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Mite Responses and Trichome Characters in a Full-Sib F_2 Family of *Lycopersicon esculentum* x *L. hirsutum*

Catherine D. Carter[1] and John C. Snyder
Department of Horticulture and Landscape Architecture, University of Kentucky, Lexington, KY 40546

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Abstract. **A full-sib family of F_2 individuals from a cross between mite-susceptible *Lycopersicon esculentum* Mill and mite-resistant *Lycopersicon hirsutum* Humb and Bonpl. was assayed for trichome characters and resistance to spider mites (*Tetranychus urticae* Koch). Mite responses primarily were associated with density of the Type IV trichome and, to a much less degree, with the leaflet surface bioassayed and phenol content of the Type VI trichome tip. Mite survival on F_2 hybrids with at least 5.6 Type IV trichomes per mm^2 was comparable to that on *L. hirsutum*.**

The wild tomato species *L. hirsutum* is highly resistant to various arthropods, including 2-spotted spider mites (*Tetranychus urticae* Koch) (1, 6, 9, 10). Arthropod resistance in *Lycopersicon* has been associated with density of the Type VI trichome (12), with antibiotic properties of the Type VI trichome tip (1, 6, 10) and with density of the Type IV trichome (3). Type IV trichomes have a single-celled tip with viscous exudate, and are present at high densities on *L. hirsutum* but are absent on *L. esculentum* Mill. (7, 11). Type VI trichome density does not differ between species, but the 4-celled tips of Type VI trichomes differ in structure and histochemistry between these species (7, 11). The F_2 generation of mite-susceptible *L. esculentum* x resistant *L. hirsutum* f. *hirsutum* segregates for Type IV trichome density and for qualitative Type VI trichome characters including morphology, lipid content, and phenol content of the glandular tip (11).

In previous bioassays conducted in this laboratory, mite responses were evaluated on a small number of *L. esculentum* x *L. hirsutum* F_2 hybrids chosen to represent the maximum available ranges of Type IV and Type VI trichome densities and a single qualitative feature of the Type VI trichome tip, the amount of red fluorescence in ultraviolet light (3). The latter presumably is associated with the lipid characteristics of the glandular tip of the Type VI trichome (13). In these bioassays, resistance was associated mainly with Type IV trichome density. Because the number of plants used in this research was small, potential existed for chance covariance between Type IV trichome density and mite resistance, even though trends were consistent across genetic and developmental sources of variance as well as consistent with mite bioassays performed on the resistant parent (10). In contrast to our findings, other authors have associated host-plant resistance of tomato with Type VI trichome density and with toxicity of the Type VI trichome tip (1, 6, 12). Phenolic components, lipophilic methyl ketones, and sequiterpenoids all have been implicated as antibiotic factors associated with Type VI trichomes (4, 5, 8). The present investigation was conducted to determine the degree to which Type IV trichome density, Type VI trichome density, and structure, phenol content, and lipid content of the tip of the Type VI trichome are associated with mite responses on leaflets of a full-sib F_2 family of *L. esculentum* x *L. hirsutum*.

Materials and Methods

Seed of *Lycopersicon hirsutum* f. *hirsutum*, P.I. 251303, were obtained from the USDA North Central Regional Plant Introduction Station, Ames, Iowa Sib crosses between 2 F_1 hybrids from *L. esculentum* 'Ace' x *L. hirsutum*, P.I. 251303, produced the F_2 population of 37 plants described herein as F_2 2002 × 2010. Ten of these hybrids died or appeared to be diseased and were not used in the assays. The remaining 27 plants were scored for structure and histochemistry of the Type VI trichome tip as previously described (11). Scores of the structure of the Type VI trichome tip ranged from 10, corresponding to the globular tips of *L. hirsutum*, to 50, corresponding to the strongly lobed tips of *L. esculentum* (11). Phenol content of the Type VI trichome tip was assayed on the basis of staining intensity following reaction with the phenol indicator $NaNO_2$ (2), where a score of 10 was comparable to the low phenol content of tips from *L. hirsutum* and 50 to the high phenol content of tips from *L. esculentum* (11). Lipid content was scored as intensity of red fluorescence in ultraviolet light (13), where a score of 10 corresponded to the low intensity of Type VI trichome tips on *L. esculentum* and 50, to the bright red fluorescence exhibited by those on *L. hirsutum* (11).

Leaflets used in the mite resistance bioassays were taken as pairs from the 3rd leaf from the apex of each plant. One leaflet of each pair served as the adaxial surface sample and the opposite leaflet as the abaxial, giving a total of 54 observations (2 leaflets × 27 plants). Type IV and Type VI trichomes were counted on each leaflet with the aid of a dissecting microscope (11). Ten teneral female spider mites were placed with a Tanglefoot ring on each leaflet. Mite resistance was assayed in terms of 4 mite responses: fecundity (number of eggs per leaflet), mortality (number of mites dead, excluding those in Tanglefoot), avoidance (mites in Tanglefoot), and survival (number alive and not in Tanglefoot). Thus, mite survival = 10 initial mites − (mortality + avoidance), per leaflet. Responses were determined at 24 and 72 hr after inoculation, as previously described (3, 10). Because results were generally similar at both times, only the data taken at 72 hr will be presented.

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[1]Present address: Dept. of Hort. and For., Cook College, Rutgers Univ., New Brunswick, NJ 08903.

Mite response variables were analyzed in relation to Type IV and Type VI trichome density, leaflet length, leaflet surface bioassayed (adaxial and abaxial surfaces were assigned values of 1 and 2, respectively), and the structure, phenol content, and intensity of fluorescence of the Type VI trichome tip. Stepwise regression analyses were used to determine the best simple and multiple variable regression models explaining the variation in mite resistance for the 54 observations in the total data set, or for 36 observations on low Type IV density leaflets (<1 Type IV trichome per mm^2) and 18 observations on high Type IV density leaflets (≥ 1 Type IV trichome per mm^2). Log transformations were used where appropriate to provide the best fitting linear regressions.

Results and Discussion

Among the F_2 individuals surveyed, qualitative characters of the Type VI trichome tip encompassed the full range of differences between *L. hirsutum* and *L. esculentum* for lipid content (UV score) and phenol content ($NaNO_2$ score). Structure scores of the Type VI trichome tip ranged from 20 to 50; i.e., none of these hybrids had Type VI trichome tips quite as globular as those of *L. hirsutum*. Type IV trichome density ranged from 0 to almost 14 trichomes per mm^2 of leaflet surface which occurred on the abaxial surface of one hybrid and was comparable to Type IV trichome density on the *L. hirsutum* parent (10).

Mite responses on leaflets of the 27 F_2 individuals were analyzed in relation to the independent variables by stepwise regression analysis. Type IV trichome density produced the best single-variable regression model for each of the mite response variables except avoidance (Table 1). The R^2 for regression of mite survival at 72 hr on Type IV trichome density was 0.49; i.e., nearly 50% of the variation in survival was associated with variation in Type IV trichome density. Mite survival declined in relation to the number of Type IV trichomes per mm^2, with $r = -0.70$, highly significant at $P = 0.001$. Variation associated with the surface bioassayed and with Type VI trichome density contributed about another 7% to the variation in mite survival (Table 1). Only Type IV trichome density contributed significantly to variation in mite mortality. Variation in avoidance at 72 hr was associated with the leaflet surface bioassayed (Table1). Avoidance was generally greater on abaxial leaflet surfaces. The mean number of mites in Tanglefoot on abaxial surfaces was 3.5, significantly greater at $P = 0.03$ than the 2.2 mites on adaxial surfaces (mean avoidance on each surface was adjusted for covariance with Type IV trichome density). Fecundity varied with Type IV trichome density and to a lesser degree with leaflet surface, although means for each surface did not differ significantly when adjusted for covariance with Type IV density. Thus, although most of the variation in mite resistance in this hybrid family was associated with the density of the Type IV trichome, mite avoidance and fecundity appeared also to be affected by additional factors associated with differences between leaflet surfaces. Variation in mite fecundity was also slightly, and inversely ($r = -0.28$, $P = 0.05$) related to leaflet length (Table 1).

To demonstrate the effects of leaflet surface and Type VI trichome density, tip structure, and histochemistry in the absence of overriding Type IV trichome density effects, the data were separated into 2 classes according to Type IV trichome density. The low density class included leaflets with less than 1 Type IV trichome per mm^2 of leaflet surface, and the high density class included leaflets with at least 1 Type IV trichome per mm^2. Stepwise regression analysis of mite responses was conducted for each class (Table 2).

On leaflets with less than 1 Type IV trichome per mm^2, most of the variation in mite survival was associated with the leaflet surface bioassayed (Table 2). Density, tip structure, and histochemistry of the Type VI trichome also contributed to the multiple-variable model for survival. Based on the slope from the regression analysis, mite survival was lower on abaxial leaflet surfaces and generally declined as Type VI trichome density increased ($r = -0.44$, $P = 0.01$) and as Type VI trichome tips became more lobed, i.e., more like those of *L. esculentum* ($r = -0.48$, $P = 0.01$). Leaflet length and the UV score of the Type VI trichome tip contributed slightly to the variation in mite survival with positive slopes between the independent variables and mite survival. Variation in mite mortality on these leaflets was not significantly associated with any of the independent variables in this model. Avoidance varied with leaflet surface, and was greater on abaxial surfaces ($r = +0.34$, $P = 0.05$). The $NaNO_2$ scores and structure of the Type VI trichome tip also contributed somewhat to the model for mite avoidance, with a tendency, based on the slope from the regression analysis, for increased avoidance in the presence of low $NaNO_2$ scores or increased lobing of the Type VI trichome tip. Fecundity was reduced on abaxial surfaces ($r = -0.42$, $P = 0.01$), and no other variables contributed significantly to this model. These surface differences for mite fecundity were similar to

Table 1. Best models from stepwise regression of mite resistance variables at 72 hr on trichome variables and leaflet surfaces of F_2 2002 × 2010.

	Single-variable models		Multiple-variable models	
Mite resistance variable	Source of variation[z]	R^2	Source of variation	R^2
Survival	IV density	0.49***	IV density, surface, VI density	0.56***
Mortality	IV density	0.34***	IV density	0.34***
Avoidance	surface	0.15**	surface	0.15**
Fecundity	IV density	0.29***	IV density, surface, leaflet length	0.46***

[z]Variables for which $P > 0.05$ are not included in the model; all other variables are given in order of the significance of their partial sums of squares. Each model was based on 54 observations.
,*Significant at $P = 0.01$ and 0.01, respectively.

Table 2. Best single (S) and multiple (M)-variable models from stepwise regression of mite resistance variables at 72 hr on trichome variables and leaflet surface of F_2 2002 × 2010 divided into low (<1 trichome per mm^2) and high (≥1 trichome per mm^2) Type IV density classes.

		<1 Type IV trichome per mm^2		≥1 Type IV trichome per mm^2	
Variable	Model	Source of variation[z,y]	R^2	Source of variation[z,x]	R^2
Survival	S	Surface	0.17**	IV density	0.59***
	M	Surface, VI density, VI structure, leaflet length, VI UV score	0.43**	IV density, surface, leaflet length	0.61**
Mortality	S	NS		IV density	0.56***
	M	NS		IV density, VI $NaNO_2$ score	0.66**
Avoidance	S	Surface	0.12*	VI $NaNO_2$ score	0.24*
	M	Surface, VI $NaNO_2$ score, VI structure	0.30**	VI $NaNO_2$ score	0.24*
Fecundity	S	Surface	0.18*	NS	
	M	Surface	0.18*	NS	

[z]Variables for which $P > 0.05$ are not included in the models; all other variables are given in order of the significance of their partial sums of squares.
[y]Models were based on 36 observations.
[x]Models were based on 18 observations.
NS,*,**,***Nonsignificant and significant at $P = 0.05$, 0.01, or 0.001, respectively.

effects associated with density of the Type V trichome on selected F_2 hybrids with low Type IV trichome density (3), and consequently, Type V trichome density may have contributed to the surface effects on mite fecundity of F_2 2002 x 2010 with less than 1 Type IV trichome per mm^2.

On leaflets with at least 1 Type IV trichome per mm^2, Type IV trichome density was associated with 59% of the variation in mite survival at 72 hr (Table 2). Most of the variation in mite mortality was associated with Type IV trichome density, with mortality increasing as Type IV trichome density increased ($r = +0.49, P = 0.001$). Variation in phenol content of the Type VI trichome tip contributed another 10% to the model for mite mortality, which, based on the slope from the regression equation, tended to decline as $NaNO_2$ score increased. Avoidance was associated exclusively with the $NaNO_2$ score of the Type VI trichome tip. Mite avoidance increased with $NaNO_2$ score ($r = +0.49, P = 0.05$), which was opposite to the direction of avoidance in relation to phenol content on leaflets with low Type IV trichome densities. That is, mites responded differently to phenol content of the Type VI trichome tip depending on whether the leaflets had high or low Type IV trichome densities. Our data are insufficient to explain this difference; however, phenol content of the Type VI trichome tip did not affect mite survival or fecundity significantly on leaflets of either Type IV trichome density class and so was of minor importance to overall resistance on these leaflets.

Mite resistance in this full-sib F_2 family was associated primarily with Type IV trichome density, in agreement with results of previous tests on selected F_2 hybrids (3). Although Type VI trichome tips from *L. hirsutum* are more toxic to mites than those from *L. esculentum* and appear to contribute to the mite resistance of *L. hirsutum* (10), structure, phenol content, and lipid content of the Type VI trichome tip had little effect on resistance of the F_2 2002 x 2010. This lack of effect might be because the toxicity of the Type VI trichome tip does not correlate with any of the variables chosen to reflect qualitative features of Type VI trichome tips, or requires a combination of characters of the Type VI trichome tip which did not appear in the hybrids bioassayed.

Differences in mite fecundity associated with the leaflet surfaces of the F_2 2002 x 2010 were similar to the effects of Type V trichome density on fecundity of selected F_2 hybrids (3). Type I, III, and V trichomes were not counted during the bioassays of F_2 2002 x 2010. If, however, the pattern of Type V trichome density differences on these hybrids was similar to that on the previously assayed, selected F_2 individuals, then Type V trichome density would be greater on abaxial surfaces than on adaxial surfaces. Type V trichome density may thus have contributed to the increased abaxial avoidance observed on leaflets of F_2 2002 x 2010. The omission of counts of Type I, Type III, and Type V trichomes during these bioassays also may account for the smaller amount of explained variation in the analyses of mite responses on F_2 2002 x 2010, as indicated by the lower coefficients of determination in Tables 1 and 2 compared to those of previous analyses (3).

Mite survival declined in relation to Type IV trichome density averaged over both surfaces of F_2 2002 x 2010 (Fig. 1). The relatively poor fit ($R^2 = 0.37$) of this regression probably is due to the large amount of scatter at low Type IV densities. As indicated by the analysis of leaflets having low Type IV trichome densities in Table 2, mite survival varied with factors other than Type IV density when Type IV trichomes were absent or scarce. The data in Fig. 1 demonstrate the overriding importance of Type IV trichome density, even when averaged over both surfaces of leaflets from F_2 individuals, above some minimal Type IV density level. If this minimal Type IV trichome density level can be determined, it may serve as a basis for selection and thereby contribute to the identification of mite resistant individuals for breeding purposes.

The minimal Type IV trichome density level for spider mite resistance on these F_2 hybrids can be projected from the regression equation of Fig. 1, using the mite survival level of the resistant *L. hirsutum* parent as the desired predicted Y value. Leaflets from the parental *L. hirsutum* plant bioassayed at the same time as these F_2 hybrids had an average mite survival of 23% after 72 hr. For mite survival of 23%, the predicted Type IV density of these F_2 leaflets, derived from the regression equation of Fig. 1, was 5.6 Type IV trichomes per mm^2 (mean Type

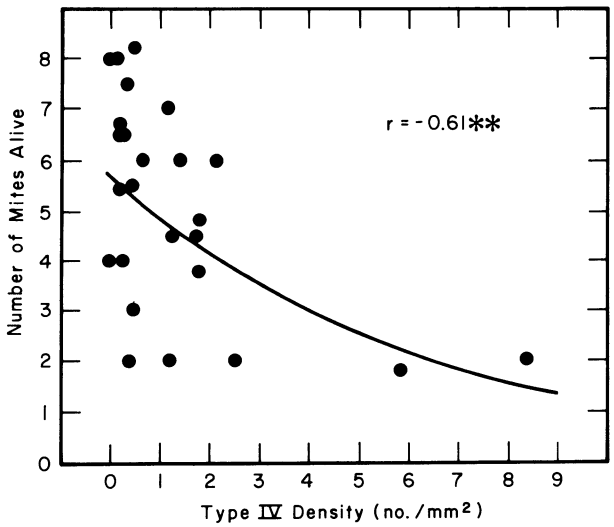

Fig. 1. Mite survival 72 hr after inoculation onto leaflets of F_2 2002 x 2010, averaged over both surfaces of each leaflet. The predicted line was obtained by regression of mean log live mites on mean Type IV trichome density, log (live mites) = 0.76 − 0.07 (Type IV trichomes/mm^2).

IV trichome density on the *L. hirsutum* leaflets was 6.4 per mm^2). If Type IV trichomes are the primary resistance factors on *L. hirsutum* and retain equal capacity in the F_2, then the mite resistance of the F_2 hybrids would be expected to be equivalent to that of *L. hirsutum* at Type IV trichome densities of 5 per mm^2 or higher. Among the total F_2 population of 67 individuals (comprising 6 families) examined to date, 9% had a mean Type IV trichome density, averaged over both surfaces of leaflets from the 3rd or 4th leaf from the apex, of at least 4.8 per mm^2. Therefore, it may be possible to select for high Type IV trichome density, and concurrently for mite resistance, from this population.

To summarize these results, resistance of a full-sib F_2 family from the cross of susceptible *L. esculentum* 'Ace' by resistant *L. hirsutum* was associated predominantly with high density of Type IV trichomes, a result in agreement with our previous findings (3, 10). The leaflet surface bioassayed, density of the Type VI trichome, and phenol content of the Type VI trichome tip also contributed slightly to mite responses, especially in the absence of high Type IV trichome densities. Type IV trichomes reduced mite survival and fecundity by producing mortality and avoidance; other independent variables produced mainly avoidance responses by mites. Although most F_2 hybrids had low Type IV trichome densities compared to that of *L. hirsutum*, mite survival on F_2 leaflets with 5.6 Type IV trichomes per mm^2 was comparable to that on *L. hirsutum*.

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