

Abiotically-Induced Plant Morphological Changes and Host-Range Expansion in Quarantine Evaluations of Candidate Weed Biocontrol Agents: The Case Study *Conchyloctenia hybrida* (Coleoptera: Chrysomelidae)

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ABSTRACT Plant morphological changes mediated by growth conditions are linked to changes in host preference of herbivores. Understanding how these morphological changes influence herbivore feeding is critical in the interpretation of results of host evaluation of candidate weed biocontrol agents in quarantine and improvement of the evaluation system. We determined the effect of plant growth conditions on leaf trichomes and host choice of *Conchyloctenia hybrida* Boheman, an insect adapted to the removal of trichomes before feeding. The study included four *Solanum* species: *Solanum lichtensteinii* Willdenow (natural host of *C. hybrida*), *Solanum mauritianum* Scopoli, *Solanum melongena* L., and *Solanum tuberosum* L. Plants were grown in either full sun, shade, a glasshouse, or in a growth-chamber. Plants grown in full sun had a higher leaf trichome density than those in shade or controlled environments. *S. mauritianum* had the highest trichome density and thickness of trichome layer. In a multiple-choice test using excised leaves, feeding by *C. hybrida* was higher on *Solanum* plants grown in the controlled environment as compared with full sun. Trichome removal from leaf surfaces of plants grown in full sun, using adhesive tape, was effective for *S. lichtensteinii*, *S. mauritianum*, and *S. melongena*, but not *S. tuberosum*. Leaf consumption by *C. hybrida* increased significantly where manual trichome removal using adhesive tape was effective. Structurally, leaves of *S. tuberosum* have simple trichomes with basal cells sunken into the mesophyll tissue. When using forceps to remove trichomes of *S. tuberosum*, mesophyll and vascular tissue remained attached to the trichomes. Generally, the type, density, and mat-thickness of leaf trichomes determined feeding by *C. hybrida*, but varied with plant species and growth conditions.

KEY WORDS growth condition, plant–herbivore interaction, *Solanum* species, trichome, tortoise beetle

Traditionally, host specificity tests of weed biocontrol agents are undertaken under laboratory conditions (Barratt et al. 2010). This approach is aimed at first determining the fundamental host range (absolute limits of a species' host range) of potential agents and then predicting how that potential will be expressed in a given target release area (realized host range; van Klinken 2000, Sheppard et al. 2005). Fundamental host range is genetically determined and can be estimated in well-designed no-choice tests (van Klinken 2000). The realized host range depends on factors including host preference and performance, time-dependent effects, spatial and temporal coincidence of herbivores and hosts, and relative availability of hosts (Courtney

and Kibota 1990, van Klinken 2000). The preference ranking of hosts can be determined in multiple-choice tests (Sheppard et al. 2005).

Laboratory choice and no-choice tests have often been reported to overestimate (false positives) and in some instances to underestimate (false negatives) field host range (Olckers et al. 1995, Heard 2000, Haines et al. 2004, Sheppard et al. 2005). The main focus in understanding the underlying cause for such ambiguous results has been on insect behavioral aspects such as disruption of host perception owing to captivity, motivational status, prior experience, and learning (Blossey et al. 1994, Olckers et al. 1995, Heard 2000, Withers et al. 2000, Sheppard et al. 2005), whereas only a few studies have focused on the aspect of changes in plant quality owing to the growth conditions (van Klinken 1999, Diaz et al. 2011) in environmental chambers, glasshouses or shade nets where test plants are grown (Olckers and Borea 2009, Hakizimana and Olckers 2013).

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Table 1. Soil chemical properties (mean \pm SEM) of full sun and shade field trial sites

Field sites	pH _(H₂O)	EC _e (mS m ⁻¹)	P (mg kg ⁻¹)	Ca (mg kg ⁻¹)	K (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Na (mg kg ⁻¹)	C (%)
Full sun	6.0 \pm 0.25	21.0 \pm 6.0	5.5 \pm 2.65	1,087.5 \pm 260.50	275.5 \pm 17.50	258.5 \pm 48.50	14.5 \pm 2.50	1.6 \pm 0.05
Shade	6.3 \pm 0.05	19.0 \pm 5.0	5.8 \pm 4.25	1,278.0 \pm 332.00	217.0 \pm 20.00	291.5 \pm 51.50	17.0 \pm 1.00	1.5 \pm 0.46
<i>t</i>	1.18	0.26	0.06	0.45	2.20	0.47	0.93	0.2
<i>P</i>	0.36	0.82	0.96	0.69	0.16	0.69	0.45	0.86

Plant-herbivore interactions are affected by morphological and chemical qualities of plants (Baraza et al. 2007), which in turn are affected by abiotic factors such as light intensity (Roberts and Paul 2006). Previous studies suggest that herbivory tends to be higher on plants grown in shade compared with conspecific plants grown in full sun (Maiorana 1981, Guerra et al. 2010). This hypothesis, referred to as the shade habitat hypothesis (Louda et al. 1987), has been supported by several studies. Muth et al. (2008) found higher herbivory by the lepidopteran herbivore *Epimecis hortaria* (F.) (Lepidoptera: Geometridae) on shaded *Lindera benzoin* L. (Lauraceae) plants as compared with plants growing in the full sun. Similar results were reported for other insect herbivores, including the larva of a *Desmia* sp. (Lepidoptera: Pyralidae) on *Psychotria horizontalis* Swartz (Rubiaceae) (Sagers 1992).

The "shade habitat hypothesis" has been explained in terms of changes in plant attributes such as increased nitrogen content, which enhance the nutritive value of the plant (Iason and Hester 1993, Moon et al. 2000), decreased content of mainly phenolics that act as secondary metabolites used for defense (Newbery and Foresta 1985, Bryant et al. 1987, Iason and Hester 1993, Dudt and Shure 1994), and increased palatability by reducing tissue toughness (Bryant et al. 1987, Sagers 1992, Guerra et al. 2010). Less attention has been given to morphological traits such as trichome density, and results often were not definitive (Bentz 2003, Guerra et al. 2010). Some studies indicated a reduction in leaf trichome density under shade conditions (Young and Smith 1980, Pérez-Estrada et al. 2000, McGuire and Agrawal 2005, Diaz et al. 2011), whereas others found an increase in density (Lill et al. 2006). Furthermore, the effect of trichomes on herbivores may be positive, negative, or nonexistent depending on the plant or herbivore species (Southwood 1986, Moran 1986).

The majority of studies on leaf trichomes and insect behavior have focused on the roles of trichome density, shape, length, and orientation (Levin 1973, Cardoso 2008, Guerra et al. 2010). These aspects may have limited impact on those herbivores adapted to trichome removal as a prerequisite for feeding, such as *Conchyloctenia* species feeding on some *Solanum* species (Olckers and Hulley 1989, Ghebremariam et al. 2014).

The genus *Conchyloctenia* belongs to the subfamily Cassidinae (Borowiec 1994), also known as tortoise beetles because of the characteristic shape of the adults. They are characterized by a narrow host range (Jolivet et al. 1988) and voracious feeding by both

the larval and adult stages (Paterson 1941, Chaboo 2007). These beetles exhibit various defense adaptive mechanisms such as eggs enclosed in papery cases (Paterson 1941, Jolivet et al. 1988); camouflage by carrying a caudal shield made of exuviae, feces, and trichomes in the larval stages; and the ability to change color in the adults (Paterson 1941, Siebert 1975, Jolivet et al. 1988, Chaboo 2007). *Conchyloctenia hybrida* Boheman is native to tropical and subtropical Africa, including South Africa, and feeds on two indigenous *Solanum* species, *Solanum lichtensteinii* Willdenow (previously confused with *Solanum incanum* L.) and *Solanum campylacanthum* Hochstetter subsp. *panduriforme* (Paterson 1941, Jaeger 1985, Borowiec 1994, Samuels 2012). Larvae and adults of *C. hybrida* selectively feed on the interveinal zones of leaves after removal of the trichomes from those areas (Ghebremariam et al. 2014).

For trichome-removing species such as *C. hybrida*, trichome density and the ease with which trichomes can be removed may influence the level of herbivory. The aim of the current study was to compare trichome density and morphology of four *Solanum* species grown under different conditions, and to assess the effect of leaf trichomes on herbivory by *C. hybrida*.

Materials and Methods

Plants and Insects. Seeds of *S. lichtensteinii* (bitter apple) and *Solanum mauritianum* Scopoli (woolly night-shade) were collected from plants growing in the wild around Pretoria (25° 45' S; 28° 14' E), whereas *Solanum melongena* L. (eggplant) 'Black Beauty' was purchased from Hygrotech Seed (Pty) Ltd, Pretoria, South Africa. Seeds of these three species were sown into seedling trays and kept in a glasshouse until ready for transplanting in the field. A field trial was set up at the Experimental Farm of the University of Pretoria (25° 74' S; 28° 25' E) in the summer of 2007–2008, and was repeated in 2008–2009. Two full-sun and two shade sites were selected from which soil samples were taken and analyzed for nutrient content (P, Ca, K, Mg, Na, and C), salinity, pH, and texture. The soils from the four sites were all sandy-clay-loam, and no significant difference in nutrient content, salinity, and pH was found between the full-sun and shade sites (Table 1). At each site, 40 6-wk-old seedlings of the aforementioned three species and 40 *Solanum tuberosum* L. (potato; 'BPI') tubers purchased from the National Potato Coop (Pty) Ltd. (Bethal, South Africa) were planted. Plants were mainly rainfed (average total rainfall was 780 mm during the growth period), but also irrigated with sprinklers during dry periods. In

addition, a pot trial was done to study leaf trichomes and their effect on herbivory by *C. hybrida* under controlled and full-sun conditions. In the pot trial, 40 seedlings of *S. lichtensteinii*, *S. mauritanum*, and *S. melongena* and 40 *S. tuberosum* tubers were transplanted individually into 5-liter pots filled with a mixture of quartz sand and coir. The pots were divided randomly into four groups of 10 plants each, and groups were randomly allocated to four sites representing different growing conditions: 1) full sun and ambient temperature (14 to 28°C [minimum and maximum]; 40–80% relative humidity [RH]), 2) glasshouse with daily temperatures ranging from 22 to 33°C (minimum and maximum) and 60–70% RH, 3) shadehouse (40% black shade net) with daily temperatures ranging from 15 to 38°C and 25–80% RH, and 4) a growth chamber set at a constant 28°C, a photoperiod of 14:10 (L:D) h and average photosynthetic photon flux density of 550 $\mu\text{mol m}^{-2}\text{s}^{-1}$, and 40–60% RH.

Plants in the pot trials were irrigated once a week and fertigated 3 d a week, to field capacity, with a standard nutrient solution prepared by mixing the products Solu-Cal (composed of 11.7% N and 16.6% Ca) and Hygroponic (6.8% N, 4.2% P, 20.8% K, and 3% Mo). Both products were purchased from Hygrotech SA (Pty) Ltd.

Trichome counts were done on plants from the field trial, and qualitative comparisons of trichome density were done on pot trial plants to compare between plants grown in full sun and controlled environments. Feeding experiments were carried out on pot trial plants. Another batch of 25 potted plants of each of the four species that were grown in full sun was used for the leaf trichome removal and feeding trials. The plant species used in the current study were identified by W.G. Welman, South African National Biodiversity Institute, Pretoria.

C. hybrida was cultured from adults and immatures collected in the field around Donkerhoek, Gauteng, South Africa (25° 52' S; 28° 31' E) from *S. lichtensteinii* and a few individuals from *S. campylacanthum* subsp. *panduriforme*. Adults were sexed according to size. Females generally are larger than males (Ghebremariam et al. 2014), as is common among the Cassidinae (Siebert 1975). Gender was confirmed after observing copulation and individuals were randomly allocated to groups consisting of three females and three males per group to obtain eggs. Each group was placed on *S. campylacanthum* subsp. *panduriforme* leaves in plastic gauze-covered jars (7 cm in diameter, 12 cm in height). Field-collected egg batches and pupae were incubated on moist filter paper in petri dishes (9 cm in diameter), which were also used to rear larvae. Insects were kept in rearing rooms under natural light and ambient room temperature ranging from 23 to 29°C, 40–50% RH, and were regularly observed for oviposition. Adult *C. hybrida* were identified by E. Grobbelaar and voucher specimens were deposited in the South African National Collection of Insects of the Agricultural Research Council–Plant

Protection Research Institute, Pretoria, South Africa (accession number: SANC COLL 00115).

Host Choice Test. A multiple-choice trial was carried out in a glass cage (50 by 50 by 30 cm) with a total of 16 excised leaves from the four *Solanum* species (used for trichome study) grown in the full sun, glasshouse, shadehouse, and growth chamber. The youngest fully expanded leaves were excised and inserted in moist rockwool randomly arranged in a circular pattern, with 16 adult *C. hybrida* released in the center. This was repeated four times, and feeding was recorded 48 h postrelease, whereupon beetles were removed and leaves were scanned, photographed, and pictures saved as bitmap images. The leaf area consumed was measured using the software package “Compu Eye, Leaf and Symptom Area” (Bakr 2005).

Leaf Trichome Counts. At flowering stage, mature and fully expanded leaves were selected for analysis from four plants of each of the four *Solanum* spp. grown under the different trial conditions. Leaf segments measuring 5 by 5 mm were excised from the interveinal area in the center of the lamina, fixed for 2 h in 2.5% gluteraldehyde and postfixed for 1 h in 0.25% aqueous osmium tetroxide, both buffered with 0.075 M sodium phosphate (pH 7.4). Samples were rinsed with distilled water and dehydrated in a graded ethanol series. The dehydrated specimens were dried to critical point in liquid CO₂ and sputter-coated with RuO₄ (Van der Merwe and Peacock 1999). Specimens were examined with a Jeol/JSM-840 scanning electron microscope (Jeol, Tokyo, Japan), and the number of nonglandular trichomes in a 0.9 by 1.1 mm surface area was counted.

Trichome Removal and Insect Feeding. The effect of trichome removal on insect feeding behavior was determined for 10 potted plants each of *S. lichtensteinii*, *S. mauritanum*, *S. melongena*, and *S. tuberosum* grown in full sun. The youngest fully expanded leaf was selected from half of each leaf, either the left or right side of the midrib, trichomes were removed by placing a piece of clear adhesive tape (Eurocel, South Africa) over the abaxial and adaxial surfaces of intact leaves and then gently removing it (Siebert 2004). An adult *C. hybrida* female was subsequently placed on the midrib of each dehaired leaf section, after which the leaf was enclosed in a sleeve cage made of a nylon cloth. After 24 h, the beetles were removed and the leaf area consumed was measured using a method similar to that described for the host-choice trial.

Trichomes Removed by Adhesive Tape. Four young, fully expanded leaves were sampled from the potted plants of the four *Solanum* species grown in full sun used for the trichome removal and feeding trial. An area of 3 by 3 mm was marked with a razor blade and the number of trichomes in the marked area was counted under a stereomicroscope. Trichomes were removed from the leaf using the adhesive tape method of Siebert (2004) described earlier and counted again to estimate the number of trichomes removed. The pieces of tape used during the experiment were then examined under a light microscope to check for de-

Table 2. Leaf area (mm²) consumed (mean \pm SEM) by adults of *C. hybrida* in a multiple-choice test using excised leaves of *Solanum* species grown in pots under full sun, shade net, glasshouse, and growth chamber

<i>Solanum</i> spp.	Sun	Shade net	Glasshouse	Growth chamber
<i>S. mauritianum</i>	0.00e	20.07 \pm 1.65abc	5.10 \pm 0.41cde	19.05 \pm 1.27abc
<i>S. lichtensteinii</i>	11.27 \pm 1.12de	37.47 \pm 2.89ab	55.35 \pm 4.60abc	5.42 \pm 0.31cde
<i>S. melongena</i>	44.40 \pm 2.90ab	46.17 \pm 2.63abc	75.95 \pm 2.93ab	62.57 \pm 1.29a
<i>S. tuberosum</i>	0.00e	0.00e	0.00e	0.00e

Means with the same letters do not differ significantly ($P < 0.0001$, Kruskal-Wallis ANOVA, followed by nonparametric LSD).

tached trichomes and to determine whether other epidermal tissue was removed from the leaves as well.

The tape technique was effective in removing trichomes from the leaves of all the *Solanum* species except *S. tuberosum*. Therefore, a further study was done on the anatomy of the trichomes of *S. tuberosum* by making free-hand sections of fresh leaf segments and mounting them in glycerin on a microscope slide and examining them under a light microscope.

A new batch of 15 singly potted *S. tuberosum* plants that were grown in full sun were used for further investigation of trichome removal. One leaflet randomly selected from the youngest fully expanded leaf was used carefully to remove the trichomes from half of the leaflet with a pair of fine-tipped forceps under a stereomicroscope. A single adult *C. hybrida* female was then placed on the midrib of each of the treated leaflets, after which each leaflet was enclosed in a sleeve cage. Leaf damage was measured after 24 h.

Data Analysis. Data were tested for normality and homogeneity of variances. Differences in trichome density among *Solanum* species as well as insect feeding after trichome removal using adhesive tape were analyzed using analysis of variance (ANOVA). Means were separated using Fisher's protected least significant difference (LSD) test. Soil characteristics of full-sun and shade sites and trichome density of plants grown in full sun and in shade of individual species were compared using Student's *t*-test. Data on host choice did not meet the assumption of normal distribution for ANOVA. Therefore, data were rank-transformed and analyzed using Kruskal-Wallis ANOVA (Conover 1999) followed by nonparametric LSD test for mean separation (Conover 1999) using PROC RANK and PROC GLM. Trichome counts before and after the adhesive tape treatments were compared using paired *t*-tests. Leaf damage by *C. hybrida* after trichome removal using forceps was analyzed using Student's *t*-test. SAS statistical software (version 9.2, SAS Inc., Cary, NC) was used for all statistical analyses.

Results

Host Preference. *C. hybrida* did not feed on *S. tuberosum* in the multiple-choice tests (Table 2). For the other *Solanum* species evaluated, feeding by *C. hybrida* was generally more extensive on leaves of plants grown under controlled conditions (shade net, glasshouse, and growth chamber) compared with full sun (Table 2). This was even more pronounced in the

case of *S. mauritianum*, where feeding was limited to leaves of plants grown under controlled conditions only. Generally, *C. hybrida* tended to prefer *S. melongena* and the natural host *S. lichtensteinii* to *S. mauritianum*. When plants were grown under controlled conditions, however, feeding on leaves of *S. mauritianum* was comparable with that on *S. lichtensteinii* and *S. melongena*.

Trichome Types. Multicellular stellate (star-shaped) trichomes (Fig. 1A) predominated on leaves of *S. lichtensteinii*, *S. melongena*, and *S. mauritianum*, with a few simple, unbranched, slender trichomes interspersed. The basal cells of both stellate and simple trichomes were raised above the epidermis, although more prominently so in the stellate than in the simple trichomes. *S. tuberosum* leaves only had simple trichomes that were morphologically distinct from those of the other *Solanum* species in that they appeared jointed and curved, with a thicker base and a pointed end (Fig. 1B).

Trichome Density. In all four *Solanum* species grown in the field, the number of trichomes per unit leaf area (mm²) was significantly higher in full-sun plants than in those raised in the shade (Table 3). A similar trend was observed for the potted plants, where individuals grown in the full sun had a higher trichome density than those grown under controlled conditions (Fig. 2A and B). *S. mauritianum* had the highest trichome density, followed by *S. lichtensteinii* and *S. melongena*. *S. tuberosum* had the lowest density of trichomes (Fig. 3).

Trichome Stalk Length. Stalk length differed with species (Fig. 2C and D). In *S. mauritianum*, the stalk length of the trichomes varied, causing the flat (parallel to the leaf surface), stellate parts of the trichomes to be arranged in different layers, causing a thick "roof" similar to a forest canopy (Fig. 2B, and D). The stalk length of *S. melongena* and *S. lichtensteinii* was shorter than that of *S. mauritianum* (Fig. 2C and D).

Trichome Removal by the Adhesive Tape Method. The effectiveness of the adhesive tape method in trichome removal, based on the difference between the trichome numbers before and after trichome removal, differed among the four *Solanum* species. The method was effective in removing a significant number of trichomes from *S. lichtensteinii*, *S. melongena*, and *S. mauritianum* leaves, but not from *S. tuberosum* (Fig. 4A).

Effect of Trichome Removal on Insect Feeding. The trichome removal treatment described above had a significant impact on feeding by *C. hybrida* ($F_{1, 98} =$

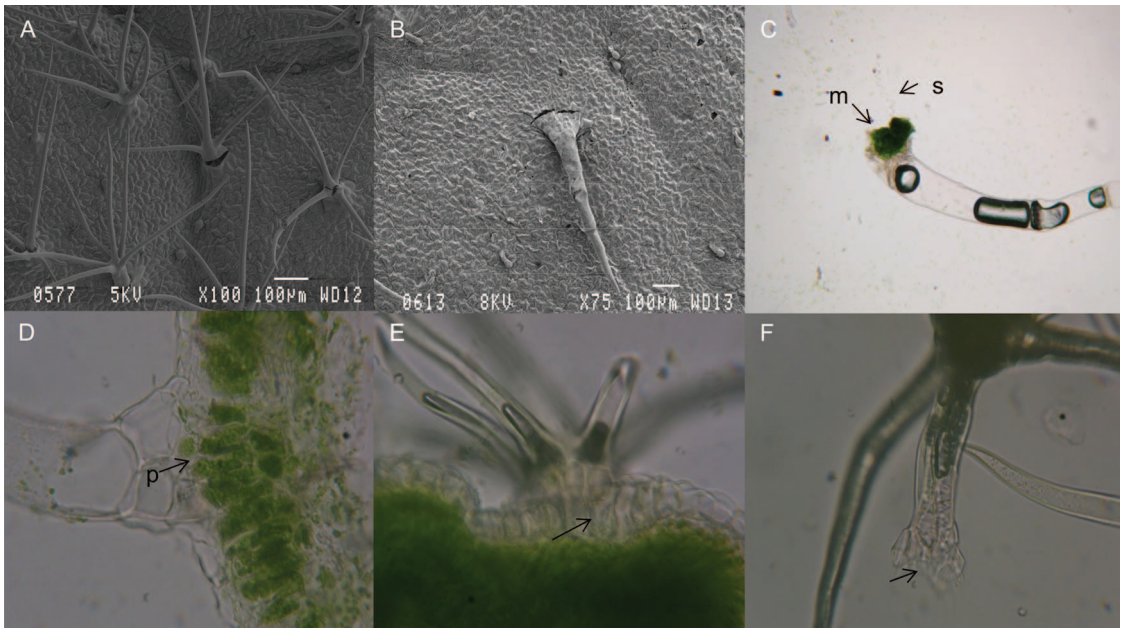


Fig. 1. Trichomes of pot-grown *S. mauritianum* and *S. tuberosum* leaves. (A) Surface view of a *S. mauritianum* leaf showing stellate trichomes; (B) surface view of a leaf of *S. tuberosum* showing a simple trichome; (C) *S. tuberosum* trichome detached from a leaf with forceps, showing the attached mesophyll tissue (m) and the spiral of a vascular element (s); (D) cross-section of a leaf of *S. tuberosum* showing the palisade tissue (p) extending to the trichome base; (E) part of an *S. mauritianum* leaf cross section showing a stellate trichome; and (F) a detached stellate trichome of *S. mauritianum* without attached subepidermal tissue. (Online figure in color.)

6.02; $P = 0.016$). Higher levels of feeding were observed on leaf portions with trichomes removed than the controls (Fig. 4B). The level of feeding also differed among *Solanum* species ($F_{2,98} = 3.12$; $P = 0.049$). Higher feeding damage occurred on *S. lichtensteinii* than on *S. mauritianum*. Feeding damage on *S. melongena* did not differ from *S. lichtensteinii* or *S. mauritianum*. There were no significant interactions between plant species and trichome removal ($F_{2,98} = 0.34$; $P = 0.712$). *S. tuberosum* was excluded from the analysis because no feeding occurred on the control leaves. Hardly any trichomes were removed by the tape, and only one leaf showed feeding damage after trichome removal.

Anatomy of *S. tuberosum* Trichomes. Because trichome removal using adhesive tape was not effective in *S. tuberosum*, trichomes were manually removed using forceps, after which feeding by *C. hybridus* tended to increase from a mean of 1.22 to 12.13 mm^2 , though this was not statistically significant ($t = 0.93$; $P = 0.36$). While removing the trichomes, it was

observed that their basal cells were partly embedded in the mesophyll and in close contact with the spiral xylem elements via the adaxial palisade cells (Fig. 1B–D); this caused the spiral elements to be extracted together with the trichomes. Trichome basal cells in the other species were not embedded in the mesophyll, and the trichomes were therefore more easily removed (Fig. 1A, E, and F).

Discussion

During the multiple-choice feeding preference trial, *C. hybridus* generally preferred leaves of plants grown under partial shade (in controlled conditions) to leaves of conspecific plants grown in full sun. Furthermore, the multiple-choice test indicated that *S. mauritianum* was not a host when grown in full sun but that it was accepted as a host plant when grown under partial shade conditions. This finding indicates that plant-related changes influenced host preference of *C. hybridus*, and provides an explanation for the ambiguous (false positive and false negative) results observed during quarantine evaluations, as was suggested by van Klinken (1999) and Diaz et al. (2011). In addition to their physiological role in reducing water loss due to transpiration (Young and Smith 1980), trichomes have been reported to play a role in plant defense (Levin 1973, Johnson 1975, Southwood 1986), although contradictory results were reported by Andres and Connor (2003). The fact

Table 3. Trichome density per square millimeter leaf area (mean \pm SEM) of field-grown *Solanum* species in full sun and shade

<i>Solanum</i> spp.	Sun	Shade	<i>t</i>	<i>P</i>
<i>S. mauritianum</i>	36.25 \pm 3.98	20.75 \pm 2.29	3.37	0.0150
<i>S. lichtensteinii</i>	25.75 \pm 2.17	16.5 \pm 0.64	4.08	0.0065
<i>S. melongena</i>	22.75 \pm 1.03	15.75 \pm 0.63	5.8	0.0012
<i>S. tuberosum</i>	5.00 \pm 0.71	1.25 \pm 0.25	5	0.0025

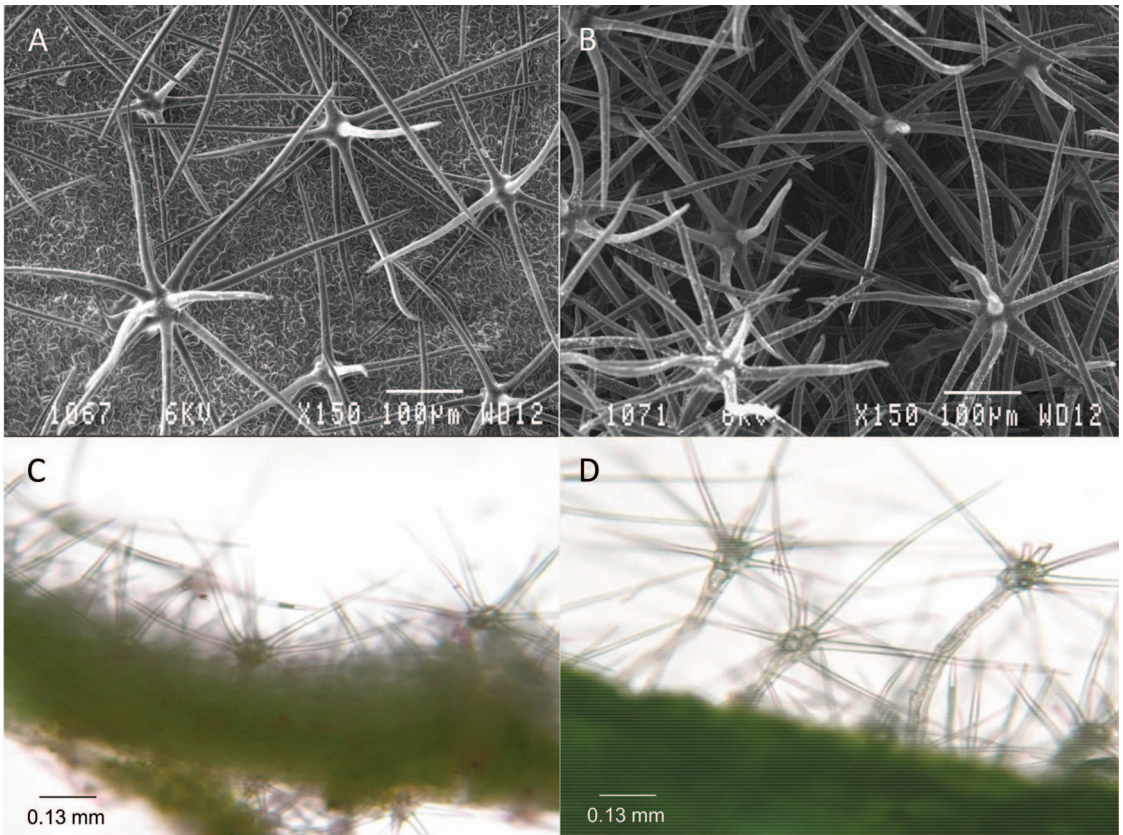


Fig. 2. Stellate trichomes. Scanning electron microscope micrographs of abaxial leaf surfaces from *S. mauritianum* plants grown in (A) a glasshouse, and (B) full sun. Micrographs of transverse hand sections of leaves from (C) *S. lichtensteinii* and (D) *S. mauritianum* grown in full sun. (Online figure in color.)

that full-sun plants of *S. mauritianum* were accepted (with minimal feeding) only under a no-choice setting (control treatment of the trichome removal trial) where there was more confinement (sleeve cages) could be attributed to changes in herbivore behavior because of captivity (Blossey et al. 1994, Olckers et al. 1995, Heard 2000).

In all four *Solanum* species tested, full-sun plants had a higher density of trichomes than shade plants.

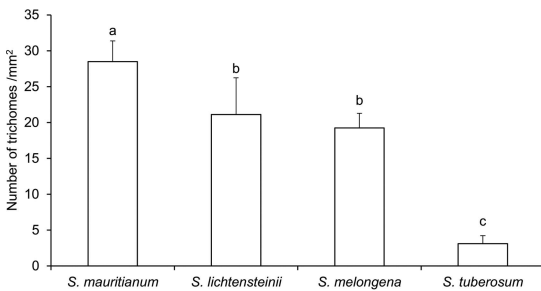


Fig. 3. Trichome density (mean \pm SEM) on leaves of four *Solanum* species grown in full sun and shade. Means with the same letters do not differ statistically (ANOVA followed by Fisher’s LSD, $P > 0.05$).

This is consistent with the findings of Young and Smith (1980), Pérez-Estrada et al. (2000), McGuire and Agrawal (2005), but contradicts those of Lill et al. (2006). In the current study, the inhibitory effect of trichomes on feeding by *C. hybrida* is shown by the significant increase of beetle feeding after trichome removal in the three *Solanum* species (*S. lichtensteinii*, *S. melongena*, and *S. mauritianum*). The interspecific variation in the level of feeding damage among the three *Solanum* species (*S. tuberosum* was the exception) is related to their trichome density. *S. mauritianum*, which had the highest trichome density, had the lowest amount of damage from insect feeding.

In addition to trichome density, trichome stalk length was higher in *S. mauritianum*, which had a thick mat of stellae, especially on the abaxial leaf surface, which would have hampered the movement of *C. hybrida* adults and prevented them from reaching the epidermis. *S. mauritianum* is a noxious alien invader species in South Africa, and native insect herbivores are likely thwarted by the trichomes. The name “woolly night-shade” for *S. mauritianum*, and its resistance to various cassidine beetles can probably be better explained by the stalk length of the stellate

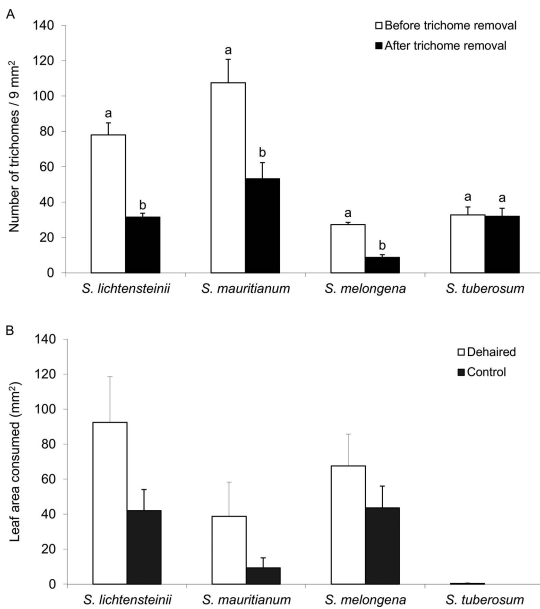


Fig. 4. (A) Number of trichomes before and after application of adhesive tape. Means (\pm SEM) with the same letters within species do not differ statistically (paired *t*-test, $P > 0.05$). (B) Intensity of herbivory of *C. hybrida* on *Solanum* leaves with trichomes removed (dehaired) and trichomes intact (control). Results of the statistical analyses are presented in the text.

trichomes rather than by their higher density, as was suggested by Olckers and Hulley (1989).

S. lichtensteinii and *S. melongena*, which have comparable trichome densities, exhibited similar feeding by *C. hybrida*. *S. tuberosum* appeared to be an exception to this trend: despite having the lowest trichome density, it also had the lowest amount of herbivore damage. However, *S. tuberosum* has a different type of trichome, and the adhesive tape technique was not effective in removing these trichomes. This led us to further investigate why the *S. tuberosum* trichomes are resistant to removal and if insect feeding will increase if trichomes are removed using other techniques. When trichomes were removed from *S. tuberosum* leaves using another technique (forceps), feeding by *C. hybrida* tended to increase but not significantly. Apparently, the intrusion of basal cells of trichomes into the mesophyll tissue in *S. tuberosum* may explain the resistance of the trichomes to removal by insects such as *C. hybrida*, which normally remove trichomes before feeding (Ghebremariam et al. 2014). Medeiros and Moreira (2005) suggested that larvae and adults of another cassidine (*Gratiana spadicica* Klug) need to remove trichomes before feeding because the head capsule width of the larvae and adults was significantly greater than the intertrichome space, thus inhibiting the insects from reaching the epidermis and feeding on the leaf.

Although the lower trichome density on leaves with trichomes removed significantly enhanced feeding by *C. hybrida*, the feeding on *S. mauritianum* (and *S.*

tuberosum) with trichomes removed was still lower than for the controls of *S. lichtensteinii* and *S. melongena*. This implies that trichome density is but one of the plants' defense mechanisms inhibiting feeding by *C. hybrida*, and it is possible that other physical or chemical factors contributed to the feeding preference results obtained in this study, such as leaf toughness and content of biominerals (calcium oxalates) and secondary metabolites such as phenolics and alkaloids.

Leaf trichome characteristics varied with plant species and growth conditions. The type, density, and thickness of trichomes influenced feeding by *C. hybrida*, suggesting that growth conditions in the field can affect acceptability of plants to herbivores and may influence the outcome of host-specificity tests in the laboratory. Plant morphological changes may thus in part explain host range differences between quarantine evaluations of weed biological control agents and field host ranges (realized host range). Understanding how these morphological changes influence herbivore feeding is critical for the interpretation of results of host plant evaluation in quarantine and improvement of the risk assessment system. Thus, when studying host specificity of herbivores, such as in weed biocontrol programs, the feeding behavior of herbivores and the nature of plant trichomes should be considered when selecting test plants. For trichome-removing herbivores, the testing of plants with trichomes that detach easily is recommended.

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