OVIPOSITION RESPONSES OF MANDUCA SEXTA TO SOLANACEOUS VOLATILE COMPONENTS

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ABSTRACT

Whole plant steam distillates were obtained from ten common solanaceous species and presented to excised *Manduca sexta* moth antennae for electroantennogram (EAG) analysis. The distillates were also used on plant surrogates to test for attraction for oviposition by gravid *M. sexta* moths. All samples were positive in both tests. The distillates from two of the species, tobacco and tomato, were fractioned with various chromatographic techniques and the fractions tested as were the whole distillates. A number of fractions retained EAG activity but none were able to induce oviposition.

INTRODUCTION

The tobacco hornworm, *Manduca sexta* (Johan.), as many other Lepidopterous insects, is oligophagous. In the wild it feeds on tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum*), occasionally horsenettle (*Solanum carolinese*) and Jimson weed (*Datura stramonium*) (Yamamoto et al. 1969). In caged situations, however, *Manduca sexta* has been shown to accept numerous solanaceous plants for both oviposition and larval food sources (Yamamoto and Fraenkel 1960 a.c.). In their study, the non-solanaceous plants tested would not induce oviposition. This suggests that the female moth is able to discriminate among potential host plants at two levels. First the moth is able to distinguish a solanaceous plant from plants of other families, and second it is able to choose its preferred host from among the available solanaceous plants (Yamamoto and Fraenkel 1960 c).

The female *M. sexta* moth is attracted to a plant (or to a leaf surrogate) for oviposition by compounds insoluble by steam distillation (Yamamoto and Fraenkel

1960 b) or water extraction (Sparks 1970). Crude isolates have been further fractionated via various chromatographic techniques and tested for their ability to attract and induce oviposition (Tichenor and Seigler 1980). These tests indicate both adult and larva (Stadler and Hanson 1978) are attracted to a specific mixture of volatiles and not to individual compounds. Electroantennogram analysis showed that these ovipositionally active mixtures were also able to stimulate antennal chemoreceptors (Tichenor and Seigler 1980).

In this paper we will examine attraction by ten members of the Solanaceae with measurements of electrophysiological and ovipositional responses to various extracts and gas chromatographic fractions.

MATERIALS AND METHODS

Living Material

The insects used in these tests were obtained as pupae from the USDA Agricultural Research Service Tobacco Research Laboratory in Oxford, North Carolina. These tobacco hornworms were reared on artificial diets which contained no solanaceous plant material. The pupae were maintained in humidity at 80% until the emergence of the adults. At this time they were placed in a cheesecloth-covered cage (1.3x1.3x1.3 meter), fed sucrose solution daily, and allowed to mate. After two or three days the insects were removed for use in various bioassays.

Both tobacco (variety "Havana" and tomato (var. "Supersonic") were grown in greenhouses at the University of Illinois. Eggplant (*Solanum melongena*), potato (*S. tuberosum*), and greenpepper (*Capsicum grossum*) were grown locally in gardens. Five non-cultivated species of Solanaceae, horsenettle, Jimson weed, ground cherries (*Physalis subglabrata*) and *P. heterophylla*, and deadly nightshade (*Solanum dulcamara*); were collected near Champaign, Illinois (USA). Identification was based on *Flora of Illinois* (Jones 1963).

CHEMICAL PROCEDURES

Steam distillates were obtained with a modified Clevinger apparatus (von Rudloff 1969). Fresh plant material was placed in a chamber directly above a boiling water reservoir. Steam and volatile compounds passed upward, were condensed, and collected in a layer of ether.

The ether layer was collected after each distillation and concentrated under nitrogen. The oils were stored under nitrogen at about -10C.

Gas chromatography (GLC) of solanaceous plant steam distillates was carried out on a nickel column (3 mm x 3 m) packed with 5% XE-60 liquid phase coated on a support (Gas Chrom Q). Chromatograms were run on a Packard-Becker model 409 gas chromatograph programmed from 50 to 220° C (5° per minute with an isothermal post injection period of five minutes). Nitrogen was used as a carrier gas in all analytical determinations (flow rate of 25 ml per minute). Capillary gas chromatography utilized a support coated open tubular (SCOT) Apiezon L column (50 foot, 0.2 inch, gas flow rate 4 ml per minute). Preparative GLC was conducted on a 10 foot, 1/4" diameter, 5% XE 60 stainless steel column with 10:1 splitting ratio. Fractions were collected in 16 gauge syringe needles cooled with dry ice.

Mass spectral data for active fractions were obtained on a Varian CH-7 sector field mass spectrometer interfaced with a B - system Varian 620/*i* computer. This system was also interfaced with a highly modified Varian gas chromatograph equipped with 3% OV-1 column (1/8 inch, stainless steel).

BIOSASSAYS

Ovipositional assays were carried out in a cheesecloth covered cage (1.3x1.3x2.5 m). Chemicals to be tested were sprayed onto polyurethane rectangles (7x20 cm) which were stapled to steel wire stems. These in turn were inserted into the corner posts of the cage. The cage was surrounded on three sides by windows which provided ample light for oviposition. A large exhaust fan maintained gentle air flow through the room.

Electroantennogram (EAG) data were obtained as previously reported (Tichenor et al. 1979).

RESULTS

Both aqueous extracts and steam distillates of tobacco and tomato have previously been shown to be ovipositionally attractive as well as active in electroantennogram analysis for female *Manduca sexta* moths (Yamamoto and Fraenkel 1960 b, Tichenor and Seigler 1980).

Steam distillates of ten solanaceous plants, five weedy species (*Datura stramonium, Solanum carolinense, Solanum dulcamara, Physalis heterophylla*) and five cultivars (tobacco, tomato, potato, green pepper, and eggplant) were prepared and tested in the flight cage. All proved attractive (Table 1) when tested with only a control (surrogate sprayed with water) present. The steam distillate from the non solanaceous plant, *Coleus blumei*, would not induce oviposition.

Electroantennogram activities for the various steam distillates were measured and all solanaceous plants proved positive. EAG response was measured in milli-volts and reported as the activity relative to an air control (Table 2).

GLC patterns of essential oils from the ten plants were obtained on nickel 5% XE-60 column (1/8" x 10") (see Figure 1). One major area of similarity is apparent in all of these chromatograms: the presence of peaks with retention times of 41-42 minutes. These peaks were collected from tobacco and tomato from a 1/4 inch 5% XE-60 column and subjected to further analysis with capillary chromatography. A typical capillary chromatogram indicated that over twenty compounds were still present in these fractions. EAG analyses of these peaks from tobacco and tomato fractions were positive. Oviposition tests were, however, negative.

Two fractions were then subjected to gas chromatographic-mass spectral analysis. Data obtained from this analysis revealed that both the tobacco and tomato fractions show an unknown compound with the unusual base peak of 173. The main components of the tobacco fraction indentified by computerized literature survey and subsequent co-chromatography with standards were methyl palmitate and methyl stearate, neither of which were EAG active.

The mass spectrum of the common compound with the base peak of 173 showed the nine major peaks to be: 173(100), 41(51), 159(41), 91(41), 55(38), 105(36), 93(35), 43(31), 79(30). The parent peak was determined to be 204 and the molecular formula $C_{15}H_{24}O$.

DISCUSSION

Manduca sexta is an oligophagous insect capable of surviving on numerous members of the Solanaceae. Its behavioral patterns however are more closely monophagous in a given geographical region. This situation appears similar to

Table 1. Oviposition Produced by Steam Distillates from Solanaceous Plants

Steam Distillate of:	eggs	H_2O control	moths
Nicotiana tabacum	211	13	2
Lycopersicon esculentum	183	6	2
Physalis subglabrata	27	11	2
Physalis heterophylla	78	3	2
Solanum carolinense	92	26	3
Solanum dulcamara	26	13	1
Datura stramonium	114	12	2
Solanum tuberosum	117	12	2
Solanum melongena	105	3	2
Capsicum grossum	50	12	2
*Coleus blumei**	0	0	2

*non solanaceous plant

Table 2. EAG Activities of Plant Steam Distillates

Material Presented:	EAG response (MV)	Relative EAG Activity
Control	1.05	1
Nicotiana tabacum	2.10	2.1
Lycopersicon esculentum	2.40	2.3
Solanum dulcamara	1.85	1.8
Solanum carolinense	1.85	1.8
Datura stramonium	1.80	1.7
Physalis subglabrata	2.60	2.5
Physalis heterophylla	2.15	2.1
Solanum tuberosum	2.60	2.5
Solanum melongena	2.30	2.2
Capsicum grossum	1.80	1.7

that of the old world swallowtail, *Papileo macheon* L. as described by Wiklund (1974). For this butterfly Wiklund suggests that the keys used by both the adult to induce oviposition and the larva to induce feeding are genetically determined. It is further stated that theses keys are distinguished by chemoreceptors in adults and larvae which are encoded on separate gene complexes so that the phytochemistry involved in the insect's responses may be different for the adult and larvae. Insect population differences may also play a role in the determination of which aspects of a plant's chemistry will induce a given behavioral response.

To make the situation more complex it seems that both the larva (Stadler and Hansen 1978) and the adult *M. sexta* (Tichenor and Seigler, 1980) respond to complex chemical mixtures and not simply to one or two compounds. Chromatographic separations to fractions of about 20 compounds give mixtures which have lost the ability to induce biological responses. Numerous individual compounds have also been shown to be discerned by excised antennae (Tichenor and Seigler, 1980; Adler and Jacobson 1972), but induce no behavioral modification in the intact insect.

M. sexta has been shown to oviposit in caged situations on all ten solanaceous species tested in this work (Yamamoto and Fraenkel, 1960). Limited field observations (in Champaign Co. and Macon Co., Illinois, USA) by the author, and by others (Yamamoto *et al.* 1969) indicate that the moth very rarely oviposits on anything but tobacco and tomato; although one large population of *Physalis subglabrata* near Decatur, Illinois was found to be heavily infested with larvae.

Electroantennogram analysis reveals that despite this essentially monophagous behavior the moth is able to detect volatiles of the remaining Solanaceae, and in caged situations will oviposite in response to them. Gas chromatographic patterns of the essential oils from these ten species show at least one peak among them is similar. Isolation of this peak from tobacco and tomato gives a strongly EAG active fraction which is, however, unable to induce oviposition. Mass spectral analysis of these tobacco and tomato fractions reveals one compound which may be the same in both plants. The compound has been determined to have the molecular formula $C_{15}H_{24}O$.

The plant-insect interactions between members of the Solanaceae and *Manduca sexta* are complex. The female moth utilizes a wide range of sensory input to determine a proper host. Numerous chemicals are sensed via olfaction as well as tactile receptors (Yamamoto et al. 1969). This information is correlated with visual perception and the moth's evaluation of the physical nature of the substrate (Sparks 1970). A combination of all these factors ultimately leads to the behavioral response commonly known as oviposition.

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REFERENCES

Adler, V.E. and Jacobson, M., 1972. *J. Econ. Ent.*, 65: 1582-1585.
Jones, G.N., 1963. *Flora of Illinois*. U. of Notre Dame Press, Notre Dame, Ind. 193 pp.
Sparks, M.R., 1970. *J. of Econ. Entomol.*, 63: 537-540.
Stadler, E. and Hanson, F.E. 1978. *Physiol. Ent.*, 3: 121-133.
Tichenor, L.H., Seigler, D.S. 1980. *J. of Insect Physiol.*, 26: 309-314.
Tichenor, L.H., Seigler, D.S. and Sternberg, J.G. 1979. *BioScience*, 29: 364-365.
von Rudloff, E., 1969. *Recent Advances in Phytochemistry* 2. (Seikel, M.K. and Runeckles, V.C., Eds.) Appleton-Century-Crofts, N.Y. 127-162.

Wiklund, C., 1974 *Ent. Scad.*, 5: 151-160.
Yamamoto, R.T., Jenkins, R.Y. and McClusky, R.K., 1969. *Ent. exp. & appl.*, 12: 504-508.
Yamamoto, R.T. and Fraenkel, G.A., 1969. *Ann. Ent. Soc. Amer.*, 53: 499-507.
_____b. 1960. *Int. Kong. Entom. Wien.*, 11: 127-133.
_____c. 1960. *Ann. Entomol. Soc. Amer.*, 53: 499-507.

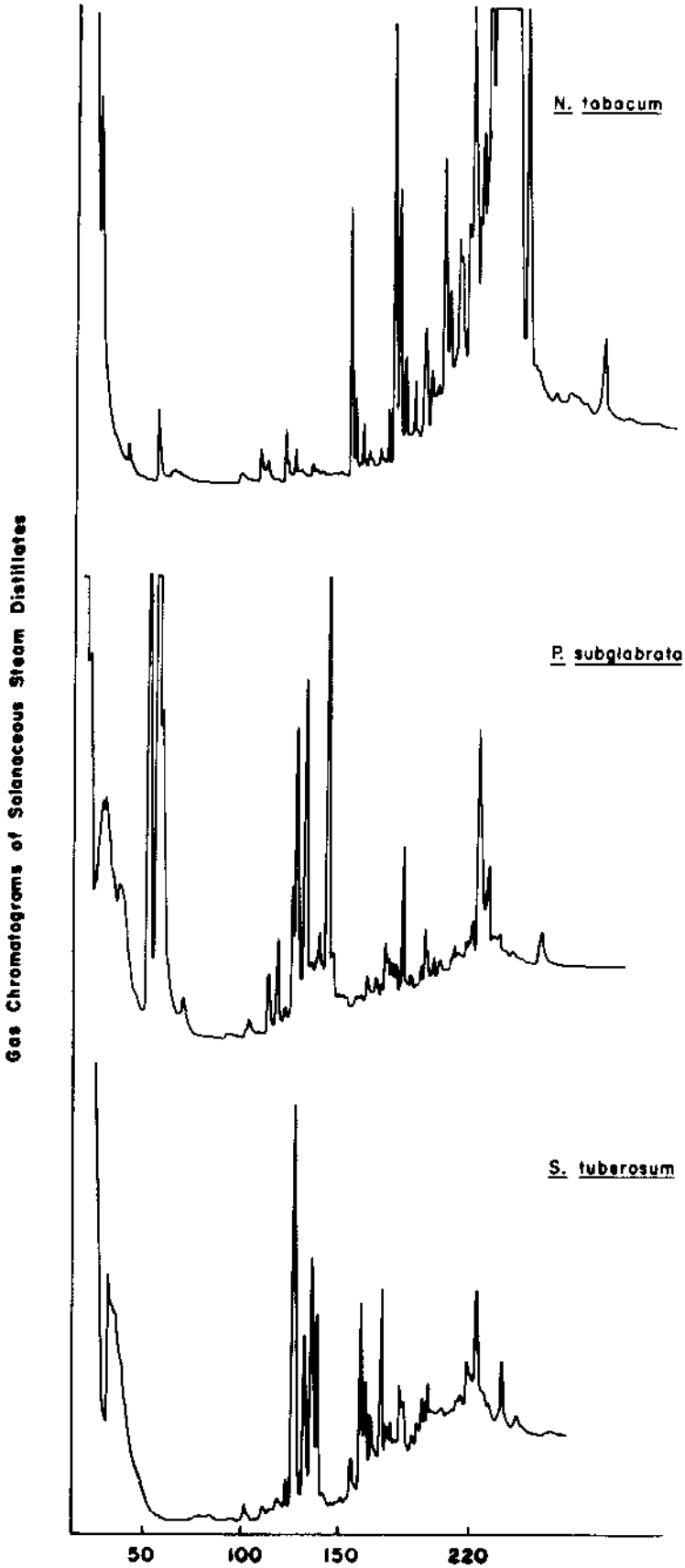

FIGURE 1. Gas Chromatograms of Solanaceous Steam Distillates
Typical chromatograms from three of the ten solanaceous species tested (run on a 2mm x 3m nickel column with 5% XE-60 on gas chrom Q, programmed from 50 to 220°C. The peak mentioned in the text appears slightly after the 220° mark.