Trichome Exudates and Resistance to Beet Armyworm (Lepidoptera: Noctuidae) in *Lycopersicon hirsutum* f. *typicum* Accessions

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ABSTRACT Three accessions of *Lycopersicon hirsutum* f. *typicum* Humb. and Bonpl. were highly resistant to beet armyworm, *Spodoptera exigua* (Hübner), as indicated by reduced survival and growth on excised leaflets, compared with leaflets of a susceptible tomato variety. Removal of trichome exudates from LA 1777 and LA 2329 eliminated resistance affecting early instars; but this treatment had no effect on the resistance of PI 126445. Primarily, lamellar factors must condition resistance in PI 126445 and contribute to resistance to later instars in LA 1777 and LA 2329. Type-VI trichome exudates of all 3 accessions were topically toxic to 1st-instar *S. exigua*, and this toxicity differed among the accessions (LA 1777 > LA 2329 > PI 126445). The lower toxicity of trichome exudates on PI 126445 apparently accounts for their minimal role in resistance in this accession. During feeding, 1st instars ruptured sufficient numbers of type-VI trichome glands to account for the observed mortality on both LA 1777 and LA 2329. Toxicities of trichome exudates from the accessions were not correlated with their sesquiterpene and total phenolic contents; the most toxic trichomes (LA 1777) had the lowest concentrations of volatiles detectable by gas chromatography, and total phenolic compounds. The important toxins remain unidentified. Identification of the lamellar factors from the accessions, and the toxins in the trichome exudates of LA 1777 and LA 2329, will facilitate the use of these accessions in breeding programs for resistance to *S. exigua* and possibly other pests of tomatoes.

KEY WORDS beet armyworm, *Lycopersicon hirsutum* f. *typicum*, trichomes, trichome exudates, tomato, host plant resistance

AS A PART OF EFFORTS to develop pest management alternatives for the beet armyworm, *Spodoptera exigua* (Hübner), on tomatoes, we have screened potential sources of host plant resistance to this pest (Eigenbrode and Trumble 1993). As expected, survival of *S. exigua* was found to be low on the known insect-resistant *Lycopersicon hirsutum* f. *glabratum* Mull. (*hir*) accession PI 134417 but it was also low on several accessions of *L. hirsutum* f. *typicum* Humb. and Bonpl. (*typ*). These 2 subspecies of *Lycopersicon* were delineated by Muller (1940) on the basis of floral characteristics and geographic distribution. They also differ in the chemical composition of their trichome exudates (Weston et al. 1989, Eigenbrode et al. 1994). *Typ* exudates do not contain the 2 insecticidal methylketones, 2-undecanone and 2-tridecanone, consistently found in *hir* exudates, and there is a considerable diversity of other exudate components among *typ* accessions. The dominant sesquiterpenes in *typ* accessions are toxic to *S. exigua* (Eigenbrode et al. 1994), but other unidentified components are also apparently toxic (Lin et al. 1987, Eigenbrode et al. 1994). Lamellar components may also contribute to resistance in *typ*, as in some accessions of *L. h.* f. *glabratum* (Farrar and Kennedy 1992). Thus, *typ* accessions potentially produce novel insect resistance factors, which, if identified, could be used to develop insect-resistant cultivated tomatoes, *L. esculentum* Mill.

Our objectives in the current study were the following: (1) confirm resistance to *S. exigua* in *typ* accessions LA 2329, LA 1777, and PI 126445; (2) determine the role of type-VI trichomes in this resistance by removing their exudates before bioassay; (3) compare the topical toxicity to *S. exigua* of individual type-VI trichomes from each accession; (4) determine if *S. exigua* larvae break these trichomes during feeding in sufficient numbers to account for the observed resistance; and (5) relate these findings to the composition of the type-VI trichome exudates of these accessions.

Materials and Methods

Plants and Insects. Seeds were obtained either from the C. M. Rick *Lycopersicon* collection at the University of California, Davis (LA 1777 and LA 2329), the USDA–NRS Germplasm Repository, Geneva, NY (PI 126445), or Petoseed (Woodland,

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CA) (insect-susceptible *L. esculentum* variety VFN 7718). Ten plants of each plant type were germinated in the greenhouse at the University of California, Riverside and transplanted into 11-liter pots containing UC mix (Matkin and Chandler 1957) potting soil. Lighting was ambient (February through May) and temperatures were ≈20°C at night and 28°C during the day. The primary terminals of each plant were clipped to encourage branching to provide sufficient numbers of young leaves for experiments. All data were obtained using leaves from the 5th node from a terminal. Experiments began when the plants were 8 wk old. Growth and development bioassays with *S. exigua* were carried out in the laboratory at 20–21°C and a photoperiod 16:8 (L:D) h.

Spodoptera exigua were from a colony maintained in continuous culture on artificial diet (Shorey and Hale 1965) at 27°C and a photoperiod of 16:8 (L:D) h. The colony was originally established from insects collected from Orange County, California, and had new genetic material added within 12 mo of the experiments. Insects used in bioassays were neonates <2 h old.

***Spodoptera exigua* Development on Leaf Tissue With and Without Trichome Exudates.** Neonate *S. exigua* larvae were reared to adults on leaflets of the 3 *typ* accessions and the susceptible VFN 7718. Ten larvae were reared individually in 30-ml plastic cups on leaflets from each of 10 plants of each plant type. Ten additional larvae were reared on similar leaflets from the same plants, wiped with a methanol-moistened tissue to remove trichome exudates without noticeably damaging the leaf surface. Leaflets were replaced daily throughout larval development. The plastic cups were partially filled with nonnutrient agar gel to help maintain moisture and plant turgor. The percentage of larvae surviving for 3, 5, 7, and 10 d to pupation and to adult, the weight of larvae on day 7, the weight of pupae (1 d after pupation), and days to pupation and adult emergence were recorded. All variables were analyzed using analysis of variance (ANOVA) with plant type and treatment (trichomes intact or exudates removed) as classification variables. Where applicable, individual means were compared using the Tukey honestly significant difference (HSD) test at the $P = 0.05$ level. Survival data were transformed to the arcsine of the square root, and weights were log-transformed. Analyses were performed using SAS procedure general linear model (GLM) (SAS Institute 1988).

Trichome Topical Toxicity. Toxicity of individual type-VI trichomes to neonate *S. exigua* was measured by rupturing the trichome glands against the dorsum of individual larvae. The larva was held on a moistened camel's-hair brush and brought into contact with individual trichomes on an intact leaflet until the glands burst. Pilot tests determined the dose series required to assess the LD_{50} (trichomes per larva) for each plant type. These were 0, 3, 5, 7, 10, 15, 20, and 40 trichomes for LA 2329; 0, 1, 2, 3, 5, 10, 20, and 40 trichomes for LA 1777; and 0, 5, 10, 15, 20, 40, and 60 trichomes for PI 126445. Toxicity of VFN 7718 trichomes was much lower than that of *typ* trichomes, but the dose series used was the highest practicable: 0, 5, 10, 20, 40, and 60. Ten larvae were treated at each dose for each of the 10 plants from each test entry. Control larvae (dose = 0) were manipulated with a camel's-hair brush to duplicate the physical trauma caused during the breaking of 60 trichomes. Treated larvae were placed on artificial diet (the same used to maintain the *S. exigua* colony) and survival was assessed after 24 h. Because within accession variation was of interest, an overall probit analysis was not conducted. Rather, an LD_{50} was estimated for each individual plant using the PROBIT procedure in SAS (SAS Institute 1988). This provided 10 independent measures of the LD_{50} for trichomes of each accession. These LD_{50} values were then treated as individual observations for an ANOVA with plant accession as the classification variable.

Trichome Rupturing By Neonate Larvae. The number of type-VI trichome glands broken by neonates on each test entry was estimated by confining individual larvae on 1-cm-diameter leaf disks of each plant type in 30-ml plastic cups for 24 h. The number of type-VI trichomes was counted before and after larvae were confined on the disks. This number was corrected for the attrition of trichome glands during 24 h on similar disks of each test entry without caterpillars. Twelve replicates were conducted for each of the plant types; no larvae died during these tests.

Trichome Characteristics. The number of type-VI trichomes per square centimeter of leaf surface was counted on a single leaflet from each of the 10 test plants from each plant type. The estimated density for each leaflet was the mean density of two 2-mm^2 areas on the upper and lower surface of each leaflet. Trichome density for each plant type was then calculated as the mean of these individual leaflet estimates from all 10 plants.

The sesquiterpene content of type-VI glands was determined by collecting the extracts from a total of 1000 trichomes from 5 leaflets of each plant. The extracts were collected by rupturing individual trichomes with a filter-paper wick (5 mm long by 2 mm wide at the widest point and tapering to a sharp point) soaked in hexane and then extracting these wicks in distilled hexane. Each wick could absorb the exudates of ≈20 glands. The 50 wicks from each plant were extracted together with three 10-ml washes of distilled hexane. The 3 washes were combined, concentrated, and analyzed with coupled gas chromatography/mass spectrometry (GC/MS) for common sesquiterpenes of *L. hirsutum* (Eigenbrode and Trumble 1993). The concentration of each sesquiterpene was estimated on a per-trichome basis for each plant. Mean concentrations were then calculated for each plant type.

Phenolic content of the glands was also estimated by extracting 100 glands from 4 leaflets from each plant using a filter-paper wick soaked in methanol acidified with 10% acetic acid to denature polyphenol oxidases that would otherwise oxidize the phenolics to quinones. The extract was reduced to 0.5 ml and combined with 0.5 ml of a 10% aqueous solution of ammonium molybdate in the cells of an ELISA plate and absorbance was measured at 350 nm. A standard absorbance curve developed using chlorogenic acid was used to estimate concentrations of phenolics.

Untransformed trichome densities, nanograms of components per trichome, and the corrected numbers of trichomes broken by neonates were analyzed using ANOVA, and the Tukey HSD for mean separation (SAS Institute 1988).

Results

Compared with the susceptible variety VFN 7718, the 3 *typ* accessions were highly resistant to *S. exigua*. Resistance was indicated by significantly reduced survival after 3 d (LA 2329 and LA 1777) or 5 d (PI 126445), and continued higher mortality throughout development, resulting in low or zero (LA 2329 and LA 1777) percentage of pupation (Fig. 1). Mortality after 3 d was greatest on LA 2329, followed by LA 1777, and then PI 126445 and VFN 7718, which were not significantly different from one another, and pupation only occurred on PI 126445. Those larvae pupating on PI 126445 had lower pupal weights and longer times to pupation and adult emergence than those on susceptible VFN 7718 (Table 1). Weights of surviving larvae at day 7 were greatly reduced on all *typ* accessions compared with the susceptible variety (Table 1).

Removal of trichome exudates from the LA 2329 and LA 1777 significantly increased survival of *S. exigua*. During the first 7–10 d, survival on these accessions without exudates was not different from survival on the susceptible VFN 7718 ($P = 0.05$). After 10 d, survival on LA 2329 and LA 1777 with exudates removed fell below survival on VFN 7718, but was significantly greater than survival on these accessions with intact trichomes (Fig. 1). Those insects surviving on LA 2329 and LA 1777 with exudates removed also had reduced pupal weights and survival to adult and longer development times than did insects on VFN 7718. Removal of exudates from PI 126445, in contrast to the result with LA 2329 and LA 1777, did not improve *S. exigua* survival.

Exudate removal increased larval weight at 7 d on all three accessions and on VFN 7718 (Table 1). Surprisingly, pupal weights on VFN 7718 and PI 126445 were reduced significantly as a result of removing trichome exudates (Table 1).

The topical toxicity of type VI trichome exudates from the 3 *typ* accessions differed significantly (Fig. 2). The LD_{50} of LA 1777 was only 5 trichomes, whereas that for PI 126445 was 30, and LA 2329 was intermediate at 16. The mean LD_{50} for VFN 7718 was >200 trichomes, but could not be determined with certainty because the value was above the highest dose applied in the bioassay and fiducial limits were rarely estimated by the PROBIT procedure (SAS Institute 1988).

The number of type VI trichome glands broken by neonate *S. exigua* in 24 h also differed significantly on the 4 plant types, and was greatest (>30) on VFN 7718 and least (14) on LA 1777 (Fig. 2) and intermediate for LA 2329 and PI 126445. The density of type VI trichomes on LA 2329 was twice as great as on the other 2 *typ* accessions, whereas the trichome density on VFN 7718 was intermediate (Fig. 2).

Trichome exudate composition differed among the accessions and the susceptible tomato (Table 2). LA 2329 and PI 126445 exudates had similar concentrations of zingiberene and γ-elemene, whereas LA 1777 had no detectable zingiberene and a lower concentration of γ-elemene. No sesquiterpenes were detected in VFN 7718 trichomes. The total concentration of volatiles, including sesquiterpenes, was greatest in LA 2329, followed by PI 126445, and then LA 1777 and VFN 7718. LA 2329 also had many more detectable volatile peaks (mean of 16) than the other accessions (4–5). The phenolic content was significantly higher in exudates of the susceptible *L. esculentum* VFN 7718 than in any of the *L. hirsutum* accessions.

Discussion

Spodoptera exigua growth and survival was greatly reduced on all 3 *typ* accessions as compared with the susceptible variety, indicating their potential as sources of resistance to this pest. The resistance mechanisms involve several factors. In LA 2329 and LA 1777, reduced survival of young larvae was completely dependent on the presence of the type VI trichome exudates, but their removal did not eliminate negative effects on the growth and survival of later instars. On PI 126445, reduced survival of young larvae did not depend on trichome exudates; their removal increased larval growth rates only slightly, but otherwise left resistance in this accession intact. Thus, lamellar factors must produce resistance to later instars in LA 2329 and LA 1777, and most of the resistance in PI 126445. In general, lamellar factors were more effective against later instars.

The acute topical toxicity of type VI trichome exudates of LA 1777 and LA 2329 can explain resistance of these accessions to early instars of *S. exigua*. The number of glands ruptured by feeding 1st instar larvae in 24 h exceeds the LD_{50} for these glands applied directly to the larval dorsum. Despite this, no mortality was observed after 24 h in the bioassay for trichome breakage by larvae or in the growth and development bioassay (data not

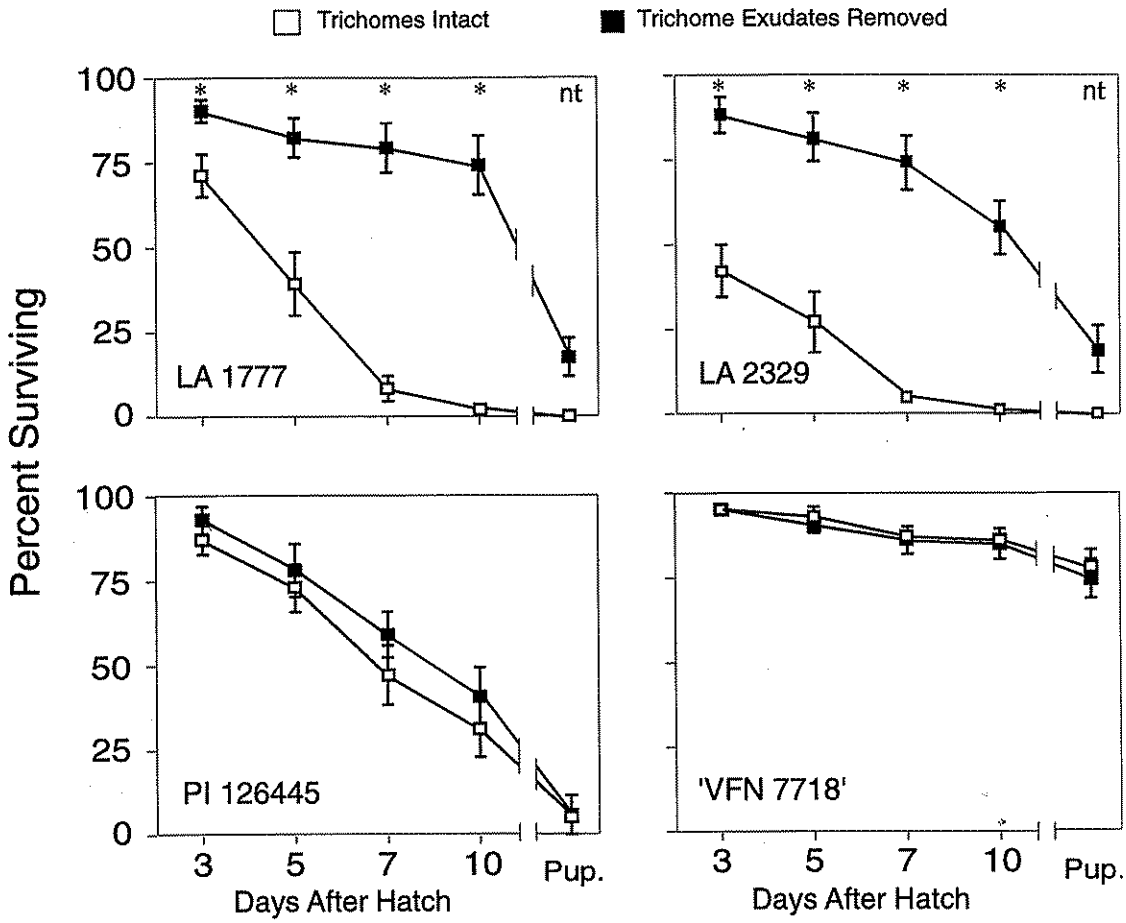

Fig. 1. Survival through 10 d and to pupation (Pup.) of *S. exigua* larvae on leaflets of three *L. hirsutum* f. *typicum* accessions and a susceptible variety of *L. esculentum*; trichome exudates were either intact or removed with methanol. Note that time to pupation differed among plant types. ° = survival on leaflets with and without trichome exudates removed differs (Tukey HSD, $P \leq 0.05$). nt = no test possible because one treatment value = zero. Bars are SEM.

Table 1. Growth and development of *S. exigua* larvae on *L. hirsutum* f. *typicum* accessions and a susceptible *L. esculentum* with trichome exudates either intact or removed with methanol

Test line	Treatment	Larval wt		Pupal wt, mg[c]	% adult survival[d]	Days to adult[e]
		at 7 d, mg[a]	Days to pupation[b]			
LA 1777	trichomes intact	0.6 ± 0.2d	—	—	0 ± 0	—
	exudates removed	2.9 ± 0.3c	26.2 ± 1.5a	64.2 ± 2.1c	14 ± 5b	34.6 ± 1.4a
LA 2329	trichomes intact	0.6 ± 0.2d	—	—	0 ± 0	—
	exudates removed	2.4 ± 0.4c	25.6 ± 1.2a	59.6 ± 3.4d	8 ± 6b	35.1 ± 1.7a
PI 126445	trichomes intact	0.7 ± 0.1d	26.8 ± 2.7a	77.8 ± 7.9b	6 ± 3b	35.5 ± 2.2a
	exudates removed	1.6 ± 0.3c	27.8 ± 1.2a	52.3 ± 2.0d	9 ± 3b	34.4 ± 1.2a
VFN 7718	trichomes intact	5.9 ± 0.9b	17.3 ± 0.4b	91.8 ± 2.2a	70 ± 4a	26.1 ± 0.4b
	exudates removed	11.5 ± 1.6a	17.3 ± 0.9b	75.8 ± 2.4b	55 ± 7a	25.9 ± 0.7b

Means ± SEM with the same letter within a column are not significantly different (Tukey HSD; $P = 0.05$).
[a] $F = 32.29$; df 7, 59; $P = 0.0001$.
[b] $F = 17.24$; df = 5, 35; $P = 0.0001$.
[c] $F = 20.52$; df = 5, 35; $P = 0.0001$.
[d] $F = 12.89$; df = 5, 33; $P = 0.0001$.
[e] $F = 21.80$; df = 5, 33; $P = 0.0001$.

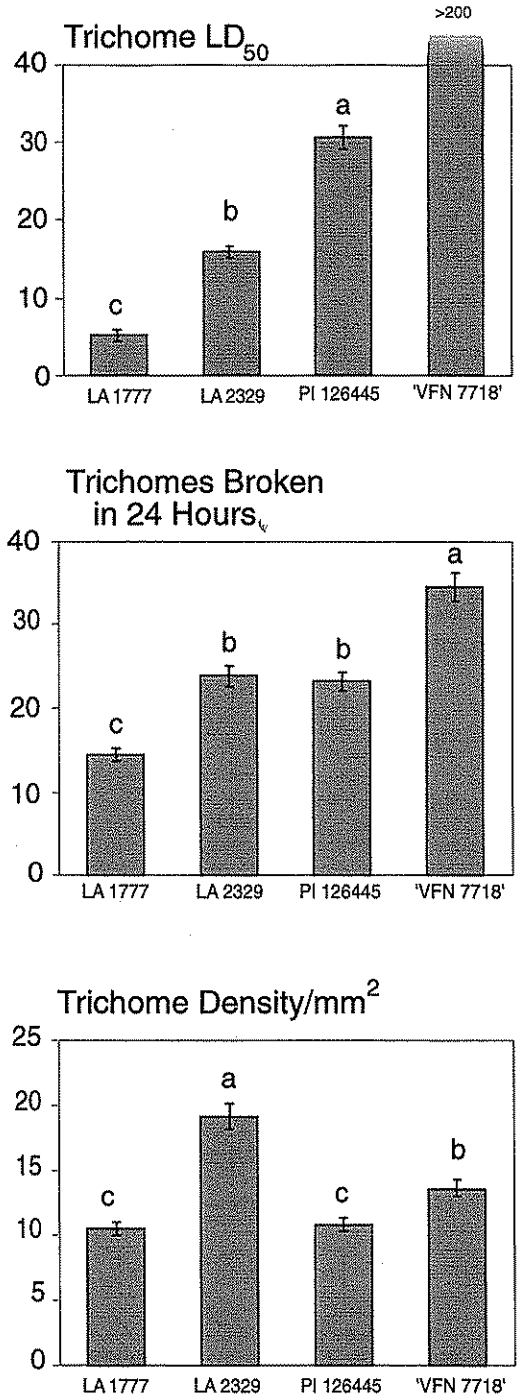

Fig. 2. Toxicity of trichome exudates (LD_{50} in number of trichomes), number of trichomes broken by feeding larvae, and density of trichomes on three *L. hirsutum* f. *typicum* accessions and a susceptible variety of *L. esculentum*. LD_{50} values are means for each of 10 plants within each accession, calculated by independent probit analyses. Bars for all variables are SEM.

Table 2. Principal sesquiterpenes, volatiles detectable by GC, and phenolics in type-VI trichome glands of 3 *L. exigua*-resistant *L. hirsutum* f. *typicum* accessions and a susceptible *L. esculentum*

	Nanograms per type-VI trichome			
	γ-Elemene[a]	Zingiberene[b]	Total GC volatiles[c]	Phenolics[d]
LA 1777	2 ± 1b	0 ± 0	4 ± 1c	3 ± 1b
LA 2329	3 ± 0.4ab	25 ± 3a	96 ± 12a	13 ± 1b
PI 126445	4 ± 0.4a	29 ± 4a	38 ± 4b	4 ± 1b
VFN 7718	0 ± 0	0 ± 0	1 ± 0.2c	63 ± 7a

Means ± SEM with the same letter within a column are not significantly different based on Tukey HSD ($P = 0.05$) or Student *t*-test.

[a] $F = 6.31$; df = 6, 31; $P = 0.0058$.

[b] Student *t*-test, $P = 0.4402$.

[c] $F = 46.90$; df = 3, 35; $P = 0.0001$; approximated from FID signal (Eigenbrode et al. 1994).

[d] $F = 67.21$; df = 3, 35; $P = 0.0001$; based on standard absorption curve of chlorgenic acid.

shown), implying that not all exudates from glands ruptured during feeding actually contact larvae. Although the exudates from LA 2329 are not as toxic as are those from LA 1777, the higher type VI trichome densities on LA 2329 result in more trichome rupture and an equivalent effect of these exudates on survival of early instar *S. exigua*. Mortality on LA 1777 and LA 2329 over several days may result from postingestive or antifeedant effects, as well as topical toxicity of their trichome exudates. These potential effects must be tested with additional bioassays.

The toxic constituents of the trichome exudates of all 3 *typ* accessions remain unidentified. Topical toxicity of the exudates is not related to the concentrations of sesquiterpenes, other unidentified volatiles, or phenolics quantified in this study (Fig. 2; Table 2). *Typ* trichome LD_{50}s contain only 0.01–0.9 μg of the sesquiterpenes (Table 2), much less than the topical LD_{50}s of the pure compounds (3–6 μg; Eigenbrode et al. 1994). The components responsible for the toxicity of the intact exudates remain unidentified. Possibilities include acyl-sugars and sesquiterpene acids, which would not be detected using our GC methods.

The trichome exudates of susceptible VFN 7718 also contain unknown factors with some minor effects on *S. exigua* growth (Table 1), but not survival (Fig. 1). These factors are likely to be phenolics, which were present in high concentrations in the exudates, and which have been shown to inhibit the growth of corn earworm, *Helicoverpa zea* (Boddie) (Duffey and Isman 1981). The reduced pupal weights on both VFN 7718 and PI 126445 with trichome exudates removed is a paradoxical result. This may have occurred because the trichomes contain factors that enhance growth or delay pupation (PI 126445 only), as well as toxins.

The number of trichomes broken in 24 h on the accessions and on VFN 7718 are only partly explained by differences in trichome density (Fig. 2).

Disproportionately more are broken on susceptible VFN 7718. This is either because larvae remain more active over 24 h on VFN 7718, thus breaking more trichomes, or because the trichomes on the susceptible plant are more fragile and easily broken, or because larvae deliberately avoid disrupting trichomes on resistant accessions because these trichomes have antifeedant properties.

Resistance in all 3 *typ* accessions depends on several factors in the trichome exudates and leaf lamellae. Further elucidation of the specific mechanisms will facilitate their use in breeding programs. The additional work should consider the influence of day length on trichome-based resistance (for example, Kennedy and Dimock 1983). The extremely toxic trichome exudates of LA 1777 appear most likely to contain a single potent factor that could condition usable resistance to *S. exigua* and possibly other insects. Lamellar factors from all 3 accessions may be valuable as an alternative to trichome-based resistance that has proven difficult because of undesirable linkages (Farrar and Kennedy, 1992).

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