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ACTIVITY OF VOLATILE COMPOUNDS IN GLANDULAR TRICHOMES OF *Lycopersicon* SPECIES AGAINST TWO INSECT HERBIVORES

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Abstract—Several major chemicals in the glandular heads of type VI trichomes of *Lycopersicon* species were identified and quantified by gas chromatography and mass spectrometry. Two normal odd-chained ketones, 2-undecanone (47 ng) and 2-tridecanone (146 ng), and one unknown sesquiterpene (5 ng), comprised approximately 95% of the contents of a gland of *L. hirsutum* f. *glabratum* Mull. In a closely related plant, *L. hirsutum* Humb. & Bonpl. (LA 361), two unknown insecticidal sesquiterpenes accounted for 6% of the gland contents. Additionally, small amounts of one unknown monoterpene and another unknown sesquiterpene were found in type VI glands of a commercial tomato variety, *L. esculentum* Mill. Bioassays comparing the gland exudate (by direct contact) and isooctane extracts of glands to neonate larvae of *Keiferia lycopersicella* (Walsingham) (Lepidoptera: Gelechiidae) and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) indicated that: (1) 2-tridecanone and 2-undecanone were the major insecticidal compounds in *L. hirsutum* f. *glabratum*, (2) the two unknown sesquiterpenes in *L. hirsutum* were acutely toxic to both species, and (3) gland contents in the commercial tomato variety provided only a physical barrier to *K. lycopersicella*, and were not detrimental to *S. exigua*. In topical bioassay trials, synthetic mixtures of 2-tridecanone and 2-undecanone (3:1) demonstrated potentiation. Concentrations of these chemicals decreased as trichomes aged. Quantities of insecticidal chemicals and density of type VI trichomes varied with plant age and location within plants.

Key Words—*Lycopersicon*, *Keiferia lycopersicella*, *Spodoptera exigua*, Lepidoptera, Gelechiidae, Noctuidae, leaf trichomes, 2-tridecanone, 2-undecanone, terpenoids, toxicity.

INTRODUCTION

Glandular trichomes of wild tomatoes in the genus *Lypersicon* have recently become the subject of considerable study because of their effectiveness in protecting tomato plants from insect damage (Rodriguez et al., 1972; Schuster, 1977; Dimock and Kennedy, 1983; Schwartz and Snyder, 1983; Snyder and Carter, 1984; Kennedy and Sorenson, 1985) and the possibility of breeding this character into the commercial tomato *L. esculentum* (Kennedy and Henderson, 1978; Fery and Kennedy, 1983; Fery et al., 1984). The secretion of glandular trichomes from one tomato accession, *L. hirsutum* Humb. & Bonpl. (PL 251301), has also proven toxic to the spider mite *Tetranychus urticae* Koch (Aina et al., 1972), suggesting that trichome-based resistance could be an effective tool in a pest management program against a wide variety of arthropod pests.

Relatively few chemical analyses of trichome contents or exudates in tomato species have been reported. Studies by Beckman et al. (1972) found phenolics in capitate cells of four-lobed trichomes of *L. esculentum*, and Duffey and Isman (1981) determined that rutin (a flavonoid glycoside) was a major constituent of the trichomes in commercial tomatoes. More recently, Kennedy and Dimock (1983) reported that 2-tridecanone (a methyl ketone) was the dominant compound in the type VI glands (characterized by Luckwill, 1943) of *L. hirustum* f. *glabratum* (PL 134417) which was acutely toxic to *Manduca sexta* (L.), *Heliothis zea* (Boddie), and *Leptinotarsa decemlineata* (Say).

Our previous studies on resistance in wild and commercial tomatoes to a specialist herbivore, the tomato pinworm (TPW), *Keiferia lycopersicella*, demonstrated that type VI glandular trichomes were the primary cause of larval mortality (Lin and Trumble, 1986). In this paper we: (1) quantify the constituents of glandular heads of type VI trichomes from selected wild and commercial tomato accessions, (2) document effects of trichome age and location within plants on chemical composition of type VI trichomes, and (3) report a bioassay procedure designed to quantify the insecticidal activity of these compounds for both *K. lycopersicella*, and a generalist herbivore, the beet armyworm (BAW), *Spodoptera exigua*.

METHODS AND MATERIALS

All plants used in the following tests were grown from seed in a glasshouse in 15-cm pots containing UC soil mix (Matkin and Chandler, 1957) and a slow-release fertilizer. Day length was maintained at 16 hr using artificial illumination. Seeds of *L. hirsutum* (LA 361) were obtained from Dr. C. Rick (University of California, Davis) and *L. hirsutum* f. *glabratum* (PL 134417) were obtained from Dr. G. Kennedy (North Carolina State University). Commercial *L.*

esculentum cv. VFN 7718 were purchased from Champion Seed Co. (529 Mercury Lane, Brea, California 92621).

Identification and Quantification of Chemicals in Type VI Glands. All gland samples were analyzed for chemical content using a capillary gas chromatograph (GC) and GC-mass spectrometer. The GC was a Packard gas chromatograph equipped with hydrogen flame ionization detector and a DB-5 60-m × 0.25-mm-ID capillary column. Temperature was programmed from 45 to 210°C at 1°C/min. The head pressure for helium was 20 pounds per square inch. The flow rates for hydrogen and compressed air were 30 and 200 ml/min, respectively. Internal standards for quantitative analyses were 2-decanone and 2-pentadecanone peak areas and retention time(s) were calculated using a Spectra-Physics Autolab System I program integrator (333 N. First St., San Jose, California). Electron-impact mass spectra were recorded from samples at 70 eV with a VG ZAB-HF mass spectrometer. Comparisons of chemical quantities in glandular trichomes were evaluated with the Student's *t* test.

Identification of the chemical contents in glandular heads of type VI trichomes was based on three samples of 100 intact mature glands which were collected from the adaxial, interveinal areas of three leaflets per plant (8th–9th leaves from the bottom) for five plants of each variety ($N = 45$ samples of 100 glands/variety; plants were 123 days postgermination). With the aid of a dissecting microscope, a glass probe was used to rupture the glands and transfer their contents directly into isooctane. The probe was rinsed in ethanol and isooctane after collecting each 100-gland sample to avoid any contamination of subsequent samples. Additional samples of 200–4500 glands were also collected for identification of other possible minor constituents. All glands were collected between 11 AM and 2 PM to minimize potential diel variation in chemical production (Turner et al., 1980).

Effect of Trichome Age and Location within Plants on Chemical Composition of Type VI Glands. Fully developed type VI trichomes exhibited two stages which were clearly discernible using a dissecting microscope. In the initial mature stage, the trichomes possessed glandular heads with a clear liquid content. The subsequent stage, referred to as aged, had yellow-orange glandular heads which appeared to have a more viscous content. In order to determine if gland age affected the chemical content, three 200 gland samples of each maturity stage were collected from four plants each of *L. hirsutum* and *L. hirsutum* f. *glabratum*, and prepared for analysis as described previously ($N = 12$ samples of 200 glands/maturity stage). Mature and aged trichomes were collected from the same leaves.

The influence of location within plants on chemical content of type VI trichomes was evaluated by collecting 200 gland samples from three locations per plant on five plants each of *L. hirsutum* and *L. hirsutum* f. *glabratum*. To standardize the test material, glands were collected weekly from 2–5-week post-emergence-old plants on: (1) the terminal leaflet of the third leaf from the bot-

tom, (2) the stem between the second and third lowest nodes, and (3) terminal leaflet of 1-week-old leaf at the plant apex. This plant stage was chosen because the tomatoes were in the critical developmental period following germination, and the specific leaves tested began to senesce at five weeks. In addition, number of type VI glands per unit leaf area was determined using a micrometer grid reticule and a dissecting microscope.

Bioassays of Gland Exudate, Gland Extracts, and Pure Compounds. TPW and BAW used in these trials were obtained from cultures initiated with larvae collected in Orange County, California and maintained on tomato plants (VFN 7718) and artificial diet (Patana, 1969), respectively. Insects were maintained at 27 ± 1°C and a photoperiod of 12:12 light–dark. Mature type VI glands were collected from terminal leaflets (N = 12 leaflets/variety) of the eighth and ninth leaves from the base of 2-month-old *L. hirsutum*, *L. hirsutum* f. *glabratum*, and *L. esculentum*. Additional type VI glands (N = 12 samples of 200/leaflet) were collected for GC quantitative analysis in order to document concentrations of key compounds in fresh plant material for comparison of isooctane gland extracts with pure compounds. Data on the toxicity resulting from topical applications of gland exudate, isooctane extracts of glands, and pure compounds to BAW and TPW were standardized by transforming all data to nanograms of chemicals per larva to allow direct comparisons between treatments. Data were then analyzed by probit analysis with the Proc Probit procedure of the Statistical Analysis System (Helwig and Council, 1979) after correction for control mortality with Abbott's (1925) formula.

First-instar larvae of BAW and TPW were exposed directly to gland exudate on fresh plant material by adhering individuals to a water-moistened 000 paint brush, and with the aid of a dissecting microscope, breaking type VI glands against the dorsal thorax. Four replicates of 15 larvae of TPW were treated at the following gland "dosage" (N = 60 larvae/dose) for the two wild tomato accessions tested: *L. hirsutum* = 1, 2, 4, 6, 8 glands/larva, and control (moistened brush treatment only); *L. hirsutum* f. *glabratum* = 1, 2, 3, 4, 5, and control. Similarly, BAW were exposed as follows: *L. hirsutum* = 20, 30, 40, 50, 60, and control; *L. hirsutum* f. *glabratum* = 12, 15, 18, 21, 24, and control. Mortality was also observed in trials with *L. esculentum*, where TPW or BAW were exposed to 20 or 60 glands, respectively, the maximum possible for the size of the larvae. Larvae were held at 26 ± 1°C, and mortality was evaluated at 24 hr. Larvae were considered dead if they were unable to move within 30 sec of being prodded.

In a related experiment, neonate larvae of both insect species were exposed to an extract of glands dissolved in isooctane. Isooctane was selected as a carrier because this solvent was used for all GC analyses. Gland contents were collected with a glass probe as described earlier, and serial dilutions of concentrate were topically applied to neonate larvae using a 0.5-μl Hamilton syringe. Be-

cause of the small size of the larvae, dosages were applied to the dorsum of the thorax in a 0.01-μl droplet for TPW, and in a 0.2-μl droplet for BAW. Larvae were treated on a glass surface instead of filter paper, since our preliminary trials indicated that efficacy was artificially reduced on filter paper as some test material was lost to absorption into the substrate. Four replicates of 15 larvae of each species were exposed to the extract of each of the five dosages and isooctane controls evaluated, and mortality was assessed at 24 hr. Dosages tested for TPW were 60, 70, 80, 90, and 100 glands of *L. hirsutum* f. *glabratum*/ μl of isooctane and 30, 40, 50, 60, and 70 glands of *L. hirsutum*/ μl of isooctane. Dosages evaluated for BAW were 60, 70, 80, 90, and 100 glands of *L. hirsutum* f. *glabratum*/ μl of isooctane and 10, 20, 30, 40, and 50 glands of *L. hirsutum*/ μl of isooctane.

The contact toxicities of the two commercially available pure chemicals, 2-undecanone and 2-tridecanone (purity 97.0%, Pfaltz & Bauer, Inc., Waterbury, Connecticut), were also evaluated. Each compound was dissolved in isooctane to a concentration of 50 mg/ml, as was a 3 : 1 mixture of 2-tridecanone and 2-undecanone. Neonate larvae of TPW and BAW were treated with five concentrations (dosages) of the test chemicals and a control (isooctane). Dosages tested for TPW were 3.6, 4.0, 4.8, 5.6, and 6.4 mg of 2-tridecanone/ml of isooctane; 2.0, 2.4, 3.2, 4.0, and 4.8 mg of 2-undecanone/ml of isooctane; and 0.1, 0.3, 0.5, 0.7, and 0.8 mg of mixture/ml of isooctane. Dosages evaluated for BAW were 12, 14, 16, 18, and 20 mg of 2-tridecanone/ml of isooctane; 12, 14, 16, 18, and 20 mg of 2-undecanone/ml of isooctane; and 6, 8, 10, 12, and 14 mg of mixture/ml of isooctane. Data were analyzed for potentiation after Finney (1971). All topical applications were made with a 0.5-μl Hamilton syringe, using the same equipment and procedures as previously described.

RESULTS AND DISCUSSION

Identification and Quantification of Chemicals in Individual Glands. GC analyses of isooctane extracts of the type VI glands of *L. hirsutum* f. *glabratum* detected three major components: 2-tridecanone, 2-undecanone, and one unknown compound in the relative proportion of 30 : 10 : 1, respectively, (Figure 1). The predominance of 2-tridecanone was consistent with previous reports documenting the presence of this chemical in foliage of *L. hirsutum* f. *glabratum* (Soost et al., 1968; Dimock and Kennedy, 1983). The relatively high proportion of 2-undecanone was unexpected. Mass spectrometric analysis of the unknown compounds produced different fragmentation patterns with identical molecular ions at *m/e* 204 (Table 1), which are consistent with the assignment of the molecular formula of a sesquiterpene (SES) ($C_{15}H_{24}$) (Silverstein et al., 1974).

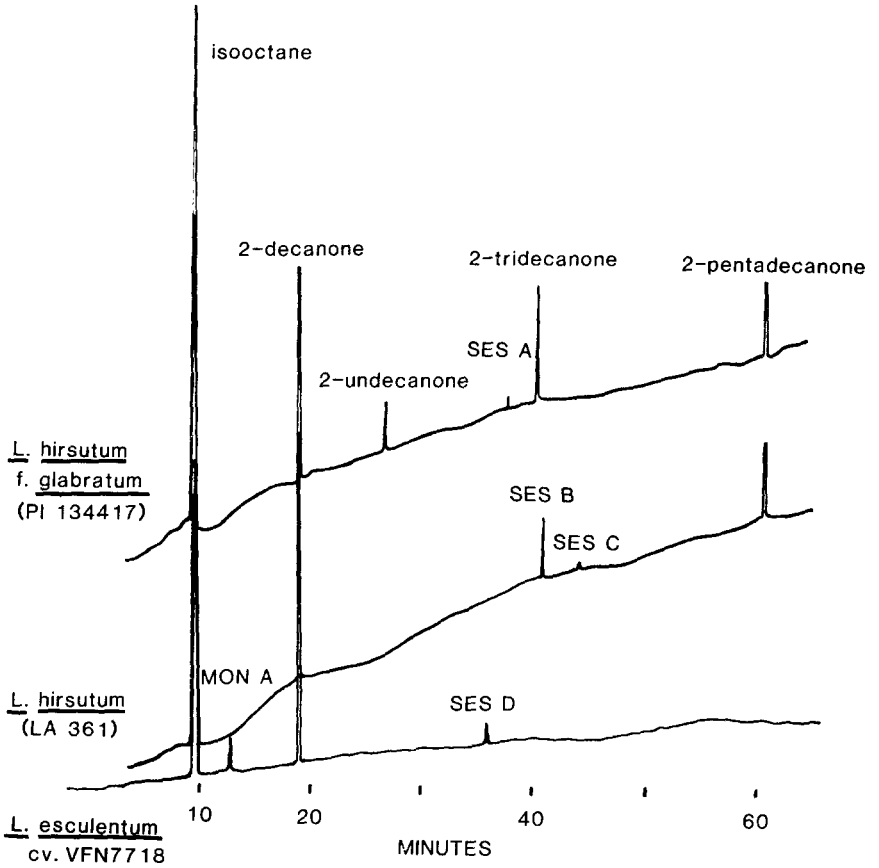

FIG. 1. Chromatograms of isooctane extracts of glandular heads of type VI trichomes from *L. hirsutum* (LA 361), *L. hirsutum* f. *glabratum* (PI 134417), and *L. esculentum* (VFN 7718) using a DB-5 0.25-mm-ID × 60-m long capillary column on a Packard gas chromatograph.

In *L. hirsutum* (LA 361), two unknown sesquiterpenes (SES B and SES C) were detected by GC-MS analysis (Table 1, Figure 1). The retention time of the unknown SES B was very similar to 2-tridecanone, but augmentation with 2-tridecanone confirmed that SES B was different. In *L. esculentum*, one unknown monoterpene and one unknown sesquiterpene (SES D, which was different from SES A, B, and C; Figure 1) were found to be the major volatile components. Although Soost et al. (1968) reported several ketone groups as the major volatile compounds in whole foliage of *L. hirsutum* and *L. esculentum* cv. Ace, our experiments demonstrated that there were neither 2-tridecanone nor other methyl ketones in the glandular heads of type VI trichomes of *L hir-*

TABLE 1. MASS SPECTRA[a] OF MAJOR UNKNOWN COMPONENTS IN GLANDULAR HEADS OF TYPE VI TRICHOMES FROM *L. hirsutum* (LA 361), *L. hirsutum* f. *glabratum* (PI 134417), AND *L. esculentum* CV. VFN 7718

Variety	Compound	*m/e* (% relative intensity)				
L. hirsutum	Sesquiterpene A[b]	204(13)	161(34)	148(31)	133(91)	120(42)
f. *glabratum*	($C_{15}H_{24}$)	119(39)	107(46)	105(60)	93(99)	91(84)
		81(41)	79(81)	77(48)	69(75)	67(41)
		53(38)	41(100)	39(42)		
L. hirsutum	Sesquiterpene B[b]	204(13)	120(12)	119(97)	105(15)	93(100)
	($C_{15}H_{24}$)	92(17)	91(34)	77(30)	69(36)	56(15)
		55(14)	41(36)	39(10)		
	Sesquiterpene C[b]	204(22)	121(100)	107(61)	105(65)	93(79)
	($C_{15}H_{24}$)	91(66)	79(65)	53(63)	41(94)	39(55)
L. esculentum	Monoterpene A[b]	136(30)	121(10)	93(100)	77(48)	68(22)
	($C_{10}H_{16}$)	53(20)	41(59)			
	Sesquiterpene D[b]	204(20)	189(11)	161(28)	148(20)	133(68)
	($C_{15}H_{24}$)	120(32)	105(43)	93(100)	79(80)	69(72)
		53(36)	41(100)			

[a] See text for GC-MS conditions. The *m/e* values for the unknowns are arranged in order of descending fragment size.
[b] Tentative compound identification based on GC and MS data.

sutum (LA 361) and *L. esculentum* cv. VFN 7718. Even though the GC retention time of the major unknown monoterpene in *L. esculentum* cv. VFN 7718 was close to limonene, which was reported as the major volatile on cv. Ace (Soost et al., 1968), the mass spectrum demonstrated that these compounds were different. The identification of these unknown terpenoids in *L. hirsutum* and *L. esculentum* is currently under investigation. To date, the quantities of glandular material have not been sufficient for qualitative analysis.

Gas chromatography revealed an average of 146 ng of 2-tridecanone, 49 ng of 2-undecanone, and 5 ng of sesquiterpene A per type VI gland of *L. hirsutum* f. *glabratum*, constituting approximately 93% of the estimated volume (Table 2). These results are in contrast to those obtained earlier by Kennedy and Dimock (1983), who reported only ca. 6.3 ng of 2-tridecanone in a glandular head of *L. hirsutum* f. *glabratum*. Such differences might occur in response to method of extraction, the GC sensitivity level, photoperiod during plant growth or, as noted in the following section, gland age, plant developmental status, or location within pl?

The relative concentrations of the volatile compounds in the other *Lycopersicon* accessions tested were considerably less than for *L. hirsutum* f. *glabratum*. The quantity of the two unknown sesquiterpenes in *L. hirsutum* was

TABLE 2. QUANTIFICATION OF CHEMICALS IN ISOOCTANE EXTRACTS OF GLANDULAR HEADS OF TYPE VI TRICHOMES FROM *L. hirsutum* (LA 361), *L. hirsutum* f. *glabratum* (PI 134417), AND *L. esculetum* CV. VFN 7718

Accession	Chemical	Amount (ng ± SD/gland[a])	% of gland content
L. hirsutum	2-Tridecanone	145.7 ± 7.6	68.1
f. *glabratum*	2-Undecanone	48.6 ± 2.5	22.1
	Sesquiterpene A	4.9 ± 0.3[b]	2.3
L. hirsutum	Sesquiterpene B	7.2 ± 2.0	5.0–6.3
	Sesquiterpene C	0.8 ± 0.2[c]	0.5–0.6
L. esculentum	Monoterpene A	3.0 ± 1.2[d]	2.1–2.6
	Sesquiterpene D	0.5 ± 0.1[d]	0.4–0.5

[a] Unless noted, 200 glands per sample, 6 samples/plant, 5 plants/variety, all plants were 123 days old; radius of type VI glands was 0.04 mm in *L. hirsutum* f. *glabratum*, 0.035 mm in *L. hirsutum*, and 0.05 mm in *L. esculentum* [density assumed to range between 0.8 g/cm^3 (Buckingham, 1982, for high tridecanone content) to 1.0 g/cm^3 for water].
[b] 3000 glands per sample.
[c] 1000 glands per sample.
[d] 4500 glands per sample.

only 8 ng/gland, while the two unknown terpenoids in *L. esculentum* accounted for only 3.5 ng of the contents of a type VI glandular head.

Effect of Trichome Age and Location within Plants on Chemical Composition of Type VI Glands. The chemical contents of type VI glands varied with trichome age in *L. hirsutum* and *L. hirsutum* f. *glabratum*: all chemical concentrations decreased significantly as the glands aged ($P \leq 0.05$, t test). In mature glands from *L. hirsutum* f. *glabratum*, 149.2 ng/gland of 2-tridecanone was detected versus 31.9 ng in an aged gland. In mature glands, the ratio of 2-tridecanone to 2-undecanone in glands of *L. hirsutum* f. *glabratum* was constant (ca. 3.4). However, neither 2-undecanone in *L. hirsutum* f. *glabratum* nor SES C in *L. hirsutum* could be detected in samples of 200 aged glands. Thus, contents of glands vary with the age of the gland as well as accession.

Chemical content of glandular heads in the type VI trichomes of *L. hirsutum* and *L. hirsutum* f. *glabratum* also varied with plant age and location within plants (Figure 2). In the glands of *L. hirsutum* f. *glabratum*, 2-tridecanone concentration remained relatively constant for leaves reaching maturity, then increased sharply when leaves became senescent (Figure 2A). A similar trend was evident for aging stems. Concentration of 2-tridecanone in glands on 1-week-old foliage increased with plant age, but was four to six times lower than in glands on mature leaves or stems. However, since the density of type

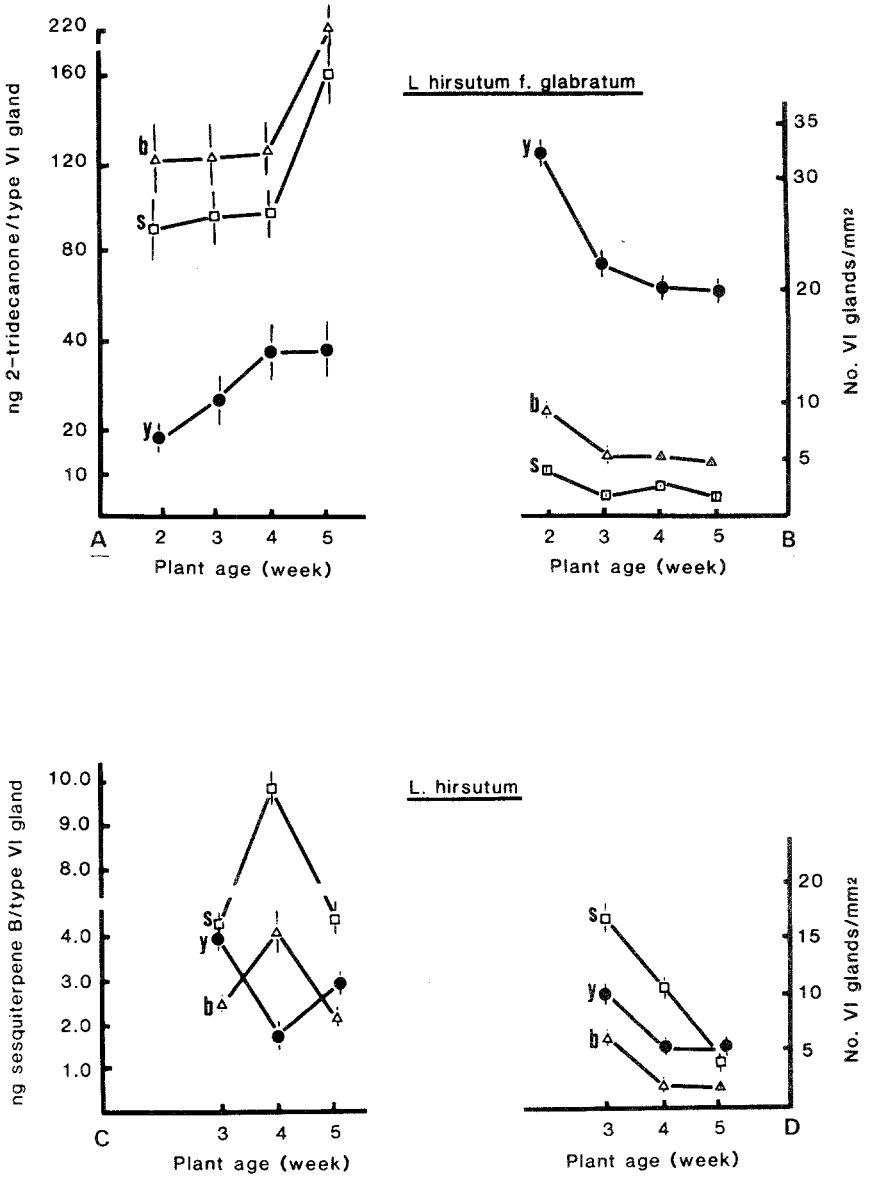

FIG. 2. Chemical contents of glandular heads of type VI trichomes as affected by age and location within plants: y = 1-week-old leaves, b = third true leaves from the bottom, and s = internodal stem between second and third nodes in *L. hirsutum* f. *glabratum* (PI 134417) and *L. hirsutum* (LA 361). Brackets on data points delineate standard errors.

VI trichomes on the young leaves was approximately four times that of the older foilage (Figure 2B), the young leaves were similarly protected.

In the mature glands of *L. hirsutum*, SES B content varied regardless of location within plants (Figure 2C). Concentrations of SES B in glands from both stems and mature leaves fluctuated in a similar pattern: SES B increased until decreasing sharply as the leaves became senescent and stems grew tougher. This pattern is consistent with studies by Croteau and Loomis (1972) documenting that terpenoid synthesis was promoted by the availability of sucrose and other photosynthates, although this pattern could also occur as a result of increased catabolism without any change in the rate of synthesis. The function of sesquiterpenes as insecticides, repellents, and insect feeding deterrents has been previously reported (Kelsey et al., 1984). Sesquiterpene content in trichome heads from immature leaves was highest when plants were youngest. Since density of type VI trichomes was highest at this time as well (Figure 2D), the youngest leaves of young plants were also well protected. The importance of this protection was demonstrated in our previous experiments, where TPW survival was greatly improved by removal of the glandular heads of type VI trichomes (Lin and Trumble, 1986).

Bioassays of Gland Exudate, Gland Extracts, and Pure Compounds. Chemicals in the glandular heads of type VI trichomes from either wild tomato accession were toxic to larvae of both herbivore species. However, BAW was approximately 20 times less susceptible than TPW, despite weighing only three times more than TPW (23.6 μg/BAW; 7.6 μg/TPW). Such differences might occur in response to either differential detoxification systems or variations in cuticular penetrability.

No acute toxicity was observed at 24 hr for either TPW or BAW exposed to glandular heads from *L. esculentum*. However, a previous study reported that TPW would ultimately starve as a result of entrapment, whereas BAW was not affected (Lin and Trumble, 1986). Thus, chemical contents in glandular heads of type VI trichomes on this commercial variety provide only a physical barrier to TPW and are not detrimental to BAW.

Toxicities of equivalent concentrations of the two volatiles from gland exudate or isooctane extracts of glandular heads of *L. hirsutum* f. *glabratum* were not significantly different within each species of herbivore (Table 3). This suggests that not only did the isooctane successfully extract key volatiles, but that the estimates of 2-tridecanone and 2-undecanone concentration determined by GC analyses of isooctane extracts were close to the actual chemical content in type VI glandular heads.

Even with the relatively small amounts found in glandular heads, the two unknown sesquiterpenes provided considerable insecticidal activity. However, the isooctane extract from glandular heads of *L. hirsutum* was more toxic than glandular exudate to both TPW and BAW. If increased toxicity of the extract is due to the isooctane acting as a more efficient carrier, this suggests that a

TABLE 3. TOXICITY OF GLANDULAR HEADS OF TYPE VI TRICHOMES, ISOOCTANE EXTRACTS OF GLANDULAR HEADS FROM WILD TOMATOES, AND PURE COMPOUNDS TO *K. lycopersicella* (TPW) AND *S. exigua* (BAW)[a]

Chemical source	LD_{50} (ng/larva)/95% confidence interval	
	TPW	BAW
L. hirsutum f. *glabratum*		
Exudate	186.9/—[b]	3056.3/2924.7–3180.4
Extract	171.9/67.3–261.7	3374.7/2941.2–3748.5
L. hirsutum		
Exudate	28.5/23.4–46.7	306.2/283.9–332.0
Extract	3.7/2.6–5.8	43.8/33.6–51.8
Pure compounds		
2-Tridecanone ($2C_{13}O$)	51.0/32.0–79.9	3440.0/2820.0–4160.1
2-Undecanone ($2C_{11}O$)	37.0/26.3–57.1	3480.0/3380.1–3619.8
Mixture (3:1) of $2C_{13}O/2C_{11}O$	4.0[c]/2.2–9.6	2080.0[c]/2000.1–2440.5

[a] LD_{50} values and 95% confidence (Helwig and Council, 1979); larvae were directly contacted with glandular heads containing 149.5 ng of 2-tridecanone and 37.4 ng of 2-undecanone per gland of *L. hirsutum* f. *glabratum* (PI 134417), and 7.3 ng of unknown sesquiterpene B per gland of *L. hirsutum* (LA 361) (from concurrent GC analyses). Four replicates of 15 larvae were tested per dose, with five doses per treatment.

[b] A single gland caused in excess of 50% mortality.

[c] Significantly more toxic than expected from an additive effect ($P < 0.05$, chi-square test).

large portion of the unidentified content of the type VI glandular heads on *L. hirsutum* might be water or some other isooctane-insoluble materials.

In bioassays with pure compounds, 2-undecanone was at least as toxic to TPW and BAW as 2-tridecanone ($P > 0.05$, chi-square) (Table 3). In a study comparing the relative toxicity of analogs of 2-tridecanone, Dimock et al. (1982) found 2-undecanone to be less acutely toxic than 2-tridecanone to *H. zea*. A comparison of their results with our own suggests that the toxicity of the two chemicals varies with insect species. The LD_{50} values for the chemical reagents were consistent with the LD_{50} values produced by gland exudates and isooctane extracts for BAW. Similar comparisons were not possible for TPW, because even a single gland caused 100% mortality. Gland extracts were less toxic than the chemical reagents to TPW. This difference may be due, in part, to the ratio of 2-tridecanone and 2-undecanone. There was more 2-tridecanone (4:1) in the extract than in the pure compounds (3:1), which might have influenced penetration of the cuticle.

Mortalities resulting from combinations of both chemicals in a ratio ap-

proximating that found in mature glands (e.g., 3:1) demonstrated significant potentiation [expected additive effect for TPW, LD_{50} = 20.0 ng/larva, observed LD_{50} = 4.0 ng/larva, $P < 0.01$; expected additive effect for BAW = 2800.0 ng/larva, observed LD_{50} = 2080 ng/larva, $P < 0.05$ (chi-square tests)]. This potentiation provides a clear advantage for those plants with glandular heads of type VI trichomes synthesizing or sequestering both chemicals. Additionally, since in a natural state 2-tridecanone is a solid (Dean, 1979), and since 2-undecanone is a liquid which evaporates readily (Dean, 1979), we suspect that the 2-undecanone serves to keep 2-tridecanone dissolved in a liquid form (and therefore more suitable for contact toxicity), while the mixture helps retard evaporation of 2-undecanone from the glands. This hypothesis is supported, in part, by our earlier observation that 2-undecanone could not be detected in aged type VI glands. This combination of chemicals probably plays a key role in physical defense (entrapment) as well. Following release of the liquid content, the rapid evaporation of 2-undecanone would leave an increasingly viscid residue attached to the insect.

CONCLUSION

The major volatile chemicals produced and/or stored in glandular heads of type VI trichomes produce acute insecticidal activities against the specialist and generalist herbivores tested. Although these chemicals proved to be more toxic to the specialist, TPW feeds for the first two instars as a leafminer (Lin and Trumble, 1985) and may thereby behaviorally avoid contact with trichome exudates. In contrast, the generalist BAW feeds on whole foliage and is therefore forced to contact and consume the glandular heads. Thus, the potential defense offered by the glandular heads of type VI trichomes may be greater for the early instars of BAW than for TPW. However, additional studies on the effects of chemicals in glandular heads on oviposition behavior are needed before the importance of chemical activities against larvae can be determined.

Since the concentrations of these volatiles and density of type VI trichomes varied with gland age, plant age, and location within plants, variations in "investment" in specific tissues may occur. Information on such chemical variation could not only offer new avenues of research for plant systematists interpreting relationships difficult to clarify by strictly morphological means, but may assist plant breeders in selecting accessions exhibiting specific chemical traits as well as morphological characteristics which impact TPW and BAW populations. Additional research on the chemical content of type VI glands will be necessary, since the onset and rate of chemical production appears to be dependent on a variety of environmental factors (Langenheim et al., 1984).

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