

Aphid Resistance and Leaf Surface Chemistry of Sugar Ester Producing Tobaccos¹

A. W. Johnson,² V. A. Sisson,³ M. E. Snook,⁴ B. A. Fortnum and D. M. Jackson⁵

Department of Entomology, Clemson University, Pee Dee Research and Education Center, 2200 Pocket Road, Florence, SC 29506 USA

J. Entomol. Sci. 37(2): 154–165 (April 2002)

Abstract Sugar ester producing tobacco lines were evaluated for aphid resistance and other surface chemicals. The cembrenoid and labdenoid diterpenes, α - and β -4,8,13-*duvatrien*-1-ols, α - and β -4,8,13-*duvatriene*-1,3-diols, (12*Z*)-labda-12,14-diene-8 α -ol (*cis*-abienol), (13*E*)-labda-13-ene-8 α ,15-diol (labdenediol), docosanol, and sugar esters were quantified using high pressure liquid chromatography and compared with aphid infestation ratings. Regression analysis of aphid [*Myzus persicae* (Sulzer)] infestation rating and leaf surface chemistry was statistically significant and showed that surface chemicals were important in explaining the observed variation in the aphid infestation ratings. A significant negative correlation was found between aphid ratings and sugar ester levels among the 62 entries evaluated ($r = -0.2758$, $P = 0.0301$). α and β monols (α - and β -4,8,13-*duvatrien*-1-ols) were also significantly correlated with aphid infestations in this study ($r = -0.2743$, $P = 0.0310$ and $r = -0.2797$, $P = 0.0109$, respectively). None of the other surface chemicals were statistically correlated with aphid resistance. Although high sugar ester levels were correlated with aphid resistance, not all tobacco entries with high levels of sugar esters, such as TI 1568 were resistant. This would suggest that there may be different types of sugar esters present in these tobaccos, and total sugar ester levels alone could not be used to predict aphid resistance. Also, some tobacco lines, like TI 1674 and TI 59 with lower sugar ester levels, were resistant in this study because of high monol levels. The ten tobacco entries with the highest levels of sugar esters in this study were TI 698, TI 675, TI 704, TI 998, TI 193, JA 389, TI 722R, TI 1092, TI 711, and TI 1007. All of these lines exhibited high levels of aphid resistance, but some also had low-to-moderate levels of monols that may have elevated the aphid resistance level. A number of these tobaccos could be used for production of natural sugar ester biorationals or used in a breeding program for development of aphid resistant cultivars.

Key Words Diterpenes, sugar esters, host plant resistance, *Nicotiana tabacum*, *Myzus persicae*, *Myzus nicotianae*, cembrane, *duvane*, aphids, labdenediol, abienol, labdane

Green peach aphids, *Myzus persicae* (Sulzer), are a major pest of tobacco, *Nicotiana tabacum* L., in most of the tobacco-producing countries throughout the world (Akehurst 1968). Development of aphid-resistant tobacco cultivars is needed as an

¹Received 03 July 2001; accepted for publication 19 September 2001. Technical Contribution No. 4703 of the S. C. Agric. Exp. Stn., Clemson University.

²All offprint requests are to be directed to A. W. Johnson (email: ajhnsn@clemson.edu)

³North Carolina State University, Oxford Tobacco Research Station, Oxford, NC 27565.

⁴University of Georgia, Russell Research Center, P. O. Box 5677, Athens, GA 30604.

⁵USDA, ARS, U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC 29414.

alternative to chemical insecticides. Thurston (1961) conducted some of the earliest research on aphid resistance in tobacco in Kentucky. He reported on resistance of several varieties, tobacco introductions (TIs), and *Nicotiana* species. Subsequently, high levels of aphid resistance were reported from the *Nicotiana* species (Thurston 1961, Thurston and Webster 1962, Burk and Stewart 1969). Other researchers have also reported tobacco lines that are resistant to aphids (Thurston and Katanyukul 1973, Abernathy and Thurston 1969, Thurston et al. 1977, Elsey and Chaplin 1978, Johnson 1978, 1980).

Johnson and Severson (1982) evaluated trichomes and surface chemistry of known aphid-resistant tobaccos. The important cuticular components they measured were the cembranoid diterpenes (=duvanes), the labdanoid diterpenes (=labdanes), sugar esters, and the primary fatty alcohol, *n*-docosanol (C_{22}) (Reid 1974, Severson et al. 1984, 1985, 1985a). The cembranes were (1*S*,4*S*,6*R*,2*E*,7*E*,11*E*)-4,6-dihydroxy cembra-2,7,11-triene and its (4*R*) epimer, trivially known as α - and β -4,8,13-duvatatriene-1,3-diols (Roberts and Rowland 1962, Jackson and Danehower 1996), and (1*S*,4*S*,6*R*,2*E*,7*E*,11*E*)-4-hydroxycembra-2,7,11-triene and its corresponding (4*R*) epimer, trivially known as α - and β -4,8,13-duvatrien-1-ols (Wahlberg et al. 1981, Jackson and Danehower 1996). The principal tobacco labdanes were (12*Z*)-labda-12,14-diene,8 α -ol, trivially known as *cis*-abienol, and (13*E*)-labda-13-ene-8 α ,15-diol, trivially known as labdenediol (Colledge and Reid 1974, Reid 1974). The sugar esters (glucose and sucrose esters) are a unique class of natural products that have been reported in the leaf-surface extracts of *Nicotiana*, *Solanum*, *Lycopersicon*, *Datura*, and *Petunia* species (Burke et al. 1987, Johnson and Severson 1982, Kays et al. 1994, King et al. 1986, 1987, 1988, King and Calhoun 1988, Severson et al. 1983, 1984, 1985, 1985a, 1991). For the sugar esters of *N. tabacum*, hydroxyl groups at the 2, 3, 4 and 6 positions of the glucosyl moiety of sucrose are esterified with low molecular weight C_2 - C_8 aliphatic (fatty) acids (Severson et al. 1985a, 1991, 1994a, b).

Johnson and Severson (1982) found that tobacco with high levels of α - and β -monols and sugar esters were resistant to aphids. Severson et al. (1985) also reported that tobaccos with high levels of duvanes, *cis* abienol, and sucrose esters have decreased aphid damage. Johnson et al. (1992) listed sources of *N. tabacum* that were resistant to aphids, tobacco budworms, *Heliothis virescens* (F.), and tobacco hornworms, *Manduca sexta* (L.), and grouped the tobaccos by surface chemistry profiles. Using stepwise regression analysis, Eckel et al. (1991) reported that the levels of α - and β -monols and sugar esters on green leaves were negatively correlated to aphid infestations on a large number of tobacco introductions in the field.

Severson et al. (1984, 1985) quantified and characterized the sugar esters in *Nicotiana* species. They showed that sugar esters were made up of mixtures of sucrose and glucose esters with various aliphatic acids esterified to two or more free hydroxyl groups of fructose and glucose (Severson et al. 1991, 1994a, b). Studies have shown that sugar ester fractions from *Nicotiana* were toxic to pests other than aphids, such as whiteflies, *Bemisia tabaci* (Gennadius), and pear psylla (Neal et al. 1994, Buta et al. 1993, Puterka and Severson 1995, Nottingham et al. 1996). The type and amount of sugar esters vary among *Nicotiana* species (Chortyk et al. 1993, Severson et al. 1994, Jackson et al. 1998). Chortyk et al. (1996) and Xia et al. (1998) demonstrated that sugar esters could be synthesized that were toxic to the tobacco aphid and sweet potato whitefly.

Although several cuticular components from tobacco are topically toxic to tobacco aphids in laboratory bioassays, sucrose esters have the lowest LC_{50} (defined as the

lethal dose per aphid needed to kill 50% of the test population when applied topically to the dorsum of tobacco aphids) (Severson et al. 1994b, Eckel et al. 1998). The toxicity of synthetic sugar esters and natural sugar esters extracted from tobacco plants to aphids when applied as topical treatments to leaves also was demonstrated (Severson et al. 1994a, b). The field efficacy of sugar esters for control of soft-bodied insects such as whiteflies and aphids has been shown (Liu and Stansly 1995, McPherson et al. 1995, Nottingham et al. 1996, Severson et al. 1994a,b). Sugar esters do not harm beneficial biological control agents, such as *Encarsia formaosa* Gahan (Bentz and Neal 1995). Because of these benefits, there is interest in the commercial development of natural and synthetic sugar esters as biorational insecticides. The sugar esters from *N. gossei* were patented as a biopesticide (Pittarelli et al. 1993). The production potential, composition, ease of extractability, and insecticidal activities of the sugar esters from different *Nicotiana* species vary considerably (Jackson and Daneshmand 1996). Chortyk et al. (1996) produced synthetic analogues of sucrose esters that were structurally similar to natural sucrose esters from *Nicotiana* spp. These analogues are much cheaper to produce than natural sugar esters. Sucrose esters with C₇ and C₈ aliphatic acids were the most effective at killing silverleaf whiteflies and aphids (Chortyk et al. 1996).

The purpose of this study was to determine if all high sugar ester producing tobaccos were resistant to aphids. We also wanted to select high sugar ester tobacco lines that could be used in a breeding program for developing aphid resistant tobacco or be evaluated as potential sources of sugar ester extracts for biorational insecticides.

Materials and Methods

This study was conducted at the Clemson University Pee Dee Research and Education Center near Florence, SC. Seeds for high sugar ester producing tobacco introductions were obtained from the U.S. tobacco germplasm collection maintained at the Oxford Tobacco Research Station in Oxford, NC. Nine tobacco breeding lines (JA) from the Clemson University germplasm collection were also included. Tobacco entries were selected based on surface chemistry data collected by Sisson et al. (1990). Only tobaccos with high sugar ester levels were selected for this study. Sixty-two *N. tabacum* entries were planted 16-18 May of each year in 1993 and 1994. Plots consisted of 1 row (1.2 m wide and 6.1 m long) with 12 plants in each row. Experimental plots were arranged in a randomized complete block design with 3 replications. The evaluated entries were planted next to each other with no skip rows. Tillam (6 EC) and Devrinol (50 WP) were applied for weed control as broadcast incorporated treatments 2 weeks prior to transplanting at rates of 6.21 L/ha and 2.2 kg/ha, respectively. One week after transplanting the tobacco was fertilized with 6-6-18 fertilizer at a rate of 757 kg/ha. Two weeks after transplanting it was side-dressed with 16-0-0 sodium nitrate at a rate of 140 kg/ha. Normal cultivation practices for flue-cured tobacco were followed, and no supplemental irrigation was applied. No chemicals were used for insect or sucker control.

In 1993 and 1994, two replications for each tobacco entry were evaluated for cuticular components 7 weeks after transplanting. One tobacco bud leaf (12 to 15 cm long) was sampled from each of 5 plants/plot. Five 2 cm-diam (3.14 cm²) leaf disks (one disk from one leaf from each of 5 plants) were removed with a cork borer from each plot. The 5 leaf disks were weighed, and each was dipped 8 times in 10 ml of

Table 1. Leaf surface chemistry and aphid infestation ratings of high sugar ester producing tobaccos. Pee Dee Research and Education Center, Florence, S.C., 1993-1994

Tobacco entry	Mean leaf surface chemical levels (µg/cm ²)*							Aphid infestation rating**	
	α-OL	β-OL	α-DIOL	β-DIOL	CIS-AB	15-OH	C ₂₂ OH		SE
TI-58	2.6 e-k	1.3 hij	48.0	31.7	0.0 f	0.0 i	1.3	26.5 b-k	2.03 c-h
TI-59	10.3 b	5.9 b-e	2.9	1.6	11.2 ef	1.3 c-g	0.5	13.3 i-n	0.20 nop
TI-62	2.2 e-k	1.7 g-j	46.0	24.5	39.7 abc	2.2 b-e	1.1	35.1 a-i	0.10 op
TI-66	0.5 g-k	0.8 ij	30.4	12.9	46.6 a	3.6 a	1.5	24.4 c-k	0.40 l-p
TI-165	0.9 g-k	1.4 hij	48.4	28.3	0.0 f	0.0 i	0.8	20.0 e-n	1.33 g-m
TI-193	1.6 f-k	2.7 e-j	58.4	35.3	0.0 f	0.0 i	1.9	44.8 abc	1.00 h-p
TI-194	1.0 g-k	1.3 hij	41.0	21.0	0.0 f	0.0 i	1.0	26.9 b-k	0.97 i-p
TI-226	1.6 f-k	4.5 c-h	45.8	21.4	0.0 f	0.0 i	0.8	20.8 d-n	0.80 i-p
TI-234	2.0 e-k	1.1 ij	35.6	18.3	0.0 f	0.0 i	0.4	15.6 g-n	1.80 d-i
TI-245	1.4 f-k	3.7 d-i	43.4	17.0	0.0 f	0.0 i	0.7	21.5 d-n	1.37 f-l
TI-248	0.9 g-k	2.8 e-j	43.5	18.5	0.0 f	0.0 i	0.9	20.1 d-n	2.40 b-f
TI-421	1.7 f-k	3.7 d-i	26.8	11.4	22.8 de	1.2 d-g	1.0	22.9 c-m	0.43 k-p
TI-424	2.0 e-k	4.7 b-g	41.9	14.4	39.0 abc	2.4 b	1.2	21.7 d-n	0.23 nop
TI-437	1.2 g-k	0.8 ij	49.2	30.7	0.0 f	0.0 i	0.9	23.4 c-l	1.23 g-n
TI-462	1.0 g-k	1.4 hij	41.0	26.2	0.0 f	0.0 i	1.2	35.6 a-h	0.43 k-p
TI-494	1.1 g-k	0.8 ij	34.8	21.6	0.0 f	0.0 i	0.9	31.3 b-i	0.33 l-p
TI-499	2.1 e-k	5.1 b-f	42.2	17.1	0.0 f	0.0 i	1.0	35.9 a-h	0.23 nop

Table 1. Continued.

Tobacco entry	Mean leaf surface chemical levels (µg/cm ²)*							Aphid infestation rating**
	α-OL	β-OL	α-DIOL	β-DIOL	CIS-AB	15-OH	C ₂₂ OH	
TI-536	1.2 g-k	2.6 e-j	27.5	9.9	0.0 f	0.0 i	1.2	19.8 e-n
TI-550	1.3 f-k	0.6 ij	30.1	14.3	0.0 f	0.0 i	0.6	18.1 f-n
TI-574	1.1 g-k	1.1 ij	40.7	22.6	30.2 bcd	1.2 d-g	1.7	35.0 a-i
TI-592	2.1 e-k	1.3 hij	38.8	22.6	0.0 f	0.0 i	1.4	23.8 c-k
TI-617	0.4 h-k	0.9 ij	22.4	8.6	33.3 a-d	2.3 bcd	0.7	24.3 c-k
TI-657	3.5 c-g	1.7 g-j	49.5	31.0	0.0 f	0.0 i	0.7	27.0 b-k
TI-675	2.9 c-k	1.6 g-j	50.6	33.4	0.0 f	0.0 i	1.8	47.7 ab
TI-691	2.7 d-k	1.5 g-j	53.7	31.8	0.0 f	0.0 i	1.4	25.5 b-k
TI-698	2.5 e-k	5.8 b-e	49.9	22.0	0.0 f	0.0 i	1.3	56.7 a
TI-704	6.0 c	13.7 a	64.8	29.3	0.0 f	0.0 i	1.4	45.1 abc
TI-708	2.7 d-k	6.5 bcd	42.3	18.1	0.0 f	0.0 i	0.9	33.1 b-i
TI-711	5.7 cd	13.6 a	61.6	30.6	0.0 f	0.0 i	1.5	40.1 a-f
TI-722R	3.3 c-i	7.9 b	47.7	24.1	0.0 f	0.0 i	1.5	41.3 a-e
TI-760	3.5 c-h	7.7 bc	51.0	18.7	0.0 f	0.0 i	1.0	34.4 a-i
TI-956	3.1 c-k	7.3 bc	45.3	18.9	0.0 f	0.0 i	0.9	34.7 a-i
TI-982	3.3 c-i	1.6 g-j	57.1	37.0	0.0 f	0.0 i	1.2	37.8 a-g
TI-998	4.4 c-f	2.5 f-j	59.3	40.3	0.0 f	0.0 i	1.3	45.0 abc
TI-999	2.1 e-k	1.3 hij	42.3	24.1	40.3 ab	1.1 f-i	0.5	31.5 b-i

Table 1. Continued.

Tobacco entry	Mean leaf surface chemical levels ($\mu\text{g}/\text{cm}^2$)*							Aphid infestation rating**
	α -OL	β -OL	α -DIOL	β -DIOL	CIS-AB	15-OH	C ₂₂ OH	
TI-1007	5.0 cde	2.3 f-j	54.8	38.7	0.0 f	0.0 i	1.1	39.8 a-f
TI-1068	1.9 e-k	1.3 hij	41.5	22.2	32.5 a-d	2.2 b-e	1.0	33.0 b-i
TI-1092	3.3 c-i	7.8 b	55.0	20.3	0.0 f	0.0 i	1.0	40.3 a-f
TI-1123	0.1 k	0.1 j	1.5	1.2	0.0 f	0.0 i	0.6	1.1 mn
TI-1132	0.6 g-k	1.3 hij	38.3	18.1	0.0 f	0.0 i	0.8	0.0 n
TI-1268	0.9 g-k	0.5 ij	49.5	24.7	0.0 f	0.0 i	1.1	36.9 a-h
TI-1275	0.7 g-k	0.6 ij	29.1	13.5	19.1 de	0.9 f-i	0.7	26.2 b-k
TI-1308	1.4 f-k	1.6 g-j	51.9	27.1	23.9 cde	0.8 f-i	1.1	29.7 b-j
TI-1309	0.6 g-k	0.7 ij	31.5	13.6	28.8 bcd	2.4 b	0.9	26.5 b-k
TI-1347	0.3 ijk	0.4 j	24.8	10.4	0.0 f	0.0 i	0.6	7.6 j-n
TI-1396	1.8 f-k	1.9 f-j	49.3	28.9	30.9 a-d	1.7 b-f	0.9	34.2 b-i
TI-1499	2.2 e-k	1.4 hij	40.7	21.3	31.0 a-d	1.1 f-i	0.8	28.1 b-k
TI-1568	1.1 g-k	0.6 ij	27.3	13.0	26.3 de	3.8 a	0.9	32.8 b-i
TI-1674	20.9 a	11.6 a	1.0	1.2	0.0 f	0.0 i	0.9	8.3 j-n
Samsun	2.1 e-k	1.8 f-j	36.5	19.0	11.3 ef	0.5 ghi	0.9	13.2 i-n
Hav-425	0.4 h-k	0.5 ij	26.9	11.2	29.5 bcd	2.3 bc	0.9	16.7 g-n
JA-228	0.3 ijk	0.3 j	14.0	7.2	0.0 f	0.0 i	0.8	7.1 k-n
JA-248	0.9 g-k	0.9 ij	49.2	29.1	2.1 f	0.1 hi	1.2	22.1 d-n

Table 1. Continued.

Tobacco entry	Mean leaf surface chemical levels ($\mu\text{g}/\text{cm}^2$)*							Aphid infestation rating**
	α -OL	β -OL	α -DIOL	β -DIOL	CIS-AB	15-OH	C ₂₂ OH	
JA-386	1.0 g-k	2.4 f-j	58.2	33.4	0.0 f	0.0 i	1.2	1.5 lmn
JA-389	0.3 ijk	0.9 ij	47.3	20.9	0.0 f	0.0 i	1.2	42.3 a-d
JA-397	0.7 g-k	1.9 f-j	59.0	31.1	0.0 f	0.0 i	1.0	22.9 c-m
JA-454	0.4 h-k	1.0 ij	44.5	18.3	1.4 f	0.0 i	0.9	20.5 d-n
JA-457	0.6 g-k	1.6 g-j	44.0	19.4	0.0 f	0.0 i	0.8	16.1 g-n
JA-460	0.2 jk	0.5 ij	20.7	10.3	0.0 f	0.0 i	0.8	7.1 k-n
JA-520	0.5 g-k	1.6 g-j	38.8	15.1	0.0 f	0.0 i	0.8	14.8 h-n
NC-2326	0.4 g-k	1.0 ij	39.4	18.0	0.0 f	0.0 i	0.7	0.0 n
NC-95	0.4 g-k	0.9 ij	35.8	13.3	0.0 f	0.0 i	0.6	0.0 n

* α -OL = α -4,8,13-*duvatrien*-1-ol, β -OL = β -4,8,13-*duvatrien*-1-ol, α -DIOL = α -4,8,13-*duvatrien*-1,3-diol, β -DIOL = β -4,8,13-*duvatrien*-1,3-diol, CIS-AB = *cis*-abienol, 15-OH = (13*E*)-*labda*-13-ene-8 α ,15-diol, C₂₂OH = *docosanol* (C₂₂OH), and SE = *sugar esters*.

** Aphid plant infestation ratings were based on a scale of 0-7 with 0 representing no aphids, and 7 representing a very heavy infestation (1994 data only).

*** Treatment means in each column followed by the same letter are not significantly different ($P = 0.05$; Tukey's HSD).

methylene chloride in a 20-ml scintillation vial. The leaves were discarded after they were dipped in the solvent. Each scintillation vial containing the methylene chloride extract was sealed with a Teflon-lined cap and stored in a freezer (-18°C) until analysis. These samples were later analyzed by glass capillary chromatography after addition of an internal standard and derivatization by BSTFA/DMF (Severson et al. 1982, 1984, 1985, 1988) to quantify the major cuticular components. Those components quantified were α - and β -monols, α - and β -diols, *cis*-abienol, labdenediol, *n*-docosanol, and sugar esters.

Tobacco entries were evaluated 8 weeks after transplanting for aphid resistance each year. Ten plants per plot were rated on a scale of 0 to 7 for aphid infestations. Plant ratings were as follows: 0, no aphid infestation; 1, very light infestation; 2, light infestation; 3, light to moderate infestation; 4, moderate infestation; 5, moderate to heavy infestation; 6, heavy infestation; and 7, very heavy aphid infestation. The ratings for 10 plants/plot were averaged for an overall plot rating.

All surface chemistry data and aphid infestation ratings were subjected to Pearson correlation analysis and linear regression analysis (SAS Institute 1990). First, each chemical parameter was regressed against the aphid field infestation rating. Chemical components that were significant ($P = 0.05$) in simple regression models were subsequently included in a multiple regression model (SAS Institute 1990). The data were also subjected to an analysis of variance, and a mean separation was conducted for the most significant factors using Tukey's HSD test.

Results and Discussion

A summary of the leaf surface chemicals and aphid infestation ratings for the 62 tobacco entries evaluated in this study are presented in Table 1. The aphid infestation data shown in Table 1 were collected in 1994. Aphid ratings were made in 1993, but populations were extremely low, and the data for 1993 were not included in the statistical analyses.

Some of the entries with high levels of sugar esters also contained high levels of α - and β -monols, *cis* abienol, and/or labdendiol, and no attempt was made to eliminate them from this study even though α - and β -monols also affect aphid resistance (Eckel et al. 1991, Johnson and Severson 1982, Jackson et al. 1989, Severson et al. 1994b). However, the data were analyzed with and without high monol and high abienol tobaccos, and the results of the overall analyses were similar.

The ten tobacco entries with the highest levels of sugar esters in this study were TI 698 ($56.7\text{ }\mu\text{g}/\text{cm}^2$), TI 675 ($47.7\text{ }\mu\text{g}/\text{cm}^2$), TI 704 ($45.1\text{ }\mu\text{g}/\text{cm}^2$), TI 998 ($45.0\text{ }\mu\text{g}/\text{cm}^2$), TI 193 ($44.8\text{ }\mu\text{g}/\text{cm}^2$), JA 389 ($42.3\text{ }\mu\text{g}/\text{cm}^2$), TI 722R ($41.3\text{ }\mu\text{g}/\text{cm}^2$), TI 1092 ($40.3\text{ }\mu\text{g}/\text{cm}^2$), TI 711 ($40.1\text{ }\mu\text{g}/\text{cm}^2$), and TI 1007 ($39.8\text{ }\mu\text{g}/\text{cm}^2$) (Table 1). A mean separation of the sugar ester levels of these entries did not reveal any significant differences ($P = 0.05$; Tukey's HSD). TI 698 and TI 675 have consistently had the highest levels of sugar esters in earlier studies (Severson et al. 1985b, 1994b). All of these tobaccos exhibited good levels of aphid resistance, but some also had low-to-moderate levels of monols that may have elevated the aphid resistance level.

A regression analysis (Unweighted Least Squares Linear Regression) of the aphid infestation ratings revealed an F value of 2.56 ($P = 0.0195$; $\text{df} = 8, 53$). This P -value indicated that at least some of the independent variables are important in explaining the observed variation in the aphid infestation ratings. The regression equation for these data was:

$$\begin{aligned} \text{Aphid rating} = & 1.4058 - 0.0049 (\alpha\text{-monol}) - 0.1044 (\beta\text{-monol}) \\ & - 0.0330 (cis\text{-abienol}) + 0.0257 (\alpha\text{-diol}) - 0.0123 (\beta\text{-diol}) \\ & + 0.6383 (\text{labdendiol}) - 0.4676 (\text{docosanol}) - 0.0243 (\text{sugar ester}). \end{aligned}$$

Correlation analysis of the data showed that sugar esters were significantly correlated with aphid ratings ($r = -0.2758$, $P = 0.0301$) (Table 2). α - and β -monols were also significantly correlated with resistance in this study ($r = -0.2743$, $P = 0.0310$ and $r = -0.3211$, $P = 0.0109$, respectively for α - and β -monols). None of the other surface chemicals were correlated with aphid resistance in this study.

If only the significant factors are considered, the regression equation gave an F value of 3.815 ($P = 0.0145$; $df = 3,58$). The regression equation for these factors was:

$$\text{Aphid rating} = 1.7311 - 0.0627 (\alpha\text{-monol}) - 0.0556 (\beta\text{-monol}) - 0.0216 (\text{sugar ester}).$$

A two-way multiple regression analysis gave a better fit when only the monols (α -monols plus β -monols) and sugar esters and were considered ($F = 5.818$; $df = 2,59$; $P = 0.0049$).

Although there was a statistical correlation between sugar esters and aphid infestation ratings, there were some tobacco entries, such as TI 1568, with high sugar esters that were susceptible. This would suggest that some tobacco entries may have different types of sugar esters, and that total sugar ester levels cannot always be used unconditionally to predict aphid resistance. Tobacco entries with low sugar ester levels were also observed that were resistant to aphids. Aphid resistance in certain low sugar ester tobaccos such as TI 1674 could be explained because of high monols. Another example of aphid resistance in a tobacco with low sugar ester levels was TI 1123. It has low cuticular chemistry and would be considered a nonsecretor.

Table 2. Correlation of surface chemicals with aphid infestation ratings in high sugar ester tobaccos. Pee Dee Research and Education Center, Florence, S.C., 1994

Chemical†	Pearson correlation coefficient (r)	P values
α -OL	-0.2743	0.0310*
β -OL	-0.3211	0.0109**
α -DIOL	0.0443	0.7326
β -DIOL	0.0320	0.8049
CIS AB	-0.0797	0.5379
labdenediol	-0.0378	0.7706
C ₂₂ OH	-0.2386	0.0618
SE	-0.2758	0.0301*

† α -OL = α -4,8,13-duvatrien-1-ol, β -OL = β -4,8,13-duvatrien-1-ol, α -DIOL = α -4,8,13-duvatriene-1,3-diol, β -DIOL = β -4,8,13-duvatriene-1,3-diol, CIS-AB = *cis*-abienol, 15-OH = (13E)-labda-13-ene-8 α ,15-diol, C₂₂OH = docosanol (C₂₂OH), and SE = sugar esters.

*, ** Significant at the 0.05 and 0.01 probability level, respectively.

Tobaccos with very low trichome exudates are known to exhibit aphid resistance (Johnson and Severson 1982, Johnson et al. 1992).

These results show a clear association between high sugar ester production in tobacco and aphid resistance. A number of the tobacco lines evaluated in this study could be used for production of natural sugar ester biorationals or used in a breeding program for development of aphid resistant tobacco cultivars.

References Cited

- Abernathy, C. O. and R. Thurston. 1969.** Plant age in relation to the resistance of *Nicotiana* to the green peach aphid. *J. Econ. Entomol.* 62: 1356-1359.
- Akehurst, B. C. 1968.** Tobacco. Tropical Agriculture Series, Humanities Press, NY, 551 p.
- Bentz, J. A. and J. W. Neal, Jr. 1995.** Effects of natural insecticide from *Nicotiana gossei* on the whitefly parasitoid *Encarsia formosa* (Hymenoptera: Aphelinidae). *J. Econ. Entomol.* 88: 1611-1615.
- Burk, L. C. and P. A. Stewart. 1969.** Resistance of *Nicotiana* species to the green peach aphid. *J. Econ. Entomol.* 62: 1115-1117.
- Burke, B. A., G. Goldsby and J. B. Mudd. 1987.** Polar epicuticular lipids of *Lycopersicon pennellii*. *Phytochem.* 26: 2567-2571.
- Buta, J. G., W. R. Lusby, J. W. Neal, Jr., R. M. Waters and G. W. Pittarelli. 1993.** Sucrose esters from *Nicotiana gossei* active against the greenhouse whitefly *Trialeurodes vaporariorum*. *Phytochem.* 32: 859-864.
- Chortyk, O. T., T. G. Pomonis and A. W. Johnson. 1996.** Syntheses and characterizations of insecticidal sucrose esters. *J. Agric. Food Chem.* 44: 51-57.
- Chortyk, O. T., R. F. Severson, H. C. Cutler and V. A. Sisson. 1993.** Antibiotic properties of sugar esters isolated from selected *Nicotiana* species. *Biosci. Biotech. Biochem.* 57: 1355-1356.
- Colledge, A. and W. W. Reid. 1974.** The phytochemistry of the genus *Nicotiana*. Part V. The biosynthesis of *cis*-abienol and phytosterols in *Nicotiana tabacum* PB. *Ann. du Tabac., Sec. 2*, 11: 177-183.
- Eckel, R. V. W., D. M. Jackson, R. F. Severson and M. G. Stephenson. 1991.** Correlations between cuticular *Nicotiana tabacum* components and resistance to the tobacco aphid, *Myzus nicotianae*. 45th Tobacco Chem. Res. Conf., Abstract No. 7, Program Vol. 45: 16.
- Eckel, R. V. W., R. F. Severson and D. M. Jackson. 1998.** Toxicity of cuticular components from tobacco on the tobacco aphid (*Myzus nicotianae* Blackman). *Tobacco Sci.* 42: 27-32.
- Elsey, K. D. and J. F. Chaplin. 1978.** Resistance of tobacco introduction 1112 to the tobacco budworm and green peach aphid. *J. Econ. Entomol.* 71: 723-725.
- Jackson, D. M. and D. A. Danehower. 1996.** Integrated case study: *Nicotiana* leaf surface components and their effects on insect pests and disease, pp. 231-254. *In* G. Kerstiens, ed., Plant Cuticles: An integrated Functional Approach. BIOS Scientific Publishers, Ltd., Oxford, England, UK.
- Jackson, D. M., O. T. Chortyk, M. G. Stephenson, A. W. Johnson, C. D. Harlow, A. M. Simmons and V. A. Sisson. 1998.** Potential of *Nicotiana* species for production of sugar esters. *Tobacco Sci.* 42: 1-9.
- Jackson, D. M., R. F. Severson and A. W. Johnson. 1989.** Effects of natural tobacco constituents on insect survival, development, and behavior. *Recent Adv. Tobacco Sci.* 15: 26-116.
- Johnson, A. W. 1978.** Evaluation of several *Nicotiana tabacum* entries for resistance to two tobacco insect pests. *Tobacco Sci.* 22: 41-43.
- 1980.** Resistance in tobacco to the green peach aphid. *J. Econ. Entomol.* 73: 707-709.
- Johnson, A. W. and R. F. Severson. 1982.** Physical and chemical leaf surface characteristics of aphid resistant and susceptible tobacco. *Tobacco Sci.* 26: 98-102.

- Johnson, A. W., R. F. Severson and D. M. Jackson. 1992.** Sources of resistance to insect pests of tobacco. CORESTA Info. Bull. 1992:3/4: 151-175.
- Kays, S. J., R. F. Severson, S. F. Nottingham, R. B. Chalfant and O. T. Chortyk. 1994.** Possible biopesticide from *Petunia* for the control of the sweetpotato whitefly (*Bemisia tabaci*) on vegetable crops. Proc. Fla. State Hort. Soc. 107: 163-167.
- King, R. R. and L. A. Calhoun. 1988.** 2,3-Di-*O*- and 1,2,3-Tri-*O*-Acylated glucose esters from the glandular trichomes of *Datura metel*. Phytochem. 27: 3761-3763.
- King, R. R., L. A. Calhoun and R. P. Singh. 1988.** 3,4-Di-*O*- and 1,2,3-tri-*O*-acylated glucose esters from the glandular trichomes of nontuberous *Solanum* species. Phytochem. 27: 3765-3768.
- King, R. R., Y. Pelletier, R. P. Singh and L. A. Calhoun. 1986.** 3,4-Di-*O*-Isobutryl-6-*O*-carprylsucrose: The major component of a novel sucrose ester complex from the type B glandular trichomes of *Solanum berthaultii* Hawkes (PL 473340). J. Chem. Soc. Chem Commun. 1986: 1078-1079.
- King, R. R., R. P. Singh and L. A. Calhoun. 1987.** Isolation and characterization of 3,3,4,6-tetra-*O*-acylated sucrose esters from the type B glandular trichomes of *Solanum berthaultii* Hawkes (PI 265857). Carb. Res. 166: 113-121.
- Liu, T.-X. and P. A. Stansly. 1995.** Toxicity and repellency of some biorational insecticides to *Bemisia argentifolia* on tomato plants. Entomol. Exp. Appl. 74: 137-143.
- McPherson, R. M., E. K. Hall, A. L. Lambert and G. A. Herzog. 1995.** Effects of a natural pesticide from *Nicotiana gossei* on controlling whiteflies (Homoptera: Aleyrodidae) on a susceptible and resistant soybean line. J. Entomol. Sci. 30: 312-317.
- Neal, J. W., Jr., J. G. Buta, G. W. Pittarelli, W. R. Lusby and J. A. Bentz. 1994.** Novel sucrose esters from *Nicotiana gossei*: effective biorationals against selected horticultural insect pests. J. Econ. Entomol. 87: 1600-1607.
- Nottingham, S. F., O. T. Chortyk and M. G. Stephenson. 1996.** Sugar esters from *Nicotiana* species as potential insecticides against the sweetpotato whitefly (Homoptera: Aleyrodidae). J. Entomol. Sci. 31: 331-339.
- Pittarelli, G. W., J. G. Buta, J. W. Neal, W. R. Lusby and R. M. Waters. 1993.** Biological pesticide derived from *Nicotiana* plants. United States Patent No. 5,260,281.
- Puterka, G. J. and R. F. Severson. 1995.** Activity of sugar esters isolated from the leaf trichomes of *Nicotiana gossei* to pear psylla (Homoptera: Psyllidae). J. Econ. Entomol. 88: 615-619.
- Reid, W. W. 1974.** Part I. The cuticular and cytoplasmic lipids of *Nicotiana tabacum*. Ann. du Tabac., Sec. 2, 11: 151-159.
- Roberts, D. L. and R. L. Rowland. 1962.** Macrocyclic diterpenes. α - and β -4,8,13-duvatriene-1,3-diols from tobacco. J. Org. Chem. 27: 3989-3995.
- SAS Institute. 1990.** SAS/STAT User's Guide. SAS Institute, Cary, NC.
- Severson, R. F., R. F. Arrendale, O. T. Chortyk, C. R. Green, F. A. Thome, J. L. Stewart and A. W. Johnson. 1985a.** Isolation and characterization of the sucrose esters of the cuticular waxes of green tobacco leaf. J. Agric. Food Chem. 33: 870-875.
- Severson, R. F., R. F. Arrendale, O. T. Chortyk, A. W. Johnson, D. M. Jackson, G. R. Gwynn, J. F. Chaplin and M. G. Stephenson. 1984.** Quantitation of the major cuticular components from green leaf of different tobacco types. J. Agric. Food Chem. 32: 566-570.
- Severson, R. F., O. T. Chortyk, M. G. Stephenson, D. H. Akey, J. W. Neal, D. M. Jackson and V. A. Sisson. 1994a.** Characterization of natural pesticides from *Nicotiana gossei*. Am. Chem. Soc. Symp. Ser. 557: 109-121.
- Severson, R. F., R. V. W. Eckel, D. M. Jackson, V. A. Sisson and M. G. Stephenson. 1994b.** Aphicidal activity of cuticular components from *Nicotiana tabacum*. Am. Chem. Soc. Symp. Ser. 551: 172-179.
- Severson, R. F., G. R. Gwynn, J. F. Chaplin and J. D. Miles. 1983.** Leaf trichome exudate associated with insect resistance in *Nicotiana tabacum* L. Tobacco Sci. 27: 82-83.
- Severson, R. F., D. M. Jackson, A. W. Johnson, V. A. Sisson and M. G. Stephenson. 1991.**

- Oviposition behavior of tobacco budworm and tobacco hornworm: effects of cuticular components from *Nicotiana* species. Am. Chem. Soc. Symp. Ser. 449: 264-277.
- Severson, R. F., A. W. Johnson and D. M. Jackson. 1985.** Cuticular constituents of tobacco: factors affecting their production and their role in insect and disease resistance and smoke quality. Recent Adv. Tobacco Sci. 11: 105-174.
- Severson, R. F., K. L. McDuffie, M. D. Jackson, A. W. Johnson, M. G. Stephenson and G. A. Herzog. 1982.** The fractionation of green tobacco leaf cuticular chemicals for insect bioassay studies. Georgia J. Sci. 40: 15.
- Severson, R. F., M. G. Stephenson, A. W. Johnson, D. M. Jackson and O. T. Chortyk. 1988.** Isolation and preparative chromatography of the major cuticular diterpenes of green tobacco. Tobacco Sci. 32: 99-103.
- Sisson, V. A., R. F. Severson and L. B. Smith. 1990.** Leaf surface chemistry of the U.S. Tobacco Introduction collection. 44th Tobacco Chem. Res. Conf., Abstract No. 13, Program Vol. 44: 17.
- Thurston, R. 1961.** Resistance in *Nicotiana* to the green peach aphid and some other tobacco insect pests. J. Econ. Entomol. 54: 946-949.
- Thurston, R. and J. A. Webster. 1962.** Toxicity of *Nicotiana gossei* Domin to *Myzus persicae* (Sulzer). Entomol. Exp. Appl. 5: 233-238.
- Thurston, R. and W. Katanyukyl. 1973.** Response of certain insects to tobacco cultivars T. I. 1298 and T. I. 1068. Tobacco Sci. 17: 162.
- Thurston, R., M. J. Kasperbauer and C. A. Jones. 1977.** Green peach aphid and tobacco flea beetle populations on tobacco cultivars and haploid-derived lines with various alkaloid levels. Tobacco Sci. 21: 22-24.
- Wahlberg, I., I. Wallin, C. Narborne, T. Nichida and C. R. Enzell. 1981.** Note on the stereostructures of thunbergol (isocembrol) and 4-Epiisocembrol. Acta Chem. Scand. Ser. B, Org. Chem. Biochem. 35: 65-68.
- Xia, Y., A. W. Johnson and O. T. Chortyk. 1998.** Toxicity of synthetic sucrose esters against the tobacco aphid (Homoptera: Aphididae). J. Entomol. Sci. 33: 292-299.