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

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Abstract:	Host selection by adult greenhouse whitefly <i>Trialeurodes vaporariorum</i> (Westwood) was assessed on two pelargonium plant cultivars, <i>Pelargonium x domesticum</i> (regal) and <i>P. x hortorum</i> (zonal) using Petri dish bioassay chambers in choice and no-choice tests. Plant characteristics which could influence the oviposition preference of the whitefly 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 \pm 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 <i>Pelargonium</i> cultivar preference and plays an important role in their host plant selection for oviposition.
Response to Reviewers:	L.155 Both experiments 1 and 2 had five replicate bioassay chambers. 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). -> 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.

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.

->

Analysis from a two-way ANOVA showed that there were significant differences in the number of eggs found between leaves per cultivar ($F = xx$, $df = x, y$, $p = yyy$) but no significant differences between leaves per position ($F = xx$, $df = x, y$, $p = yyy$) after being exposed to whitefly adults for 24 h or 48 h.

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 (\pm 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 \pm 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 \pm SE of trichomes / leaf cultivar" to "Mean number \pm 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

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1 For: Applied Entomology and Zoology

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4 **Influence of leaf trichome type, and density on the host plant selection by the greenhouse**

5 **whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)**

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33 **Abstract**

34 Host selection by adult greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) was
35 assessed on two pelargonium plant cultivars, *Pelargonium x domesticum* (regal) and *P. x*
36 *hortorum* (zonal) using Petri dish bioassay chambers in choice and no-choice tests. Plant
37 characteristics which could influence the oviposition preference of the whitefly i.e., type and
38 density of trichomes on the abaxial leaf surface was determined. A strong host preference was
39 observed for the regal compared to the zonal pelargonium by the adult whiteflies. In no-choice
40 tests, adults laid a significantly higher number of eggs on regal than on zonal leaves both at 24
41 and 48 hours post-exposure, respectively. After exposure to the adult whitefly, the number of
42 eggs in choice tests were similar between cultivars at 24 hours, but were higher for regal at 48
43 and 72 hours. The total number of trichomes (sng: straight non-glandular + sg: straight
44 glandular) per 0.50 cm² was significantly less on regal (Mean ± SE sng + sg; 43.1 ± 1.5) than on
45 zonal leaves (60.5 ± 1.2); however, the sng trichomes were significantly higher on the zonal
46 (49.4 ± 0.96) than the regal leaves (28.6 ± 1.00). Also, the number of sg trichomes was slightly
47 higher for the regal cultivar leaves compared to the zonal, being 14.4 ± 1.2 and 11.2 ± 0.5,
48 respectively. Results suggest that the trichome density, type and the ability to express glandular
49 exudates can affect adult whitefly *Pelargonium* cultivar preference and plays an important role in
50 their host plant selection for oviposition.

51
52 **Keywords:** *Pelargonium*; choice tests; no-choice tests; anacardic acid; glandular trichome; non-
53 glandular trichome

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6 **55 Introduction**

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8 56 The genus *Pelargonium* is one of the most popular ornamental plants grown worldwide (García-
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10 57 Sogo et al. 2012). It consists of more than 250 species of plants, currently grouped into 16
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12 58 sections, which differ in their anatomy and morphology (van der Walt 1993). The two cultivars
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14 59 *Pelargonium* species, *P. x hortorum* and *P. x domesticum*, both belong to the section Ciconium
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16 60 and are among the most economically important bedding and pot plants in Europe as well as in
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18 61 North America with yearly sales greater than \$100 million (Canadian) (Mamba and Wahome
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20 62 2010; Mithila et al. 2001). The zonal pelargonium, *P. x hortorum*, which is the highest selling
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22 63 potted flowering plants (Kessler 2007), is derived from crossing *P. zonale* with *P. inquinans*. It is
23
24 64 a plant with extensive branching that grows to a height of 15 cm (Laughner 1993). The leaves are
25
26 65 rounded (Fig. 1), pale to mid-green, with a dark reddish anthocyanin-containing zone which
27
28 66 gives rise to the botanical name, zonale (van der Walt 1993). The regal pelargonium, *P. x*
29
30 67 *domesticum* mainly derived from crossing *P. culcullatum*, *P. fulgidium* and *P. grandiflorum*, is a
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32 68 plant bearing broadly ovate to palmate, toothed, mid-green leaves (Fig. 1), and growing to a
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34 69 height of 38 - 70 cm. These floral crops are sold for their beauty, and the mere presence of any
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36 70 pest or damage detracts from its value. Insect pests, which utilize ornamental plants as a source
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38 71 of nutrition or site for oviposition, can have a major impact on the aesthetic value, economic
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40 72 quality and marketability of the plant crop. Therefore, zero-tolerance for such pests or their
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42 73 damage (to keep them blemish free) requires multiple, routine pesticide applications.
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52 74 The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera:
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54 75 Aleyrodidae), is a common pest of *Pelargonium* species (Avery 2002; Simmonds 2002),
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56 76 especially regal pelargoniums which are widely cultivated in the Mediterranean area and other
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6 77 parts of the world (Castañé and Albajes 1992; Gilrein 2004; Lis-Balchin 2002). This whitefly
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8 78 can also infest zonal pelargoniums, although reports indicate that certain cultivars are more
9
10 79 susceptible than others (Gilman and Howe 1999; Sanderson and Ferrentino 1993). Walters et al.
11
12 80 (1989a) found after analyzing both the morphological and chemical differences between the
13
14 81 insect-resistant and -susceptible zonal pelargoniums, that the tall glandular trichomes and the
15
16 82 exudate they produced were the most important factors in pest resistance. In addition, the ability
17
18 83 of the plant to express a delta-9 (omega-5) unsaturated fatty acid from the omega-5 unsaturated
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20 84 alkyl anacardic acid as an exudate on the glandular trichome head exterior was lacking in the
21
22 85 susceptible lines (Schultz et al. 1996).

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28 86 Insect-plant interactions involve complex behavioral responses of the insect to physical
29
30 87 and chemical characteristics of the host plant. The plant's physical resistance mechanisms, such
31
32 88 as trichomes and leaf morphology, have been reported to influence the interactions between
33
34 89 various phytophagous insects and their host plants (Campos et al. 2003; Malakar and Tingey
35
36 90 2000; Medeiros and Tingey 2006; Simmons et al. 2003, 2006). In particular, trichome density
37
38 91 has been shown to affect oviposition selection for whitefly species and is a major factor in
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40 92 determining preference for various host plants, including *P. x domesticum* (Chu et al. 2000;
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42 93 Heinz and Parrella 1994; McAuslane, 1996; Riley 1995; Sánchez-Peña et al. 2006). However,
43
44 94 little information is available about other morphological characters of *P. x domesticum* that might
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46 95 influence host selection by whitefly for oviposition. In developing an effective biological control
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48 96 management program for the greenhouse whitefly on *Pelargonium* cultivars, a critical stage is
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50 97 determining which host plant is most preferred for feeding and oviposition. Thus, the objective
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52 98 of the current study was two-fold: 1) to determine host preference of greenhouse whitefly
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99 between *P. x domesticum* and *P. x hortorum* and 2) to determine if these cultivars differed in the
100 types, and density of trichomes on the abaxial leaf surface.

102 **Materials and methods**

103 **Plants and insects**

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

114 **Bioassay chambers and protocol**

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

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

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

140 Host preference tests

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

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

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150 *No-choice tests*

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

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163 *Choice tests*

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6 164 Plant cultivar leaves that were healthy in appearance and similar in size were used for each
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8 165 experiment. Leaves ranging in width from 4.0 cm to 9.0 cm from cuttings were selected from
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10 166 several different stock plants of each cultivar. Each chamber containing a regal and a zonal leaf
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13 167 was considered a replicate (block). Experiments 1 and 2 each had fifteen replicates and
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15 168 experiment 3 had 19 replicates. After either 24 h (experiment 1), 48 h (experiment 2), or 72 h
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18 169 (experiment 3) exposure by the whitefly adults, the chambers were opened and the total number
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20 170 of eggs deposited on the abaxial surface of each leaf were counted.
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25 172 Type of trichomes on *Pelargonium* cultivar leaves
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28 173 To differentiate between the type of trichomes found on each cultivar, leaf samples were
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30 174 prepared prior to being observed under the scanning electron microscope (SEM). Excised leaf
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32 175 samples of each cultivar were fixed in 0.1M sodium cacodylate, 2% paraformaldehyde and 2.5%
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34 176 glutaraldehyde (pH 7.0) at 6°C for 2 h. Fixed leaves were then rinsed first with 0.1M sodium
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37 177 cacodylate buffer (pH 7.0) at 6°C for 30 min (2X), fixed using 1% OsO₄ buffer (pH 7.0) at
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40 178 ~22°C for 1 h and then rinsed with water at ~22°C for 10 min twice. The samples were
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42 179 dehydrated with 50% alcohol at ~22°C for 30 min, 70% alcohol overnight at 6°C, 90% alcohol at
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45 180 ~22°C for 10 min, 100% alcohol at ~22°C for 4 min (4X), dry absolute alcohol at ~22°C for 10
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47 181 min and then subjected to critical point drying using CO₂. The dehydrated leaves were then
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50 182 mounted on SEM stubs, sputter coated with gold to a thickness of 15 mÅ twice for 4 min at
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52 183 ~22°C, and viewed using a JEOL CF-35 (JSM 35) scanning electron microscope (JOEL Ltd.,
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54 184 Hertfordshire, UK).
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186 Trichome assessment

187 Fifteen leaves from 5-10 stock plants of each cultivar were excised and the total number of tall
188 straight non-glandular (sng; Fig. 3 and 4) and straight glandular (sg; Figs. 3 and 4) trichomes on
189 the abaxial side of each leaf were counted with a stereomicroscope (50X). To account for
190 possible variation in trichome density due to leaf size, a random equal selection of leaves ranging
191 in width from 4.0 to 9.0 cm were used for this study. To determine the trichome density, each
192 leaf was subdivided into two areas, A (outside) and B (inside) for both cultivars (Fig. 1). Within
193 each area between the secondary and tertiary veins, five disks (0.50 cm²) in each zone were
194 punched out with a cork borer from each leaf cultivar (10 disks total / leaf). The dark reddish
195 anthocyanin-containing zone subdivided areas A and B for the *P. x hortorum* leaf; the secondary
196 and tertiary veins subdivided areas A and B for the *P. x domesticum* leaf. Area A was towards
197 the leaf edge, whereas area B was between the secondary and below the tertiary veins. The total
198 number of sng and sg trichomes were counted per leaf.

199
200 Statistical analysis

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

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6 208 one cultivar was preferred by the adult greenhouse whitefly, the difference in the mean number
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8 209 of eggs per replicate per leaf cultivar was statistically analyzed using a Wilcoxon signed-rank
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10 210 test ($\alpha = 0.05$). The difference in the mean number of sng and sg trichomes and the combined
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12 211 number of sng+sg trichomes on the leaves of the two cultivars were statistically analyzed using a
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14 212 one-way ANOVA with a Sheffe's F -test ($\alpha = 0.05$) to determine if there were differences
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16 213 between cultivars.
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23 215 **Results**

25 216 No-choice tests

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28 217 The adult greenhouse whitefly laid significantly more eggs (4.3 and 7.7 times,
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30 218 respectively) on regal than on zonal leaves both at 24 h ($F = 7.83$; $df = 1, 8$; $p = 0.023$) and 48 h
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32 219 ($F = 18.2$; $df = 1, 8$; $p = 0.003$), respectively (Table 1). Analysis from a two-way ANOVA
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34 220 showed that there were significant differences in the number of eggs found between leaves per
35
36 221 cultivar ($F = 32.3$; $df = 1, 9$; $p < 0.001$, $F = 11.9$; $df = 1, 9$; $p = 0.006$), but no significant
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38 222 differences between leaves per position for regal ($F = 3.762$; $df = 4, 9$; $p = 0.089$, $F = 0.224$; $df =$
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40 223 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
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42 224 being exposed to whitefly adults for 24 h or 48 h, respectively.
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50 226 Choice tests

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52 227 There was a significant difference in the number of greenhouse whitefly eggs laid on
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54 228 leaves of the two cultivars in the choice test study (Table 2). In all the three experiments, higher
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56 229 numbers of eggs were deposited on leaves of regal cultivars than zonal leaves after 24 h ($Z = -$
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6 230 1.934; $df = 1, 29; p = 0.054$), 48 h ($Z = -2.89; df = 1, 29; p = 0.004$) and 72 h ($Z = -5.24; df = 1,$
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8 231 $37; p < 0.001$) post-exposure to the whitefly. The mean number of eggs laid on the regal leaves
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10 232 after 24, 48 and 72 h post-exposure was ~2.1, 2.5, and 1.5 times that found on the zonal leaves,
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13 233 respectively.

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18 235 Trichome density

20 236 The number of sng trichomes per 0.50 cm^2 of leaf area was significantly much higher on
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23 237 the zonal (Mean \pm SE; $49.4 \pm 0.96; n = 150$) than the regal ($28.6 \pm 1.00; n = 225$) ($F = 173.1; df =$
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25 238 $= 1, 298; p < 0.001$) leaves, whereas the number of sg trichomes was significantly higher on the
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28 239 regal cultivar ($F = 5.56; df = 1, 298; p = 0.019$) compared to the zonal, being 14.4 ± 1.2 and 11.2
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30 240 ± 0.5 , respectively (Table 3). The total trichomes (sng + sg) was considerably less ($F = 85.5; df =$
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33 241 $1, 298; p < 0.01$) on regal leaves (Mean \pm SE sng + sg; $43.1 \pm 1.5; n = 150$) than on zonal leaves
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35 242 ($60.5 \pm 1.2; n = 225$).

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40 244 Discussion

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42 245 Host selection behavior of the greenhouse whitefly adults has been divided into 3 phases: 1) host
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45 246 plant selection before landing, 2) after landing, and 3) selection of feeding and oviposition sites
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47 247 within the plant (van Lenteren and Noldus 1990). In this study, greenhouse whitefly adults were
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50 248 placed directly on a non-selected cultivar leaf surface, which eliminated some effect of factors
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52 249 that might influence host choice prior to landing. Therefore, host plant selection of regal or zonal
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55 250 leaf cultivars was based primarily on the last two phases.

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

259 Based on the number of eggs laid on the different cultivars in both choice and no-choice
260 tests, there was a definite preference for the regal leaves for oviposition by the adult greenhouse
261 whitefly. The lowest total trichome density found on regal leaves compared to zonal, may have
262 influenced and contributed to the oviposition preference of the greenhouse whitefly adults. Other
263 plant-whitefly interaction studies conducted using poinsettia (Bilderback and Mattson 1977), and
264 several species of the genus *Cucumis* (Kowalewski and Robinson 1977) also confirm that
265 trichomes play an important role in host plant acceptance by greenhouse whitefly adults. In a
266 later study using the spiraling whitefly (*Aleurodicus disperses*), Wen et al. (1994) concluded that
267 the feeding preference seemed to be affected by the leaf structure of the host plant. However,
268 there are many other factors that may have influenced the greenhouse whitefly host acceptance
269 of the pelargonium cultivars. Some of these include: 1) the chemical compounds in the plant leaf
270 tissue detected by the insect's receptors which can result in different feeding responses (Lei and
271 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

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6 273 inserted e.g. is one kind of cell more suitable as an anchor or conduit for water (Byrne et al.
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8 274 1990). Also, other general plant-mediated interactions between whiteflies are reviewed (Inbar
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10 275 and Gerling 2008). However, in this present study, we focused primarily on the influence of leaf
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13 276 trichome type and density on egg deposition of the greenhouse whitefly for each pelargonium
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15 277 cultivar.

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18 278 The greenhouse whitefly deposited more eggs on leaves of the regal cultivar than on the
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20 279 zonal in both tests, indicating that the cultivar with a lower trichome density was the preferred
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23 280 host. In a similar study, Dabrowski (1972) found in choice caged tests using whole plants (3-5
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25 281 fully developed leaves), that the greenhouse whitefly females laid an average of 97.5 eggs on *P.*
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27 282 *x domesticum* compared with a range of 0.3-1.1 eggs on *P. x hortorum* and *P. x peltatum* after
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30 283 nine days post-release. Further tests also revealed that when females were released on *P. x*
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32 284 *domesticum* and *P. x hortorum* that feeding was much more intense on *P. x domesticum*. In this
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35 285 study we used a detached leaf bioassay and our findings were similar indicating that this
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37 286 bioassay technique was comparable to using whole plants. Walters et al. (1989b) found that
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40 287 susceptible zonal cultivar lines, which were the preferred plant host for mites and insects,
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42 288 possessed a lower trichome density compared to the resistant lines. Trichome density has been
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45 289 noted to affect plant host preference of whitefly species on wild potato (Boiteau and Singh
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47 290 1988), cotton (Butler Jr. et al. 1991), hibiscus (Meagher and Estrada 1994), poinsettia
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50 291 (Bilderback and Mattson 1977; Heinz and Parrella 1994), cucumber, pepper, okra (Aslam and
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52 292 Gerba 1995), melon (Riley 1995), soybean (Lambert et al. 1995; McAuslane 1996), and
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54 293 Egyptian henbane (Salem 1995). Castañé and Albajes (1992) also noted after investigating
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57 294 preference by the greenhouse whitefly adults between regal cultivars that adults preferred those
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6 295 with fewer trichomes, especially in the first hours after release; however, in the following hours,
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8 296 trichome density was less important in their host choice. Therefore, the different types of
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10 297 trichomes on the leaf surface may also play a role in the greenhouse whitefly oviposition
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13 298 preference per cultivar.

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15 299 The presence of both types of trichomes on pelargonium leaves can influence egg
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18 300 deposition, adult or nymphal distribution and host preference of the greenhouse whitefly.
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20 301 Straight non-glandular (sng) trichomes alone may act as a physical barrier against adult
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23 302 movement and deter the female whitefly from resting on the leaf surface, thus decreasing their
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25 303 preference and ability to oviposit on the cultivar. Ovipositional resistance to whiteflies on
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27 304 *Lycopersicon* hybrids (Erb et al. 1994), *Solanum-berthaultii* (Boiteau and Singh 1988), *Nicotiana*
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29 305 *tabacum* (Neal Jr. et al. 1987) and *Cucumis melo* (Soria et al. 1996) has been attributed to
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31 306 variation in the numbers of sng and sg trichomes present on the leaves.

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35 307 Also in this study, the zonal cultivar was the least preferred by the greenhouse whitefly
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37 308 for oviposition. The lack of preference for the zonal cultivar may be attributed to the deterrent
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39 309 effect of anacardic acids produced by the sg trichomes in combination with the high density of
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41 310 sng trichomes present on the leaf (Fig 3b). Dabrowski (1972) proposed that a chemical factor
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43 311 must be present on *P. x hortorum* that acts as a feeding deterrent to the greenhouse whitefly and
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45 312 a physiological inhibitor must be operative as well. The exudate expressed from sg trichomes on
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47 313 the surface of some zonal cultivars has been suggested as a primary factor in the two spotted
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49 314 spider mite, *Tetranychus urticae* (Koch) resistance mechanism (Gerhold et al. 1984). Walters et
50
51 315 al. (1989a, b) demonstrated that the sg trichome exudate expressed in the zonal cultivar was a
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53 316 critical factor in *Pelargonium* resistance to the foxglove aphid, *Acyrtosiphon solani*
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317 (Kaltenbach) and noted that resistant lines have higher densities of sg trichomes that express the
318 anacardic acids as exudates on the trichome exterior. In another study, Salem (1995) found a
319 high degree of resistance with Egyptian henbane, *Hyoscyamus muticus* (Solanaceae) by another
320 whitefly, *Bemisia tabaci* and the effect was associated with exudates produced by trichomes on
321 the leaf surface. This resistant characteristic associated with glandular exudates has been utilized
322 for developing plant cultivars more resistant to the greenhouse whitefly and other aleyrodids
323 (Maliepaard et al. 1995). Lastly, based on genetic studies, the gene responsible for the production
324 of omega-5 anacardic acids, a class of secondary compounds derived from fatty acids and
325 expressed only on the sg trichome exterior of zonal cultivars, has been shown to be necessary for
326 pest resistance (Schultz et al. 1996).

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

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

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350 populations from Biological Crop Protection Ltd, UK. *Pelargonium* leaves were prepared for
351 SEM and photographed by Gwen Nneji, EM technician at Birkbeck, University of London.

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6 476 **Figure captions:**

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9 478 Figure 1. Diagram of leaf bioassay setup for each cultivar, *Pelargonium x hortorum* (Zonal leaf)
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11 479 and *P. x domesticum* (Regal leaf). A Gilson pipette tip (p) was secured to the end of each
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13 480 trimmed cultivar petiole and tamped with cotton prior to being inserted into the Petri dish
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16 481 bottom. Pipette tip with water illustrates how the leaf petiole will remain wet during each test.
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18 482 Each blade was subdivided into areas A (outside) and B (inside) where leaf discs (0.05 cm²)
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21 483 were removed for determining trichome densities per cultivar. The dark reddish (color version
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23 484 illustrated on-line) anthocyanin-containing zone subdivided areas A and B for the *P. x hortorum*
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26 485 leaf; the secondary and tertiary veins subdivided areas A and B for the *P. x domesticum* leaf.
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28 486 Area A was towards the leaf edge, whereas area B was between the secondary and below the
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31 487 tertiary veins.

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35 489 Figure 2. Side view of a sealed bioassay chamber (two Petri dish bottoms) used to determine
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38 490 selectivity of *Trialeurodes vaporariorum* on *Pelargonium* cultivars placed on a 50 mL tri-corner
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40 491 polypropylene beaker filled with enough water to allow the tips to be partially immersed. To
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43 492 minimize condensation within the chamber and possible drowning of the whitefly adults, a strip
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45 493 of filter paper (~270 mm x 8.0 mm) was placed inside each dish. Prior to sealing the two halves
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48 494 (Petri dish bottoms with leaf secured) of the chamber together, 10 randomly selected whitefly
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50 495 adults (unknown ratio of male and female) were introduced into the chamber. Adults were placed
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53 496 on the abaxial side of a randomly selected leaf (Orientation I or II). Leaves randomly chosen
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55 497 (Orientation I or II) were used to minimize oviposition effects of the whitefly adults relative to
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57 498 the light source located above the individual bioassays when placed in the growth chamber. All
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499 bioassays were oriented perpendicular to the door of the growth chamber, only the leaf side
500 (Orientation I or II) was randomly designated prior to sealing the bioassay chamber. Figure is
501 drawn to scale and color version illustrated on-line.

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

507
508 Figure 4. a) SEM photomicrograph of straight glandular (sg) trichome. Note lack of exudate
509 expression on sg trichome. b) SEM photomicrograph of straight non-glandular (sng) trichomes
510 surrounding a straight glandular trichome found on abaxial side of *Pelargonium x domesticum*.

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Figure 1
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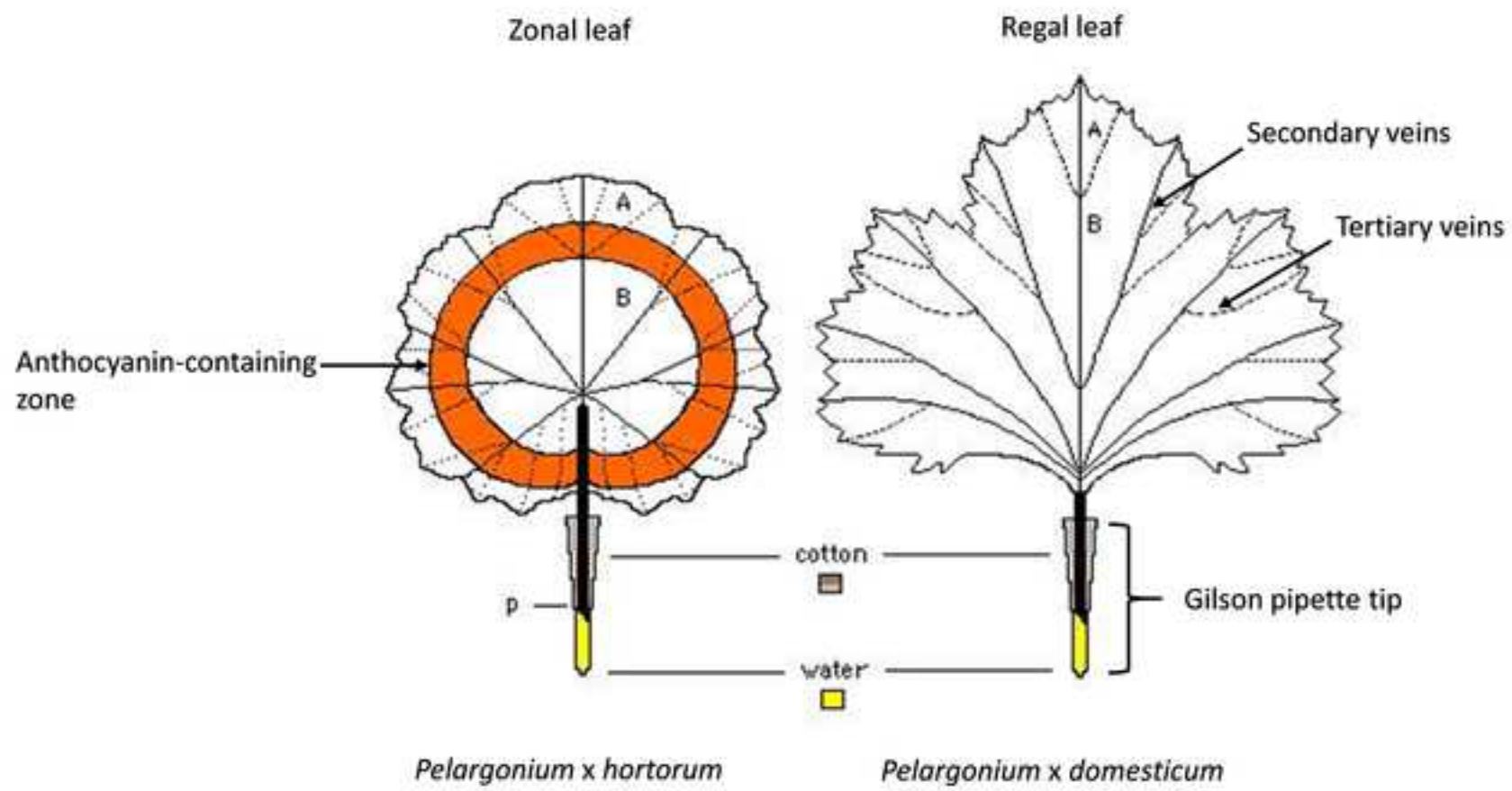


Figure 2
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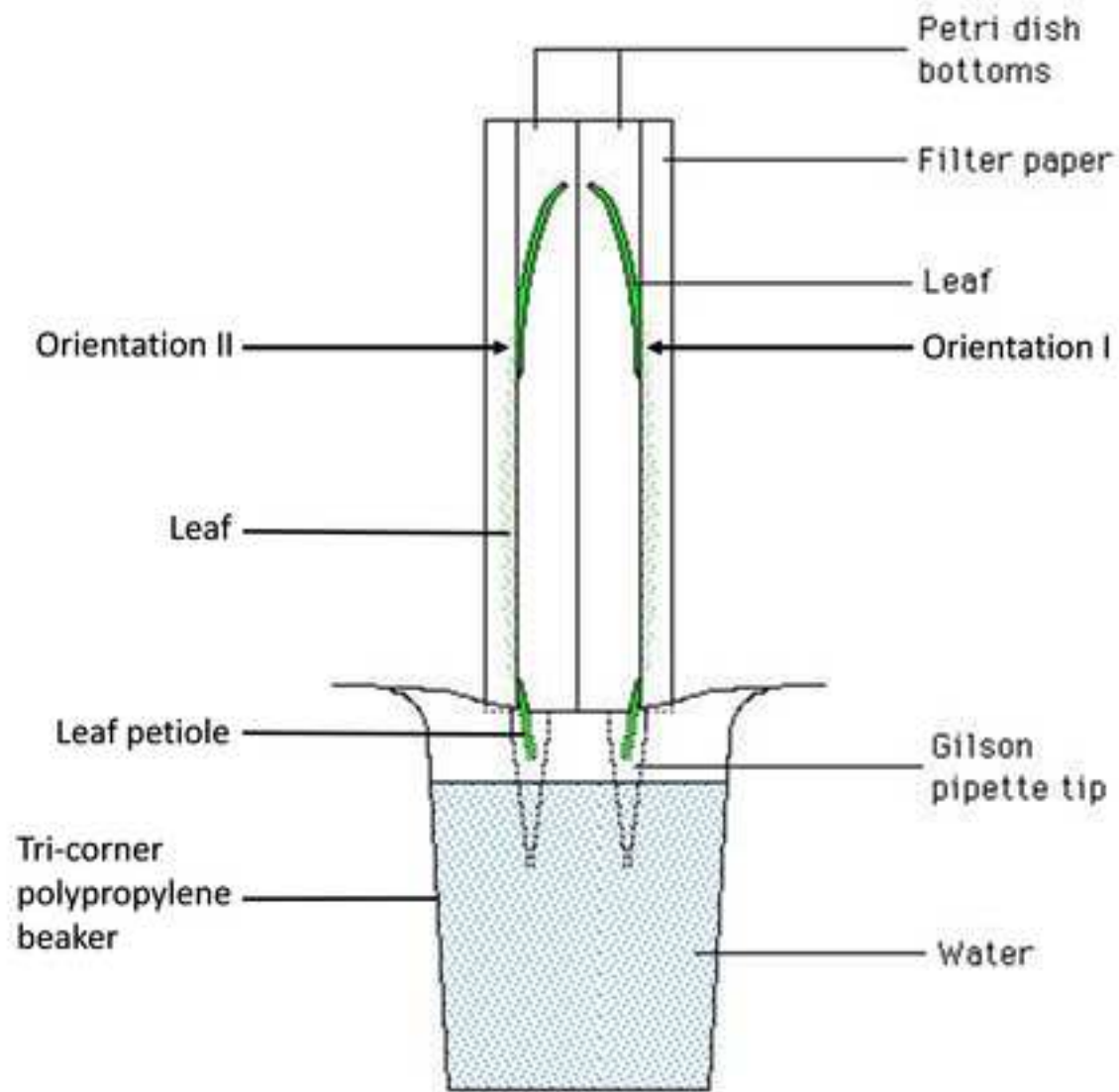


Figure 3
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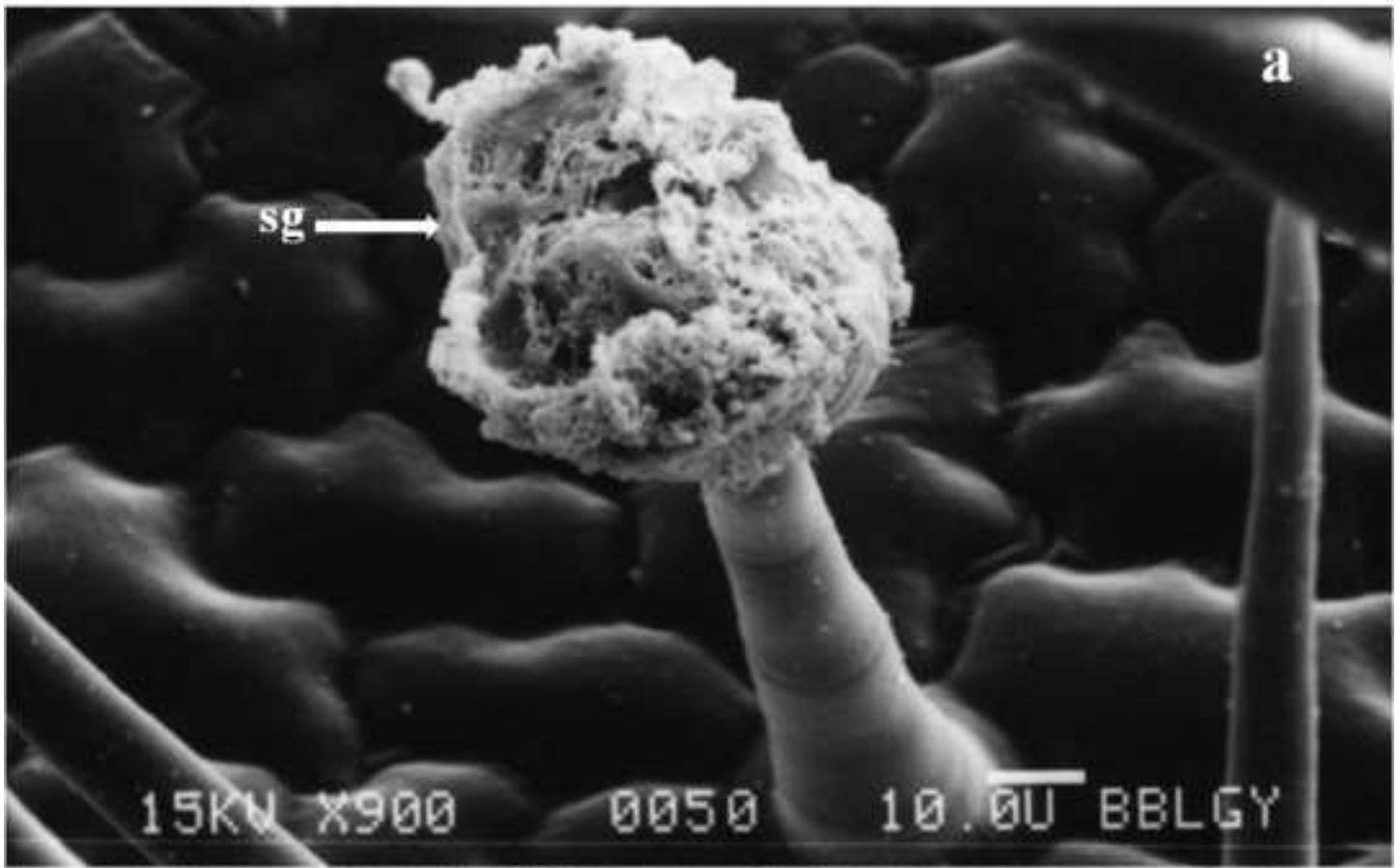


Figure 4
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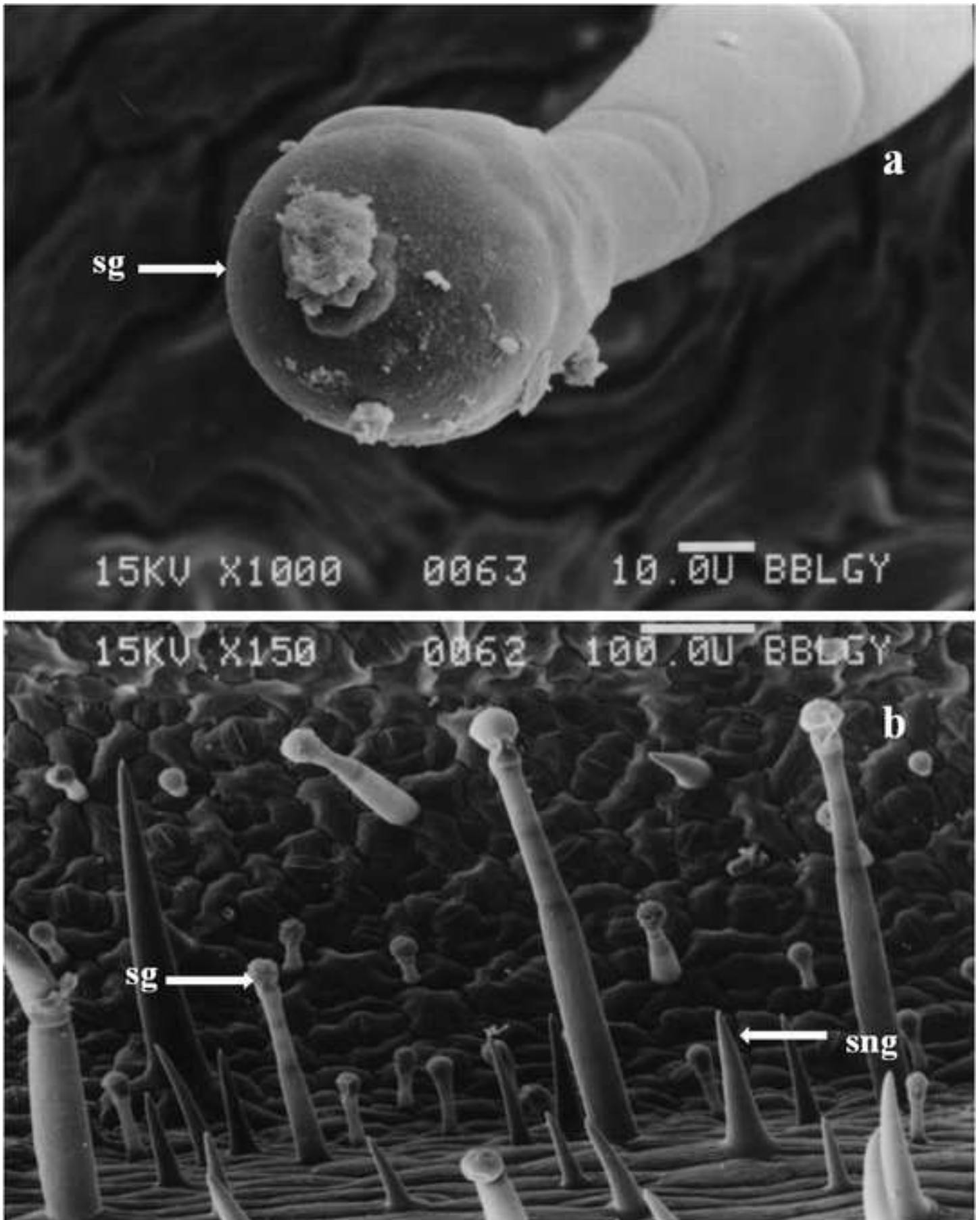


Table 1. Mean total number (\pm SE) of eggs deposited for no-choice tests by whitefly adults on excised Regal vs. Zonal *Pelargonium* leaves in bioassay chambers after being exposed for 24 (Experiment 1) or 48 (Experiment 2) hours (h).

Cultivar	Mean total number \pm SE of eggs deposited/chamber	
	Experiment 1 (24 h, $n = 5$)	Experiment 2 (48 h, $n = 5$)
Regal	19 \pm 4	37 \pm 8
Zonal	4 \pm 1	5 \pm 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 (\pm 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).

Cultivar	Mean number \pm SE of eggs deposited / leaf cultivar		
	Experiment 1 (24 h, $n = 15$)	Experiment 2 (48 h, $n = 15$)	Experiment 3 (72 h, $n = 19$)
Regal	7.6 \pm 2.7	11.7 \pm 4.5	37.8 \pm 6.8
Zonal	3.7 \pm 1.4	4.7 \pm 1.6	25.1 \pm 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 (\pm SE) per 0.05 cm² 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.

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