic tree *E. globulus* Labillardière hosts three species of Australian psyllids (Brennan 2000). The glaucous juvenile leaves are resistant to *Ctenarytaina spatulata* Taylor and *Glycaspis brimblecombei* Moore, and the glossy adult leaves are resistant to *C. eucalypti* Maskell. The epicuticular waxes present on the juvenile leaves are relatively absent on the glossy adult leaves (Hallam & Chambers 1970; Baker 1982). *Ctenarytaina spatulata* and *G. brimblecombei* survived longer on 'de-waxed' (rubbed) juvenile leaves than on 'waxy' (normal) juvenile leaves (Brennan 2000; Brennan & Weinbaum in press). Furthermore, because these two species settled more on 'de-waxed' than 'waxy' juvenile leaves, Brennan hypothesised that the wax may reduce adhesion of the psyllids to juvenile leaves. The present study tests this hypothesis.

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Our objectives were to assess the role of epicuticular wax on adhesion of *C. eucalypti*, *C. spatulata*, and *G. brimblecombei* to adult and juvenile ('waxy' and 'de-waxed') leaves of *E. globulus*; and to determine if functional and morphological differences in the tarsi of these psyllids may influence adhesion to plant surfaces.

MATERIALS AND METHODS

All experiments were conducted in July and August 1999 under the shade of *E. globulus* trees in El Cerrito, California. The reproductively mature psyllids used in each experiment were collected from wild populations of *C. eucalypti*, *C. spatulata* and *G. brimblecombei* on *Eucalyptus* trees. The psyllids were held in cages for 30 min to 2 h before use.

Experiment 1: Adhesion of the *Ctenarytaina* spp. to juvenile and adult leaf pieces

The objective was to compare adhesion of *C. eucalypti* and *C. spatulata* to the adaxial surfaces of glossy (non-'waxy') adult leaves, 'waxy' (glaucous) juvenile leaves, and 'de-waxed' juvenile leaves. A cotton swab was used to gently remove the wax from the juvenile leaves. Our use of the terms 'waxy' and 'de-waxed' refer only to epicuticular wax. Leaf pieces (4 by 4 mm) were cut from expanding leaves with a razor blade a few minutes before each trial. The two species were evaluated in pairs. Each psyllid was attached individually at the notum of the thorax to a 0.5-mm-wide by 10-mm-long strip of double-stick adhesive tape attached to the tip of a toothpick (Fig. 1). Only female psyllids were used because they are larger and, thus, easier to attach to toothpicks; but preliminary trials indicated that males and females responded similarly to the treatments. To avoid damaging the psyllids while attaching them to the toothpicks, the toothpick with the tape attached was gently pressed onto the notum of the psyllid as it walked on a layer of nylon stocking stretched over the mouth of a Petri dish. To ensure that the psyllid was securely attached to the toothpick, another toothpick was used to attach its wings to the tape. The toothpick and attached psyllid was then attached to a compass (MC-1, Suunto, Espoo, Finland) that contained a clinometer. A leaf piece was placed with its adaxial surface facing down to form a 'bridge' between two plastic supports that were 3.5 mm apart. The psyllid was raised up to the leaf piece from below until its tarsi contacted the centre of the leaf piece and the leaf piece was lifted off the plastic supports. While balancing the leaf piece on its legs, the psyllid was slowly tilted from the upside down position to the left (i.e. in the direction of the psyllid's right legs) at approximately 5–10°s^{-1}. On the clinometer we recorded the angle at which the psyllid either dropped the leaf piece or 'walked' to the edge of it. We also recorded whether the psyllid continued to adhere to the centre of the leaf piece when it was rotated to 180° from its starting position. Pairs of *C. eucalypti* and *C. spatulata* were tested once with three leaf pieces of each of the three leaf treatments, and different psyllids and leaf pieces were used for each replication. The approximate fresh weights of juvenile ('waxy' and 'de-waxed') and adult leaf pieces were 5 mg and 6 mg, respectively. The experiment was replicated eight times between 07.00 and 18.00 hours. The order of the treatments and psyllid species were randomised in each replicate.

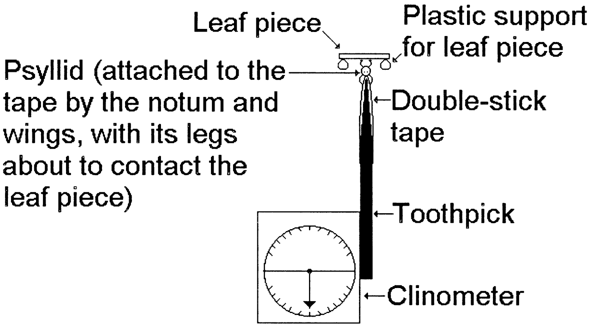

Fig. 1. Apparatus used in experiment 1 to test psyllid adhesion to pieces of juvenile leaves ('waxy' and 'de-waxed') and adult leaves of *Eucalyptus globulus*. The angle of the leaf piece shown here is 0° (psyllid upside down).

For each replicate we compared the adhesion ability of the two species, based on the angle at which the psyllids either dropped the leaf piece or walked to the edge, and the ability of the psyllids to hold the leaf piece at 180°. We rated adhesion in the following declining order: 'held' the leaf piece at 180° > 'walked' to the edge of the leaf piece > 'dropped' the leaf piece. To statistically compare adhesion as a function of leaf type and psyllid species we calculated exact binomial probabilities. Replicates in which both species performed equally well were disregarded in the analysis.

Experiment 2: Climbing ability of the *Ctenarytaina* spp. on 'de-waxed' versus 'waxy' juvenile leaves

The objective was to compare the abilities of *C. eucalypti* and *C. spatulata* to climb 'de-waxed' and 'waxy' regions of the abaxial and adaxial surfaces of vertically inclined, expanded juvenile leaves. A juvenile leaf was attached with either its abaxial or adaxial surface facing outwards to a vertically orientated sheet of clear plexiglas by two 1-cm-wide plastic strips (Fig. 2). A psyllid was released onto the plastic release strip and allowed to climb onto the leaf. We recorded the time when the psyllid stepped off of the release strip onto the 'de-waxed' region of the leaf; the time that it reached the transition line between the 'de-waxed' and 'waxy' leaf surfaces; and the time that it crossed the finish line in the 'waxy' region of the leaf. We also recorded psyllid behaviour (i.e. jumped, fell off leaf, stopped etc.) at the transition line. Each psyllid was observed for up to 2 min, and psyllids that walked to the edge of the leaf before reaching the transition line were excluded. The experiment assessed three levels of climbing ability including an inability to cross the transition line; an ability to cross the transition line but not proceed

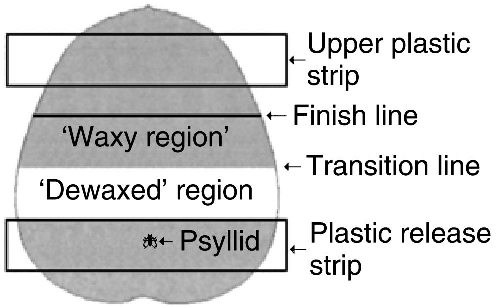

Fig. 2. Diagram of a juvenile leaf (abaxial side facing out) used to test the ability of psyllids to climb on 'de-waxed' and 'waxy' regions of juvenile leaves. The leaf tip is at the top of the diagram.

further; and an ability to cross both the transition and finish lines. One pair of male or female *C. eucalypti* and *C. spatulata* was tested in each replicate and we randomised the order that they were tested. On each leaf surface we replicated the experiment 22 times using three leaves, and 12 pairs of females and 10 pairs of males. Different pairs of psyllids were tested on each leaf surface. Initial analyses showed that both sexes responded similarly on the 'de-waxed' and 'waxy' regions of the leaf so we pooled the data for males and females for each leaf surface. We used chi-squared analysis in SAS (SAS Institute Inc., Cary, NC, USA) to compare the abilities of two species to cross the transition and finish lines on each leaf surface. We calculated the average climbing speed (mm s^{-1}) of the psyllids on the 'de-waxed' and 'waxy' regions and used two-sided paired *t*-tests to compare these speeds within each psyllid species.

Experiment 3: Climbing ability of the *Ctenarytaina* spp. on glossy versus 'waxed' adult leaves

The objective was to compare the abilities of *C. eucalypti* and *C. spatulata* to climb glossy versus artificially 'waxed' regions of the adaxial surfaces of vertically inclined, expanded adult leaves. Adult leaves were 'waxed' by gently pressing two 2-cm-wide strips of juvenile leaf onto the adaxial surface of an adult leaf. The upper 1 cm of the juvenile leaf strip contained the normally 'waxy' surface and the lower 1 cm of the leaf was 'de-waxed' prior to pressing. This resulted in a 1-cm upper strip of juvenile wax on the adult leaf and a lower 1 cm naturally glossy strip. The climbing ability of psyllids was evaluated as in experiment 2. The experiment was replicated 38 times on three leaves, with equal pairs of males and females. Analysis of results was as for experiment 2.

Experiment 4: Climbing ability of *G. brimblecombei* on the 'de-waxed' versus 'waxy', juvenile leaves

The objective was to determine if *G. brimblecombei* could climb up 'de-waxed' and 'waxy' regions of the abaxial and adaxial surfaces of vertically inclined, expanded juvenile leaves. This experiment was otherwise identical to experiment 2 and included 12 replicates of equal numbers of males and females on two leaves on both leaf surfaces.

Experiment 5: Climbing ability of *G. brimblecombei* on the glossy versus 'waxed' adult leaves

The objective was to determine if *G. brimblecombei* could climb up glossy and artificially 'waxed' regions of the adaxial surface of vertically inclined, expanded adult leaves. This experiment was identical to experiment 3 but it evaluated climbing ability of *G. brimblecombei* and included 12 replicates with equal numbers of males and females on two leaves.

Tarsal morphology and function

To compare the functional morphology of tarsi of the three species we examined the tarsi using light microscopy. We observed the tarsi at low magnifications (×10–30) as the psyllids climbed up the sides of a clear vial cage and upside down onto a microscope slide lid. For observations at higher magnifications (×40–1000) live psyllids were attached by their dorsal thorax to pieces of double-stick tape on microscope slides and glass cover slips were gently rested on the legs of the psyllids. We then observed the tarsi adhering to the cover slips under a compound microscope. We measured the length and width of the circular or oval-shaped pulvilli (i.e. adhesive pads covered in setae) on the tarsi of three forelegs, midlegs and hindlegs for three males and females of each species. To facilitate the taking of these measurements a few drops of 70% ethanol were added under the cover slip to kill the psyllids while their tarsi remained attached to the cover slip. Using these measurements we calculated the approximate total pulvillar area of males and females of each psyllid species. To obtain an average live body weight we weighed three males and females of each species.

To further assess adhesion and walking and climbing behaviour, we observed the 'foot prints' that psyllids left on microscope slides coated with a thin layer of soot. One side of each microscope slide was coated in soot by passing it several times through a candle flame. When a psyllid began walking on a sooty slide we slowly tilted the slide vertically until the psyllid fell off. The 'foot prints' on the sooty slide indicated the number of pairs of legs used in walking and climbing, the parts of the tarsi contacting the substrate, and the amount of exfoliation (casting off) of soot that occurred during walking or climbing.

RESULTS

Experiment 1: Adhesion of the *Ctenarytaina* spp. to juvenile and adult leaf pieces

The two *Ctenarytaina* species adhered equally well to the adult ($P = 0.13$) and the 'de-waxed' juvenile leaves ($P = 0.13$). *Ctenarytaina eucalypti*, however, adhered better than

C. spatulata to the 'waxy' juvenile leaves ($P \le 0.01$) (Table 1). The majority of psyllids of both species 'walked' to the edge of the pieces of adult and 'de-waxed' juvenile leaves as the angle of inclination increased. In contrast, the majority of the psyllids dropped the pieces of 'waxy' juvenile leaves as the angle of inclination increased. Across all leaf treatments *C. eucalypti* adhered to the leaf pieces at consistently higher inclination angles than *C. spatulata*.

Experiment 2: Climbing ability of the *Ctenarytaina* spp. on 'de-waxed' versus 'waxy' juvenile leaves

Ctenarytaina eucalypti and *C. spatulata* differed significantly in their abilities to climb from the 'de-waxed' to 'waxy' regions on adaxial ($\chi^2 = 36.7$; d.f. = 1; $P \le 0.001$) and abaxial ($\chi^2 = 27.1$; d.f. = 1; $P \le 0.001$) surfaces of juvenile leaves (Table 2). Similarly there were significant differences in the abilities of *C. eucalypti* and *C. spatulata* to cross the finish line in the 'waxy' region on the adaxial ($\chi^2 = 33.4$; d.f. = 1; $P \le 0.001$) and abaxial leaf surfaces ($\chi^2 = 30.5$; d.f. = 1; $P \le 0.001$; Table 2). On both leaf surfaces the majority of *C. eucalypti* crossed the transition line (i.e. from the 'de-waxed' to 'waxy' region) and also crossed the finish line. In contrast none of the *C. spatulata* crossed the transition line on adaxial leaf surfaces and only a few did so on abaxial surfaces. On abaxial surfaces the few *C. spatulata* that crossed the transition and finish lines climbed mainly on the midrib that protruded approximately 0.5–1 mm above the leaf surface. The average climbing speed of *C. eucalypti* was significantly greater on 'de-waxed' than on the 'waxy' regions of the adaxial ($t = 16.7$; d.f. = 16; $P < 0.001$) and abaxial ($t = 6.6$; d.f. = 21; $P < 0.001$) juvenile leaf surfaces. The average climbing speed of *C. spatulata* was significantly greater on 'de-waxed' than on 'waxy' regions of abaxial juvenile leaf surfaces ($t = 5.2$; d.f. = 2; $P < 0.05$).

Experiment 3: Climbing ability of the *Ctenarytaina* spp. on glossy versus 'waxed' adult leaves

Ctenarytaina eucalypti and *C. spatulata* did not differ significantly in their abilities to climb across the transition line of the adult leaves ($\chi^2 = 3.1$; d.f. = 1; $P = 0.08$). *Ctenarytaina eucalypti*, however, crossed the finish line significantly more often than *C. spatulata* ($\chi^2 = 23.6$; d.f. = 1; $P \le 0.001$) (Table 3). All of the *C. eucalypti* crossed the transition and finish lines on the adult leaves. In contrast, although the majority of the *C. spatulata* crossed the transition line, approximately half of them fell off the 'waxed' region before reaching the finish line. The average climbing speed was significantly greater on glossy than on waxed regions for *C. eucalypti* ($t = 6.9$; d.f. = 37; $P < 0.001$) and *C. spatulata* ($t = 8.4$; d.f. = 19; $P < 0.001$).

Table 1 Behaviour of the *Ctenarytaina* species with square pieces of juvenile and adult leaves of *Eucalyptus globulus*

Psyllid behaviour	*C. eucalypti*			*C. spatulata*		
	'Waxy' juvenile leaves	'De-waxed' juvenile leaves	Adult leaves	'Waxy' juvenile leaves	'De-waxed' juvenile leaves	Adult leaves
Dropped leaf piece	83%	4%	0%	100%	12.5%	12.5%
	(59 ± 7°)	(75 ± 0°)	(–)	(34 ± 3°)	(62 ± 8°)	(47 ± 12°)
'Walked' to edge of leaf piece	17%	71%	79%	0%	75%	87.5%
	(62 ± 9°)	(78 ± 5°)	(89 ± 14°)	(–)	(64 ± 5°)	(70 ± 9°)
Held leaf piece at 180°	0%	25%	21%	0%	12.5%	0%

Percentages represent the proportion of replicates in which psyllids either dropped the leaf piece, 'walked' to the edge of the piece, or held onto the middle of the piece as it was rotated from 0° (psyllid upside down) to 180° (psyllid right side up). Numbers in parentheses are the mean angle (± SE) at which the psyllids either dropped the leaf piece or 'walked' to the edge of the leaf piece.

Table 2 Climbing ability of the *Ctenarytaina* species on 'de-waxed' and 'waxy' regions of vertically inclined juvenile leaves of *Eucalyptus globulus*

Psyllid behaviour	Adaxial leaf surface		Abaxial leaf surface	
	C. eucalypti	*C. spatulata*	*C. eucalypti*	*C. spatulata*
Crossed transition line	91%	0%	100%	27%
Crossed finish line	86%	0%	100%	18%
Climbing speed on 'de-waxed' region (mm s^{-1})*	4.0 ± 0.3	3.2 ± 0.4	3.1 ± 0.3	2.0 ± 0.2
	$n = 17$	$n = 22$	$n = 22$	$n = 21$
Climbing speed on 'waxy' region (mm s^{-1})*	1.5 ± 0.2	NA	2.0 ± 0.2	1.2 ± 0.3
	$n = 17$		$n = 22$	$n = 3$

*Climbing speeds are means ± SE; NA, not applicable.

Experiments 4 and 5: Climbing ability of *G. brimblecombei* on 'de-waxed' and 'waxy' juvenile leaves, and glossy versus 'waxed' adult leaves

The waxiness of the juvenile and adult leaves affected the climbing ability of *G. brimblecombei* (Table 4). It was unable to cross the transition line on the adaxial surfaces of juvenile leaves. In contrast, approximately half of the *G. brimblecombei* crossed the transition line and most of these eventually crossed the finish line on the abaxial surfaces of juvenile leaves and the adaxial surfaces of adult leaves. *Glycaspis brimblecombei* usually climbed on the protruding leaf midrib on 'waxy' regions of the abaxial surfaces of juvenile leaves. The climbing speeds on 'de-waxed' regions of juvenile leaves were significantly greater than the speed on 'waxy' regions ($t = 3.1$; d.f. = 4; $P < 0.05$). The climbing speed was also significantly greater on the glossy than on 'waxed' regions of adult leaves ($t = 2.9$; d.f. = 5; $P < 0.05$).

Table 3 Climbing ability of the *Ctenarytaina* species on glossy and 'waxed' regions of the adaxial surface of adult leaves of *Eucalyptus globulus*

Psyllid behaviour	*C. eucalypti*	*C. spatulata*
Crossed transition line	100%	92%
Crossed finish line	100%	53%
Climbing speed on glossy region (mm s^{-1})*	4.4 ± 0.4 *n* = 38	3.4 ± 0.3 *n* = 38
Climbing speed on 'waxed' region (mm s^{-1})*	3.2 ± 0.2 *n* = 38	1.1 ± 0.3 *n* = 20

*Climbing speeds are means ± SE.

Tarsal morphology and function

The tarsi of the three psyllid species differed morphologically and functionally (Fig. 3; Table 5). *Ctenarytaina eucalypti* walked and climbed with all legs adhering to the substrate (i.e. leaves, clean glass and sooty glass). In contrast *C. spatulata* and *G. brimblecombei* generally walked and climbed using only the front two pairs of legs, and appeared to drag the hind pair of legs. *Ctenarytaina eucalypti* and *G. brimblecombei* walked and climbed with the basal tarsomeres in contact with the substrate while the apical tarsomeres (that bear the tarsal claws) oscillated or vibrated in the air. In contrast *C. spatulata* walked and climbed with both the basal and apical tarsomeres in contact with the substrate. The basal tarsomeres of all three species contained pulvilli covered in setae that appeared to be adhesive. The total pulvillar area of all legs used in walking by *C. eucalypti* and *G. brimblecombei* were similar, whereas the total pulvillar area of *C. spatulata* was several times smaller (Table 5). Relative to bodyweight, *C. eucalypti* had a total pulvillar area several times greater than that of the other two species.

All three species adhered to and walked upside down on clean microscope slides. *Ctenarytaina eucalypti* also adhered well to and walked normally on horizontal and vertically orientated sooty slides. In contrast *C. spatulata* and *G. brimblecombei* walked with great difficulty on the horizontal sooty slides. There were clear differences in the 'foot prints' of the

Table 4 Climbing ability of *Glycaspis brimblecombei* on 'de-waxed' and 'waxy' regions of juvenile leaves, and glossy and 'waxed' regions of adult leaves of *Eucalyptus globulus*

Psyllid behaviour	Experiment 4		Experiment 5
	Adaxial juvenile leaf surface	Abaxial juvenile leaf surface	Adaxial adult leaf surface
Crossed transition line	0%	42%	67%
Crossed finish line	0%	42%	50%
Climbing speed on 'de-waxed' juvenile and glossy adult leaf regions (mm s^{-1})*	14.3 ± 2.7 *n* = 12	5.4 ± 1.3 *n* = 12	9.3 ± 1.3 *n* = 12
Climbing speed in 'waxy' juvenile and 'waxed' adult leaf regions (mm s^{-1})*	NA	1.4 ± 0.4 *n* = 5	3.8 ± 1.5 *n* = 6

*Climbing speeds are means ± SE; NA, not applicable.

Table 5 Pulvillar charactersistics in relation to individual body weights of *Ctenarytaina eucalypti* that colonises 'waxy' (glaucous) juvenile shoots, and *C. spatula* and *Glycaspis brimblecombei* that colonise glossy adult shoots of *Eucalyptus globulus*

Characteristic	*C. eucalypti*		*C. spatulata*		*G. brimblecombei*	
	Males	Females	Males	Females	Males	Females
Pairs of legs with pulvilli	3	3	2	2	2	2
Pulvillar area per psyllid ($mm^2 \times 10^{-3}$)	15	20	2	2	13	13
Mean live body weight (μg ± SE)	146 ± 2	286 ± 9	163 ± 4	314 ± 13	566 ± 55	922 ± 14
Pulvillar area/body weight ($mm^2\ g^{-1}$)	103	70	12	6	23	14

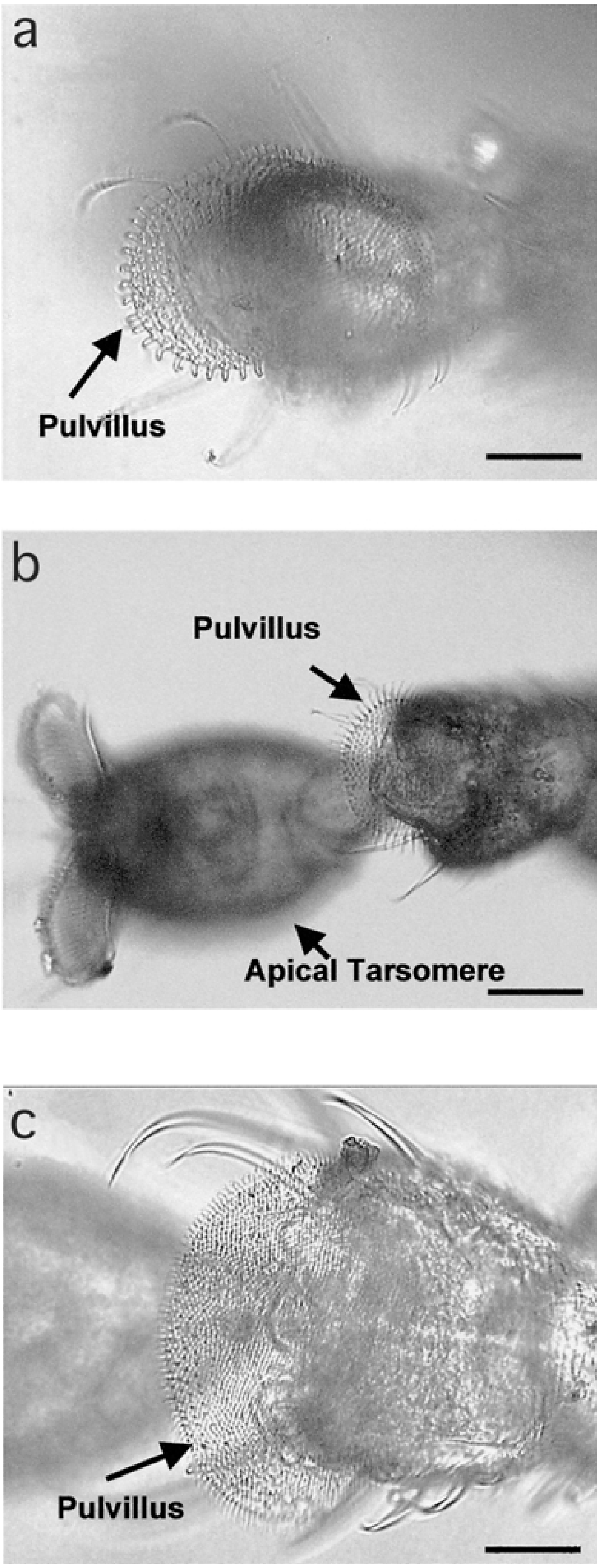

Fig. 3. Photomicrographs of the pulvilli on the basal tarsomeres of the middle legs of females of **(a)** *Ctenarytaina eucalypti*, **(b)** *C. spatulata* and **(c)** *Glycaspis brimblecombei*. Bar = 20 μm

psyllids on the sooty slides. *Ctenarytaina eucalypti* left distinct, circular to oval-shaped indentations where the pulvilli touched the soot, and seldom scratched soot off of the glass. In contrast *C. spatulata* and *G. brimblecombei* slipped while trying to walk on the sooty surface, scratched off large amounts of the soot and rarely left pulvillar indentations.

DISCUSSION

The present study demonstrated that juvenile epicuticular leaf wax reduced adhesion of all three psyllid species, and that *C. eucalypti* adhered better than *C. spatulata* and *G. brimblecombei* to the 'waxy' juvenile leaves. These results are consistent with previous findings (Brennan 2000; Brennan & Weinbaum in press) that *C. spatulata* and *G. brimblecombei* colonised only the glossy adult shoots, oviposited primarily on adult shoots, and survived longer and initiated more stylet tracks on adult and 'de-waxed' juvenile leaves than on 'waxy' juvenile leaves. These findings support the hypothesis of Eigenbrode and Espelie (1995) that ovipositional preferences for glossy plants may be related in part to the ability of insects to adhere to such plants.

The morphological and functional differences in the tarsi of the three psyllid species may help explain their differing abilities to adhere to the glaucous juvenile leaves of *E. globulus*. The mechanisms of adhesion by insects and vertebrates to smooth surfaces may involve cohesive forces between adhesive setae (like those we observed on the three psyllid species) and the substrate (Ruibal & Ernst 1965; Nachtigall 1974; Stork 1980a, 1983; Ishii 1987). We are unaware of studies on the mechanisms of insect adhesion to 'waxy' surfaces but we speculate that they are similar to those for a smooth surface because *C. eucalypti* used the same adhesive regions of the tarsi to adhere to smooth (i.e. glass) and slippery surfaces (i.e. 'waxy' leaf or sooty glass). Although all three psyllid species had pulvilli on their tarsi, *C. eucalypti* had eight and 11 times more total pulvillar area per unit of body weight than did *C. spatulata*, and four and five times more total pulvillar area than did *G. brimblecombei*, for males and females, respectively. These differences may explain the greater adhesion of *C. eucalypti* on 'waxy' juvenile leaves and sooty glass. This assumes that the three species have an equal density of adhesive setae per area of pulvilli and, if so, the contact area (and hence cohesion force) between the tarsi of *C. eucalypti* and the substrate would be several times greater than that of *C. spatulata* or *G. brimblecombei*. To our knowledge *C. eucalypti* is one of the first reported examples of an herbivorous insect with tarsi that are adapted to adhere to and walk on a flat 'waxy' leaf surface. The morphology and function of psyllid tarsi have received little attention (Carver 1987).

Adhesion by *C. spatulata* and *G. brimblecombei* to 'waxy' surfaces apparently depended upon whether the psyllids could clasp the leaf. Both species were able to adhere to the 'waxy' juvenile abaxial leaf surfaces only by clinging to protruding leaf veins (experiments 2 and 4). Similarly, Shah

(1982) found that coccinellid larvae moved effectively only on protruding leaf veins and leaf edges of glaucous brassicas. Both *C. spatulata* and *G. brimblecombei* were able to climb on the edges of 'waxy' juvenile leaves. Edge-walking may allow chewing insects to feed on 'waxy' hosts (Bodnaryk 1992a,b) but this is unlikely with psyllids and other Homoptera that generally feed from veins away from the leaf edge. Furthermore, even if *C. spatulata* or *G. brimblecombei* adults could survive on leaf edges, we question whether their navigational precision would enable them to land there.

Adhesion of insects and vertebrates to smooth surfaces has interested biologists for centuries (Blackwall 1833; Stork 1983) but, to our knowledge, our study is the first to assess adhesion by homopterans. Due to the small size and jumping ability of psyllids many previously described methods for measuring adhesion were impractical. For example, rather than measuring the angle at which an insect fell off an increasingly angled surface (Gillet & Wigglesworth 1932; Edwards & Tarkanian 1970; Stork 1980b), we measured the angle at which a leaf piece fell off of the psyllid. Although this method does not measure the force required to detach a psyllid from a surface (Stork 1980a; Walker *et al.* 1985; Eigenbrode *et al.* 1999), it shows that both *C. eucalypti* and *C. spatulata* supported up to 20 times their bodyweight while adhering to the glossy surface of adult leaves, and *C. eucalypti* supported up to 15 times its body weight on the 'de-waxed' juvenile leaf. Similarly, Ishii (1987) showed that a ladybird beetle supported a load that was approximately five times its bodyweight.

Studies on the effects of epicuticular wax on insects typically compare insect responses to glossy and glaucous plants (Stork 1980b), or on 'de-waxed' versus 'waxy' regions of glaucous surfaces (i.e. Bodnaryk 1992b; Blua *et al.* 1995; Federle *et al.* 1997). To our knowledge the present study is the first to transfer wax from glaucous to glossy leaf surfaces and in doing so increase leaf slipperiness and reduce insect adhesion. Although transferring the wax may have altered its properties, the fact that the transferred wax gave the normally glossy adult leaves a glaucous bloom like that of the normal juvenile leaves suggests that the wax's physical structure was somewhat maintained during the transfer. Other workers (Knoll 1914, cited in Eigenbrode 1996) increased the slipperiness of a surface by applying various crystalline compounds. Wax crystals may reduce insect adhesion by exfoliating as the insect walks on the 'waxy' surface (Juniper & Burras 1962; Eigenbrode 1996). Our novel sooty slide method provides evidence that *C. eucalypti* may be able to adhere to a slippery surface by depressing and adhering to surface deposits and minimising exfoliation. In contrast, *C. spatulata* and *G. brimblecombei* appeared to slip on such surfaces due to their inability to adhere to the soot without scratching it off the surface.

We speculate that leaf epicuticular waxes may mediate interactions with numerous other insects associated with *Eucalyptus* in its native range and as an exotic, especially considering the biodiversity of the approximately 700 *Eucalyptus* (Williams & Brooker 1997) and associated herbivores in Australia (i.e. > 150 species of psyllids (Majer *et al.* 1997)). For example it is likely that the parasitoid (*Psyllaephagus pilosus* Noyes) of *C. eucalypti* (Dahlsten *et al.* 1998) has specialised tarsi that adhere well to the juvenile leaves of *E. globulus*, but the ants and ladybird beetles that we have observed associated with *C. spatulata* only on the adult leaves of *E. globulus* may lack these adaptations.

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