# Petroleum spray oils-Lubricating the path to IPM: Part 2. How do PSOs deter oviposition of *Helicoverpa spp. on cotton plants*

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## Introduction

Petroleum spray oils (PSOs) are now an essential part of many integrated pest management (IPM) programs in agricultural crops (Beattie et al. 1995; Mensah et al. 1995). Despite these benefits, the use of PSOs has been limited in cotton due to a perceived risk of PSO-induced phytotoxicity and the fact that PSOs do not have a quick kill effect. Recent research on citrus and a range of other horticultural crops had led to the development of PSO additives such as UV light absorbers that can reduce the risk of phytotoxicity. The additives also enhance the persistence and activity of UV sensitive products such as biological insecticides to improve their efficacy and also can be mixed with synthetic insecticides to improve efficacy against a wide range of pests (see Mensah et al this proceedings; Beattie et al. 1995; Mensah, *et al.,* 1995; Jeyakumar and Gupta, 1999).

The mode of action of PSOs appears to be multifaceted. Recent studies by Mensah *et al.* (2001, 2000) have shown that application of PSOs can affect *Helicoverpa* spp. egg lays on a range of host plants. Deterrence of oviposition by any compound or product against any pest should have a significant effect on the pests' population by reducing the number of eggs deposited by pests on the plant leading to a reduced pest population (Hagen et al 1971). For cotton growers to utilize the oviposition deterrent activity of PSOs in their pest management program, they need to understand the mechanisms underlying the oviposition deterrent activity of PSOs. This will give growers a detail understanding of PSO use pattern effective against cotton pests particularly *Helicoverpa* spp.

The aim of this study was to determine the mechanism that may be involved in the oviposition deterrence activity of PSO against *Helicoverpa* spp.

## 2. Methodology

### 2.1 *Plant and insect materials*

Unless otherwise stated, all experiments were conducted in the Phytopharmacie laboratory at the Institut National Recherchez Agronomique (INRA) in Versailles in France.
*H. armigera* adults were used in all the experiments on cotton and were from laboratory reared colonies at the Australian Cotton Research Institute in Narrabri. The pupae of

these insects were transported to INRA in France from Narrabri. All experimental data were subjected to ANOVA (Instat 2.03; Graphpad Instat software Inc., San Diego, California). The Tukey-Kramer Multiple Comparisons Test was used to separate the means.

### 2.2 *Temporal change in H. armigera oviposition on cotton plants after PSO treatment (No-choice tests)*

The experiment was conducted in a mesh cage (25cm x 35cm x 40cm) using treatments representing 0 DAT (i.e. same day plants was treated with PSO), 1 DAT, 2 DAT, 3 DAT, 4 DAT, 5 DAT, 6 DAT, 7 DAT and control (water). The "no-choice" test was measured in the laboratory by the number of eggs laid by the adult female moths placed in separate cages with the treated plants.

In this experiment, one cotton plant from each treatment was enclosed in a mesh cage and four pairs of *H. armigera* adults were released into the cage. Each treatment was applied to run-off using a small hand held pressure sprayer. The control plants were sprayed with water. Each treatment was replicated four times. After four days, plants were removed from the cage and the number of eggs laid per plant was counted. Number of eggs per plant was used to calculate an oviposition deterrent index (ODI) for each treatment as follows: ODI = 100 x (C-T)/(C + T), Where C represents the total number of eggs per plant in the control, and T the total number of eggs on the treated plants. An ODI significantly greater than zero (repeated measures ANOVA) indicates *H. armigera* preferred to lay on control plants, an ODI not significantly different from zero indicates no preference between control and treated plants, and an ODI significantly less than zero indicates a preference for laying on treated plants relative to the control.

### 2.3 *Wind Tunnel bioassay test on H. armigera females on cotton plants sprayed with PSO at different dates*

The treatments evaluated were 0 DAT (plants sprayed same day with PSO), 1 DAT, 2 DAT, 3 DAT, 4 DAT, 5 DAT and control (water treated) plants. For each treatment, 10 mated female moths were introduced individually into a 5.5 cm x 8 cm wire mesh cylinder, placed on a 10 cm high platform with the open end of the cylinder upwind. Treated cotton plants were placed individually 120 cm downward from the mated adult female in the wind tunnel. The times taken for the insect to take-off after release and to locate and land on a plant were recorded. The mean take-off, flight, and landing times for each insect per treatment were calculated and analysed.

In addition, the PSO treated and the water-treated (control) plants were placed together 120 cm downward from the mated females in the wind tunnel to determine the preference of the female moths. The number of female moths that landed on the treated and control plants was recorded. The positions of the treated plants were interchanged after every two moths tested. In all, ten mated female moths were tested (10 replicates) and the mean and percentage number of female moths that landed on each treated plants were calculated and analysed.

2.4 *Effect of PSOs on airborne volatiles released by treated cotton plants (SPME tests)*

Based on the results of the wind tunnel tests, 0 DAT, 4 DAT and control plants were selected for this experiment. A solid phase micro extraction (SPME) fibre (65µm polymethylsilixane-divinylbenzene) was introduced into a plastic cage enclosing a cotton leaf from each treated plant. The exposure time of the fibre in the plastic cage was 24 hours. The fibre was then directly desorbed to a Gas Chromatograph (GC) injector at 240°C. A GC analysis of cotton leaf volatiles from each treatment was performed.

## 3.0 Results

3.1 *Days after PSO treatment (DAT) on H. armigera oviposition on cotton plants (No choice preference tests)*

Significantly fewer ($P<0.001$) eggs were recorded on 0 to 3 DAT plants than 4 to 7 DAT and control plants (table 1). The number of eggs per plant recorded on 4 to 7 DAT plants was not significantly different ($P>0.05$) among treatments and control plants (table 1). ODI values calculated for 0 to 3 DAT plants were significantly higher ($P<0.001$) than zero indicating deterrence activity against *H. armigera*. In contrast the ODI values for the 4 to 7 DAT plants were not significantly different ($P>0.05$) from zero indicating no oviposition deterrence activity against *H. armigera* females (table 1).

*Table 1. Temporal effects of 2% PSO application on oviposition by H. armigera females on cotton plants under no-choice conditions in the laboratory at INRA, Versailles, France, June 2003.*

| Treatments | No. eggs/plant | Mean Oviposition Deterrent Index (ODI) |
|---|---|---|
| Control (water) | 75.25 ± 10.19 a | 0.00 a |
| 0 Day after treatment (DAT) | 19.00 ± 1.35 b | 59.68 b |
| 1 Day after treatment (DAT) | 29.50 ± 2.56 b | 43.68 b |
| 2 Days after treatment (DAT) | 18.50 ± 3.25 b | 60.53 b |
| 3 Days after treatment (DAT) | 32.23 ± 8.23 b | 40.03 b |
| 4 Days after treatment (DAT) | 69.25 ± 43.50 a | 4.15 a |
| 5 Days after treatment (DAT) | 65.25 ± 21.30 a | 7.12 a |
| 6 Days after treatment (DAT) | 78.45 ± 8.95 a | -2.08 a |
| 7 Days after treatment (DAT) | 85.25 ± 12.56 a | -6.23 a |

Means within column followed by the same letter are not significantly different ($P> 0.05$) Tukey-Kramer Multiple Comparison Test.

### 3.2 *Wind Tunnel bioassay test on H. armigera females ovipositing on cotton plants sprayed with PSO at different dates*

Significant difference was found among the times taken by *H. armigera* mated females to take off and land on the treated plants in the wind tunnel (table 2). In the case of 0-3DAT plants, the female moths took 54 - 88 seconds to take off after release in the wind tunnel. After taking off, the insects were in flight for 47 to 58 seconds but did not land on the 0 and 2 DAT plants. Only 10 per cent landed on the 3 DAT plants (tables 2). In contrast, the female moths took off within 23- 26 seconds after they were released in the wind tunnel containing 4 and 5 DAT and control plants. The insects landed on the 4 to 5 DAT and control plants after having been in flight for 12 – 20 seconds. Among the 10 insects released in each test all the female moths landed on the control plants and 40-60 per cent on the 4 to 5 DAT plants (table 2).

*Table 2. Wind tunnel test of the flight behaviour (take-off and landing times) of H. armigera females on cotton plants sprayed with 2% v/v PSO in the laboratory at INRA, Versailles, France, July 2003 (Mean of 10 females).*

| Treatments | Mean time taken to take-off (Secs) | Mean time taken to land on the plant (Secs) |
|---|---|---|
| Control (water) | 26.80 ± 1.98 c | 12.80 ± 1.22 c |
| 0 Day after treatment (DAT) | 88.50 ± 7.11 a | 58.00 ± 4.23 a |
| 1 Day after treatment (DAT) | 73.50 ± 8.66 ab | 47.00 ± 4.73 ab |
| 2 Days after treatment (DAT) | 54.50 ± 3.20 bc | 47.50 ± 2.39 b |
| 3 Days after treatment (DAT) | 54.00 ± 3.86 c | 46.50 ± 2.26 b |
| 4 Days after treatment (DAT) | 32.80 ± 2.04 c | 20.00 ± 1.13 c |
| 5 Days after treatment (DAT) | 23.80 ± 1.72 c | 13.50 ± 1.00 c |

Means within column followed by the same letter are not significantly different (P> 0.05) Tukey-Kramer Multiple Comparison Test).

### 3.3 *Effect of PSOs on airborne volatiles released by cotton plants (SPME tests)*

The quantity of volatiles released by cotton plants treated with water (control) and 2% v/v PSO at 0 DAT and 4 DAT (figures 1-3) was different. The lowest quantity of volatiles was released by 0 DAT plants (Figure 1) followed by the 4 DAT (Figure 2) and then the control plants (figures 3). However the quantity of volatiles released by the 4 DAT plants (Figure 2) was the same as the control (water) treated plants (figure 3) indicating that any suppression of volatiles by the oil lasted for 4 days (see figures 1, 2 and 3).

The lower quantity of volatiles released by the 0 DAT plants corresponded to a higher ODI (81.4 and 59.7) for both choice and no choice tests respectively (tables 2 and 3) compared to a lower ODI (13.5 and 4.2) on the 4 DAT and (ODI = 0) on the control (water) treated plants indicating that the suppression of the volatiles by the PSO sprays may be responsible for the reduction of *H. armigera* eggs deposited on the 0 DAT plants.

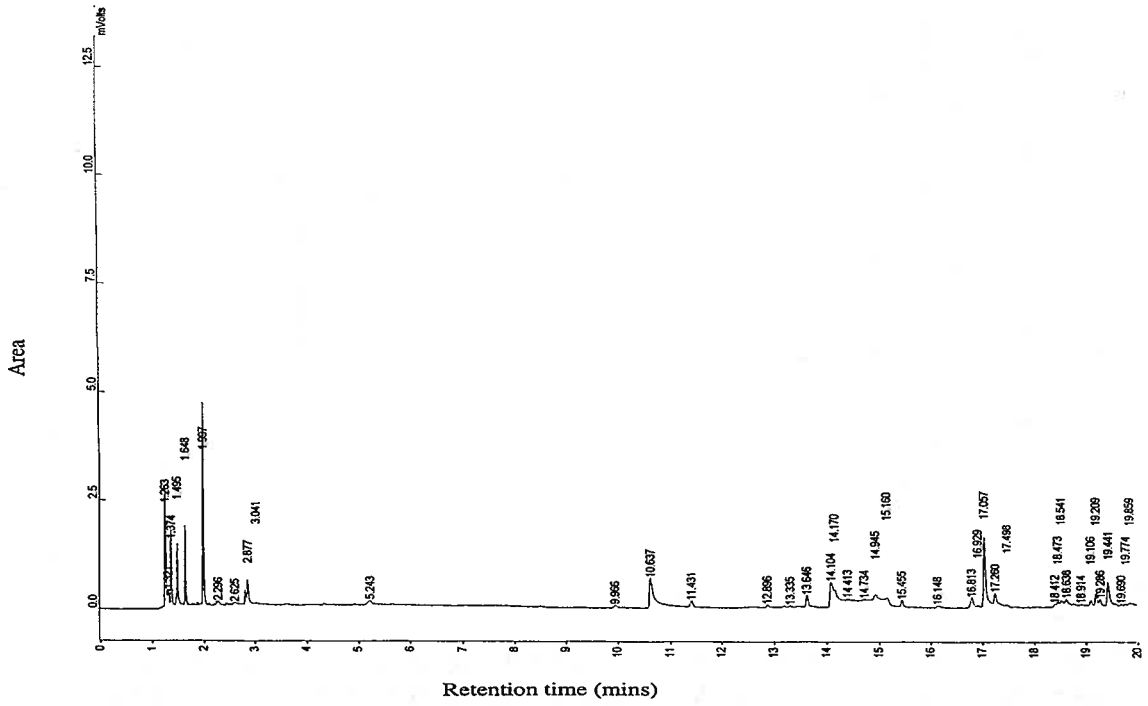


Figure 1. Chromatographic analyses of SPME sampled from 0 DAT plants in the glasshouse at INRA, Versailles, France, July 2003. (column DB wax 30m; 0.32 ID; Temp: 50°C to 120°C; 20°C/min; Injector splitless =240°C; Detector FID = 260°C

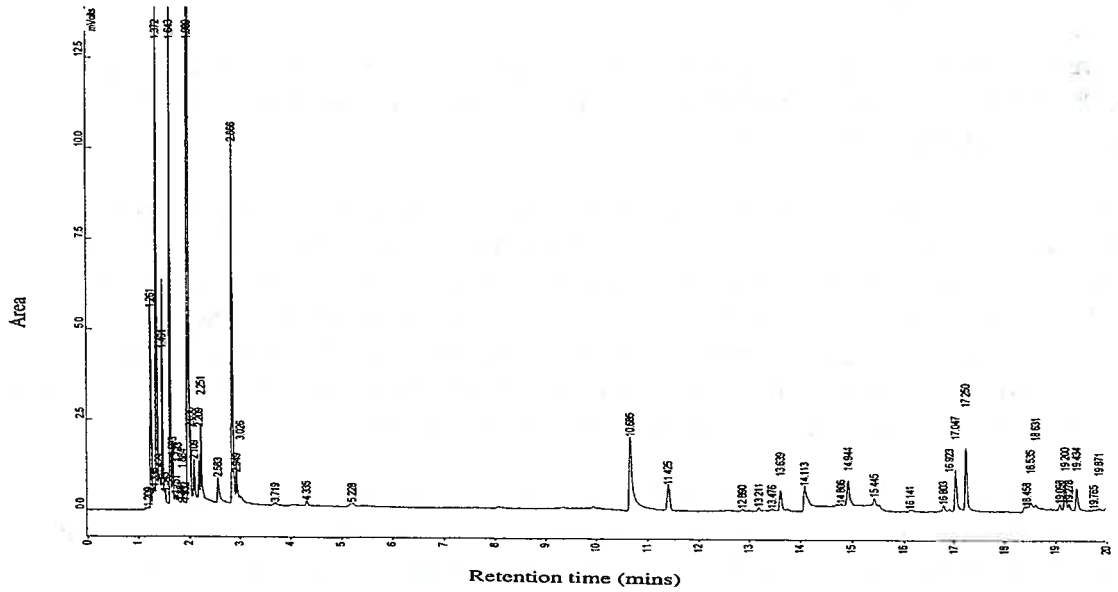


**Figure 2.** Chromatographic analyses of SPME sampled from 4 DAT plants in the glasshouse at INRA, Versailles, France, July 2003. (column DB wax 30m; 0.32 ID; Temp: 50°C to 120°C; 20oC/min; Injector splitless =240°C; Detector FID = 260°C

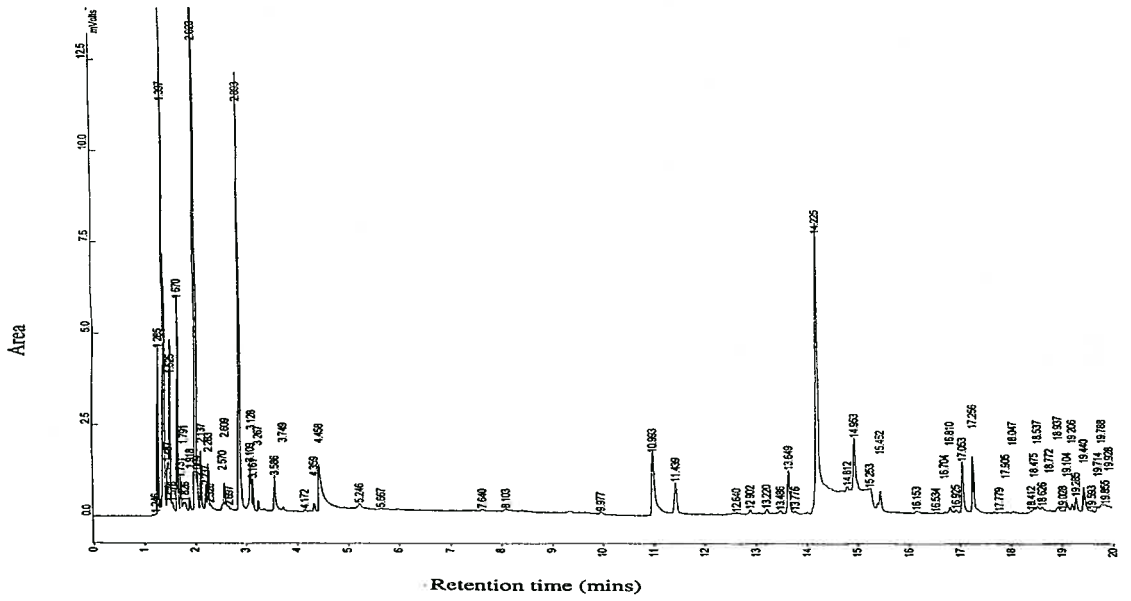


**Figure 3.** Chromatographic analyses of SPME sampled from control (water) treated plants in the glasshouse at INRA, Versailles, France, July 2003. (column DB wax 30m; 0.32 ID; Temp: 50°C to 120°C; 20°C/min; Injector splitless =240°C; Detector FID = 260°C

## 4.0 Discussion

Our study shows that PSOs applied to cotton plants can deter oviposition of *H. armigera* on cotton. Under no-choice conditions in the laboratory, cotton plants treated with PSO suppressed *H. armigera* egg lay.

The results also showed that the oviposition deterrence activity of a single application of the PSO against *H. armigera* females lasted for 4 to 5 days. Thus, for a grower to take advantage of the oviposition deterrence activity of the PSO, multiple application of the PSO is crucial. To achieve good results, growers may have to mix the PSO in with every spray such as insecticides, growth regulators and foliar fertilizers that are applied to cotton crops. This may enhance the build up of oil residues on the leaves and allow oviposition deterrence activity to persist longer on the cotton leaves.

The solid phase micro-extraction (SPME) tests showed that airborne volatiles released by the cotton plants may play a role in *H. armigera* female's ability to choose between PSO-treated and untreated cotton plants. The quantity of airborne volatiles released by the plants may assist the female moths to detect and select appropriate plants for oviposition. The quantity of airborne volatiles released by 0 DAT (same day treated) plants were far less than that released by the 4 DAT and control (water) plants indicating that the PSO may be suppressing the quantity of volatiles released by the cotton plants treated with PSO. The low quantity of volatiles emitted by the 0 DAT plants might have resulted in a

longer time taken for the moths to respond and locate the plants in the wind tunnel test. In addition, the lower quantity of volatiles might also have resulted in a reduced number of eggs laid on the 0 DAT plants compared to the 4 DAT and water treated plants. Thus the underlying mechanisms of oviposition deterrence activity of PSO against *H. armigera* may be due to the PSO suppressing the quantity of airborne volatiles released by the plants. The effect of this suppression activity lasted for 4 to 5 days after a single application of the PSO.

The use of airborne volatiles by moths to detect their host plants has been reported by many researchers. For example, Mitchell *et al.* (1991) reported that many moths use airborne volatiles emitted from plants to locate their host in contrast to visual cues such as colour, shape and size of the host plant. Fitt (1989) also reported that since *H. armigera* female adults migrate and lay most of their eggs at night, it is possible that they may utilise airborne volatiles to locate cotton plants. Hence, the quantity of volatiles released by the plants may influence host selection and the amount of eggs *H. armigera* lay on the selected host plant.

In conclusion, the study has demonstrated that PSOs can be used to manage *H. armigera* on cotton. This can be achieved by deterring egg lay by these pests on their target crops but this deterrence activity may last for only 4 days in a single PSO application. Addition of PSO in every spray product applied to the cotton crops may assist to extend the oviposition deterrent activity of the PSOs. Thus PSOs have the potential to be integrated into programmes to assist in the control of *H. armigera* on cotton and *O. nubilalis* on maize.

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