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Arthropod Resistance in a Petunia Ecotype with Glabrous Leaves

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***Abstract*. A novel ecotype of *Petunia integrifolia* subsp. *integrifolia* var. *depauperata* with glabrous leaves was found near the town of Torres, Brazil, and hybridized with *P.* ×*hybrida*. In the F_1 and F_2 generations, the glabrous leaf trait was quantitatively inherited with high heritability. The absence of trichomes was not associated with the decrease in the resistance of the glabrous-leaved species and hybrids to aphids, but was correlated with a lower resistance to spider mites.**

The garden petunia (*Petunia* ×*hybrida*) is a complex hybrid of *P. integrifolia* (Hook.) Schinz et Thell. and *P. axillaris* (Lam.) B.S.P. that originated ≈1825 (Sink 1984). *Petunia axillaris* is a uniform species with white flowers, while *P. integrifolia* is a diverse purple-flowered species with many ecotypes. Several ecotypes within the *P. integrifolia* complex have been given independent taxonomic status (Ando and Hashimoto, 1993, 1994, 1995, 1996, 1998; Smith and Downs, 1964, 1966; Tsukamoto et al., 1998; Wijsman, 1982).

Ecotypes in the *P. integrifolia* species complex have unique characteristics that could be useful in petunia improvement, such as glabrous leaves. Garden petunias have leaves and stems that are covered with glandular trichomes that have a sticky and foul smelling exudate. These trichomes produce an exudate that is composed of several sugar ester compounds that are very effective deterrents against arthropods (Chortyk et al., 1997; Isman et al., 1997). We collected an unusual ecotype of *P. integrifolia* subsp. *integrifolia* var. *depauperata* growing on the coastal sand dunes in the state of Rio Grande do Sul near the town of Torres, Brazil, that had glabrous leaves. The objective of this project was to introduce the glabrous leaf trait into the garden petunia. This paper describes the inheritance of this glabrous leaf trait and its effect on arthropod resistance.

Materials and Methods

Plant material. A single plant of *P. integrifolia* subsp. *integrifolia* var. *depauperata* was collected from the coastal sand dunes of the state of Rio Grande do Sul near the town of Torres, Brazil. This plant (‘Torres Ecotype’) had glabrous leaves and was self- incompatible. A genetically marked plant of the garden petunia *P.* ×*hybrida* ‘Red Magic Inbred’ (Griesbach, 1996) was used in breeding with the ‘Torres Ecotype’. All plants were vegetatively propagated by shoot-tip cuttings and were grown and pollinated using standard horticultural practices in greenhouses at Beltsville, Md.

Genetic analysis. An interspecific F_1 hybrid population of 25 plants was created between the ‘Torres Ecotype’ and ‘Red Magic Inbred’. Chromosomal pairing in meiotic cells was observed in five plants of this population using standard techniques (Snow, 1955), and analyzed using the Jackson Model (Jackson, 1991) to predict chiasmata distribution. Five individual plants from the F_1 population were intercrossed in all combinations. The seeds from these crosses were bulked to create the F_2 population. A random sample of 20 F_2 plants was selected for further analysis.

Leaf trichome density and resistance to the green peach aphid (*Myzus persicae* Sulzer) and the twospotted spider mite (*Tetranychus urticae* Koch) were characterized for all of 20 F_2 plants. Trichome density (i.e., number of trichomes per 0.25-cm^2 leaf area) was determined using a dissecting microscope (Zeiss Stemi DRC, New York). Measurements were taken from a single leaf from five plants of each clone.

Data from a replicated (five replicates) clonal trial of the 20 F_2 plants were used to determine the broad sense heritability [$H = \sigma^2_G / (\sigma^2_G + \sigma^2_E)$] of trichome density using the method of Burton and deVane (1953). The benefit of a clonal trial lies in the immediate fixation of all genetically determined characteristics. This allows for a significantly lower sample size than is typically used. From the analysis of variance (ANOVA), we obtained the error mean square that estimated σ^2_E, and the clones mean square that estimated $\sigma^2_E + n\sigma^2_G$ where n is the level of replication used.

Arthropod screening. Leaves of ‘Red Magic Inbred’, ‘Torres Ecotype’, a single randomly selected F_1 hybrid, and five individual glabrous-leaved F_2 hybrids were screened for resistance against the green peach aphid and the twospotted spider mite. *Calibrachoa linoides* (Sendtn.) Wijsman, syn. *Petunia linoides* Sendtn. (Wijsman, 1990), was used as the susceptible control when testing for the aphid studies and *Solanum melongena* var. *esculentum* (L.) Nees. (eggplant) was used as the susceptible control for the spider mite studies. A single leaf from each treatment plant was placed within a 5-cm petri dish, with five petri dishes per plant. Either one adult female spider mite or two adult female aphids were released into each petri dish. The petri dishes were maintained on a laboratory bench at room temperature and with cool-white fluorescent room lighting.

Green peach aphid. The number of dead adults, the total number of nymphs produced, and the number of dead nymphs were counted after 72 h. The experiment was performed three times. For each petri dish, adult mortality was scored as 100% if both aphids were dead, as 50% if only one aphid was dead, and as 0 if both were alive at 72 h. Adult mortality and the total number of dead nymphs in the petri dish were analyzed as dependent variables.

Data for adult mortality were analyzed using ANOVA, with plant (i.e., treatment) as the main effect and experimental replicate as the block effect. An iterated weighted-least squares technique was included in the analyses to correct for lack of homogeneity of variances. Data for nymphal mortality were square-root transformed before analysis to correct for lack of normality. The total number of nymphs that developed within the petri dishes was included in the analysis as a covariate to correct for differences in this number among petri dishes ($F = 154.4$, $P < 0.01$). The type III mean square for experimental replicate by plant interaction was used as the error term in tests of hypotheses and in the calculation of SE and probabilities.

For all analyses, tests of significance were made at $\alpha < 0.05$. Means for each dependent variable, for each plant treatment, were obtained using the LSMEANS statement within SAS (SAS Institute, 1996). Means for nymph mortality were adjusted for the covariate. Means were compared using the Bonferroni *t* test with an adjusted $P = 0.005$.

Twospotted spider mite. Each female was identified as alive or dead and the number of eggs laid was recorded after 48 h. The experiment was performed 16 times. For each petri dish, adult mortality was scored as 100% if the mite was dead and as 0 if alive at 48 h. Data for eggs oviposited were square-root transformed before analysis to correct for lack of normality.

Mortality of spider mites and the number of eggs laid were analyzed using a mixed model ANOVA (Proc Mixed, SAS Institute, 1996) with the plant (i.e., treatment) as the main effect, and experimental replicate and experimental replicate by plant interaction as random effects. The experiment was treated as a randomized complete-block design with unbalanced data.

For all analyses, tests of significance were made at $P < 0.05$. Means for each dependent

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Table 1. The number of different meiotic associations at diakinesis and metaphase I within a single F_1 hybrid plant between *P. integrifolia* subsp. *integrifolia* var. *depauperata* 'Torres Ecotype' and *P. ×hybrida* 'Red Magic Inbred'. The model of Jackson (1991) was used to predict chiasmata distribution. For NR: $\chi^2 = 0.0382$, $P = 0.975$. For R: $\chi^2 = 158.2$, $P \leq 0.005$.

No.	Observed			Expected (NR)[z]			Expected (R)		
Cells	I[y]	cI	oII	I	cII	oII	I	cII	oII
38	2	213	52	0	215	51	87	128	94

[z]NR = normal, nonrandom chiasmata distribution; R = mutant, random chiasmata distribution.
[y]I = univalent; cII = chain bivalent with 1 chiasmata; oII = circle bivalent with two chiasmata.

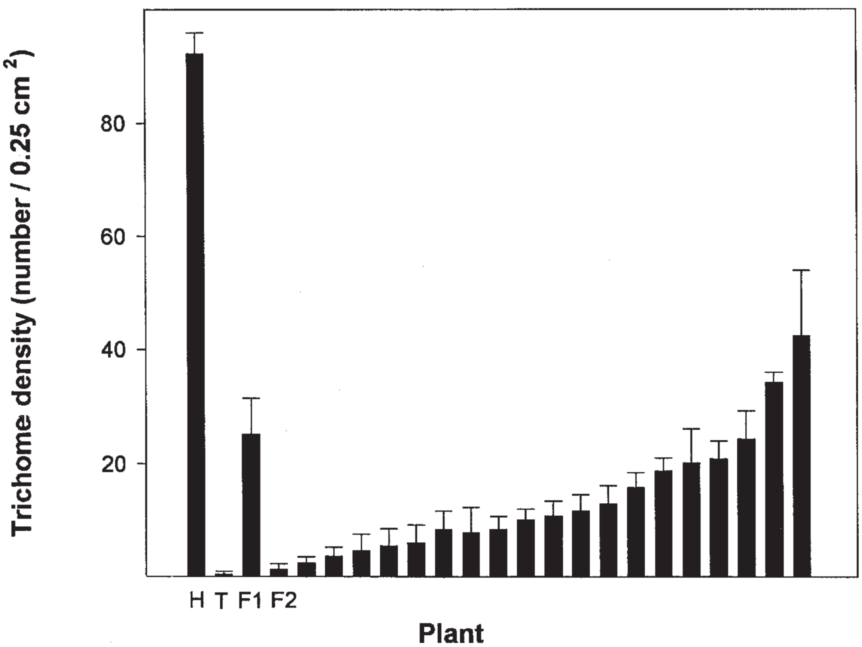

Fig. 1. The mean trichome density of *P.* × *hybrida* 'Red Magic Inbred' (H), *P. integrifolia* subsp. *integrifolia* var. *depauperata* 'Torres Ecotype' (T), a single F_1 hybrid plant (F_1), and 20 F_2 segregants (F_2). Each measurement was from a single leaf from five plants of each clone. The error bars indicate SD of the mean.

Table 2. Resistance of *P. ×hybrida* 'Red Magic Inbred', *P. integrifolia* subsp. *integrifolia* var. *depauperata* 'Torres Ecotype', the single F_1 hybrid plant, five glabrous-leaved F_2 plants, and the susceptible *C. linoides* to aphid (*Myzus persicae*) infestation. Means within each column that are followed by the same letter are nonsignificant (Bonferroni *t* test; adjusted $P = 0.05$).

Plant	Adult mortality (%)	Nymphal mortality[z] (#)
P. ×hybrida	100 a	1.5 ± 0.2[y] a
P. integrifolia	94.2 ± 3.0 a	1.5 ± 0.2 a
C. linoides	22.5 ± 5.3 b	0 b
F_1	97.1 ± 2.6 a	1.7 ± 0.2 a
F_2-1	100 a	2.0 ± 0.2 a
F_2-2	100 a	2.0 ± 0.2 a
F_2-3	96.5 ± 2.6 a	2.0 ± 0.2 a
F_2-4	100 a	1.8 ± 0.2 a
F_2-5	100 a	1.6 ± 0.2 a

[z]Square-root (x + 0.5)
[y]Mean ± SE.

Table 3. Resistance of *P. ×hybrida* 'Red Magic Inbred', *P. integrifolia* subsp. *integrifolia* var. *depauperata* 'Torres Ecotype', a single F_1 hybrid plant, five glabrous-leaved F_2 plants, and the susceptible *S. melongena* to the twospotted spider mite (*Tetranychus urticae*). Means within each column that are followed by the same letter are nonsignificant (contrasts at $P = 0.05$).

Plant	Adult Mortality (%)	Oviposition[z] (#)
P. ×hybrida	94.6 ± 7.0[y] a	0.9 ± 0.2 c
P. integrifolia	43.4 ± 7.0 d	1.6 ± 0.2 b
S. melongena	20.8 ± 7.3 e	2.8 ± 0.2 a
F_1	58.7 ± 6.8 c	1.5 ± 0.2 b
F_2-1	63.0 ± 6.8 bc	1.5 ± 0.2 b
F_2-2	74.0 ± 6.8 b	1.1 ± 0.2 c
F_2-3	72.5 ± 6.8 b	1.5 ± 0.2 b
F_2-4	48.4 ± 6.8 cd	1.7 ± 0.2 b
F_2-5	67.2 ± 6.8 bc	1.5 ± 0.2 b

[z]Square root (x + 0.5).
[y]Mean ± SE.

variable, for each plant (i.e., treatment), were obtained using the LSMEANS statement within SAS (SAS Institute, 1996). Means were compared using contrasts at $P = 0.05$.

Results and Discussion

Five F_1 hybrids between 'Torres Ecotype' and 'Red Magic Inbred' were analyzed. All five plants exhibited the same leaf morphology and not significantly different trichome number (data not shown). A single plant was randomly selected for further studies. This F_1 plant was completely fertile and exhibited no preferential chromosomal pairing (Table 1). Thus, we assumed unrestricted gene flow between the wild species and the garden petunia.

Leaf trichome density appeared to be quantitatively inherited (Fig. 1). The F_1 hybrid between the 'Torres Ecotype' and 'Red Magic Inbred' was intermediate between the parents. However, the F_1 mean (≈25) was closer to the mean of the 'Torres Ecotype' (≈1) than to the mean of the 'Red Magic Inbred' (≈90). This suggests that the glabrous trait was partially dominant. For the variable F_2 population, the mean trichome density was 14 trichomes per 0.25 cm^2, the genetic variance was 112 and the heritability was 0.88.

Mortality of aphid adults ($F = 26.3$; df = 8,124; $P < 0.01$; N = 135) and of nymphs ($F = 11.0$; df = 8,107; $P < 0.01$; N = 135) was not significantly different among the 'Red Magic Inbred', 'Torres Ecotype', the single F_1 hybrid plant, and the five glabrous-leaved F_2 plants (Table 2). The glabrous-leaved plants had the same level of resistance to aphids as plants of the hirsute-leaved garden petunia.

Mortality of female spider mites ($F = 12.8$; df = 8,115; $P < 0.01$; N = 643) and oviposition ($F = 16.0$; df = 8,116; $P < 0.01$; N = 644) were significantly different among the 'Red Magic Inbred', 'Torres Ecotype', the single F_1 hybrid plant, and the five glabrous-leaved F_2 plants (Table 3). Mortality was significantly higher on 'Red Magic Inbred' than on the others. In addition, those females that remained alive on 'Red Magic Inbred' laid significantly fewer eggs than females on the other plants.

The glabrous-leaved plants were less resistant to spider mite infestation than plants of the garden petunia. Among the F_2 plants, significant differences occurred in spider mite mortality that could not be directly attributed to trichome density since all the F_2 plants had a similar trichome density. Adult spider mite mortality on the F_2-2 and F_2-3 segregants was significantly higher than that on F_2-4 segregant (Table 3).

The introgression of the glabrous-leaved trait from *P. integrifolia* subsp. *integrifolia* var. *depauperata* 'Torres Ecotype' into the garden petunia could improve the horticultural value of the crop without adversely affecting aphid resistance. The introgressed petunias, however, might be more susceptible to spider mite infestation.

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