Sugar Esters from *Nicotiana* Species as Potential Insecticides Against the Sweetpotato Whitefly (Homoptera: Aleyrodidae)[1]

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ABSTRACT Eight *Nicotiana* species and three species accessions were grown and their sugar esters were isolated. *Nicotiana trigonophylla, N. palmeri,* and *N. glutinosa* gave the highest sugar ester yields. Sugar ester isolates were bioassayed at concentrations from 1.0 to 0.05 mg/ml against nymphs of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). The highest mortalities were observed with isolates from *N. gossei* and *N. langsdorffii.* As the sugar ester isolate from *N. gossei* contained both sucrose esters and glucose esters, these were bioassayed separately and both caused high mortality. The isolates of three *N. glutinosa* accessions varied in sucrose ester and labdane terpenoid content; labdane fractions were less toxic than sucrose ester fractions. *Nicotiana glutinosa* 24, *N. langsdorffii,* and *N. trigonophylla,* in addition to *N. gossei,* showed good potential as sources of biorational insecticide against whitefly.

KEY WORDS *Bemisia tabaci, Nicotiana gossei, Nicotiana glutinosa, Nicotiana* species, sugar esters, biorational insecticide

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius), has been a pest of tropical crops for many years (Greathead 1986), but was not considered to be of major agricultural importance in North America until the 1980s, when massive outbreaks occurred in greenhouse poinsettia in Florida (Faust 1992). This whitefly was referred to as the poinsettia strain and, subsequently, *B. tabaci* strain B. Some workers have described this biotype as a new species, the silverleaf whitefly, *Bemisia argentifolli* N. (Bellows et al. 1994) but it is still unclear whether all *B. tabaci* B strain can be so reclassified (e.g., Brown et al. 1995). *Bemisia tobaci* B strain has spread rapidly and is now a major pest on a diverse range of crops throughout the U.S., including tomato, cole, cucurbits, cotton, lettuce, peanut, sugar beet, and ornamentals (Faust 1992, Gill 1992). Control of *B. tabaci* has become more difficult because of the development of resistance to a variety of insecticides (Prabhaker et al. 1985). Annual losses in

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the USA, due to this insect, now exceed $200 million (Faust 1992). The search for novel biorational control agents has, therefore, become one of the priority areas of whitefly research (Faust 1992, Henneberry et al. 1994).

Plants in the Solanaceae family, including *Nicotiana,* have trichomes that exude a gummy material containing terpenes and sugar esters (Severson et al. 1991). The sugar esters may consist only of sucrose esters or of a mixture of sucrose and glucose esters, with various aliphatic acids esterified to two or more of the free hydroxyl groups of sucrose or glucose (Severson et al. 1994a). Certain *Nicotiana* species have natural resistance to aphids and whiteflies (Thurston and Webster 1962, Neal et al.1987). Sugar ester fractions of one of these resistant species, *Nicotiana gossei,* and extracts from other *Nicotiana* species, are toxic to early-instar nymphs of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) and *B. tabaci* (Buta et al. 1993, Neal et al. 1994). A sugar ester extract from *N. gossei* has shown promising results as a biorational insecticide in field trials on cotton against *B. tabaci* (Severson et al. 1994a). A sugar ester extract from the related Petunia also appears to be effective against *B. tabaci* (Kays et al. 1994).

In this study, the relative efficacy of sugar ester isolates of *N. gossei* and nine other *Nicotiana* species or species accessions were assessed in bioassays against nymphs of *B. tabaci.* It is known that both type and amount of sugar esters vary among *Nicotiana* species (Chortyk et al. 1993, Severson et al. 1994a). The selection of *Nicotiana* species was based on a combination of factors, including leaf levels of sugar esters (Severson et al. 1991), aphid resistance (Burk and Stewart 1969, Thurston 1961), aphicidal activity (Severson et al. 1994b), antibiotic activity (Chortyk et al. 1993), and whitefly toxicity (Neal et al. 1987). The *Nicotiana* species chosen represent a range of subgenera and sections, following the classification of Goodspeed (1954), and include species closely related to *N. gossei* and others with reported insect resistance (Thurston et al. 1966). Sugar ester isolates from these selected *Nicotiana* species were bioassayed against *B. tabaci* to identify those species with the most potential as plant sources for biorational control agents against whitefly.

The sugar ester isolate of *N. gossei* consists of two major types of glucose esters (2,3 di-*O*-acyl-1-acetyl glucose and 2,3-acyl-glucose) and two major types of sucrose esters (2,3 di-*O*-acyl-1'-acetyl sucrose and 2,3-di-*O*-acyl-1',6'-di-*O*-acetyl sucrose) (Severson et al. 1994a). The sugar ester isolate has been extensively investigated (Buta et al. 1993), and the two sucrose ester compounds have been patented (Pittarelli et al. 1993). The two major acyl groups on the above sugar esters have been determined to be 5-methylhexanoyl and 5-methylheptanoyl (Pittarelli et al. 1993). In the present study, glucose and sucrose ester fractions from the *N. gossei* isolate were sprayed separately against nymphs to assess the contribution of each to toxicity against *B. tabaci.* The sugar esters in the sugar ester isolates of the other species have yet to be fully identified.

The *N. glutinosa* accession isolates were unique in having labdane terpenes in addition to sugar esters (Arrendale et al. 1990). None of the other *Nicotiana* species contained significant amounts of labdanes (Severson et al. 1991). Sugar ester and labdane fractions from the *N. glutinosa* 24A isolate were bioassayed separately to determine whether labdane terpenes contributed significantly to whitefly toxicity.

Materials and Methods

Plant cultivation. The *Nicotiana* species grown for leaf surface extractions were *N. glutinosa* L. (Oxford, NC, lab accesisons 24, 24A and 24B; National Plant Germplasm Accessions, PI 555 507, PI 555 510 and PI 241 768, respectively), *N. trigonophylla* Dunal, *N. palmeri* Gray, *N. langsdorffii* Weinmann, *N. benthamiana* Domin., *N. gossei* Domin., *N. amplexicaulis* Burb. and *N. hesperis* Burb. The plants were grown in replicated field plots (300 plants total each entry) under flue-cured tobacco production conditions at three sites: University of Georgia Coastal Plains Experimental Station, Tifton, GA; the Crops Research Laboratory, Oxford, NC; and the Pee Dee Research and Education Center, Clemson University, Florence, SC. All species were grown at each site and the extracts of each from different sites were combined for the purpose of bioassays.

Sugar Ester Isolates. Cuticular extracts were obtained by dipping whole, cut-off plants into methylene chloride or isopropyl alcohol (1.5 L/kg of plant material) in the field, on ten sampling dates, from 28 May to 5 September 1993, as previously described (Severson et al. 1994a). Initial use of methylene chloride was discontinued due to possible health hazards. Plants were allowed to regrow and were harvested 3 or 4 times for extraction. Sugar ester isolates were obtained from the cuticular extracts by a previously described solvent partitioning procedure (Severson et al. 1985, Severson et al. 1994a). This scheme was designed to remove alkaloids (including all nicotine compounds) with an aqueous tartaric acid solution, leaving an acetonitrile fraction that contained the purified sugar esters, termed SE isolate (sugar ester isolate). The sugar esters of *N. gossei* were separated by liquid chromatography on Sephadex LH-20 into its sucrose and glucose esters (Severson et al. 1993a). Individual compounds were dissolved in acetone to prepare stock solutions. Aliquots of stock solution were then used to prepare the bioassay concentrations (1.0, 0.50, 0.10, and 0.05 mg/ml) in 5% acetone-water.

The separation of the *Glutinosa* 24A SE isolate into its terpenoid and sugar ester compounds was accomplished by silicic acid column chromatography. A 1-g sample was chromatographed on 30 g of Unisil silicic acid (Clarkson Chemical Co. Inc., Williamsport, PA). Sequential elution of the column with increasing percentages of acetone (1%, 5%, 8%, 20%) in methylene chloride eluted the labdane terpenes and then the pure sucrose ester compounds. Sucrose esters were characterized by gas chromatography-mass spectrometry (GC/MS) by our usual procedure of converting them to violatile trimethylsilyl derivatives and separating them on SE-54 or DB-5 glass capillary GC columns (Arrendale et al. 1990).

Bioassay. A culture of *B. tabaci* B strain, isolated from a greenhouse in Tifton, GA on March 1994, was established on sweet potato (Jewel cultivar) in a muslin-tented area, with a clear polyethylene roof, in a greenhouse at the USDA Richard B. Russell Agricultural Research Center, Athens, under natural (summer) light conditions. Four-to six leaf sweetpotato plants were placed in the culture cage for 2 to 3 days, to be infested with eggs, and then removed to a whitefly-free section of the greenhouse. After 2 to 3 weeks, when nymphal stages were present, abaxial leaf surfaces were sprayed to run-off with sugar ester isolates, from 10-cm distance, using Spritzer applicators (Bel-Art Products, Pequannock, NJ). Leaves were removed from plants 6 days after treatment and examined

under a binocular microscope. A number 14 cork borer (defining a 3.15 ± 0.09 cm^2 leaf disc area) was used to mark basal leaf areas. Three leaf discs per treatment were scored, each taken from a different leaf, and the percent mortality determined by counting live and dead nymphs within leaf disc areas. Dead nymphs were distinguished by their brown dehydrated appearance. Leaf disc counts were pooled and treatments were replicated on different dates.

Statistical analysis. Kruskal-Wallis one-way ANOVA by ranks (Seigel and Castellan 1988) was done on the whitefly mortalities for the ten *Nicotiana* sugar ester isolates (without control) at the four concentrations, and Kolmogrov-Smirnov two-sample, one-tailed, tests were conducted for nymph mortality at 0.10 and 0.05 mg/ml to compare sugar ester isolates to the control. Chi-squared tests were done to prepare mortality due to sucrose and glucose ester fractions of the *N. gossei* sugar ester isolate and sucrose ester and labdane terpene fractions from the *N. glutinosa* 24A sugar ester isolate.

Results

Large differences were observed in the yields of sugar ester isolates per kilogram of green plant material obtained from the *Nicotiana* species grown in 1993. *Nicotiana trigonophylla, N. palmeri,* and *N. glutinosa* 24 and 24A produced the highest yields of sugar ester isolates, 2.0 to 2.8 g sugar ester/kg of green plant material. *Nicotiana gossei* gave 0.2 g/kg, while *N. hesperis* gave particularly low yields (0.01g/kg). The other species yielded intermediate amounts (0.1-0.9g/kg).

All of the sugar ester isolates produced high mortalities at the two highest concentrations (Table 1). No differences between the ten sugar ester isolates were observed for nymph mortality at the 1.0, 0.50, and 0.10 mg/ml concentrations, but differences occurred at 0.05 mg/ml (Kruskal-Wallis ANOVA, $P = 0.05$); *N. langsdorffii, N. benthamiana, N. glutinosa* 24, *N. glutinosa* 24B, and *N. gossei* produced the highest mortalities and *N. hesperis* the lowest mortality (Table 1). At 0.10 mg/ml nymph mortality due to *N. hesperis* was not significantly different from the control, while at 0.05 mg/ml *N. hesperis, N. amplexicaulis, N. glutinosa* 24A, *N. palmeri,* and *N. trigonophylla* did not differ from the control (Kolmogrov-Smirnov two-sample tests, $P = 0.05$).

The fractions of two sucrose esters and two glucose esters from the *N. gossei* sugar ester isolate all gave high mortalities, being significantly higher than the control at concentrations above 0.05 mg/ml ($P < 0.05, \chi^2$) (Table 2). 2,3-di-O-acyl glucose gave lower mortality at 0.50 mg/ml than the other sugar esters (Table 2; $P < 0.05$, χ^2), but further work needs to be done on the relative efficacy of individual sugar esters.

In contrast to the other *Nicotiana* species' sugar ester isolates, the sugar ester isolates of the three accessions of *N. glutinosa* contained labdane terpenoid components (Table 3). The accessions also differed in their sucrose ester content, but none contained significant amounts of glucose esters. The sucrose ester fraction from *N. glutinosa* 24A caused a significantly higher nymph mortality than the labdane terpene fraction at the 1.0 and 0.5 mg/ml concentrations ($P < 0.05$, χ^2) (Table 4). The labdane fractions of the other two *N. glutinosa* accessions were not tested.

Table 1. Mean percentage mortality of *Bemisia tabaci* nymphs sprayed with four concentrations of sugar ester isolates from *Nicotiana* species and species accessions.

Nicotiana species	n*	Concn. mg/ml 1.0	0.50	0.10	0.05
N. amplexicaulis	3	97 (82-100)**	91 (44-97)	31 (29-44)	8 (3-20)
N. benthamiana	3	100 (99-100)	92 (81-99)	61 (48-72)	39 (11-76)
N. glutinosa 24	6	92 (63-100)	91 (66-97)	59 (38-85)	36 (5-82)
N. gultinosa 24A	4	96 (94-100)	89 (76-100)	60 (19-92)	28 (3-86)
N. glutinosa 24B	4	96 (92-100)	90 (70-99)	49 (3-81)	40 (11-82)
N. gossei	3	98 (93-100)	93 (84-98)	85 (50-93)	37 (8-52)
N. hesperis	3	91 (89-93)	85 (71-93)	8 (4-16)	3 (2-4)
N. langsdorffii	3	99 (96-100)	86 (77-91)	85 (67-94)	59 (44-80)
N. palmeri	5	78 (32-95)	68 (15-86)	20 (6-50)	7 (1-20)
N. trigonophylla	3	98 (96-100)	95 (88-99)	17 (14-23)	13 (9-17)
Control	11	5 (1-19)			

* Number of replications.
** Mean (range).

Discussion

In recent years, *N. gossei* has been grown on large plots and its sugar ester isolate has been used as a biorational insecticide in selected, small field trials. In the present study, high mortality was observed with the *N. gossei* sugar ester isolate (also called *N. gossei* biorational). The *N. gossei* biorational contains approximately a 50:50 mixture of glucose esters: sucrose esters (Severson et al. 1994a). In our tests, both the glucose and sucrose esters of *N. gossei* caused high mortality of *B. tacaci* and both, therefore, appear to contribute to the high bioactivity of the *N. gossei* sugar ester isolate. In addition, extracts from many of the other *Nicotiana* species also caused levels of whitefly mortality comparable to that produced by extracts of *N. gossei* in this and previous studies (e.g., Severson et al. 1994a). Therefore, some of these other *Nicotiana* species may be more suitable as sources of biorational insecticide, considering the very low yield of sugar ester isolate for *N. gossei*.

The sugar ester isolates of *N. glutinosa* contained labdane terpenes, in addition to sucrose esters (Table 3). The *N. glutinosa* 24A isolate was bioactive, although containing only a low percentage (11%) of sucrose esters and a high percentage (85%) of labdanes (sclareol, 13-epi-sclareol, and labdene diol). This suggested that the labdane terpenes might also be bioactive. Previous work (Severson et al.

Table 2. Mean percentage mortality of *Bemisia tabaci* nymphs sprayed with two glucose ester, (i) 2,3,-di-*O*-acyl-*O*acetyl glucose and (ii) 2,3-di-*O*-acyl-glucose, and two sucrose esters, (iii) 2,3-di-*O*-acyl-1'-*O*-acetyl sucrose and (iv) 2,3-di-*O*-acyl-1'6'-di-*O*-acetyl sucrose, silated from *N. gossei*..

		Concn. mg/ml			
	n*	1.0	0.50	0.10	0.05
glucose esters					
(i)	2	74 (70-77)**	70 (56-83)	24 (8-40)	17 (2-31)
(ii)	2	62 (35-88)	28 (3-52)	15 (6-23)	16 (1-30)
sucrose esters					
(iii)	2	84 (76-91)	81 (71-90)	23 (9-36)	22 (12-31)
(iv)	2	96 (96)	92 (91-92)	10 (8-11)	2 (0-3)
Control	2	2 (1-2)			

* Number of replications.
** Mean (range).

Table 3. Percent distribution of labdanes and sucrose esters in *N. glutinosa* 24, *N. glutinosa* 24A, and *glutinosa* 24B.

Nicotiana Species	Labdanes	Sucrose Esters	Unidentified Compounds
N. glutinosa 24	43	55	2
N. glutinosa 24A	85	11	4
N. glutinosa 24B	6	89	5

Table 4. Mean percentage mortality of *Bemisia tabaci* nymphs sprayed with labdane and sucrose ester fractions of the N. glutinosa 24A isolate.

		Concn. mg/ml			
	n*	1.0	0.50	0.10	0.05
labdane	4	38 (7-79)**	21 (17-38)	30 (5-55)	24 (0-56)
sucrose ester	4	83 (71-98)	63 (43-94)	34 (7-92)	35 (11-86)
Control	2	12 (11-12)			

* Number of replications.
** Mean (range).

1994b) had shown that one of the labdane compounds, cis-abienol, had considerable activity against aphids. Therefore, it was possible that equal toxicity would be shown by the albdane fraction of *N. glutinosa* against whiteflies. However, the labdane terpene fraction at 1 mg/ml caused relatively low mortality, indicating that labdane terpenes probably do not make a major contribution to the toxicity of the *N. glutinosa* 24A sugar ester isolate.

Neal et al. (1994) have recently shown, in bioassays with young nymphs of the greenhouse whitefly, *T. vaporariorum*, that *N. gossei, N. benthamiana* and *N. bigelovii* (Torrey) Watson were the most active of 17 *Nicotiana* species sugar ester isolates tested. They observed differences between *Nicotiana* species at 1 mg/ml concentrations, with *N. glutinosa* and *N. langsdorffii* causing only intermediate mortalities at this concentration (mean mortality 45% and 33%, respectively), while some species had no activity compared to control. With *B. tabaci*, in the present data, all sugar ester isolates caused high mortalities at 1 mg/ml and differences between *Nicotiana* isolates, and loss of activity by certain species, was only observed at 0.10 mg/ml. Therefore, *B. tabaci* nymphs my be more susceptible than *T. vaporariorum* nymphs to sugar esters, which interfere with cuticular lipid production to cause desiccation, as suggested by Neal et al. (1994).

Nicotiana sugar ester isolates did not cause egg mortality in *T. vaporariorum* (Neal et al. 1994). Preliminary experiments with *B. tabaci* also showed that eggs were unaffected by 1 mg/ml sprays of sugar ester isolates (Unpubl. data).

Since *N. palmeri* has relatively low toxicity, the top three potential biorational insecticide producers appear to be *N. trigonophylla, N. glutinosa* 24, and *N. langsdorffii.* As for the quantity of plant biomass per hectare produced by the three species, no agronomic data are yet available, but a future 2 to 3 year replicated study would reveal which species would consistently yield the most sugar ester per kilogram of biomass, taking into account year to year variations due to weather conditions. From our observations and Goodspeed's description (Goodspeed, 1954), *N. glutinosa* 24 produces the largest plant at 1 to 2.5 m with large, 5 to 20 cm long, cordate pubescent leaves. *Nicotiana langsdorffii* is 1 to 1.5 m

high with 15 to 30 cm leaves which are oblancolate or ovate lancolate. *Nicotiana trigonophylla* is the smallest of the three, ranging in height from 0.5 to 1 m with leaves 5 to 20 cm long which maintain their length nearly to the inflorescence, but are generally smaller than *N. langsdorffii. Nicotiana trigonophylla* is a limited perennial which may be a factor in its vigorous regrowth and with its many small leaves, the reason for its high sugar ester and biomass production.

Climate, soils, agronomic methods, and responses of the different species to such conditions will all be factors to be considered. For example, *N. gossei* and other species from the Suaveolentes section, a group of Australian origin (Goodspeed, 1954), are originally from an arid environment, and having no leafspot or stem rot disease resistance, did poorly during the wet summer of 1994 in Georgia. In contrast, *N. trigonophylla,* from the arid area of the southwest Pacific Coast of the U.S., and *N. glutinosa* 24, from a semi-arid area of western Peru, had more disease resistance and flourished. However, these *Nicotiana* species may respond differently under other conditions.

In conclusion, *Nicotiana* species in addition to *N. gossei,* particularly *N. glutinosa* 24, *N. langsdorffii,* and *N. trigonophylla,* are potentially promising large-scale producers of biorational insecticides that should be evaluated in field tests against whiteflies and possibly other insect pests.

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References Cited

Arrendale, R. F., R. F. Severson, V. A. Sisson, C. E. Costello, J. A. Leary, D. S. Himmelsbach and H. Van Halbeek. 1990. Characterization of the sucrose ester fraction from *Nicotiana glutinosa*. J. Agric. Food Chem. 38: 75-85.

Bellows, T. S. Jr., T. M. Perring, R. J. Gill and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 87: 195-206.

Brown, J. K., D. R. Frolich and R. C. Rosell. 1995. The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex? Ann. Rev. Ent. 40: 511-534.

Burk, L. G. and P. A. Stewart. 1969. Resistance of *Nicotiana* species to the green peach aphid. J. Econ. Entomol. 62: 1115-1117.

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*. Phytochemistry 32: 859-864.

Chortyk, O. T., R. F. Severson, H. G. Cutler and V. A. Sisson. 1993. Antibiotic properties of sugar esters isolated from selected *Nicotiana* species. Biosci. Biotech. Biochem. 57: 1355-1356.

Faust, R. M. (ed.). 1992. Conference report and 5-year action plan for development of management and control methodology for the sweetpotato whitefly. Houston, Texas Feb. 18-21, USDA-ARS.

Gill, R. J. 1992. A review of the sweetpotato whitefly in Southern California. Pan-Pacific Entomologist 68: 144-152.

Goodspeed, T. H. 1954. The genus *Nicotiana*. Chronica Botanica. 16, no. 1-6: 1-536. Chronica Botanica Co., Waltham, Mass., USA.

Greathead, A. H. 1986. Host plants, Pg 17-25. *In* M. W. J. Cock (ed.) *Bemisia tabaci* - a literature survey on the cotton whitefly with an annotated bibliography. CAB Intern. Inst. Biol. Control, Silwood Park, Ascot, England.

Henneberry, T. J., N. C. Toscano, R. M. Faust and J. R. Coppedge (eds.). 1994. Silverleaf whitefly (formerly sweetpotato whitefly, strain B): 1994 Supplement to the 5-year National Research and Action Plan - second Annual Review Meeting, Orlando, Florida, Jan. 24-27, 1994. USDA-ARS no. 125, 288 pp.

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.

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.

Neal, J. W. Jr., G. W. Pittarelli and K. M. Gott. 1987. *Nicotiana* species with high resistance to greenhouse whitefly, *Trialeurodes vaporariorum.* Tob. Sci. 31: 61-63.

Pittarelli, G. W., J. G. Buta, J. W. Neal, Jr., W. R. Lusby and R. M. Waters. 1993. Biological pesticide derived from *Nicotiana* plants. US Patent # 5,260,281.

Probhaker, N., D. L. Coudriet and D. E. Meyerdirk. 1985. Insecticide resistance in the sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. 78: 748-752.

Severson, R. F., R. F. Arrendale, O. T. Chortyk, C. R. Green, F. A. Thome, J. L. Stewart and A. W. Johnson. 1985. Isolation and characterization of the sucrose esters of the cuticular waxes of green tobacco leaf. J. Agric. Food Chem. 33: 871-875.

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, Pg. 264-277. *In* Hedin, P. A. (ed.), Naturally occurring pest bioregulators, ACS Symposium Series No. 449.

Severson, R. F., O. T. Chortyk, M. G. Stephenson, D. H. Akey, J. W. Neal Jr., D. M. Jackson and V. A. Sisson. 1994a. Characterization of natural pesticides from *Nicotiana gossei,* Pg. 109-121. *In* Hedin, P. A. (ed.), Bioregulators for crop protection and pest control. ACS Symposium Series No. 557.

Severson, R. F., E. V. W. Eckel, D. M. Jackson, V. A. Sisson and M. G. Stephenson. 1994b. Aphicidal activity of cuticular components from *Nicotiana tabacum*, Pg. 172-179. *In* Hedin, P. A., J. J. Menn, R. M. Holingworth (ed.), Natural and engineered pest management agents, ACS Symposium Series No. 551.

Siegel, S. and N. J. Castellen, Jr. 1988. Nonparametic statistics for the behavioral sciences. 2nd Ed. McGraw-Hill, Singapore.

Thurston, R. 1961. Resistance in *Nicotiana* to the green peach aphid and some other tobacco pests. J. Econ. Entomol. 54: 946-949.

Thurston, R., J. C. Parr and W. T. Smith. 1966. The phylogeny of *Nicotiana* and resistance to insects. Pro. 4th Intern. Tobacco Science Cong., Athens, Greece.

Thurston, R. and J. A. Webster. 1962. Toxicity of *Nicotiana gossei* Dominot to *Myzus perscicae* (Sulzer). Entomol. Exp. Appl. 5: 233-238.