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Influence of leaf trichome type, and density on the host plant selection by the greenhouse whitefly, Trialeurodes vaporariorum (Hemiptera: Aleyrodidae)

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Abstract:	Host selection by adult greenhouse whitefly Trialeurodes vaporariorum (Westwood) was assessed on two pelargonium plant cultivars, Pelargonium x domesticum (regal) and P. x hortorum (zonal) using Petri dish bioassay chambers in choice and no-choice tests. Plant characteristics which could influence the oviposition preference of the whitely i.e., type and density of trichomes on the abaxial leaf surface was determined. A strong host preference was observed for the regal compared to the zonal pelargonium by the adult whiteflies. In no-choice tests, adults laid a significantly higher number of eggs on regal than on zonal leaves both at 24 and 48 hours post-exposure, respectively. After exposure to the adult whitefly, the number of eggs in choice tests were similar between cultivars at 24 hours, but were higher for regal at 48 and 72 hours. The total number of trichomes (sng: straight non-glandular + sg: straight glandular) per 0.50 cm2 was significantly less on regal (Mean ± SE sng + sg; 43.1 ± 1.5) than on zonal leaves (60.5 ± 1.2); however, the sng trichomes were significantly higher on the zonal (49.4 ± 0.96) than the regal leaves (28.6 ± 1.00). Also, the number of sg trichomes was slightly higher for the regal cultivar leaves compared to the zonal, being 14.4 ± 1.2 and 11.2 ± 0.5, respectively. Results suggest that the trichome density, type and the ability to express glandular exudates can affect adult whitefly Pelargonium cultivar preference and plays an important role in their host plant selection for oviposition.
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Response: revised as above in text

L.220
Analysis from a two-way ANOVA showed per cultivar, that there were no significant differences in the number of eggs found between leaves per position (I or II) after being exposed to whitefly adults for 24 h or 48 h.
->
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Response: revised as below in text

Analysis from a two-way ANOVA showed that there were significant differences in the number of eggs found between leaves per cultivar ($F = 32.3$; $df = 1, 9$; $p < 0.001$, $F = 11.9$; $df = 1, 9$; $p = 0.006$), but no significant differences between leaves per position for regal ($F = 3.762$; $df = 4, 9$; $p = 0.089$, $F = 0.224$; $df = 4, 9$; $p = 0.914$) and zonal ($F = 0.750$; $df = 4, 9$; $p = 0.598$, $F = 0.864$; $df = 4, 9$; $p = 0.543$) after being exposed to whitefly adults for 24 h or 48 h, respectively.

Table 1
Remove the footnote, "Mean values within a column per experiment followed by a different letter are significantly different (ANOVA; $p < 0.05$)" and the letters "a" and "b" in the table. Change "Statistical Analysis" to "ANOVA statistics". Change "Experiment 1 (24 h)" and "Experiment 2 (48 h)" to "Experiment 1 (24 h, n = 5)" and "Experiment 2 (48 h, n = 5)", respectively. Remove the column to show "n".

Response: revised as requested above

Table 2
Remove the footnote, "Mean values (± SE; experiments 1 & 2, n = 15; experiment 3, n = 19). Values within a column followed by a different letter are significantly different (Wilcoxon signed-rank test, $p \leq 0.06$)" and the letters "a" and "b" in the table. Change "Statistical Analysis" to "Wilcoxon's test statistics". Change "Experiment 1 (24 h)", "Experiment 2 (48 h)", and "Experiment 3 (72 h)" to "Experiment 1 (24 h, n = 15)", "Experiment 2 (48 h, n = 15)", and "Experiment 3 (72 h, n = 19)", respectively.

Response: revised as requested above

Table 3
Remove the footnote, "Mean values ± SE within a column followed by the same letter are not significantly different (ANOVA plus Sheffe's F-test; $p < 0.05$). n = 150 samples per cultivar" and the letters "a" and "b" in the table. Change "Statistical Analysis" to "ANOVA statistics". Change "Mean number ± SE of trichomes / leaf cultivar" to "Mean number ± SE of trichomes / leaf cultivar (n = 150)" in the table.

Response: revised as requested above

Cited references in the text should be ordered alphabetically, as we pointed out in the previous decision letter. For example, citations at L.79 should be (Gilman and Howe 1999; Sanderson and Ferrentino, 1993), not (Sanderson and Ferrentino, 1993: Gilman and Howe 1999). Please check and revise all the citations very carefully.

Response: revised as requested above

Figs. 1 and 2 are illustrated in color. They will appear in color in an electronic PDF document but in grayscale halftone with black/white in a printed document. Please confirm that your figs can represent your idea even in grayscale.

Response: confirmed

For: Applied Entomology and Zoology

Influence of leaf trichome type, and density on the host plant selection by the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)

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Abstract

Host selection by adult greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) was assessed on two pelargonium plant cultivars, *Pelargonium* x *domesticum* (regal) and *P.* x *hortorum* (zonal) using Petri dish bioassay chambers in choice and no-choice tests. Plant characteristics which could influence the oviposition preference of the whitely i.e., type and density of trichomes on the abaxial leaf surface was determined. A strong host preference was observed for the regal compared to the zonal pelargonium by the adult whiteflies. In no-choice tests, adults laid a significantly higher number of eggs on regal than on zonal leaves both at 24 and 48 hours post-exposure, respectively. After exposure to the adult whitefly, the number of eggs in choice tests were similar between cultivars at 24 hours, but were higher for regal at 48 and 72 hours. The total number of trichomes (sng: straight non-glandular + sg: straight glandular) per 0.50 cm^2 was significantly less on regal (Mean ± SE sng + sg; 43.1 ± 1.5) than on zonal leaves (60.5 ± 1.2); however, the sng trichomes were significantly higher on the zonal (49.4 ± 0.96) than the regal leaves (28.6 ± 1.00). Also, the number of sg trichomes was slightly higher for the regal cultivar leaves compared to the zonal, being 14.4 ± 1.2 and 11.2 ± 0.5, respectively. Results suggest that the trichome density, type and the ability to express glandular exudates can affect adult whitefly *Pelargonium* cultivar preference and plays an important role in their host plant selection for oviposition.

Keywords: *Pelargonium*; choice tests; no-choice tests; anacardic acid; glandular trichome; non-glandular trichome

Introduction

The genus *Pelargonium* is one of the most popular ornamental plants grown worldwide (Garćia-Sogo et al. 2012). It consists of more than 250 species of plants, currently grouped into 16 sections, which differ in their anatomy and morphology (van der Walt 1993). The two cultivars *Pelargonium* species, *P. x hortorum* and *P. x domesticum*, both belong to the section Ciconium and are among the most economically important bedding and pot plants in Europe as well as in North America with yearly sales greater than $100 million (Canadian) (Mamba and Wahome 2010; Mithila et al. 2001). The zonal pelargonium, *P. x hortorum*, which is the highest selling potted flowering plants (Kessler 2007), is derived from crossing *P. zonale* with *P. inquinans*. It is a plant with extensive branching that grows to a height of 15 cm (Laughner 1993). The leaves are rounded (Fig. 1), pale to mid-green, with a dark reddish anthocyanin-containing zone which gives rise to the botanical name, zonale (van der Walt 1993). The regal pelargonium, *P. x domesticum* mainly derived from crossing *P. culcullatum*, *P. fulgidium* and *P. grandiflorum*, is a plant bearing broadly ovate to palmate, toothed, mid-green leaves (Fig. 1), and growing to a height of 38 - 70 cm. These floral crops are sold for their beauty, and the mere presence of any pest or damage detracts from its value. Insect pests, which utilize ornamental plants as a source of nutrition or site for oviposition, can have a major impact on the aesthetic value, economic quality and marketability of the plant crop. Therefore, zero-tolerance for such pests or their damage (to keep them blemish free) requires multiple, routine pesticide applications.

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), is a common pest of *Pelargonium* species (Avery 2002; Simmonds 2002), especially regal pelargoniums which are widely cultivated in the Mediterranean area and other

parts of the world (Castañé and Albajes 1992; Gilrein 2004; Lis-Balchin 2002). This whitefly can also infest zonal pelargoniums, although reports indicate that certain cultivars are more susceptible than others (Gilman and Howe 1999; Sanderson and Ferrentino 1993). Walters et al. (1989a) found after analyzing both the morphological and chemical differences between the insect-resistant and -susceptible zonal pelargoniums, that the tall glandular trichomes and the exudate they produced were the most important factors in pest resistance. In addition, the ability of the plant to express a delta-9 (omega-5) unsaturated fatty acid from the omega-5 unsaturated alkyl anacardic acid as an exudate on the glandular trichome head exterior was lacking in the susceptible lines (Schultz et al. 1996).

Insect-plant interactions involve complex behavioral responses of the insect to physical and chemical characteristics of the host plant. The plant's physical resistance mechanisms, such as trichomes and leaf morphology, have been reported to influence the interactions between various phytophagous insects and their host plants (Campos et al. 2003; Malakar and Tingey 2000; Medeiros and Tingey 2006; Simmons et al. 2003, 2006). In particular, trichome density has been shown to affect oviposition selection for whitefly species and is a major factor in determining preference for various host plants, including *P. x domesticum* (Chu et al. 2000; Heinz and Parrella 1994; McAuslane, 1996; Riley 1995; Sánchez-Peña et al. 2006). However, little information is available about other morphological characters of *P. x domesticum* that might influence host selection by whitefly for oviposition. In developing an effective biological control management program for the greenhouse whitefly on *Pelargonium* cultivars, a critical stage is determining which host plant is most preferred for feeding and oviposition. Thus, the objective of the current study was two-fold: 1) to determine host preference of greenhouse whitefly

between *P. x domesticum* and *P. x hortorum* and 2) to determine if these cultivars differed in the types, and density of trichomes on the abaxial leaf surface.

Materials and methods

Plants and insects

Pelargonium x domesticum var. *Dubbonet Sport* (regal) and *Pelargonium x hortorum* (zonal) cultivars were obtained from Dr. M. Lis-Balchin (School of Applied Science, South Bank University, London, UK) and grown at the Royal Botanic Gardens, Kew. Plants used in these experiments were transferred from Kew to Birkbeck College, University of London. Cuttings taken from the stock plants in the greenhouse were all grown in John Innes soil type No. 3 (John Innes Manufacturers Association, Theale, Reading, Berkshire, UK) in growth chambers maintained at 23 - 25 °C under a 16:8 hour (h) light: dark (L:D) photoperiod. Greenhouse whitefly adults reared on *Abutilon* sp. (house lime) for more than 25 generations were obtained from Royal Botanic Gardens, Kew.

Bioassay chambers and protocol

To conduct the whitefly preference test, a novel bioassay chamber was developed. Each completed bioassay chamber consisted of two Petri dishes (15 mm x 100 mm each) bottoms held together by cellophane tape to form a container (Fig. 2). The following is a description of how the bioassay chamber was constructed. First the petioles of similar-size leaves were detached from either stock plant cultivar and trimmed at approximately a 45° angle with a razor blade. Each leaf petiole was then made secure in the Gilson pipette tip (~200 µL) by tamping cotton

wool around it (Figs. 1-2). Each leaf with the petiole in the pipette tip tightly secured was allowed to absorb water in a small beaker until the cotton wool was saturated prior to placing in the Petri dish bottom. Next a small hole (~5 mm) was made in the side of a Petri dish bottom for the pipette tip to be inserted. Prior to being inserted tightly, the leaf was positioned so that when the chamber was sealed, the abaxial side would face inward towards the abaxial side of the other leaf. To minimize condensation within the chamber and prevent possible drowning of the whitefly adults, a strip of filter paper (~270 mm x 8.0 mm) was placed on the inside of each dish towards the outer edge of each dish bottom.

Prior to sealing the two halves of the chamber together, 10 randomly selected whitefly adults (unknown ratio of male and female, but most of the individuals in the selected population were females) were introduced into the chamber. Adults were placed on the abaxial side of a randomly selected leaf (I or II). The two Petri dish bottoms were sealed together with clear cellophane tape and placed on 50 ml tri-cornered polypropylene beakers filled with enough water to allow the tips to be partially immersed (Fig. 2). Each sealed bioassay chamber in the beaker was transferred to a growth chamber and then placed perpendicular to the growth chamber door in a randomized completed block design with the light source located above The sealed bioassay chambers were maintained in the growth chamber for either 24, 48 or 72 h depending on the test conducted at 23 ± 2 °C under a 16 h photophase with overhead lighting at 21, 000 lux.

Host preference tests

The leaves in the sealed bioassays were randomly assigned to position I or II with reference to the layout inside the growth chamber for all tests. The whitefly adults were given the opportunity

to oviposit on either leaf inside the bioassay chambers in two no-choice tests for 24 and 48 h (experiments 1 and 2, respectively) and three choice tests between cultivars for 24, 48 and 72 h (experiments 1, 2 and 3, respectively). After the appropriate time interval allowed for oviposition, the whitefly adults were removed from the bioassay chambers, and the number of eggs deposited on either leaf was recorded. Experiments were repeated at least two times on separate occasions.

No-choice tests

Two leaves of either regal or zonal cultivars that were healthy in appearance and similar in size and age, ranging in width from 4.0 to 9.0 cm for both cultivars, were randomly selected from several different plant cuttings. This attempt to standardize leaves was undertaken to minimize the effect of leaf vigor and size on the results. Each bioassay chamber containing two leaves, designated as either I or II, was considered as a single replicate (block). After either 24 (experiment 1) or 48 h (experiment 2), the chambers were opened and the number of eggs deposited on the abaxial surface of each leaf was counted using a stereomicroscope (40X). Both experiments 1 and 2 had five replicate bioassay chambers. Preliminary experiments had shown that eggs were laid predominately on the abaxial surface; therefore, only eggs on the abaxial surface were recorded. Host preference was determined by the highest mean number of eggs deposited per cultivar.

Choice tests

Plant cultivar leaves that were healthy in appearance and similar in size were used for each experiment. Leaves ranging in width from 4.0 cm to 9.0 cm from cuttings were selected from several different stock plants of each cultivar. Each chamber containing a regal and a zonal leaf was considered a replicate (block). Experiments 1 and 2 each had fifteen replicates and experiment 3 had 19 replicates. After either 24 h (experiment 1), 48 h (experiment 2), or 72 h (experiment 3) exposure by the whitefly adults, the chambers were opened and the total number of eggs deposited on the abaxial surface of each leaf were counted.

Type of trichomes on *Pelargonium* cultivar leaves

To differentiate between the type of trichomes found on each cultivar, leaf samples were prepared prior to being observed under the scanning electron microscope (SEM). Excised leaf samples of each cultivar were fixed in 0.1M sodium cocolydate, 2% paraformaldehyde and 2.5% glutaraldehyde (pH 7.0) at 6^{o}C for 2 h. Fixed leaves were then rinsed first with 0.1M sodium cocolydate buffer (pH 7.0) at 6^{o}C for 30 min (2X), fixed using 1% OsO_4 buffer (pH 7.0) at ~22^{o}C for 1 h and then rinsed with water at ~22^{o}C for 10 min twice. The samples were dehydrated with 50% alcohol at ~22^{o}C for 30 min, 70% alcohol overnight at 6^{o}C, 90% alcohol at ~22^{o}C for 10 min, 100% alcohol at ~22^{o}C for 4 min (4X), dry absolute alcohol at ~22^{o}C for 10 min and then subjected to critical point drying using CO_2. The dehydrated leaves were then mounted on SEM stubs, sputter coated with gold to a thickness of 15 mÁ twice for 4 min at ~22^{o}C, and viewed using a JEOL CF-35 (JSM 35) scanning electron microscope (JOEL Ltd., Hertfordshire, UK).

Trichome assessment

Fifteen leaves from 5-10 stock plants of each cultivar were excised and the total number of tall straight non-glandular (sng; Fig. 3 and 4) and straight glandular (sg; Figs. 3 and 4) trichomes on the abaxial side of each leaf were counted with a stereomicroscope (50X). To account for possible variation in trichome density due to leaf size, a random equal selection of leaves ranging in width from 4.0 to 9.0 cm were used for this study. To determine the trichome density, each leaf was subdivided into two areas, A (outside) and B (inside) for both cultivars (Fig. 1). Within each area between the secondary and tertiary veins, five disks (0.50 cm^2) in each zone were punched out with a cork borer from each leaf cultivar (10 disks total / leaf). The dark reddish anthocyanin-containing zone subdivided areas A and B for the *P.* x *hortorum* leaf; the secondary and tertiary veins subdivided areas A and B for the *P.* x *domesticum* leaf. Area A was towards the leaf edge, whereas area B was between the secondary and below the tertiary veins. The total number of sng and sg trichomes were counted per leaf.

Statistical analysis

In the no-choice tests, a one-way ANOVA ($\alpha = 0.05$) was conducted to determine if there were any differences in the mean number of eggs deposited by the greenhouse whitefly adults on leaves in any of the bioassay chambers for either cultivar after 24 h and 48 h post-exposure. To determine if there was any effect of leaf orientation (I or II) preference by the whiteflies inside the sealed bioassays with respect to the light source in the growth chamber , the total number of eggs deposited per leaf within each bioassay chamber were (n + 1) log transformed and then statistically compared using a two-way ANOVA ($\alpha = 0.05$). In the choice tests, to determine if

one cultivar was preferred by the adult greenhouse whitefly, the difference in the mean number of eggs per replicate per leaf cultivar was statistically analyzed using a Wilcoxon signed-rank test ($\alpha = 0.05$). The difference in the mean number of sng and sg trichomes and the combined number of sng+sg trichomes on the leaves of the two cultivars were statistically analyzed using a one-way ANOVA with a Sheffe's *F*-test ($\alpha = 0.05$) to determine if there were differences between cultivars.

Results

No-choice tests

The adult greenhouse whitefly laid significantly more eggs (4.3 and 7.7 times, respectively) on regal than on zonal leaves both at 24 h ($F = 7.83$; df = 1, 8; $p = 0.023$) and 48 h ($F = 18.2$; df = 1, 8; $p = 0.003$), respectively (Table 1). Analysis from a two-way ANOVA showed that there were significant differences in the number of eggs found between leaves per cultivar ($F = 32.3$; df = 1, 9; $p < 0.001$, $F = 11.9$; df = 1, 9; $p = 0.006$), but no significant differences between leaves per position for regal ($F = 3.762$; df = 4, 9; $p = 0.089$, $F = 0.224$; df = 4, 9; $p = 0.914$) and zonal ($F = 0.750$; df = 4, 9; $p = 0.598$, $F = 0.864$; df = 4, 9; $p = 0.543$) after being exposed to whitefly adults for 24 h or 48 h, respectively.

Choice tests

There was a significant difference in the number of greenhouse whitefly eggs laid on leaves of the two cultivars in the choice test study (Table 2). In all the three experiments, higher numbers of eggs were deposited on leaves of regal cultivars than zonal leaves after 24 h (Z = -

1.934; df = 1, 29; $p = 0.054$), 48 h ($Z = -2.89$; df = 1, 29; $p = 0.004$) and 72 h ($Z = -5.24$; df = 1, 37; $p < 0.001$) post-exposure to the whitefly. The mean number of eggs laid on the regal leaves after 24, 48 and 72 h post-exposure was ~2.1, 2.5, and 1.5 times that found on the zonal leaves, respectively.

Trichome density

The number of sng trichomes per 0.50 cm^2 of leaf area was significantly much higher on the zonal (Mean ± SE; 49.4 ± 0.96; $n = 150$) than the regal (28.6 ± 1.00; $n = 225$) ($F = 173.1$; df = 1, 298; $p < 0.001$) leaves, whereas the number of sg trichomes was significantly higher on the regal cultivar ($F = 5.56$; df = 1, 298; $p = 0.019$) compared to the zonal, being 14.4 ± 1.2 and 11.2 ± 0.5, respectively (Table 3). The total trichomes (sng + sg) was considerably less ($F = 85.5$; df = 1, 298; $p < 0.01$) on regal leaves (Mean ± SE sng + sg; 43.1 ± 1.5; $n = 150$) than on zonal leaves (60.5 ± 1.2; $n = 225$).

Discussion

Host selection behavior of the greenhouse whitefly adults has been divided into 3 phases: 1) host plant selection before landing, 2) after landing, and 3) selection of feeding and oviposition sites within the plant (van Lenteren and Noldus 1990). In this study, greenhouse whitefly adults were placed directly on a non-selected cultivar leaf surface, which eliminated some effect of factors that might influence host choice prior to landing. Therefore, host plant selection of regal or zonal leaf cultivars was based primarily on the last two phases.

According to van Lenteren and Noldus (1990), the fecundity of the greenhouse whitefly is highly variable and influenced by many factors which includes the experimental setup and physiological state of the host plant. However, in this study, the number of eggs deposited over time (24 -72 h) on cultivar leaves in either position (I or II) with respect to the light source in the growth chamber per sealed bioassay was similar and did not vary significantly. In addition, the random placement of the bioassays in the growth chamber did not appear to affect the results over time. Therefore, the experimental setup and physiological state of the host plant did not appear to influence the oviposition of the greenhouse whitefly adults in these studies.

Based on the number of eggs laid on the different cultivars in both choice and no-choice tests, there was a definite preference for the regal leaves for oviposition by the adult greenhouse whitefly. The lowest total trichome density found on regal leaves compared to zonal, may have influenced and contributed to the oviposition preference of the greenhouse whitefly adults. Other plant-whitefly interaction studies conducted using poinsettia (Bilderback and Mattson 1977), and several species of the genus *Cucumis* (Kowalewski and Robinson 1977) also confirm that trichomes play an important role in host plant acceptance by greenhouse whitefly adults. In a later study using the spiraling whitefly (*Aleurodicus disperses*), Wen et al. (1994) concluded that the feeding preference seemed to be affected by the leaf structure of the host plant. However, there are many other factors that may have influenced the greenhouse whitefly host acceptance of the pelargonium cultivars. Some of these include: 1) the chemical compounds in the plant leaf tissue detected by the insect's receptors which can result in different feeding responses (Lei and Xu 1995), 2) ability or inability of the insect to probe the plant tissue and reach the phloem (Lei et al. 2001; Xu et al. 1994), and 3) water availability in certain plant cells in which eggs are

inserted e.g. is one kind of cell more suitable as an anchor or conduit for water (Byrne et al. 1990). Also, other general plant-mediated interactions between whiteflies are reviewed (Inbar and Gerling 2008). However, in this present study, we focused primarily on the influence of leaf trichome type and density on egg deposition of the greenhouse whitefly for each pelargonium cultivar.

The greenhouse whitefly deposited more eggs on leaves of the regal cultivar than on the zonal in both tests, indicating that the cultivar with a lower trichome density was the preferred host. In a similar study, Dabrowski (1972) found in choice caged tests using whole plants (3-5 fully developed leaves), that the greenhouse whitefly females laid an average of 97.5 eggs on *P.* x *domesticum* compared with a range of 0.3-1.1 eggs on *P.* x *hortorum* and *P.* x *peltatum* after nine days post-release. Further tests also revealed that when females were released on *P.* x *domesticum* and *P.* x *hortorum* that feeding was much more intense on *P.* x *domesticum*. In this study we used a detached leaf bioassay and our findings were similar indicating that this bioassay technique was comparable to using whole plants. Walters et al. (1989b) found that susceptible zonal cultivar lines, which were the preferred plant host for mites and insects, possessed a lower trichome density compared to the resistant lines. Trichome density has been noted to affect plant host preference of whitefly species on wild potato (Boiteau and Singh 1988), cotton (Butler Jr. et al. 1991), hibiscus (Meagher and Estrada 1994), poinsettia (Bilderback and Mattson 1977; Heinz and Parrella 1994), cucumber, pepper, okra (Aslam and Gerba 1995), melon (Riley 1995), soybean (Lambert et al. 1995; McAuslane 1996), and Egyptian henbane (Salem 1995). Castañé and Albajes (1992) also noted after investigating preference by the greenhouse whitefly adults between regal cultivars that adults preferred those

with fewer trichomes, especially in the first hours after release; however, in the following hours, trichome density was less important in their host choice. Therefore, the different types of trichomes on the leaf surface may also play a role in the greenhouse whitefly oviposition preference per cultivar.

The presence of both types of trichomes on pelargonium leaves can influence egg deposition, adult or nymphal distribution and host preference of the greenhouse whitefly. Straight non-glandular (sng) trichomes alone may act as a physical barrier against adult movement and deter the female whitefly from resting on the leaf surface, thus decreasing their preference and ability to oviposit on the cultivar. Ovipositional resistance to whiteflies on *Lycopersicon* hybrids (Erb et al. 1994), *Solanum-berthaultii* (Boiteau and Singh 1988), *Nicotiana tabacum* (Neal Jr. et al. 1987) and *Cucumis melo* (Soria et al. 1996) has been attributed to variation in the numbers of sng and sg trichomes present on the leaves.

Also in this study, the zonal cultivar was the least preferred by the greenhouse whitefly for oviposition. The lack of preference for the zonal cultivar may be attributed to the deterrent effect of anacardic acids produced by the sg trichomes in combination with the high density of sng trichomes present on the leaf (Fig 3b). Dabrowski (1972) proposed that a chemical factor must be present on *P.* x *hortorum* that acts as a feeding deterrent to the greenhouse whitefly and a physiological inhibitor must be operative as well. The exudate expressed from sg trichomes on the surface of some zonal cultivars has been suggested as a primary factor in the two spotted spider mite, *Tetranychus urticae* (Koch) resistance mechanism (Gerhold et al. 1984). Walters et al. (1989a, b) demonstrated that the sg trichome exudate expressed in the zonal cultivar was a critical factor in *Pelargonium* resistance to the foxglove aphid, *Acyrthosiphon solani*

(Kaltenbach) and noted that resistant lines have higher densities of sg trichomes that express the anacardic acids as exudates on the trichome exterior. In another study, Salem (1995) found a high degree of resistance with Egyptian henbane, *Hyoscymaus muticus* (Solanaceae) by another whitefly, *Bemisia tabaci* and the effect was associated with exudates produced by trichomes on the leaf surface. This resistant characteristic associated with glandular exudates has been utilized for developing plant cultivars more resistant to the greenhouse whitefly and other aleyrodids (Maliepaard et al. 1995). Lastly, based on genetic studies, the gene responsible for the production of omega-5 anacardic acids, a class of secondary compounds derived from fatty acids and expressed only on the sg trichome exterior of zonal cultivars, has been shown to be necessary for pest resistance (Schultz et al. 1996).

In summary, this study is the first account where trichomes of *P.* x *domesticum* are photographed and quantified per leaf area and *P.* x *hortorum* trichomes are quantified per area. There was a strong host preference for the regal leaf by the adult greenhouse whitefly, and trichome density and type on the leaf cultivar appeared to have some influence on their oviposition preference. The preference of the whiteflies to the regal cultivar appears to be related directly to the lower density of sng + sg trichome, because no exudate is being produced by the sg trichomes. However in contrast, more research needs to be conducted to determine if the lack of preference for the zonal leaf cultivar was due to its greater sng+sg trichome density and /or toxic effect from exudates expressed from the sg trichomes. Also, these results, based on laboratory leaf bioassays, need to be confirmed under greenhouse and field conditions. The findings of this study are important in that an effective strategy for managing the greenhouse whitefly may be related to the pubescence on the plant, especially the type and density of

trichomes present on the plant. Based on the results obtained from the current study and previous work on zonal cultivars, we speculate that the zonal cultivars bearing sg trichomes with an expressed exudate (known to be highly resistant and toxic to other arthropods) can be used as part of an integrated strategy for management of the greenhouse whitefly. From a breeding perspective, perhaps further research is now warranted to hybridize the regal cultivar with the zonal to express the sg trichome exudate, which could play an important role as well.

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Figure captions:

Figure 1. Diagram of leaf bioassay setup for each cultivar, *Pelargonium* x *hortorum* (Zonal leaf) and *P.* x *domesticum* (Regal leaf). A Gilson pipette tip (p) was secured to the end of each trimmed cultivar petiole and tamped with cotton prior to being inserted into the Petri dish bottom. Pipette tip with water illustrates how the leaf petiole will remain wet during each test. Each blade was subdivided into areas A (outside) and B (inside) where leaf discs (0.05 cm^2) were removed for determining trichome densities per cultivar. The dark reddish (color version illustrated on-line) anthocyanin-containing zone subdivided areas A and B for the *P.* x *hortorum* leaf; the secondary and tertiary veins subdivided areas A and B for the *P.* x *domesticum* leaf. Area A was towards the leaf edge, whereas area B was between the secondary and below the tertiary veins.

Figure 2. Side view of a sealed bioassay chamber (two Petri dish bottoms) used to determine selectivity of *Trialeurodes vaporariorum* on *Pelargonium* cultivars placed on a 50 mL tri-corner polypropylene beaker filled with enough water to allow the tips to be partially immersed. To minimize condensation within the chamber and possible drowning of the whitefly adults, a strip of filter paper (~270 mm x 8.0 mm) was placed inside each dish. Prior to sealing the two halves (Petri dish bottoms with leaf secured) of the chamber together, 10 randomly selected whitefly adults (unknown ratio of male and female) were introduced into the chamber. Adults were placed on the abaxial side of a randomly selected leaf (Orientation I or II). Leaves randomly chosen (Orientation I or II) were used to minimize oviposition effects of the whitefly adults relative to the light source located above the individual bioassays when placed in the growth chamber. All

bioassays were oriented perpendicular to the door of the growth chamber, only the leaf side (Orientation I or II) was randomly designated prior to sealing the bioassay chamber. Figure is drawn to scale and color version illustrated on-line.

Figure 3. a) SEM photomicrograph of straight glandular (sg) trichome found on abaxial side of *Pelargonium* x *hortorum*. Note exudate present and expressed on sg trichome. b) SEM photomicrograph of straight non-glandular (sng) trichomes surrounding a straight glandular trichome.

Figure 4. a) SEM photomicrograph of straight glandular (sg) trichome. Note lack of exudate expression on sg trichome. b) SEM photomicrograph of straight non-glandular (sng) trichomes surrounding a straight glandular trichome found on abaxial side of *Pelargonium* x *domesticum.*

Figure 1
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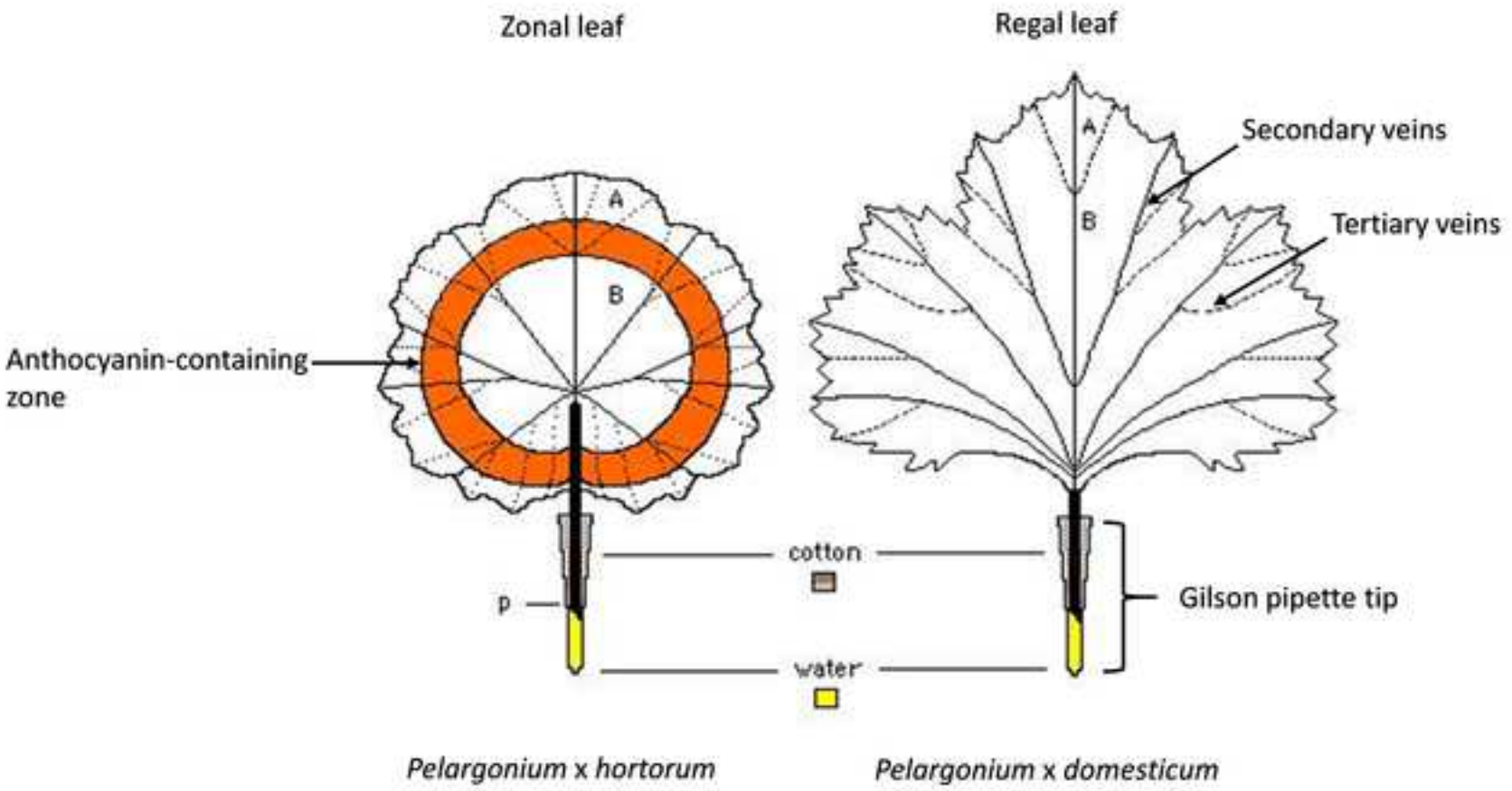

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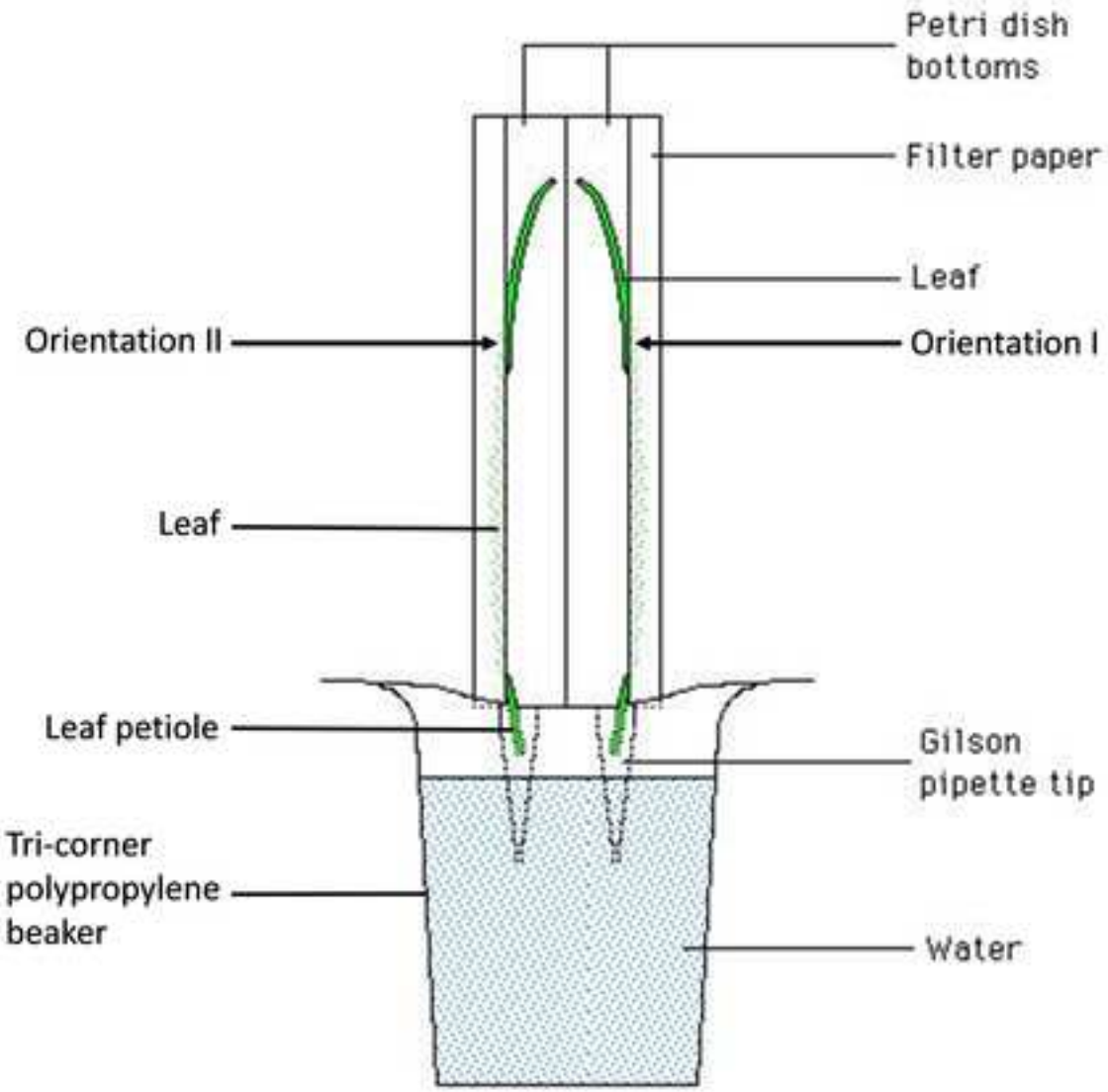

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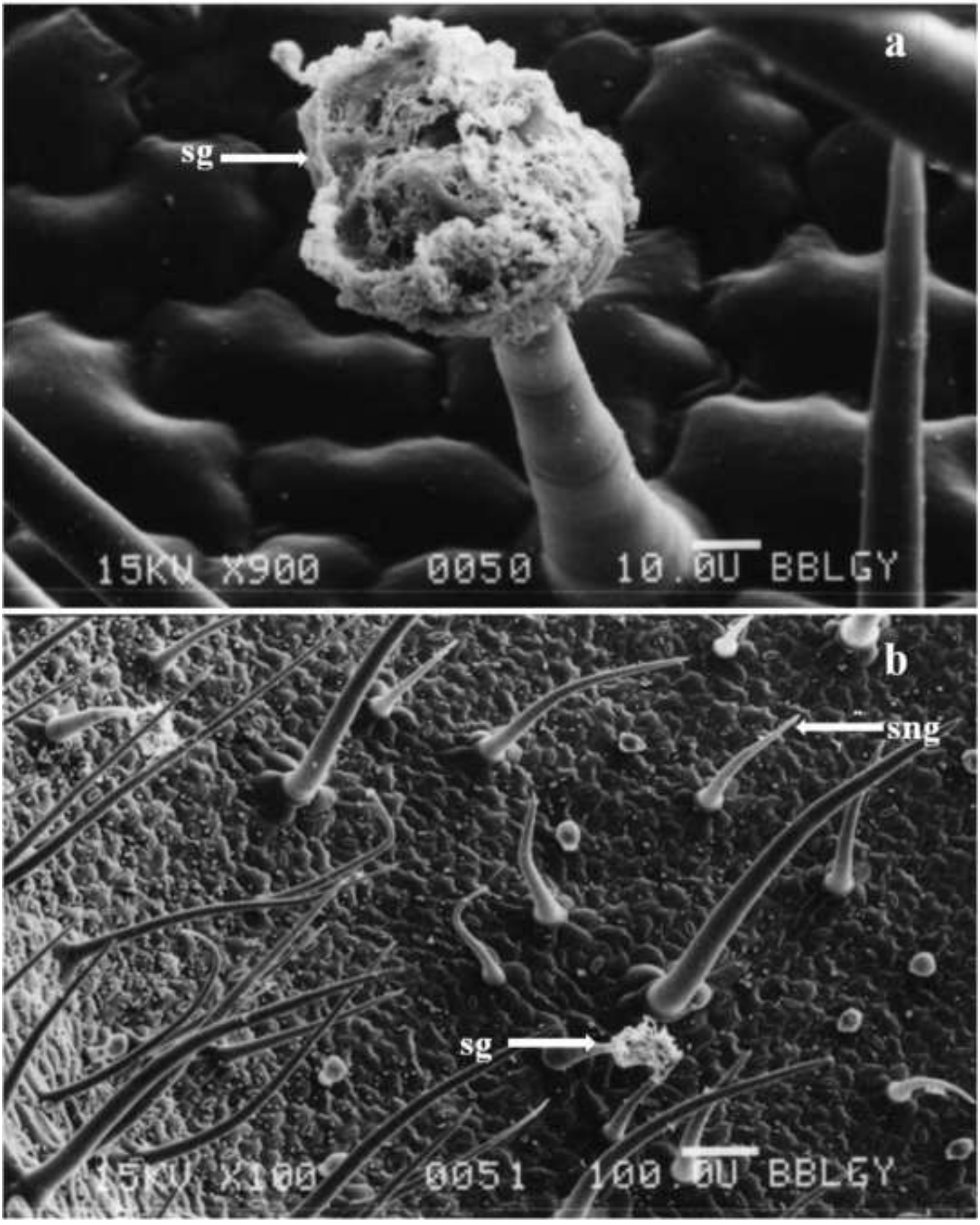

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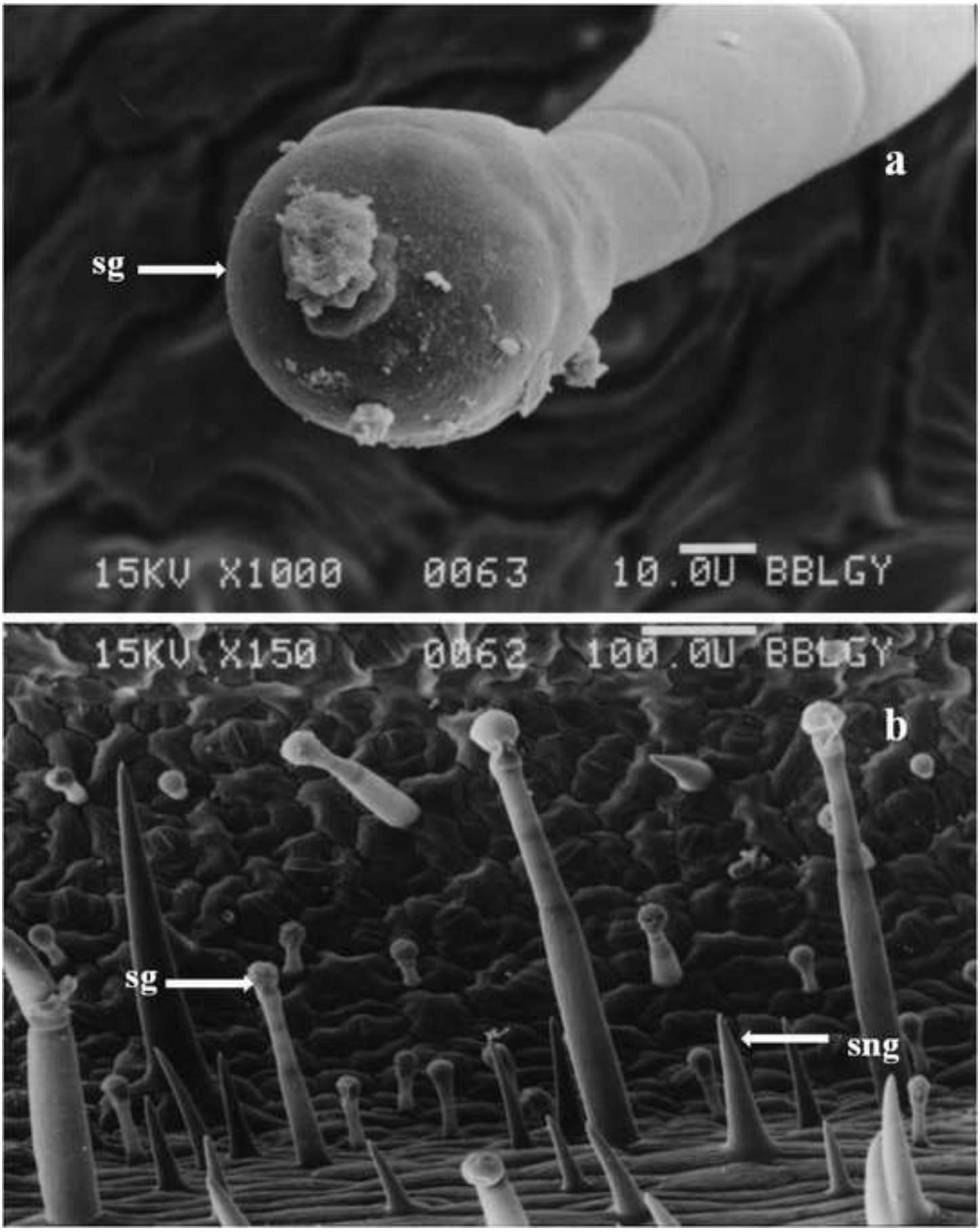

Table

Table 1. Mean total number (± SE) of eggs deposited for no-choice tests by whitefly adults on excised Regal vs. Zonal *Pelargonium* leaves in bioassay chambers after being exposed for 24 (Experiment 1) or 48 (Experiment 2) hours (h).

	Mean total number ± SE of eggs deposited/chamber	
Cultivar	Experiment 1 (24 h, $n = 5$)	Experiment 2 (48 h, $n = 5$)
Regal	19 ± 4	37 ± 8
Zonal	4 ± 1	5 ± 2
ANOVA Statistics	$F = 7.83$; df = 1, 8; $p = 0.023$	$F = 18.2$; df = 1, 8; $p = 0.003$

Table 2. Mean number (± SE) of eggs deposited for choice bioassay tests by whitefly adults on excised leaves of *Pelargonium* cultivars after being exposed for 24 (Experiment 1), 48 (Experiment 2) or 72 (Experiment 3) hours (h).

	Mean number ± SE of eggs deposited / leaf cultivar		
Cultivar	Experiment 1 (24 h, n = 15)	Experiment 2 (48 h, n = 15)	Experiment 3 (72 h, n = 19)
Regal	7.6 ± 2.7	11.7 ± 4.5	37.8 ± 6.8
Zonal	3.7 ± 1.4	4.7 ± 1.6	25.1 ± 11
Wilcoxon's test statistics	Z = -1.934; df = 1, 29; p = 0.054	Z = -2.89; df = 1, 29; p = 0.004	Z = -5.24; df = 1, 37; p < 0.001

Table 3. Mean number (± SE) per 0.05 cm^2 of straight non-glandular (sng), straight glandular (sg) and straight non-glandular plus straight glandular (sng+sg) trichomes found on Regal (*Pelargonium* x *domesticum*) and Zonal (*P.* x *hortorum*) leaf cultivars.

	Mean number ± SE of trichomes / leaf cultivar (n = 150)		
Cultivar	sng	sg	sng+sg
Regal	28.6 ± 1.0	14.4 ± 1.2	43.1 ± 1.5
Zonal	49.5 ± 1.4	11.2 ± 0.5	60.5 ± 1.2
ANOVA statistics	F = 173.2; df = 1, 298; $p < 0.001$	F = 5.56; df = 1, 298; $p = 0.019$	F = 85.5; df = 1, 298; $p < 0.001$