shelf-life of bananas may, however, pose a major constraint in practically launching transgenic bananas as edible vaccines. The banana varieties which abundantly regenerate *in vitro* and in which the 'transgene products' can be stored for long, are an ideal system for the expression of therapeutic protein genes. It is well known that proteins retain structural integrity and functionality during the dehydration process. Further, the therapeutic protein 'Hepatitis-B surface antigen', is reported to be thermostable[16]. Rajeli seems to be a suitable choice from both these points of view, since the therapeutic proteins can possibly be specifically expressed in maturing bananas and shelved for long in dried form. Such a facility of extended storage either in the dried form or via any other suitable method such as banana juice[17], would offer benefit to researchers and manufacturers for assaying, processing and distributing the therapeutic proteins to remote destinations at convenient time.

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Characterization of oviposition attractants of *Helicoverpa armigera* in two solanaceous plants, *Solanum viarum* and *Lycopersicon esculentum*

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The role of host plant chemicals in oviposition of *Helicoverpa armigera* Hübner (Lepidoptera : Noctuidae) was studied in two solanaceous plants, *Solanum viarum* Dunal. and *Lycopersicon esculentum* Mill. Plant volatiles as well as chemicals extracted from leaves were bioassayed for oviposition attraction in a two-choice olfactometer, where mated adults were given equal opportunity for oviposition either on plant extract side or solvent check side. Two fractions of microwave-assisted extracts from leaves of both plants elicited strong oviposition response. Normal alkanes, 13,17,21-trimethylheptatriacontane and octacosane were the only chemicals present in these fractions of *S. viarum*, whereas besides these chemicals, few other *n*-alkanes and related primary alcohols and aldehydes were present in tomato foliage. Oviposition attractants were also present in volatiles of both plants. Two fractions from *S. viarum* containing several small molecular weight alkanes elicited strong ovipositional response. One of the two fractions of tomato volatiles that elicited moderate oviposition response contained predominantly 3-nitrobenzyl alcohol and minor amounts of 3-nitrobenzaldehyde, whereas the other that showed strong oviposition deterrent activity contained 3-nitrobenzyl alcohol and small amounts of docosane and trimethyldecane, in addition to an unknown compound.

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Although several chemicals in both plants were active in oviposition of *H. armigera*, we were not able to ascertain whether all or only certain specific chemicals are involved in this process. However, constancy in most of the identified compounds, especially *n*-alkanes in the two different genera *Solanum* and *Lycopersicon* could have formed the basis for host selection by *H. armigera*.

Keywords: *Helicoverpa armigera*, *n*-alkanes, plant extract, plant volatiles, *Solanum viarum*.

HOST plant selection is primarily a function of gravid females in many phytophagous insects, especially in Lepidoptera in which the newly hatched larvae cannot migrate over long distances[1]. Host selection by these insects is considered primarily influenced by a set of stimuli specific to the hosts[2,3]. There is growing evidence that host-finding in moths is largely guided by secondary plant metabolites[4–6], particularly volatile compounds. A few key compounds mediate long-range attractions, which are perceived by specialized chemoreceptors in insects[7–10]. Thus, host plant chemicals are probably the most important source of information contributing to the final decision by an insect to oviposit or not.

Helicoverpa armigera Hübner (Lepidoptera : Noctuidae), a highly mobile noctuid moth is a pest of tomato and numerous other crops, including cotton, corn, chickpea, pigeonpea, sorghum, sunflower, soybean and groundnut[11,12]. The ability of gravid females to locate and utilize a wide range of hosts from a number of families for oviposition is one of the major factors contributing to the pest status of this moth[12,13]. However, despite its importance, the host selection behaviour of this moth is still poorly understood[13,14].

In spring 1998, we observed heavy loads of *H. armigera* eggs and larvae on foliage of *Solanum viarum* Dunal., which was never reported to be the host of this noctuid. Subsequent studies revealed that *H. armigera* overwhelmingly preferred to lay eggs on *S. viarum* over tomato, its normal host plant[15,16]. Microwave-assisted hexane extract of *S. viarum* leaves and hexane extract of plant volatiles attracted *H. armigera* adults for oviposition[16]. In this study, we investigated the possible role of phytochemicals from *S. viarum* and tomato in attraction of *H. armigera* for oviposition.

Leaves of 10–12-week-old potted plants of *S. viarum* and tomato (cv L390) were used for preparing extracts. Whole plants were used for collecting volatiles. Larvae of *H. armigera* were reared on a meridic diet of a polyphagous insect, *Spodoptera exigua* Hübner (Bio-Serve French Town, NJ, USA; Product No. F 9219 B) at 27 ± 1°C, and 70 ± 10% rh, L14 : D10 until pupation. On pupation, they were sexed and placed in acrylic cylinders (30 cm long and 15 cm diameter) for adult emergence. Some of the emerged adults were used for experimentation and the rest were returned for multiplication. Adults were maintained on a 10% (wt : vol) sugar solution dispensed on cotton wool placed inside the cylinders.

Foliage extracts were made using microwave-assisted extraction technique[17]. Four or five clean, healthy, medium-sized leaves from the middle canopy of ten-week-old *S. viarum* plants were excised and immediately placed in a glass container with 600 ml hexane for 30 s. The container was then placed inside a household microwave oven (800 W) and the oven was run for 30 s. The hexane extract was dried by filtering through a plug of anhydrous sodium sulphate, concentrated to 2 ml and stored at –20°C until used in bioassays. The same procedure was followed for extraction of chemicals from tomato leaves.

An airtight chamber measuring 55 × 50 × 60 cm was made from clear acrylic sheets. Two small fans were fixed to the opposite ends of the parallel walls. The top of the chamber had one inlet and one outlet hole. The glass tube fixed in the inlet hole opened halfway inside the chamber. The outlet was connected by Teflon tubing to the inlet of a tall aspirator containing 700 ml hexane. The outlet of the aspirator was connected to a vacuum source. Four potted plants of *S. viarum* or tomato were placed inside the acrylic chamber with running fans; the chamber was attached to a vacuum source and plant volatiles from inside the chamber were sucked through the hexane column continuously for one week. The plants and hexane were replaced once every 24 h. All hexane extracts were pooled and concentrated to 3 ml and stored at –20°C until used for bioassays.

This test was conducted in a 4.3 m long and 15 cm diameter two-choice olfactometer constructed out with two 2 m long acrylic cylinders fastened on each open end of central detachable 30 cm long acrylic tube (Figure 1). A hole was made in the centre of the 30 cm detachable portion of the olfactometer to suck the air out. Flexible nylon netting was used to close both open ends of the olfactometer. The plant extract to be bioassayed was blotted on a filter paper and after all solvent had evapourated, the filter paper was stapled in one piece of nylon net. The net in turn was wrapped tightly around one open end of the olfactometer, making sure that the filter paper was in the centre and inner side of the olfactometer. On the opposite side, a filter paper coated with an equal amount of solvent only was attached in a similar manner. Two gravid females along with males were placed at the centre of the detachable portion and two arms of the olfactometer. Air from within the olfactometer was sucked out continuously through the hole in the centre of the olfactometer. The sucked air came from over the filter papers at either end, giving *H. armigera* adults equal opportunity to fly to either direction to lay eggs. The entire olfactometer was covered with black cloth. After 48 h, the number of eggs laid on the filter paper, nylon net and the walls of acrylic cylinders to a length of 50 cm from either end was recorded. Each such experiment was repeated four times with a new set of insects and plant extract every time.

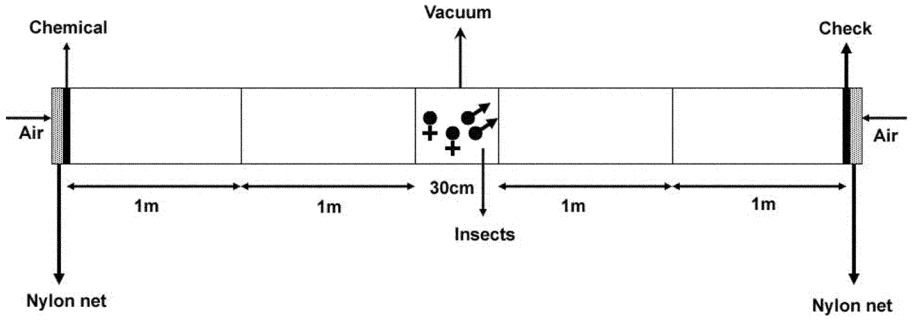

Figure 1. Two-choice olfactometer for oviposition bioassay of plant extracts against *Helicoverpa armigera.*

Table 1. Oviposition response of *Helicoverpa armigera* exposed to various fractions of extract of foliage of *Solanum viarum* and tomato plants

Fraction	*Rf* value*	Oviposition index
S. viarum microwave-assisted extract		
A	0.86	0.00
B	0.57	+ 0.19
C	0.43	+ 0.14
D	0.29	+ 0.58
E	0.14	– 0.60
F	0.06	+ 0.83
S. viarum volatiles		
A	0.41	+ 1.00
B	0.40	+ 0.90
C	0.33	– 0.40
D	0.20	0.00
Tomato microwave-assisted extract		
A	0.91	– 1.00
B	0.82	+ 0.79
C	0.57	+ 0.43
D	0.19	+ 0.44
Tomato volatiles		
A	0.39	0.00
B	0.22	+ 0.69
C	0.19	– 0.94

*Silica Gel 60, 0.25 mm layer, mobile phase : hexane : acetone 10 : 3.

The crude plant extracts that proved to be active as oviposition attractants were added on the top of a Silica Gel 60 column (25 cm × 1 cm diameter) and eluted with a 10 : 3 mixture of hexane and acetone. The eluant was collected in 1–2 ml fractions with a fraction collector. A drop of each fraction was blotted on Silica Gel GF254 TLC plate and visualized under UV light at 365 nm wavelength. Fractions that showed positive fluorescence were plated on 0.25 mm thick Silica Gel G60 TLC plates, developed with a 10 : 3 hexane : acetone mobile phase, and visualized by iodine vapour confined in a glass chamber. Fractions with identical *Rf* values were combined and bioassayed for ovipositional activity.

Preference for oviposition on the side of the plant chemicals or the check was judged by an 'Oviposition Index' (OI)[18]. It is derived from subtracting the number of eggs laid on the control side from the number laid on the chemical side and dividing the difference by the sum of total number of eggs laid on both sides. A positive OI indicates preference to the chemical and a negative OI for non-preference or deterrence. Data for ovipositional preference were analysed by paired *t* test.

Based on the results of ovipositional bioassay, the active fractions were sent to the analytical services facilities of the National Cheng Kung University Instrument Center, Tainan, Taiwan, for GC–MS analysis. Identification of the chemicals was performed on a Shimatzu QP2010 ion trap mass spectrometer (ionizing voltage 70 eV) interfaced with a GC 2010 gas chromatograph. Separation of the chemicals was achieved using a thermal programme running from initial temperature of 100°C for 4 min to final temperature of 270°C for 8 min, at a rate of 10°C/min. The column flow was 1.24 ml/min.

Column chromatographic separation of foliage extract (*S. viarum*) resulted in isolation of six fractions with *Rf* values of 0.86, 0.57, 0.43, 0.29, 0.14, and 0.06 on TLC (Table 1), which were designated as A, B, C, D, E, and F respectively, for the sake of ease in discussion. Fraction A was inactive, because *H. armigera* did not lay eggs on either side of the olfactometer. Fraction E had very high negative OI, but the number of eggs laid on the check side was too few (one) to consider it as a repellent. The remaining four fractions had positive OI values; all had substantial number of eggs laid on the plant extract side of the olfactometer. However, additional bioassays of both D and F fractions showed significantly greater number of eggs on plant extract side ($t = 3.12$; d.f. = 3; $P < 0.05$ for 'D', and $t = 5.40$; d.f. = 3; $P < 0.01$ for 'F') than on the check side (Table 2). GC–MS analysis of these fractions showed the presence of one pure chemical in each; the chemical in fraction D was 13,17,21-trimethylheptatriacontane and the one in fraction F was octacosane, which were confirmed with the mass spectra of standards from the GC–MS library.

The whole plant volatile collection had four groups of chemicals with *Rf* values 0.41, 0.40, 0.33, and 0.20 on TLC, which were designated as A, B, C, and D respectively. Fractions A and B were very active with OI values of + 1.00 and + 0.90 respectively (Table 1). Fraction C had moderately negative OI and fraction D was inactive.

Table 2. Oviposition response of *H. armigera* exposed to various active fractions of extracts of foliage of *S. viarum* and tomato plants (replicated trials) and active compounds

Active fraction	Mean number of eggs laid (± SE)		Compounds
	Fraction	Check	
S. viarum foliage extract			
D	52.00 ± 21.69	18.50 ± 12.91	13,17,21-Trimethylheptatriacontane
F	48.25 ± 9.20	11.00 ± 3.29	Octacosane
S. viarum volatiles			
A	84.25 ± 49.05	21.25 ± 11.19	Tricosane, docosane, pentacosane, hexacosane, 2-methyl octadecane, eicosane, 2-methyl eicosane and an alcohol, 6-tridecanol
B	76.50 ± 10.21	31.25 ± 14.49	2,6,11-Trimethyldodecane, eicosane, 2-methyl eicosane and 2-methyl octadecane
Tomato foliage extract			
B	94.00 ± 25.94	18.25 ± 13.21	Pentacosane, hexacosane, octacosane, 2-methyl eicosane, hexatriacontane, 13,17,21-trimethylheptatriacontane, caryophyllene, α-caryophyllene and 2-nitro-1,3-bis-octyloxy-benzene
D	30.25 ± 7.35	4.75 ± 2.93	Tricosane, docosane, hexacosane, heptacosane, octacosane, heneicosane, 13,17,21-trimethyl heptatriacontane
Tomato volatiles			
B	62.30 ± 18.67	8.00 ± 3.61	(1,2,3) Triazolo (1,5-a) pyrazine, 3-nitrobenzaldehyde, 3-nitrobenzyl alcohol
C	1.33 ± 0.88	38.30 ± 8.41	docosane, 2,3,7-trimethyldecane, 3-nitrobenzyl alcohol and 6-methyl-1-(6-methyl-1,3,5-cycloheptatrien-1-yl)

Prior to identification of the chemicals, when fractions A and B were again tested, insects laid significantly more eggs on the plant extract side than on solvent checks ($t = 3.16$; d.f. = 3; $P < 0.05$ for 'A' and $t = 3.75$; d.f. = 3; $P < 0.05$ for 'B') (Table 2).

GC–MS analysis of fraction A revealed the presence of significant quantities of seven *n*-alkanes, namely docane, tricosane, 2-methyl octadecane, pentacosane, hexacosane, 2-methyl eicosane and eicosane, present in roughly similar quantities. Minor amounts of an alcohol, 6-tridecanol were also present in fraction A[19]. Similarly, fraction B also had some *n*-alkanes, such as 2,6,11-trimethyldodecane, eicosane, 2-methyl eicosane, and 2-methyl octadecane.

Four fractions of foliage extract (tomato) that were positive for the presence of chemicals, had *Rf* values 0.91, 0.82, 0.57, and 0.19 on TLC, which were designated as A, B, C, and D respectively. Fractions A and C were inactive because few eggs (3 and 0 respectively) were laid when evaluated in the olfactometer. Fractions B and D were considered active because insects laid more eggs (94 and 30 respectively) on the sample side in the olfactometer. In further tests with fraction B, out of 112 eggs laid, 84% was laid on the plant extract side and only 16% on the check side of the olfactometer; the difference was statistically significant ($t = 2.74$; d.f. = 3; $P < 0.05$, Table 2). For fraction D, out of 35 eggs laid, over 88% was found on the plant extract side ($t = 4.28$; d.f. = 3; $P < 0.05$; Table 2). GC–MS analysis of fraction B showed the presence of two major chemicals, hexatriacontane and an unknown one. In addition, chemicals such as caryophyllene, alpha caryophyllene, pentacosane, 2-methyl eicosane, hexacosane, octacosane, 13,17,21-trimethyl heptatriacontane and 2–nitro-1,3-*bis*-octyloxy-benzene were also present. Fraction D had 13,17,21-trimethyl heptatriacontane as the major compound. Other chemicals included octacosane, heneicosane, docosane, tricosane, octacosane, 1-hexacosane and heptacosane.

In whole plant volatiles, three distinct fractions with *Rf* values 0.39, 0.22, and 0.19, were found. They were designated as A, B and C. In oviposition attraction bioassay, fraction A had no effect on oviposition; fraction B had positive OI (+ 0.69) and fraction C had strong negative OI (– 0.94; Table 1). In additional tests with fraction B, significantly more eggs (62) were laid on the plant extract side (8) than on the check side of the two-choice olfactometer ($t = 3.02$; d.f. = 3; $P < 0.05$; Table 2). Fraction C had significantly more eggs on the check side (38) than on the plant sample side (2) ($t = 4.90$; d.f. = 3; $P < 0.05$; Table 2), indicating strong oviposition repellent activity. GC–MS analysis of fraction B revealed the presence of 3-nitrobenzyl alcohol as the predominant chemical in this fraction. Other compounds in less quantity were (1, 2, 3) triazolo (1,5-a) pyrazine and 3-nitrobenzaldehyde. Fraction C had two major compounds, 3-nitrobenzyl alcohol and an unknown one. This fraction also had small amounts of docosane and 2,3,7-trimethyldecane. Siloxane compounds like 1,1,1,5,7,7,7-heptamethyl-3,3-*bis*(trimethyl siloxy) tetrasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl octasiloxane and 2,2,4,4,6,6,8,8,10,10,12,12,14,14 tetradecamethyl cyclohepta siloxane were also found in fractions B and C, but they may have come from the GC column as contaminants.

Involvement of plant volatiles, especially those produced during reproductive stage, in pheromone production and oviposition has been demonstrated in several moth species[20,21]. However, little information is available on the oviposition chemistry of *H. armigera* on tomato or other solanaceous host-plants. More eggs of *H. armigera* were found on *S. viarum*, a previously unknown solanaceous host of *H. armigera*, where the larvae feed exclusively on

leaves irrespective of plant growth stages, than on tomato plant in flowering stage[15]. Further, it was reported that oviposition of *H. armigera* on *S. viarum* and tomato is being mediated by the presence of oviposition attractants[16].

In our study, two fractions from microwave-assisted extract of *S. viarum* and two of tomato elicited strong oviposition preference response. Most of the chemicals identified in these fractions were *n*-alkanes or related primary alcohols and aldehydes. It has been proven that *n*-alkanes ranging from C_{20} to C_{37}, present on the leaf surface of maize plants elicited oviposition in *Ostrinia nubilalis* (Hübner)[18]. Like *O. nubilalis*, *H. armigera* is also a serious pest of maize inflicting similar nature of damage. Similarly, aldehydes such as benzaldehyde and phenylacetaldehyde also elicit strong response in *H. armigera*[22].

Oviposition attractants were also present in volatiles from *S. viarum* and tomato. Two fractions from *S. viarum* elicited strong oviposition attraction activities with OI of +1.00 and +0.90. Both fractions contained series of *n*-alkanes; however, they were different and had smaller chain length than those found in the active fraction of microwave-assisted hexane extract of the *S. viarum* leaves. It is possible that the chemicals in microwave-assisted extract retrieved from the leaf surface or from underneath leaf epidermis are metabolized to shorter chain molecules, which are readily volatilized from leaf surface, which attract *H. armigera* moths for oviposition. Among volatile fractions of tomato, one was strong repellent to *H. armigera*, one moderately attractant and one neutral. The attractant or the repellent compounds were closely related and both contained 3-nitrobenzyl alcohol.

It must be pointed out that though we have found several chemicals in the two solanaceous plants that are possibly involved in attraction of *H. armigera* for oviposition, we have not yet identified a specific chemical or group of chemicals directly involved in the process. However, constancy in most of the identified compounds, especially *n*-alkanes in the two different genera, *Solanum* and *Lycopersicon*, could have formed the basis for host selection by *H. armigera*. This could have considerable implications in our understanding and formulation of pest management strategies to control this moth. The use of host volatiles has been proposed as a potential lure for the female insects, and as a means of monitoring and forecasting populations[3,23]. We are pursuing this research, especially with strong attractants from *S. viarum* and the sole deterrent from tomato.

Addendum: The mass spectra of the chemicals found in both plant species and the standard are included, Srinivasan, R.[19].

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