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Chimeric Tomato Plants Show that Aphid Resistance and Triacylglucose Production Are Epidermal Autonomous Characters

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Graft chimeras were generated using *Lycopersicon pennellii* and *L. esculentum* to determine the contribution of the three meristem layers (L1, L2, and L3) to trichome density, sugar ester production, and aphid resistance. Sugar esters, in the form of triacylglucoses, have been implicated in the aphid resistance of *pennellii*. One chimera possessed the epidermal layer (L1) of *pennellii* and the internal tissues (L2 and L3) of the aphid-susceptible *esculentum*. The second chimera had both the L1 and L2 of *pennellii* and the L3 of *esculentum*. Type IV trichome densities did not differ significantly among the chimeras and *pennellii*. Both chimeras accumulated sugar esters with similar sugar and fatty acid composition as *pennellii*. The concentration of epicuticular sugar ester on the chimeras was also comparable with that of *pennellii*. Leaf cage and feeding studies demonstrated that both chimeras are as resistant to aphids as is *pennellii*. The resistance could be reduced similarly on all three types of plants by removal of the type IV trichome exudate. These results indicate that the presence and density of the type IV trichomes and the amount and type of sugar esters produced are features determined by the genotype of the epidermis. These epidermal features are sufficient to account for the aphid resistance observed in *pennellii*.

INTRODUCTION

Genetically mosaic organisms are useful in examining some of the questions that relate to mechanisms of cell differentiation. Because these organisms are a combination of cells with different genotypes, they permit a distinction between those events that are determined by the genotype of the individual cell (autonomous events) and those events that are determined by the genotypes of other cells (nonautonomous events). In plants, genetically mosaic organisms can arise spontaneously in nature or can be generated by a variety of methods (Tilney-Bassett, 1986). X-irradiation of plants can result in the formation of clonal sectors of cells lacking a chromosome arm. In the dominant *Knotted* mutant of maize, the surface cells of the leaf divide abnormally to produce a tumor-like knot. Hake and Freeling (1986) generated sectors by X-irradiation that lacked the *Knotted* gene in the surface cell layer, but the underlying layers contained the mutation. Abnormal divisions in the epidermis still occurred and a knot was formed, demonstrating that the mutation behaves in a nonautonomous manner.

Genetic mosaics can also be generated by grafting together genetically different plants and regenerating new shoots from the graft union (Marcotrigiano, 1986). For species having apical meristems organized into three layers, these shoots may yield stable periclinal chimeras in which one of the three apical layers is genetically different from the other two. By analyzing the contribution of the genetically marked meristem layers to the tissues and organs of the shoot, it has been established that the L1 surface of the meristem generates the epidermis, the L2 layer generates the subepidermal tissue, and the L3-derived cells generate the central tissue (Satina and Blakeslee, 1941). In a graft-generated periclinal chimera having the L1 of *Camellia sasanqua* and the L2 and L3 of *C. japonica*, the expression of pigments in the epidermal cells of the petals is identical to that in *C. sasanqua* and is not influenced by the *C. japonica* cells in the subepidermal layers (Stewart et al., 1972). The development of petal color in *Camellia* is, therefore, an autonomous event. In graft-generated chimeras of tomato, the identity of L3-derived cells determines the position and development of the flower pedicel abscission zone; the L1- and L2-derived cells differentiate in response to the genotype of the L3 cells (Szymkowiak and Sussex, 1989). Therefore, epider-

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mal characters can develop in either an autonomous or a nonautonomous manner.

We have generated periclinal graft chimeras between *pennellii*, an aphid-resistant wild tomato species, and *esculentum*, the aphid-susceptible cultivated tomato species, to determine the tissue basis of this resistance. The aphid resistance of *pennellii* has been attributed to epidermal characters (Gentile and Stoner, 1968a). *Pennellii* possesses high densities of type IV glandular trichomes on the leaf, stem, sepal, and fruit epidermal surfaces (Lemke and Mutschler, 1984). These trichomes consist of a short, multicellular stalk and secrete droplets of sticky exudate at the tip (Luckwill, 1943). The exudate of the type IV trichomes of *pennellii* alters the feeding behavior of the potato aphid (Goffreda et al., 1988). This exudate chiefly contains 2,3,4-triacylglucoses (Burke et al., 1987; Goffreda et al., 1989), which affect aphid settling (Goffreda et al., 1988) and are correlated with field resistance to aphids (Goffreda et al., 1990). Type IV trichomes are completely absent from *esculentum* (Luckwell, 1943).

Although epidermal cells, including type IV trichomes, are derived from the L1 of the shoot meristem, aphids feed by inserting their proboscis into sieve tube elements, which are derived from internal (L2 and L3) cell layers. Two types of periclinal chimeras were generated between *pennellii* and *esculentum* to address the role of *pennellii*'s epidermis in aphid resistance and to determine whether glucose ester production in *pennellii* is an epidermal autonomous character or whether it requires interactions between epidermal and subepidermal cells.

RESULTS

Identification of Chimeras

Two different stable periclinal chimeras were generated from cut graft junctions between *pennellii* and *esculentum*. Chimeric shoots were identified by the presence of novel combinations of the cell layer markers. The *esculentum* cells carry the mutation Xa-2, which causes yellow/green plastids, whereas the *pennellii* cells are wild type for Xa-2 and have green chloroplasts. Based on this color difference, the genetic identity of the L2 and L3 layers in the leaves of young shoots was easily determined. The L1 identity of the shoots was determined by the character of the epidermal trichomes; the presence or absence of type IV trichomes was used to determine the identity of the L1 of the chimeras as *pennellii*- or *esculentum*-like, respectively.

Several shoots having chimeric sectors (mericlinal chimeras) were obtained. By selectively pruning these shoots, stable periclinal chimeras were generated as described in Methods. One chimera, designated PEE, has the L1 of *pennellii* and the L2 and L3 of *esculentum*. This chimera has the yellow color of the mutant *esculentum* and the type IV trichomes of *pennellii*. A second chimera, designated PPE, has the L1 and L2 of *pennellii* and the L3 of *esculentum*. This chimera has leaves with green margins and slightly yellow centers and the epidermis has L1-derived *pennellii* type IV trichomes. Both chimeras have been stably propagated vegetatively for 4 years.

The cell layer identity of the chimeras was further verified. All progeny of self-pollinated PPE were *pennellii*. This confirms the identity of the L2 as *pennellii* because gametes are usually derived from the L2. PEE has not been self-pollinated. Decapitated PEE and PPE plants regenerated many *esculentum* and *pennellii* shoots, confirming the presence of both species in the chimeras. Analysis of the isozymes phosphoglucose isomerase and triosephosphate isomerase also supports the cell layer composition of the chimeras (data not shown). Superficial peels (including the epidermis with some contamination by subjacent tissues) of the PPE petiole possessed only *pennellii* isozymes, whereas the internal tissue possessed only *esculentum* isozymes. Superficial peels of the PEE petiole possessed both *pennellii* and *esculentum* isozymes, whereas the cortex tissue had only *esculentum* isozymes.

Both PEE and PPE have been independently obtained from three different grafts. Small sectors of EPP, with *esculentum*-type epidermal characters over *pennellii* internal tissues, have been observed. These sectors were restricted to small fractions of leaflets. EPP sectors were obtained on upper, lower, or both leaf surfaces. These sectors had no type IV trichomes. Because these sectors never fully crossed a leaf axis, no stable EPP periclinal chimeras were obtained from axillary buds.

Trichome Densities

Type IV trichome densities on *pennellii* and the chimeras PEE and PPE were 267, 225, and 265 trichomes/10 mm^2, respectively. Figure 1 shows the trichomes of the species and chimeras and illustrates that *esculentum* did not possess type IV trichomes. Type IV trichome densities did not differ significantly among the chimeras and *pennellii*. The F_1 hybrid between *esculentum* and *pennellii* has a type IV trichome density between 25% and 50% of that of *pennellii* (Lemke and Mutschler, 1984; Goffreda and Mutschler, 1989). In contrast to the densities of type IV trichomes, the type VI trichome densities were 20.5, 2.5, 3.4, and 2.3 trichomes/10 mm^2 on *esculentum*, *pennellii*, and the chimeras PEE and PPE, respectively. These type VI trichome densities did not differ significantly among the chimeras and *pennellii* but were significantly higher on *esculentum*.

Sugar Ester Content and Composition

Both chimeras and *pennellii* secreted glucose esters and did not secrete detectable levels of sucrose esters. The concentration of glucose esters on the leaflets of PEE,

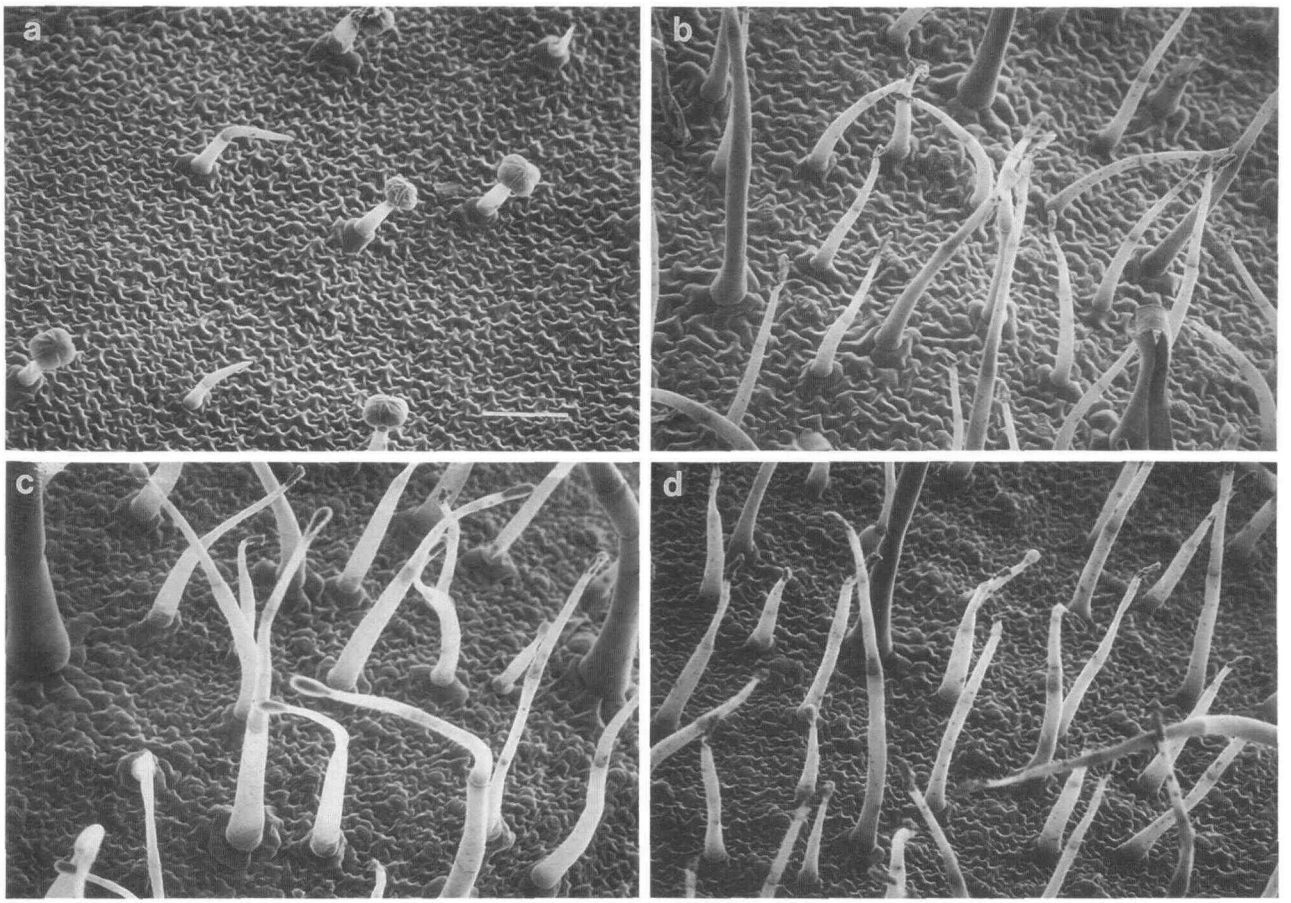

Figure 1. Scanning Electron Micrograph of Adaxial Surfaces of Terminal Leaflets.

Segments (2 mm^2) of terminal leaflets from the fourth leaf below the shoot apex longer than 2 cm were fixed for 3 hr with 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2. The tissue was dehydrated with dimethoxymethane for 10 min, transferred to absolute ethanol, and then critical point dried with CO_2 (critical point drier, Polaron, Watford, Herts., United Kingdom), sputter-coated with platinum and gold (sputter-coater, Structure Probe, Inc., Westchester, PA), and observed in an ISI-SS40 scanning electron microscope (International Scientific Instruments, Inc., Santa Clara, CA) with accelerating voltage of 10 kV. The bar is 1 μm.
(a) *esculentum*.
(b) *pennellii*.
(c) PEE.
(d) PPE.

PPE, and *pennellii* were 781.6, 620.7, and 814.3 nmol/cm^2, respectively. Leaflets of *esculentum* did not have detectable levels of glucose esters.

The sugar esters of PEE, PPE, and *pennellii* were very similar in the percent of long-chain fatty acids (36%, 36%, and 39%, respectively). The sugar esters of the chimeras and *pennellii* were also very similar for fatty acid composition, except that the chimeras had higher 8-methylnonanoic and lower decanoic and dodecanoic acids than *pennellii* as a percent of the total long-chain fatty acids. In contrast, the fatty acid profile of *esculentum* × *pennellii* F_1 plants are quite different from those of *pennellii*; the relative proportions of the fatty acids present in both the F_1 and *pennellii* are extremely different (J.C. Steffens, personal communication). The sugar esters of the PEE and PPE chimeras are also glucose esters, unlike the nearly equal mixture of sucrose and glucose esters produced by *esculentum* × *pennellii* F_1 plants (Goffreda et al., 1990).

Aphid Responses

Results concerning aphid settling, fecundity, and mortality in leaf cages are summarized in Table 1. Aphid settling on

Table 1. Aphid Settling, Fecundity, and Mortality in Leaf Cages on *esculentum*, *pennellii*, and the Chimeras PEE and PPE[a]

Plant Type	% Aphid Settling			Fecundity, 48 hr	% Adult Mortality, 48 hr	% Nymph Mortality, 48 hr
	12 hr	24 hr	36 hr			
esculentum	90y[b]	70y[c]	67y[b]	2.9x[b]	10z[b]	17z[b]
PEE	2z	35z	32z	1.7y	55x	90y
PPE	2z	27z	18z	1.1zy[d]	62x	98y
pennellii	5z	27z	16z	1.6z	27y[e]	87y

[a] Treatment means followed by the same letter (z, y, or x) within a column do not differ significantly ($P > 0.05$) by orthogonal partitioning. Fecundity is expressed as number of nymphs produced per living adult aphid in each 12-hr period.
[b] Contrast between mean response on *esculentum* and the average mean response on *pennellii* and the chimeras is significant at $P = 0.001$.
[c] Contrast between mean response on *esculentum* and the average mean response on *pennellii* and the chimeras is significant at $P = 0.01$.
[d] Contrast between mean response on PEE and the mean response on PPE is significant ($P < 0.05$).
[e] Contrast between mean response on *pennellii* and the average mean response on the chimeras is highly significant ($P < 0.01$).

pennellii and the chimeras was significantly less than that on *esculentum* at 12 hr, 24 hr, and 36 hr. Aphid settling did not differ significantly between the two chimeras, nor between *pennellii* and the average of the two chimeras. Mortality of the adult aphids and nymphs was significantly higher on the chimeras and *pennellii* than on *esculentum*. Surprisingly, mortality of adult aphids was also significantly higher on the chimeras than on *pennellii*. Fecundity was significantly higher on *esculentum* than on *pennellii* and the chimeras. Washing the terminal leaflets with three washes of 95% ethanol effectively removed the type IV trichome exudate without causing visible phytotoxic effects. Table 2 presents the effect of the ethanol rinse on aphid settling and fecundity. Removal of the exudate increased aphid settling and fecundity on leaflets of *pennellii* and the two chimeras but did not increase settling to the level observed on *esculentum*. Fecundities were comparable in all ethanol-treated plants.

Table 2. Aphid Settling and Fecundity after 24 hr in Leaf Cages on *esculentum*, *pennellii*, and the Chimeras after the Removal of the Type IV Trichome Exudate with Ethanol[a]

Plant Type	% Aphid settling, 24 hr		Fecundity, 24 hr	
	Intact Droplets	Removed Droplets	Intact Droplets	Removed Droplets
esculentum	67.5y[b]	80.0y[c]	2.0y[b]	1.9z
PEE	15.0z	47.5z	0.2z	1.4z
PPE	5.0z	62.5z	0.3z	1.5z
pennellii	12.5z	37.5z	0.4z	1.4z

[a] Treatment means followed by the same letter (z or y) within a column do not differ significantly ($P > 0.05$) by orthogonal partitioning. Fecundity is expressed as number of nymphs produced per living adult in each 12-hr period. Removal of the trichome exudate from *pennellii* and the chimeras significantly increased aphid settling and fecundity ($P < 0.0001$). Ethanol wash did not significantly affect settling and fecundity on *esculentum* ($P > 0.05$).
[b] Contrast between mean response on *esculentum* and the average mean response on *pennellii* and the chimeras is significant at 0.001.
[c] Contrast between mean response on *esculentum* and the average mean response on *pennellii* and the chimeras is significant at 0.01.

Aphid feeding responses on *esculentum*, *pennellii*, and the chimeras as measured by electronic feeding monitoring (EFM) are summarized in Table 3. Feeding responses on *pennellii* and the chimeras were characterized by an increase in the proportion of aphids that did not probe, a delay in the time to first probe, and decreases in both the number of probes and the percent of time spent probing, as compared with *esculentum*. None of the feeding parameters differed significantly between the two chimeras, nor did any of the parameters indicate that *pennellii* was more resistant than the chimeras. Removal of the type IV trichome exudate from *pennellii* and both chimeras with 95% ethanol increased aphid feeding, with a decrease in the time to first probe and an increase in both the number of probes and the percent of time the aphids spent probing (data not shown).

DISCUSSION

The glandular trichomes of *pennellii* have been implicated in insect resistance (Gentile and Stoner, 1968a, 1968b; Gentile et al., 1968, 1969; de Ponti et al., 1975; Juvik et al., 1982). The 2,3,4-triacylglucose esters secreted by type IV trichomes (Goffreda et al., 1989) reduced aphid settling and are correlated with field resistance to aphids (Goffreda et al., 1989, 1990). In the experiments reported here, we generated two graft chimeras between *pennellii* and *esculentum* having only the epidermis (L1) of *pennellii* or the

Table 3. Mean Aphid Feeding Response on *esculentum*, *pennellii*, and the Chimeras PEE and PPE as measured by EFM[a]

Plant Type	% Non-probing Aphids	Time to 1st Probe (min)	No. of Probes	% Time Probing	No. of Falls
esculentum	0z[b]	5.7z[b]	2.7y[b]	67.7x[b]	0.0z[b]
PEE	70y	22.9y	0.4z	15.9z	1.9y
PPE	75y	25.4y	0.5z	8.0z	2.1y
pennellii	55y	20.4y	0.8z	28.7y[c]	1.1y

[a] Treatment means followed by the same letter (z, y, or x) within a column do not differ significantly ($P > 0.05$) by orthogonal partitioning.
[b] Contrast between mean response on *esculentum* and the average mean response on *pennellii* and the chimeras is very highly significant ($P < 0.001$).
[c] Contrast between mean response on *pennellii* and the average mean response on the chimeras is significant ($P < 0.05$).

L1 and L2 of *pennellii*. The results of the EFM and leaf cage studies agree that the PEE and PPE chimeras are at least as resistant as *pennellii*. Therefore, these results confirm that aphid resistance in *pennellii* is a result of epidermal characters and is controlled by the epidermal genotype. The resistance does not rely on component(s) of the subepidermal layers even though aphids feed from these tissues. Clayberg (1975) created a single putative periclinal chimera. He identified the chimera as having the L1 layer of *pennellii*, based on trichome types present, and the L2 of *esculentum*, based on leaflet shape and markers affecting flower and fruit traits. He assumed that the L3 layer was also *esculentum*. The density of type IV trichomes on this chimera was 34.4/mm^2, which is very similar to that of PPE and PEE. Because his chimera supported numbers of aphids intermediate to those on his *esculentum* and *pennellii* controls under greenhouse conditions, Clayberg concluded that aphid resistance of *pennellii* was due to subepidermal factors. The reason for the discrepancy between our results and those of Clayberg is uncertain but may involve the type of resistance assay and the number of replicates used by Clayberg. It is unlikely that the different *esculentum* line used to generate our chimeras had a major impact on resistance of the chimeras because this *esculentum* line was susceptible to the aphids in these studies.

The morphology of the epidermis, as defined by the presence and density of type IV glandular trichomes, is the same in the two chimeras as in *pennellii*. The type IV glandular hairs present on both chimeras secreted 2,3,4-triacylglucoses similar in composition and amount to those secreted by *pennellii*. The presence and density of type IV hairs and the production of their secretory products in PPE and PEE are dependent on the genotype of the epidermis and are, therefore, cell layer autonomous. Although there are slight quantitative differences in the fatty acid composition of the sugar esters of the chimeras relative to *pennellii*, these changes are small compared with the major qualitative and quantitative differences in the sugar and fatty acid compositions of the sugar esters of the F_1 compared with those of *pennellii*. This is important because the energy or materials required for sugar ester production is probably derived from layer 2. It is also possible that the fatty acids for sugar ester biosynthesis could have been synthesized in layer 2; in that case, the fatty acid constitution of the sugar esters of the chimeras would have been different from those of *pennellii*, and this was not observed. Because removal of sugar esters similarly reduced the level of aphid resistance in both chimeras and *pennellii*, the association between sugar ester production and aphid resistance (Goffreda and Mutschler, 1989; Goffreda et al., 1990) is strongly supported.

METHODS

Generation of Chimeras

Seed stocks of *pennellii* P.I. 246502 (LA 716) and *esculentum* Xa-2/+ (LA 2471) were obtained from the Tomato Genetic Stock Center courtesy of C.M. Rick. Seedlings were grown in 4-inch pots of Metro Mix 2000 for 6 weeks in the greenhouse and fertilized biweekly with Peters 15-16-17 Peat Lite Special. Plants of *pennellii* and *esculentum* were reciprocally slant grafted at the second or third node above the cotyledons. The grafts were sealed with Parafilm "M" and placed in intermittent mist for 1 week. Four weeks from the time of grafting, a cut was made through the graft region so that both the scion and stock tissue were exposed on the cut surface. Callus formed on the cut surface and shoots arose from the callus.

The shoots were visually screened for chimeric sectors in which a developing meristem had incorporated cells from both stock and scion by identifying novel combinations of genetic markers. Shoots arising from the cut graft surfaces having sectors of yellow tissue with *pennellii*-type hairs or green tissue with *esculentum*-type hairs could be identified as mericlinal chimeras. These shoots were removed from the graft, rooted under intermittent mist, and grown as described. Stable periclinal chimeras were obtained by selectively decapitating plants above a node containing a chimeric sector and allowing the axillary bud at that node to grow.

Estimation of Trichome Densities

Type IV and VI trichome densities were determined on the abaxial surface of the fourth leaf from the apex using a Wild M-8 stereomicroscope (80×). Counts were made on a total of five leaves from one plant of each *esculentum*, *pennellii*, and the chimeras. Density determinations were made on a 6.4-mm^2 interveinal area on the two leaflets adjacent to the terminal leaflet, averaged, and analyzed by an analysis of variance. Mean trichome densities were separated by Scheffé's test for multiple comparisons (Snedecor and Cochran, 1980).

Estimation of Sugar Ester Content

Five leaflets from the fourth node were placed in a glass scintillation vial with 10 mL of methylene chloride, and the vial was inverted 15 times. Leaflets were removed, and the surface area was determined by a LI-COR model LI 3000 area meter. The extraction was replicated four times for each of the four genotypes. Their sugar ester content was determined on three subsamples/vial using the colorimetric method previously developed (Goffreda et al., 1990). Sugar concentrations less than 75 μg/mL were not within the linear portion of the standard curve and were scored as not having detectable levels of the sugar ester. The three determinations were averaged and analyzed by an analysis of variance. Mean glucose ester content and leaf area were separated by Scheffé's test for multiple comparisons (Snedecor and Cochran, 1980).

Plant and Aphid Culture for Aphid Studies

Plant materials used in aphid studies were grown to maturity (about 1 m tall) in a greenhouse under natural lighting conditions in the summer of 1988. Plants were not exposed to pesticides. Potato aphids (red biotype, *Macrosiphum euphorbiae*) were obtained from a colony that was initiated from a single apterae collected from tomato in Ithaca, NY, in September 1985. Aphids used in all studies were reared on excised potato leaves for at least one generation to minimize any conditioning to tomato. Aphids were reared in a growth chamber maintained at 20°C and illuminated with approximately 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ in a 16-hr light/8-hr dark photophase.

Leaf Cage Experiments

Leaf cages (Tingey, 1986) were constructed on the terminal leaflets of *pennellii*, *esculentum*, and the chimeras. Three adult apterae were placed in each cage within 24 hr of their first reproduction. The proportion of living aphids settled on the leaf and adult mortality were recorded at 12 hr, 24 hr, and 36 hr. After 48 hr, the experiment was terminated and adult fecundity and mortality and nymph mortality were recorded. There were 10 replicates per plant type (species or chimera), arranged in a randomized complete block design, blocked by the day in which the cages were constructed. Aphid settling and mortality data were transformed by taking the arc sine square root of the proportion. Data were analyzed by an analysis of variance using orthogonal partitioning to contrast treatment means. Because the genotype × block interaction was not significant, it was pooled with the residual error. Tables present the untransformed treatment means.

In a separate experiment to determine whether the type IV exudate affected aphid settling and fecundity, the type IV glandular exudate was removed from intact terminal leaflets of *pennellii* and the two chimeras by three successive washes with 95% ethanol before leaf cage attachment. Similarly treated *esculentum* leaflets and untreated leaflets of all four genotypes were included as controls. Leaf cages were constructed as previously described (Tingey, 1986) and aphid settling and fecundity were recorded at 12 hr and 24 hr. There were 10 replicates of each of the eight treatments (four genotypes ± ethanol wash) arranged in a completely randomized design. Data were analyzed by an analysis of variance with orthogonal partitioning of treatment means within an alcohol/control treatment and by planned contrasts between the alcohol-washed and unwashed control treatments within a plant type.

Electronic Monitoring of Feeding Behavior

Aphid feeding behavior was examined by EFM using a modification of previous designs (McLean and Kinsey, 1964; Brown and Holbrook, 1976). Within 24 hr of their first reproduction, aphids were wired (Goffreda et al., 1988) and starved for 30 min before monitoring. An aphid was placed on the terminal leaflet of the plant and a 2-V, 20-Hz AC signal was applied. Feeding behavior was recorded for 30 min, then the aphid was removed and discarded. The leaflet was briefly dipped (1 sec to 3 sec) in three successive volumes of 95% ethanol to remove the type IV trichome exudate and then allowed to dry for 30 min. Evaporation of the ethanol was hastened by briefly directing a blow-dryer toward the leaflet to remove the excess ethanol. Another wired aphid was placed on the leaflet and its feeding was monitored for an additional 30 min.

Five parameters were used to characterize aphid feeding behavior: (1) percent of aphids that did not probe, (2) time to first probe, (3) number of probes, (4) percent of time the aphid spent probing, and (5) number of times the aphid fell from the plant. A probe was defined as the time from stylet insertion until stylet withdrawal. Aphid behavior was monitored on a single plant of *esculentum*, *pennellii*, and each of the chimeras with 20 replicates per genotype arranged in a completely randomized design. Data were analyzed by an analysis of variance with orthogonal partitioning of treatment means. Because treatment variances were heterogeneous for the percent nonprobing aphids and the number of times the aphid fell from the plant, the standard errors of comparisons with these parameters were estimated (Snedecor and Cochran, 1980).

The effect of the removal of the type IV exudate by the ethanol wash was measured by the difference between the initial feeding data and the data obtained following the ethanol wash. Four parameters were used in analysis: (1) time to first probe, (2) number of probes, (3) percent of time probing, and (4) number of times the aphid fell from the plant. Differences were analyzed by a paired *t* test.

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