Inheritance of Biochemical and Morphological Characters Associated with Two-spotted Spider Mite Resistance in *Pelargonium ×hortorum*

Richard Grazzini¹

Intercollege Graduate Program in Genetics, The Pennsylvania State University, University Park, PA 16802

Donald Walters²

DEKALB Plant Genetics, 62 Maritime Drive, Mystic, CT, 06355

Jody Harmon²

Department of Entomology, The Pennsylvania State University, University Park, PA 16802

David J. Hesk³

Schering Plough, 2015 Galloping Hill Road, Kenilworth, NJ 07233

Diana Cox-Foster⁴

Department of Entomology, The Pennsylvania State University, University Park, PA 16802

June Medford⁵

Department of Biology, The Colorado State University, Fort Collins, CO 80523

Richard Craig⁶

Department of Horticulture, The Pennsylvania State University, University Park, PA 16802

Ralph O. Mumma⁷

Department of Entomology, The Pennsylvania State University, University Park, PA 16802

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ABSTRACT. Diploid zonal geraniums (*Pelargonium ×hortorum*) are able to resist attack by small arthropod pests such as the two-spotted spider mite (*Tetranychus urticae* Koch) when exudate produced by tall glandular trichomes contains a high percentage of ω^5 -unsaturated anacardic acids. Trichomes of susceptible plants exude primarily saturated anacardic acids. Inbred mite-resistant and -susceptible geraniums were reciprocally crossed and the F_1 , F_2 , and backcross generations were examined for anacardic acid composition and trichome density. Selected F_2 plants were bioassayed for resistance to two-spotted spider mites. High concentrations of ω^5 -unsaturated anacardic acids in resistant plants are conditioned by a single dominant allele. We propose that inheritance of tall glandular trichome density can be controlled by a small number of loci (possibly as few as one) exhibiting codominance. F_2 , with low densities of tall glandular trichomes and producing ω^5 -unsaturated anacardic acids, displayed effective resistance to two-spotted spider mites as measured by mite mortality and fecundity. A genetic model for the biosynthesis of anacardic acids is proposed.

The garden geranium (*Pelargonium ×hortorum* L.H. Bailey, 2n = 18, Geraniaceae) is an interspecific hybrid complex of unknown parentage, although many believe it to have been developed in cultivation >250 years ago (Laughner, 1992) from members of the section *Ciconium*, primarily *P. inquinans* (L.) L'Herit. and *P. zonale* (L.) L'Herit. (Moore and Hyppio, 1982; van der Walt, 1977). Other *Pelargonium* species likely to have been involved in

the development of *P. ×hortorum* include *P. acetosum* (L.) L'Herit., *P. frutetorum* R.A. Dyer, and *P. stenopetalum*, also referred to as *P. Burtoniae* Bolus. (Moore and Hyppio, 1982).

Many of the *Pelargonium* species believed to contribute to *P. ×hortorum* possess glandular trichomes as part of their epidermis (Grazzini et al., 1995; Grossman, 1982; van der Walt, 1977). The presence of multicellular glandular trichomes on the leaf surface has been associated with arthropod resistance in a number of crop species (Duffey, 1986; Kelsey et al., 1984; Stipanovic, 1983). Glandular trichomes synthesize and secrete a variety of allelochemicals (Duffey, 1986; Fahn, 1979). Small-pest resistance in geraniums has been associated with glandular trichome density (Craig et al., 1986; Walters et al., 1989a, 1989b). The *P. ×hortorum* that we examined possessed two distinct classes of glandular trichomes, short (30 to 60 μ m in high) and tall (100 to 400 μ m high). There was no apparent relationship in short glandular trichome density between resistant and susceptible inbreds. Of the five related diploid lines examined, resistant and susceptible

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¹Centre Analytical Laboratories, 3048 Research Dr., State College, PA, 16801.

²Research scientist.

³Principal scientist.

⁴Associate professor of entomology.

⁵Assistant professor of biology.

⁶Professor of plant breeding; to whom requests for reprints should be addressed.

⁷Distinguished professor of environmental quality.

Fig. 1. Molecular structures of the four major AnA from glandular trichome exudate of *Pelargonium* inbreds.

inbreds differed in the density of tall glandular trichomes (TGT), with resistant inbreds possessing a high density and susceptible inbreds a low density.

TGT of resistant inbreds produced a sticky exudate, while TGT of susceptible inbreds produced no exudate or an amorphous, non-sticky exudate (Walters et al., 1989a). Walters et al. (1989b, 1990a) demonstrated that the physical aspect of the anacardic acid (AnA) mediated resistance mechanism was critical to its effectiveness against foxglove aphids (*Acyrtosiphon solani* Kalténbach). The chemical composition of the trichome exudate could be used to predict the bioassay response of the plant examined. Plants that had an exudate composition like that of the resistant parent—in this case, the F_1 —were resistant to foxglove aphid herbivory as were the original resistant inbreds.

Gerhold et al. (1984) demonstrated that TGT exudate from resistant inbred geraniums consisted mainly of AnA possessing 22- and 24-carbon atoms. AnA are amphipathic phenolic acids (alkylated salicylic acids) characteristically present in many of the Anacardiaceae, including cashew (*Anacardium occidentale* L.) (Tyman et al., 1984), pistachio (*Pistachia vera* L.) (Yalpani and Tyman, 1983), and *Rhus* (Bhattacharya et al., 1987). AnA have been identified in only a few plant species outside of the Anacardiaceae, including *Ginkgo biloba* L. (Ginkgoaceae) (Gellerman and Schlenk 1968), *Knema elegans* Warb (Myristaceae) (Spencer et al., 1980), and *Amphypterygium adstringens* Schiede ex Schect (Julianaceae) (Navarette et al., 1989). Gerhold et al. (1984) demonstrated that AnA were the biologically active components of the TGT exudate of geraniums exhibiting resistance to two-spotted spider mites (*Tetranychus urticae* Koch) and that the TGT exudate from resistant inbreds exhibited a physical resistance mechanism, acting as a sticky trap, and a biochemical resistance mechanism, acting as an arthropod toxicant. Subsequent investigations by Walters et al. (1989a) of resistant and susceptible inbreds indicated that resistant plants produce mostly ω^5 -unsaturated AnA, while susceptible lines produce mostly saturated AnA (Fig. 1). We use the omega (ω) nomenclature for these AnA for convenience, since the position of the double bond appears to be consistently at the ω^5 position in the alkyl chains of the resistant TGT exudate, regardless of chain length. Walters et al. (1990c) demonstrated that resistant and susceptible inbreds were true-breeding for AnA composition and that the F_1 between resistant and susceptible parents developed a TGT exudate with an AnA composition characteristic of the resistant parent with respect to the presence of ω^5 -AnA. AnA are biosynthesized from fatty acid precursors, and unsaturated AnA arise from unsaturated fatty acids

(Hesk et al., 1992; Walters et al., 1990b). The ω^5 -unsaturated AnA presumably are derived from the unique ω^5 -unsaturated 16- and 18-carbon fatty acids located only in the TGT of resistant genotypes (Grazzini, 1993; Walters et al., 1990b; Yerger et al., 1992).

The inheritance of trichome density has been examined in a small number of crops. In cotton (*Gossypium*), five loci condition the presence of nonglandular trichomes (Lee, 1985). Six of the seven alleles identified at these five loci are expressed as dominant alleles reducing the density of nonglandular trichomes. In

common bean (*Phaseolus vulgaris* L.), either a single major gene or duplicate recessive epistatic genes control the presence or absence of pubescence (nonglandular trichomes) on the abaxial leaf surface (Zaiter et al., 1990). Trichome density in those bean plants possessing trichomes is a quantitative trait. In tobacco (*Nicotiana tabacum* L.), partially dominant alleles at three loci are responsible for the absence of glandular trichomes (Johnson et al., 1988; Nielsen et al., 1982) so that a recessive homozygote at any of the three loci results in the presence of trichomes. In red clover (*Trifolium pratense* L.), a single recessive nuclear gene was determined to be responsible for the absence of nonglandular trichomes (Bowley and Lackey, 1990) and was independent of the inheritance of glandular trichomes. In soybean (*Glycine max* (L.) Merr), a single recessive gene confers a glabrous condition, resulting in the absence of glandular and nonglandular trichomes. A similar single recessive gene mutation resulting in the absence of nonglandular trichomes from stems was induced by Marks and Feldman (1989) in *Arabidopsis thaliana* L.. The inheritance of glandular trichomes has also been examined in potato (Mehlenbacher et al., 1983) and tomato (Lemke and Mutschler, 1984).

Using leaf-disk bioassays, Winner (1975) determined that resistance to the two-spotted spider mite in one genotype of *P. hortorum* was genetically controlled, and, subsequently, we have used this leaf disk bioassay to assess mite resistance. Genetic studies were begun in 1988 by D.S.W. to investigate the inheritance of biochemical and morphological components of the AnA mediated small-pest-resistance phenomenon in *Pelargonium*. The results reported herein are a continuation of those studies. In this report, we use highly inbred, resistant and susceptible parental lines and examine F_1 , F_2 , and backcross generations to determine inheritance of ω^5 -unsaturated AnA, TGT densities, and the expression of mite resistance in geraniums. Furthermore, we use selected F_2 differing in AnA composition and TGT density to examine the effects of these variables on spider mite mortality and fecundity.

Materials and Methods

PLANTS. Individual selections of inbred diploid geraniums previously determined by bioassay to be two-spotted spider mite resistant (71-17-7) or susceptible (71-10-1) (Walters et al., 1990c) were crossed reciprocally to produce F_1 . The inbred parents were true-breeding selections from the Penn State geranium genetics program. F_1 were self-pollinated to produce an F_2 generation and backcrossed to the resistant and susceptible parents to produce

BCR and BCS generations, respectively. Backcrosses used in these studies were produced using the identical $F_1(R \times S)$ selection as the pollen parent. The susceptible inbred parent (71-10-1) was the same as that used by Winner (1975) and was maintained clonally. The resistant inbred parent (71-17-7) was a sibling of that used by Winner (71-18-6); subsequent research (Walters et al., 1991) indicated that 71-18-6 was a temperature-sensitive, resistant line, displaying a susceptible phenotype at $>25.5^\circ\text{C}$. The resistant inbred parent used in this study (71-17-7) was temperature stable at the range of temperatures maintained during this study and generates a chemically homogeneous TGT exudate under such conditions.

PLANT PRODUCTION AND GROWTH CONDITIONS. Seeds of all generations were manually scarified, sown in commercial 50-slot plug trays filled with once-leached commercial growing media (Metro Mix 215; W.R. Grace Co., Fogelsville, Pa.), irrigated, and placed under artificial illumination in growth chambers for 1 week before being moved into the greenhouse. Seedlings were maintained in the plug trays throughout the experiment (6 to 8 weeks) and were fertilized and irrigated as needed. Plug trays were randomized on the greenhouse bench daily to minimize the effects of environmental variation. Within the generations described here, about half of the plants were analyzed for trichome density first and chemical composition about 2 weeks later. The remainder were analyzed in the reverse order, with the chemical composition analysis performed first.

CHEMICAL ANALYSES. AnA composition of both parents and all progeny was examined by gas-liquid chromatography (GLC), using the method of Walters et al. (1989a). The third and fourth fully expanded leaves below the visible shoot apex were used for analysis, choosing leaves with a healthy appearance and of moderate size.

The two leaves removed for chemical analysis were cut into small pieces and placed in a 20-mL vial. Thirteen milliliters of 0.008 M carbonate–0.077 M bicarbonate, pH 9, buffer was added and the leaves were placed in an ultrasonication bath for 20 min to remove the exudate from the leaf exterior. The leaf fragments were then removed from the buffer with forceps, and the excess buffer was squeezed into the vial. The buffer containing the exudate was acidified to pH <2.0 using concentrated HCl, and the exudate was partitioned into 5 mL of dichloromethane (DCM). The solvent was evaporated to dryness and ethereal diazomethane was added for 10 min to form AnA methyl esters. The samples were then transferred to a disposable test tube and again evaporated to dryness. Each sample was redissolved in 50 mL DCM and immediately injected into a gas chromatograph using the conditions of Walters et al. (1989a). Walters (1988) previously determined that the bicarbonate buffer extraction used in these studies provided a representative sample of the total AnA composition as determined by total extraction with DCM. The chemical structure of the ω^5 -AnA had previously been determined by mass spectral analysis of the dimethyl disulfide derivatives (Walters et al., 1989a). AnA in the resistant genotype were cleanly resolved by GLC; however, some peaks were overlapping in the susceptible genotype.

CLASSIFICATION BY ANA COMPOSITION: RESISTANT VS. SUSCEPTIBLE PHENOTYPES. Peak areas (GLC) were determined for the four major AnA (22:0, 22:1 ω^5 , 24:0, 24:1 ω^5) found in geranium TGT exudate. Although we made every attempt to standardize the plant growing conditions, the extraction procedures, and the GLC analysis to make quantitative comparisons possible, the amount of exudate recovered from different leaves on the same plant differed even when analyzed simultaneously. As a result, the phenotypic determinations used for the genetic analyses presented in this

report were made in terms of relative (percent) composition. In most populations, there was greater variability within the 24-carbon components than within the 22-carbon AnA. Therefore, we used the percentage of 22:1 ω^5 -unsaturated AnA (of all 22-carbon AnA) as a means to distinguish susceptible from resistant phenotypes in the F_2 and backcross progenies. Based on comparison of the AnA composition of the parent, plants whose TGT exudate contained $\geq 60\%$ 22:1 ω^5 -unsaturated AnA were classified as resistant; plants with $<60\%$ 22:1 ω^5 -unsaturated AnA in their TGT exudate were classified as susceptible. The 60% boundary was chosen arbitrarily; however, no plant classified as either resistant or susceptible contained percentages close to 60%.

CLASSIFICATION BY TGT DENSITY. TGT densities of the parents and F_1 were determined using the third and fourth fully expanded leaves below the visible shoot apex. A thin plastic template with a 2-mm-wide slot was placed across the widest part of the upper and lower leaf surfaces. A dissecting microscope was used to count the TGT in the exposed area. TGT that occurred within 4 mm of the leaf edges or on the major leaf veins were not counted since both areas tended to have high TGT densities and including these areas could have biased the results. The width of the leaf at the examined transect was determined so that TGT density could be analyzed on an area basis if appropriate. TGT analyses of the F_2 and backcross generations were performed in essentially the same manner, except that the 2-mm-wide slot was divided into alternating 10-mm-wide open, 5-mm-wide occluded areas so that only two-thirds of the leaf area transect was counted in this analysis. The subdivision of the survey transect into discrete units facilitated the laborious task of TGT counting, while producing comparable results when corrected for the proportion of the transect counted. Parental ranges were distinct and non-overlapping, with the resistant parent possessing a high TGT density (93–170 TGT per unit area) and the susceptible parent a low density (13–59 TGT per unit area). Segregating generations exhibited continuous variation. Thus, data for TGT density were examined for quantitative inheritance patterns. Means and variances were calculated, expected means were computed for the segregating generations (Mather and Jinks, 1977), and the observed values compared to the expected by Student's t test (Steel and Torrie, 1980).

TWO-SPOTTED SPIDER MITE BIOASSAYS. Bioassays were performed on four classes of F_2 plants selected for high vs. low ω^5 -AnA composition and high (resistant-like) vs. low (susceptible-like) TGT densities as previously described. Five individuals were selected from each class. The high ω^5 -AnA progeny possessed an AnA composition resembling that of the resistant parent; the low ω^5 -AnA progeny, an AnA composition like that of the susceptible parent. The high TGT density progeny had densities of >94 per unit area; the low TGT density progeny had <50 per unit area.

The experiment was a randomized complete-block design with four treatments (progeny classes), three replications (blocks) from each of the five individuals within a treatment, and six observations within each replication. Each experimental unit consisted of a single 2-cm leaf disk excised with a cork borer from the third or fourth leaf below the visible shoot apex. Six disks (one replication) from each experimental plant were placed on moistened filter paper in a 100 mm $\times$ 15-mm disposable petri dish. A single adult female mite (*Tetranychus urticae* Koch) reared on 'Henderson' bush lima bean (*Phaseolus lunatus* L.) and maintained under a 25°C/12 h photoperiod/12 h nyctoperiod regimen was transferred to the abaxial surface of the leaf disk and subsequently was maintained at 21°C/12 h photoperiod/12 h nyctoperiod. After 72 h, adult mite mortality was determined by counting the number of dead mites per replication. Missing mites were arbitrarily counted

as half dead and half alive, thus accounting for all mites in each experimental unit. The number of eggs deposited during this 72 h was recorded and used as an indication of mite fecundity. Data were transformed as $Y' = \text{SQRT}(Y + 0.5)$, to compensate for the dependence of the variance on the mean (Steel and Torrie, 1980). Analysis of variance used transformed data, although the original data are reported, with each datum reporting the sum of all observations within a replication.

Results and Discussion

The AnA composition of the parents and their reciprocal F_1 is reported in Table 1. The resistant parent is chemically homogeneous as shown by the small SE. Greater variability in AnA composition is displayed by the susceptible parent. Now, we are aware that other minor components may co-elute with C22 ω^5 -AnA in TGT extract of susceptible plants (Hesk et al., 1991).

Table 1. Composition of individual AnA components of parents and F_1 as determined by gas-liquid chromatography of AnA methyl esters.

	No.	Total AnA' (%)			
		22:0 (sat)	22:1 (ω^5)	24:0 (sat)	24:1 (ω^5)
Susceptible (S)	8	33.2 (7.7)	6.4 (7.1)	52.6 (7.8)	7.8 (6.8)
Resistant (R)	8	4.6 (1.3)	33.1 (1.0)	5.4 (2.7)	56.9 (3.7)
F_1 (R $\times$ S)	8	8.6 (2.6)	47.5 (6.4)	9.6 (2.6)	34.3 (4.2)
F_1 (S $\times$ R)	10	7.9 (2.4)	50.3 (3.5)	8.8 (3.2)	33.0 (2.6)
F_1 pooled	18	8.2 (1.8)	49.1 (3.5)	9.2 (2.1)	33.6 (2.4)
t statistic ^y		2.05	4.64	1.81	9.88
Probability		0.06	<0.001	0.09	<0.001
Significance		NS	***	NS	***

^zSE in parentheses.

^yTwo-tailed t test, with 24 degrees of freedom. Null hypothesis (H_0) is that the pooled F_1 value is equal to that of the resistant parent.

Table 2. Phenotypic distribution of parents, F_1 , F_2 , and BCS generations based on AnA composition. Resistant phenotypes contain high amounts (> 60%) of ω^5 -AnA in the exudate of TGT.

	No.	Phenotype by AnA composition		Model tested	χ^2 ^z	Probability
		Resistant	Susceptible			
Resistant (R)	8	8	0	1:0	0.00	P = 1.00
Susceptible (S)	10	0	10	0:1	0.00	P = 1.00
F_1 (R $\times$ S)	8	8	0	1:0	0.00	P = 1.00
F_1 (S $\times$ R)	10	10	0	1:0	0.00	P = 1.00
Pooled F_1	18	18	0	1:0	0.00	P = 1.00
F_2 [F_1 (R $\times$ S) $\otimes$]	79	65	15	3:1	1.67	P $\geq$ 0.21
F_2 [F_1 (S $\times$ R) $\otimes$]	80	54	26	3:1	2.40	P $\geq$ 0.13
Pooled F_2	159	119	41	3:1	0.03	P $\geq$ 0.86
BC _R [R $\times$ F_1 (R $\times$ S)]	42	42	0	1:0	0.00	P = 1.00
BC _S [S $\times$ F_1 (R $\times$ S)]	35	15	20	1:0	0.71	P $\geq$ 0.43

^z χ^2 analysis of two-class data used Yate's correction factor (Steel and Torrie, 1980).

Table 3. AnA composition by individual AnA components of BCS as determined by gas-liquid chromatography of AnA methyl esters.

	No.	Total AnA (%) ^z			
		22:0 (sat)	22:1 (ω^5)	24:0 (sat)	24:1 (ω^5)
Susceptible (S)	10	29.4 (10.8)	5.1 (6.8)	58.7 (14.8)	6.8 (6.6)
BCS (susceptible-like)	20	44.1 (2.8)	8.9 (1.7)	37.6 (2.5)	9.4 (1.2)
t statistic ^y		1.73	0.72	1.94	0.53
Significance		NS	NS	NS	NS
F_1 (R $\times$ S)	8	8.6 (2.6)	47.5 (6.4)	9.6 (2.6)	34.3 (4.2)
BCS (F_1 -like)	15	11.8 (1.5)	36.6 (3.4)	11.8 (2.3)	39.9 (2.8)
t statistic ^x		1.15	1.66	0.60	1.14
Significance		NS	NS	NS	NS

^zValues in table are means with standard errors in parentheses.

^yTwo-tailed t test, with 24 degrees of freedom. Null hypothesis (H_0) is that the mean values of the susceptible-like BCS are equal to those mean values of the susceptible parent.

^xTwo-tailed t test, with 21 degrees of freedom. Null hypothesis (H_0) is that the mean values of the F_1 -like BCS are equal to those mean values of the F_1 examined.

Reciprocal F_1 variances for each AnA component were compared and shown to be homogeneous (5% level). The F_1 (R $\times$ S) and F_1 (S $\times$ R) values, therefore, were pooled for subsequent comparisons. The resistant parent and the reciprocal F_1 were substantially different from the susceptible parent for each of the AnA. TGT exudate composition of the F_1 was similar to that of the resistant parent (82.7 vs. 90%); however, the individual 22:1 ω^5 vs. 24:1 ω^5 AnA were significantly different.

Two groups of F_2 were evident based on the AnA composition of the TGT exudate. The presence of high concentrations of ω^5 -unsaturated AnA in the TGT exudate was characteristic of the resistant parental phenotype; low concentrations were characteristic of the susceptible parental phenotype. An examination of the F_2 distributions based on AnA composition (Table 2) indicates an acceptable fit to a 3 resistant : 1 susceptible model ($P \geq 0.86$ for the pooled F_2 data), suggesting monogenic inheritance and dominance for the expression of high ω^5 -AnA concentrations.

The BCR (F_1 $\times$ resistant parent) and BCS (F_1 $\times$ susceptible parent) distributions provide an acceptable fit to the distributions expected from a monogenic model with dominance for high ω^5 -AnA (Table 2). There was no phenotypic segregation among the progeny of the backcross to the resistant parent; all plants were classified as resistant. The ω^5 -AnA composition of the backcross to the susceptible parent, BCS, displayed an acceptable fit to a 1 resistant : 1 susceptible phenotypic pattern ($P \geq 0.43$), supporting the hypothesis of monogenic inheritance for ω^5 -AnA composition in the TGT exudate.

Further evidence for mono-

genic inheritance is obtained by examining the AnA composition of the phenotypic classes within the BCS (Table 3). Of the 35 BCS analyzed, 15 individuals possessed high amounts of ω^5 -AnA (F_1 -like) and 20 had low ω^5 -AnA content (susceptible-like) (Table 3). The means for each of the four major AnA components are not significantly different (5% level) between the two distinct BCS subpopulations and the corresponding F_1 or susceptible parental means. Subsequent analyses of 39 BCS, albeit slightly different individuals, using an improved analytical technique using high performance liquid chromatography (Hesk et al., 1991) identified 16 as being F_1 -like and 23 susceptible, further supporting the GLC results and suggesting a monogenic model of inheritance.

Evidence that ω^5 -AnA are derived from $\Delta 11(\omega^5)$ and $\Delta 13(\omega^5)$ fatty acid precursors has been supported by biosynthetic studies (Hesk et al., 1992; Walters et al., 1990b). As a result, we propose that the locus under investigation be described as a fatty acid desaturase acting on the ΔX position. This desaturase could be a $\Delta 11$ desaturase that acts on a 16-carbon fatty acid, or it could act on a shorter chain fatty acid with subsequent chain elongation. Since these studies do not clearly determine the substrate for the enzyme, we propose to abbreviate the enzyme as $\text{fad}\Delta X$. Using the symbol fad to indicate a fatty acid desaturase is consistent with precedent established in *Arabidopsis* by Browse et al. (1986; 1989). Thus, the two inbred parents used in these studies would be represented by:

The detailed examination by Walters et al. (1989a) of the densities of the various classes of trichomes present on the leaf surface of resistant and susceptible genotypes demonstrated a positive relationship between aphid resistance and the presence of TGT. No other class of trichome present on the geranium surface differentiated resistant and susceptible genotypes.

The resistant parent showed a relatively high TGT density (130.1 per unit area) while the susceptible parent had a relatively low density (38.9 per unit area) (Table 4 and Fig. 2). The F_1 and

Table 4. Tall glandular trichome density distributions of parents, F_1 , F_2 , and BCS generations.

Generation	No.	Tall glandular trichomes/unit area (no.)					<i>t</i> value	Probability
		High value	Low value	Observed mean	Variance	Expected mean		
Resistant (R)	33	170.0	93.0	130.1	338.9			
Susceptible (S)	12	58.5	13.5	38.9	139.94			
Midpoint						84.5		
F_1 (R $\times$ S)	19	115.0	49.5	81.2	327.61	84.5	-0.80 ^{ns}	0.23
F_1 (S $\times$ R)	15	145.0	50.5	90.8	690.64	84.5	0.93 ^{ns}	0.17
F_1 pooled	34			85.4	510.31	84.5	0.23 ^{ns}	>0.25
F_2 [F_1 (R $\times$ S) $\otimes$]	80	148.0	12.5	85.8	856.73	84.5	0.40 ^{ns}	>0.25
F_2 [F_1 (S $\times$ R) $\otimes$]	87	134.5	30.0	72.6	440.58	84.5	-5.29 ^{***}	<0.005
F_2 pooled	167			78.9	683.30	84.5	-2.76 ^{***}	<0.005
BCR [R $\times$ F_1 (R $\times$ S)]	48	169.5	41.5	109.9	567.87	107.3	0.76 ^{ns}	0.23
BCS [S $\times$ F_1 (R $\times$ S)]	41	117.0	25.0	74.8	612.07	61.7	3.02 ^{***}	<0.005

Fig. 2. Comparison of means and ranges of parental inbreds, F_1 , F_2 , and backcross generations based on the number of glandular trichomes/unit area.

reciprocal F_1 had similar TGT densities, with means of the F_1 near to the midpoint of the parental means (81.2 and 90.8 vs. 84.5). The population variances of the F_1 and reciprocal F_1 were homogeneous ($P \geq 0.10$); therefore, the populations were pooled for comparison purposes. The pooled F_1 mean (86.0 trichomes/unit area) was not significantly different ($P \geq 0.25$) from that of the parental midpoint (84.5), suggesting that genetic control of TGT density may exhibit no dominance.

Estimating the average effect of a gene substitution (Falconer, 1989) is calculated as the difference between the parental midpoint (84.5) and either parental mean. This value, 45.6, is an estimate of the effect of a single gene substitution if the phenomenon is under the control of a single locus, and the respective parents are homozygous for alternate allelic states. For the *Pelargonium*

inbreds examined in these studies, this value is an estimate of the number of TGT that each allele (assuming a single locus) contributes to the base genotype. Thus, if the susceptible parent represents the base, the F_1 mean would be estimated by the base value (38.9) plus 45.6, or 84.5 TGT per unit area. The resistant parent value then would be estimated by the estimate of the F_1 mean (84.5) plus 45.6, or 130.1 TGT per unit area.

Ranges of TGT densities of the parents were distinct and non-overlapping (Fig. 2). Distribution of the F_1 hybrids was within that of the parental extremes. A single transgressive segregant was observed in the $F_2(R \times S)$ in which a single data point (12.5 trichomes) fell below the lowest parental value recorded (13.5 trichomes). All other observations of TGT density were within the range of parental values.

The $F_2(R \times S)$ (Fig. 2) had a mean (85.8) similar to the pooled F_1 mean (85.4) and to the parental midpoint (84.5). These slight differences were not significant ($P > 0.25$). However, the reciprocal $F_2(S \times R)$ mean (72.6) was significantly lower than the $F_2(R \times S)$ mean (85.8, $P \leq 0.001$) and the pooled F_1 mean (85.4, $P \leq 0.001$). The mean trichome density distribution of the backcross to the resistant parent [$R \times F_1(R \times S)$] is not significantly different from that expected, i.e., from the mean of the resistant parent and the $F_1(R \times S)$ (109.9 vs. 107.3, $P \geq 0.23$). However, the mean of the backcross to the susceptible [$S \times F_1(R \times S)$] is significantly higher than expected (74.8 vs. 61.7, $P \leq 0.001$). This result suggests that there may be a cytoplasmic effect on TGT density in *Pelargonium*. In every case in which the low-TGT density susceptible parent was used as a seed parent, the progeny differed significantly from that expected.

Qualitative and quantitative models were examined. Both are possibilities, but the simplest explanation consistent with most of these observations is that a small number of loci (possibly as few as one) exhibiting codominance condition the expression of TGT. Each "dose" or complement from the resistant inbred contributes ≈ 45 TGT to the base genotype.

Once the AnA composition and the TGT densities of the segregating F_2 populations were determined, it was possible to examine extreme combinations of both characters by bioassay. We observed the survival (mortality) and fecundity of two-spotted spider mites placed on leaf disks from F_2 plants selected for alternate combinations of high and low ω^5 -AnA content, and high and low TGT densities. These examinations permitted us to determine the relative importance of each character in the resistance mechanism (Tables 5).

Mites reared on the resistant parent exhibited >83% mortality, while only 5.5% of the mites reared on the susceptible parent died. Regardless of the TGT density of the host plant, mites reared on F_2

with high concentrations of ω^5 -AnA exhibited high mortality. In contrast, mites reared on F_2 with susceptible-like exudate composition showed low mortality, even on those plants with high TGT densities. It is clear that within the range of TGT densities displayed by the parents, high concentrations of ω^5 -AnA elicited a clear resistance response (i.e., high mite mortality). Correspondingly, even at high TGT densities, high concentrations of saturated AnA produced a susceptible response. Among the F_2 , the density of TGT appears to be less critical to the expression of mite-resistance than the concentration of ω^5 -AnA in the TGT exudate. Based on the apparent sticky-trap mechanism of this type of small arthropod resistance (Stark, 1975; Walters et al., 1989b), we presume that a lower threshold value for TGT density must exist. The segregating F_2 and backcross appear to possess TGT densities above that threshold level, even at the lowest densities observed.

In contrast to mortality, AnA composition and TGT density affected mite fecundity (Table 5). We observed differential fecundity responses in those mites that survived for the entire 72 h test. Mites from the high TGT density, high ω^5 AnA F_2 laid the fewest eggs per mite (0.92), similar to results observed for the resistant parent. Mites reared on F_2 with a TGT density and AnA composition resembling those of the susceptible parent (low density, low ω^5 AnA content) produced the most eggs on the F_2 selections (1.86), closely corresponding to the response of the susceptible parent.

The number of eggs oviposited in the two nonparental groups was almost intermediate between the two parental genotypes; however, both groups were equal (1.24 vs. 1.29) with respect to fecundity. The similar responses probably result from two causes. In the low density-high ω^5 group, we propose that the mites may survive long enough to produce and lay eggs. In the high density-low ω^5 group, it is hypothesized that mites may be less likely to oviposit on geranium leaves with high densities of TGT, even if the TGT do not produce a sticky exudate. This type of resistance, in which the TGT presents a mechanical or physical barrier to feeding or oviposition, has been widely observed in other pest-plant interactions (Hoffman and McEvoy, 1986; Khan et al., 1986; Talekar et al., 1988). These results suggest that one possible effect of sublethal doses of AnA is to decrease mite fecundity (Grazzini, 1993; Stark, 1975). A TGT density effect would be expected in such a circumstance: the more opportunities to contact the AnA-rich exudate, the greater the potential effect on fecundity. These results also suggest that high densities of TGT may interfere with mite egg production and deposition, regardless of exudate composition.

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Table 5. An examination of the interactions among AnA composition, TGT density, mite mortality, and mite fecundity using selected F_2 as bioassay hosts.⁴

Generation	Mean trichome density	Relative trichome density	Relative ω^5 ANA content	Dead mites (no.)	Mortality (%) ^y	Mite eggs laid/survivor (no.) ^x
Resistant (R)	130	High	High	75	83.3	0.65
Susceptible (S)	38	Low	Low	5	5.5	2.02
F_2	94	High	High	53	58.9	0.92
F_2	46	Low	High	61	67.7	1.24
F_2	108	High	Low	18	20.0	1.29
F_2	38	Low	Low	20	22.2	1.86

⁴Parental inbreds were included for comparison only and were not part of the experimental design.

^yAverage of all observations within class.

^xAverage number of eggs laid per surviving mite within each experimental treatment.

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