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The Swire Group Churchill Fellowship to study the underlying mechanisms of Oviposition Deterrence of Ultra-Violet protected Petroleum Spray Oils against Bollworms in Agricultural crops with special emphasis on cotton

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1.0 **Precis and Acknowledgement**

This report outlines the findings from a Swire Group Churchill Fellowship visit to the Institut National de la Recherche Agronomique (INRA) in Versailles in France to investigate the underlying mechanisms of the oviposition deterrence activity of Ultra-Violet protected Petroleum spray oils (PSOs) against bollworms on agricultural crops particularly cotton. Specific objectives of the study included;

- assessing the oviposition deterrence activity of PSOs against *Helicoverpa armigera* (bollworm) on cotton and *Ostrinia nubilalis* (European corn borer) on maize
- identifying the optimum PSO concentration for larval efficacy and deterrence against *O. nubilalis* egg lay
- identifying the mechanisms involved in the oviposition deterrence activity against *H. armigera*

My visit to study this project in France and the experience and understanding I have gained in the effective use of PSOs in integrated pest management systems in agricultural crops would not have been possible without;

- the financial assistance given to me by the Swire Group Churchill Fellowship through the Winston Churchill Memorial Trust. Additionally, the Churchill Fellowship has improved my academic standing within the scientific community and also in my organization (NSW Agriculture).
- the support given to me by NSW Agriculture by giving me the permission to travel and undertake the Fellowship and also the Australian Cotton Industry for their continuous support and confidence they continue to have in my scientific research and their willingness to support my application which enabled me to gain this Fellowship from a highly competitive applicants.
- the kind hospitality of INRA staff particularly my host scientist Prof. Brigitte Frerot and her staff and family who willingly assisted me in every aspect of my studies in her laboratory and my stay in France.
- the kind support given by Caltex Australia Pty Ltd for providing me the Ultra-violet protected PSO (Canopy® oil) for the studies.
- my family Vida, Obed, Samuel and Stephanie for their love, encouragement and for travelling to join me in France so that I was not homesick but allowed me to concentrate fully on the project and achieve excellent results for the Agricultural industry in Australia.

2.0 Executive Summary

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FELLOWSHIP PROJECT: *Study the Underlying Mechanisms of oviposition deterrence of Ultra-Violet protected Petroleum spray oils (PSOs) against bollworms on agricultural crops with special emphasis on cotton.*

This report outlines the findings of the PSO project conducted at the Phytopharmacie Department of the Institut National Recherche Agronomique (INRA) in Versailles in France from the 28th May until 10th August 2003. The project was funded by the Swire Group Churchill Fellowship through the Winston Churchill Memorial Trust in Australia.

The purpose of the study was to determine the underlying mechanisms involved in the oviposition deterrence activity of UV-protected Petroleum spray oils (PSOs) against bollworms on agricultural crops with special emphasis on cotton. Specific objectives of the project were (1) assess the oviposition deterrence activity of PSOs against *Helicoverpa armigera* (cotton bollworm) on cotton and *Ostrinia nubilalis* (European corn borer) on maize, (2) identify the optimum PSO concentration for larval efficacy and oviposition deterrence activity against *O. nubilalis* on maize, (3) identify the mechanisms involved in the oviposition deterrence activity of PSOs against *H. armigera* on cotton plants. The UV-protected oil used in this study was Canopy® oil developed and supplied by Caltex Australia Pty Ltd.

The results of the study showed that UV-protected PSOs can deter oviposition of *H. armigera* and *O. nubilalis* on cotton and maize plants respectively. Under free and no choice tests in the laboratory, cotton and maize plants treated with different concentrations of the UV-protected PSO suppressed egg lays of *H. armigera* and *O. nubilalis*. In the case of *H. armigera*, applications of 2% v/v of the PSO to cotton plants deterred its oviposition whereas in *O. nubilalis* the optimum rate at which the PSO could deter oviposition was determined as 5% v/v.

The study also found that the oviposition deterrence activity of the PSO against the moths lasted for only 4-5 days indicating that the PSO needed to be applied every 4-5 days to maintain the oviposition deterrence activity of the oil. However, applying the product at

4-5 days interval will not be cost effective to the grower. In contrast, mixing lower rates (i.e. 1% v/v rate) of the PSO in every product (insecticides, growth regulators, foliar fertilizers etc) the grower may apply to his/her crops may be cost effective and assist in maintaining the oviposition deterrence activity of the oil.

Wind tunnel bioassay tests showed that airborne volatiles released by the cotton plants play an important role in *H. armigera* female's ability to detect and choose between PSO-treated and untreated cotton plants. A solid phase micro-extraction (SPME) tests also found that application of PSOs to cotton plants reduced or suppressed the quantity of airborne volatiles released by the plants. The reduction of airborne volatiles on the PSO treated plants prevented the female moths from detecting and accepting the plants for egg lay. Hence, the 0 - 3 DAT (days after treatment) cotton plants had significantly fewer eggs laid on them compared to 4 and 5 DAT and water-treated (control) plants. Thus, the underlying mechanism to the oviposition deterrence activity of PSO against adult female moths was identified as the suppression of the quantity of airborne volatiles released by the plant by the PSO. This suppression activity lasted for 4 to 5 days after PSO treatment.

In conclusion, the study has demonstrated the oviposition deterrence activity of UV-protected PSO (Canopy® oil) against *H. armigera* and *O. nubilalis* on cotton and maize crops respectively by suppressing the quantity of airborne volatiles released by the plants. This indicates that UV-protected PSOs have the potential to be integrated into programmes to assist in the control of *H. armigera* on cotton and *O. nubilalis* on maize.

3.0 Introduction

Cotton crops world wide are attacked by a wide range of pests particularly *Helicoverpa* (heliiothis) spp., aphids, mirids, white flies, mites etc. In Australia, the key pests attacking cotton crops are *Helicoverpa* (heliiothis) spp., green mirids, aphids and two spotted mites. The control of these pests depends exclusively on the use of synthetic insecticides. Similarly in France, insecticides remain the most recommended control method of *O. nubilalis* (European corn borer) on maize crops (S. Derridj pers. comm.). Over-reliance on insecticides can cause problems of insecticide resistance, disruption of the natural enemies of the pests and environmental concerns. Additionally, most of the insecticides are very expensive, increasing the cost of pest control and reducing gross margins, profitability and sustainability of agricultural industries.

In order to protect the future sustainability of agricultural industries particularly cotton and maize, there has been a strong push by growers to adopt an integrated pest management (IPM) programs as an alternative control option. UV-protected Petroleum spray oils (PSOs) could be a key IPM tool both in the cotton and maize industries but their use in these industries is limited due to a perceived risk of PSO-induced phytotoxicity. However, historical research has shown that this can be minimized when a number of key base oil properties are considered in good practice PSO formulation (Riehl, 1969). Furthermore, recent research on citrus and a range of other horticultural crops had led to the development of a technology in PSOs such as the use of UV light absorbers which can reduce the risk of phytotoxicity and also enhance the persistence and activity of UV sensitive products particularly biological insecticides (Bull *et al.*, 1976; Krieg, *et al.*, 1980; Beattie, 1995; Mensah, *et al.*, 1995; Jeyakumar and Gupta, 1999).

Caltex Australia Pty Ltd and Spray Adjuvants Company of Australia (SACOA) Pty Ltd have developed a PSO formulation which incorporates UV-light absorbing compounds. For example Canopy® oil used in this study has UV absorbers incorporated in the formulation. Recent studies by Mensah *et al.* (2001, 2002) have shown that application of 2% Canopy® oil to cotton plants can reduce *Helicoverpa* spp. egg lays on these plants. The reduction in egg lay by PSO is very crucial because it can substantially reduce bollworm infestations, support IPM and reduce synthetic insecticide use on maize and cotton. However, for growers to take advantage of the oviposition deterrent activity of PSOs against pests, there is the need to understand the mechanism by which PSOs deter or discourage egg lay on cotton. Knowledge of the oviposition deterrence mechanism of PSOs against bollworms will inevitably enable a better use of PSOs against pests infesting agricultural crops such as cotton.

Despite these benefits, no studies have been conducted to determine the mechanism involved in the oviposition deterrence activity of PSOs against bollworms on cotton crops. Furthermore, there have been no reported studies on the efficacy of PSOs against *O. nubilalis* on maize even though several millions of dollars of control costs are directed at *O. nubilalis* every year in France (S. Derridj, pers comm.). Thus, the successful use of PSOs against *H. armigera* and *O. nubilalis* on both cotton and maize crops will improve profitability, competitiveness and sustainability of cotton and maize industries in both Australia and France.

The objective of this project was to study the mechanisms involved in the oviposition deterrent activity of UV-protected PSOs against *H. armigera* on cotton and *O. nubilalis* on maize. In order to achieve the project objectives, several questions were asked based on the amount of information that already exist in the literature on the impact of PSOs on the oviposition of adult moths in agricultural crops.

Mensah *et al.* (2001, 2002) reported that PSO can deter oviposition of *H. armigera* on cotton and determined the optimum oviposition deterrent rate as 2 % v/v. Thus, the questions asked in relation to PSO and *H. armigera* oviposition were (1) do *H. armigera* females lay on cotton plants treated with 2% v/v PSO? (This to confirm the results of Mensah et al (2001, 2002), (2) if no, how long after PSO treatment will the oviposition deterrence activity last?, (3) can *H. armigera* females detect and select between PSO treated and untreated cotton plants? (4) do volatiles released by cotton plants play a role in *H. armigera* female's decision to select and lay on a cotton plants? (5) if yes, do PSOs applied to plants have effect on the quantity of volatiles released by the plants? and (6) how long after PSO treatment will this suppression effect lasts?

In the case of *O. nubilalis*, and the fact that no studies have been conducted on the oviposition deterrence activity of PSO against this pest on maize, the questions that were asked were (1) can PSO deter oviposition of *O. nubilalis* on maize and is it efficacious against *O. nubilalis* larvae and (2) what is the optimum oviposition deterrence and larval efficacy rates of PSO against *O. nubilalis*?

These questions were answered through choice, no choice, wind tunnel and plant volatile tests (solid phase micro-extraction (SPME) using cotton and maize crops in the laboratory.

4.0 Methodology

4.1 Plant and insect materials

Unless otherwise stated, all experiments were conducted in the Phytopharmacie laboratory at the Institut National Recherche Agronomique (INRA) in Versailles in France. The experimental plants used for the studies on cotton were "CA 222" (a normal leaf) variety; and for maize the "Dea" variety. Plants were grown from seeds in pots in a glasshouse. In the case of cotton, plants were grown up to 6-true leaf stage (young cotton plants) and squaring stage (mature plants). For maize, plants were grown up to four true-leaf stage. The climatic conditions in the glasshouse were 28±1°C.

H. armigera adults were used for all the experiments on cotton. The adults were from laboratory reared colonies at the Australian Cotton Research Institute in Narrabri in Northwest New south Wales in Australia. The pupae of these insects were transported to INRA in two batches of pupae (950 and 700 pupae respectively) in June and July 2003. *O. nubilalis* adults used in all the studies were from first generation colonies reared in INRA in Versailles in France on artificial diet composed of maize flour. The pupae of the

insects were kept at $25 \pm 1^\circ\text{C}$ in the same laboratory in INRA where the experiments were conducted. Different sizes of cages were used for the trials and this explains the different ratios of moths per plant in the various experiments particularly the maize experiments. All experimental data were subjected to ANOVA (Instat 2.03; Graphpad Instat software Inc., San Diego, California) and Tukey-Kramer Multiple Comparisons Test was used to separate the means.

4.2 *Canopy oil (PSO) and H. armigera oviposition on cotton*

4.2.1 *Do H. armigera females lay on cotton plants treated with 2% v/v PSO? (Free choice preference tests)*

The free choice ovipositional preference of *H. armigera* females to PSO (Canopy® oil) was measured by counting the number of eggs laid by *H. armigera* on cotton crops. The experiment was conducted in a mesh cage (100cm x 50 cm x 70 cm) in the laboratory on June 6, 2003 when the cotton plants were at 8 true-leaf stage. Four treatments representing concentrations of 0 (control), 1%, 2% and 5% (v/v) were evaluated. These concentrations were chosen to confirm the results of the Australian study by Mensah *et al.* (2001 and 2002). Four cotton plants were randomly allocated to each treatment and each plant was labelled with a plastic card. Sprays were applied to run-off using a small hand held pressure sprayer. The control plants were sprayed with water. The treated plants were transferred into the mesh cage and arranged in four rows in a completely randomized block design. Fifty pairs of *H. armigera* adults were released into the cage. Each treatment was replicated five 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:

$$\text{ODI} = 100 \times (\text{C}-\text{T})/(\text{C} + \text{T})$$

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 (Mensah *et al.*, 2000; Renwick and Radke, 1985; Renwick *et al.*, 1989; Dimock and Renwick, 1991).

4.2.2 *How long after PSO treatment will the oviposition deterrent activity against H. armigera last? (Free choice preference tests)*

Based on the results of experiment 4.2.1, a 2% v/v Canopy® oil concentration was used to conduct experiment 4.2.2. The experiment was conducted in a mesh cage similar to those used in 4.2.1 in the laboratory. The experiment was conducted in June 16, 2003

using cotton plants which were at eight true-leaf stage. Nine treatments consisting of 0 DAT (cotton plants treated with Canopy® oil on the same day), 1 DAT (1 day after PSO treatment), 2 DAT, 3 DAT, 4 DAT, 5 DAT, 6 DAT, 7 DAT and control (plants treated with water) were evaluated. Four cotton plants were randomly allocated to each treatment and each plant was labelled with a card. All treated plants were transferred into the mesh cage and arranged in a completely randomized design. Plants were placed so that the leaves do not touch each other. Each treatment was replicated four times. 100 pairs of *H. armigera* females were released into the cage. The number of eggs per plant per treatment was assessed after four days and an ODI was calculated for each treatment.

4.2.3 *How long after PSO treatment will the oviposition deterrent activity against H. armigera last? (No choice preference tests)*

The experiment was conducted in a mesh cage (25cm x 35cm x 40cm) using treatments representing 0 DAT, 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 produced by the adult female moths placed in separate cages with the treated plants.

The experiment commenced in June 23, 2003. One cotton plant from each treatment was enclosed in the mesh cage and four pairs of *H. armigera* adults were released into each cage. Each treatment was replicated four times. Number of eggs per plant per treatment was assessed after four days and an ODI was calculated.

4.2.4 *Can H. armigera females detect and select between PSO treated and untreated cotton plants?*

A wind tunnel bioassays were used to investigate the responses of mated *H. armigera* adult females to cotton plants treated with PSO at different dates (i.e. different days after treatments, (DAT)). The treatments evaluated were 0 DAT, 1 DAT, 2 DAT, 3 DAT, 4 DAT, 5 DAT and water treated (control) plants. In this study 10 mated adult females were introduced individually in 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. Each treated cotton plants were placed individually 120 cm downward from the mated adult female in the wind tunnel. Ten different mated females were tested for each treatment. The times taken for the insect to perceive plant odour and then take-off towards the exposed plant in the wind tunnel as well as the time taken for the insect to fly, locate and land on the 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 at 120 cm downward from the mated females in the wind tunnel. The number of female insects landing on either the treated or control plants were recorded. The positions of the treated and the control plants were interchanged after every two insects tested. In all, ten adult females were tested (10 replicates) and the mean and percentage number of insect landings on each treated and control plants were calculated and analysed.

4.2.5 *Do volatiles released by cotton plants play a role in H. armigera female's decision to select and lay on a cotton plants, do PSOs have effect on the quantity of volatiles released by the plants and how long after PSO treatment will this effect lasts?*

Based on the results of the wind tunnel tests, 0 DAT, 4 DAT and control (water) treated plants were used for this experiment. A solid phase micro extraction (SPME) fibre (65µm polymethylsilixane-divinylbenzene) was introduced into a plastic enclosing a cotton leaf of 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 injector at 240°C. The cotton leaf volatile analysis from each treatment was performed by the GC.

4.3 *Canopy oil (PSO) and O. nubilalis oviposition on maize crops*

4.3.1. *Can PSO deter oviposition of O.nubilalis on maize and what is the optimum oviposition deterrent rate of PSO against O. nubilalis (Free choice tests)*

The free choice of ovipositional preference of *O. nubilalis* females to PSO was measured by counting the number of egg masses and eggs per plant laid by *O. nubilalis* on maize crops.

The experiment was conducted in a mesh cage (100 cm x 50 cm x 70 cm) in the laboratory in June 12, 2003 when the maize plants were at the four-leaf stage. Four treatments representing concentrations of 0 (control), 1%, 2%, 3%, 5% and 10% v/v were evaluated. Four maize plants were randomly allocated to each treatment and each plant was labelled with a plastic card. Sprays were applied to run-off using a small hand held pressure sprayer. The control plants were sprayed with water. The treated plants were transferred into the mesh cage and arranged in a completely randomized block design. Twenty pairs of *O. nubilalis* adults were released in the dark to mate and oviposit on the plants. Each treatment was replicated five times.

After four days, the plants were removed from the cage and the number of egg masses and eggs per plant were counted under a stereomicroscope. Number of eggs per plant was used to calculate an oviposition deterrent index (ODI) for each treatment as previously described.

4.3.2 *Can PSO deter oviposition of O. nubilalis on maize and what is the optimum oviposition deterrent rate of PSO against O. nubilalis (No choice tests)*

Based on the results of the free choice tests in experiment 4.3.1, three different no-choice experiments were conducted on 19 June, 23 June and 25 June 2003 respectively using treatments representing concentrations of 0 (control), 1%, 2%, 3%, 5% and 10%. Each of the “no-choice” tests was measured in the laboratory by the egg production of the moths placed in separate cages with the treated plants.

In each of the three experiments, four plants from each treatment were enclosed in a mesh cage (25cm x 35cm x 40cm) and five pairs of *O. nubilalis* were released into each cage. Each treatment was replicated five times. Number of egg masses per plant, and eggs per plant were recorded after 4 days. An ODI was calculated for each treatment.

4.2.3 *Is PSO efficacious against O. nubilalis larvae?*

In all, 3 experiments were conducted using first, second and third stage larvae of *O. nubilalis* from 4 June until 7 July 2003. The larvae used for the experiments were established from colonies reared in the Phytopharmacie laboratories in INRA.

The PSO concentrations evaluated were 1%, 2%, 3%, 5% and 10% (v/v). Water was used as a control. For each concentration, I sprayed a total of 100 larvae per treatment (i.e. 25 larvae/replicate) on filter papers in a plastic tray until run-off. In addition, I mixed 1 ml of each concentration (treatment) with a soybean-based artificial diet in a 35 ml clear plastic container (P101M; Solo, Urbana, Illinois, USA). After spray application, the larvae from each treatment were transferred and kept separately in the 35 ml clear plastic containers containing the mixture of the soybean-based artificial diet and 1 ml solution of the same treatment. Each treatment was replicated four times. The number of dead larvae was recorded daily until all the live larvae had pupated, and the percentage mortality was calculated.

5.0 Results

5.1 *Canopy oil (PSO) and H. armigera oviposition on cotton*

5.1.1. *Do H. armigera females lay on cotton plants treated with 2% v/v PSO? (Free choice preference tests).*

Significant differences ($P < 0.001$) in oviposition-detering activity of the PSO against *H. armigera* females was detected among the different concentrations tested (table 1). The oviposition deterring activity of the PSO at 2% and 5% v/v on cotton plants were significantly higher ($P < 0.001$) than when the product was sprayed at 1% v/v (table 1). There was no significant difference in the number of eggs laid on plants treated with 1% v/v PSO and control (water) treated plants. The ODI for 1% v/v PSO treatment was not significantly different from the control indicating that the PSO applied at 1% v/v cannot significantly deter *H. armigera* egg lay. In contrast, the ODI for 2 and 5% v/v treatments were significantly greater than zero indicating that the product applied at these two rates can strongly deter *H. armigera* oviposition (table 1).

Table 1. Oviposition deterring activity of Petroleum spray oil (Canopy® oil) against H. armigera on cotton plants under free choice conditions in the laboratory at INRA, Versailles, France, June 2003.

Treatments	No. eggs/plant	Mean Ovipositional Deterrent Index (ODI)
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Control (water)	92.50 ± 3.25 a	0 a
1% v/v Canopy oil	88.25 ± 8.15 a	2.35 a
2% v/v Canopy oil	41.75 ± 7.75 b	37.80 b
5% v/v Canopy oil	38.50 ± 7.25 b	41.22 b

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

5.1.2 *How long after PSO treatment will the oviposition deterrent activity against H. armigera last? (Free choice preference tests)*

Significant differences ($P < 0.001$) was found among the cotton plants treated with 2% v/v PSO at different dates (different days after treatment (DAT))(table 2). The number of eggs per plant recorded on 0 to 3 DAT plants was significantly lower ($P < 0.001$) from 4 to 7 DAT and control plants (table 2). The number of eggs per plant recorded on 4 to 7 DAT plants were not significantly different ($P > 0.05$) from the control (water) treated plants indicating that the deterrent activity of the PSO can last for approximately 4-5 days after treatment (table 2).

Table 2. Ovipositional response of H. armigera females to cotton plants treated at different dates with 2% v/v Canopy oil under free choice conditions in the laboratory at INRA, Versailles, France, June 2003.

Treatments	No. eggs/plant	Mean Ovipositional Deterrent Index (ODI)
Control (water)	244.25 ± 53.19 b	0.00 a
0 Day after treatment (DAT)	25.00 ± 9.35 a	81.42 b
1 Day after treatment (DAT)	39.50 ± 11.56 a	72.16 b
2 Days after treatment (DAT)	38.50 ± 17.25 a	72.77 b
3 Days after treatment (DAT)	58.23 ± 14.23 a	61.50 b
4 Days after treatment (DAT)	186.35 ± 43.50 b	13.47 a
5 Days after treatment (DAT)	175.25 ± 21.30 b	16.45 a
6 Days after treatment (DAT)	206.50 ± 18.95 b	8.38 a
7 Days after treatment (DAT)	221.25 ± 42.56 b	4.94 a

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

The ODI calculated for the 0 to 3 DAT plants were significantly higher ($P < 0.001$) than the 4 to 7 DAT and control plants indicating strong deterrence of oviposition against *H. armigera* females. The ODI calculated for 4 to 7 DAT plants, were not significantly different from zero indicating the oviposition deterrence activity of the PSO diminishes within 4 to 5 days (table 2).

5.1.3 *How long after PSO treatment will the oviposition deterrent activity against H. armigera last? (No choice preference tests)*

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

Table 3. Ovipositional response of *H. armigera* females to cotton plants treated at different dates with 2% v/v Canopy oil under no-choice conditions in the laboratory at INRA, Versailles, France, June 2003.

Treatments	No. eggs/plant	Mean Ovipositional 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).

5.1.4 Can *H. armigera* females detect and select between PSO treated and untreated cotton plants?

Significant differences was found among the times taken by *H. armigera* mated adult females to respond and take off and also locate and land on exposed 0-3 DAT plants in the wind tunnel compared to 4 to 5 DAT and control plants (table 4). The insects took 54 - 88 seconds to respond and take off towards the 0-3 DAT plants. The insects were in flight in the wind tunnel for 47 to 58 seconds but none of them landed on the 0 and 2 DAT plants and only 10 per cent landed on the 3 DAT plants (tables 4 and 5). In contrast, the insects took 23- 26 seconds to respond and take-off towards the 4 and 5 DAT and control plants. They were in flight for only 12 – 20 seconds and then landed on the 4 to 5 DAT and control plants (tables 4 and 5). In all 100 per cent of the female moths landed on the control plants and 40-60 per cent on the 4 to 5 DAT plants (table 5).

Table 4. Wind tunnel bioassay of *H. armigera* females in relation to days after treatment of cotton plants with 2% v/v Canopy oil 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	12.8 ± 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).

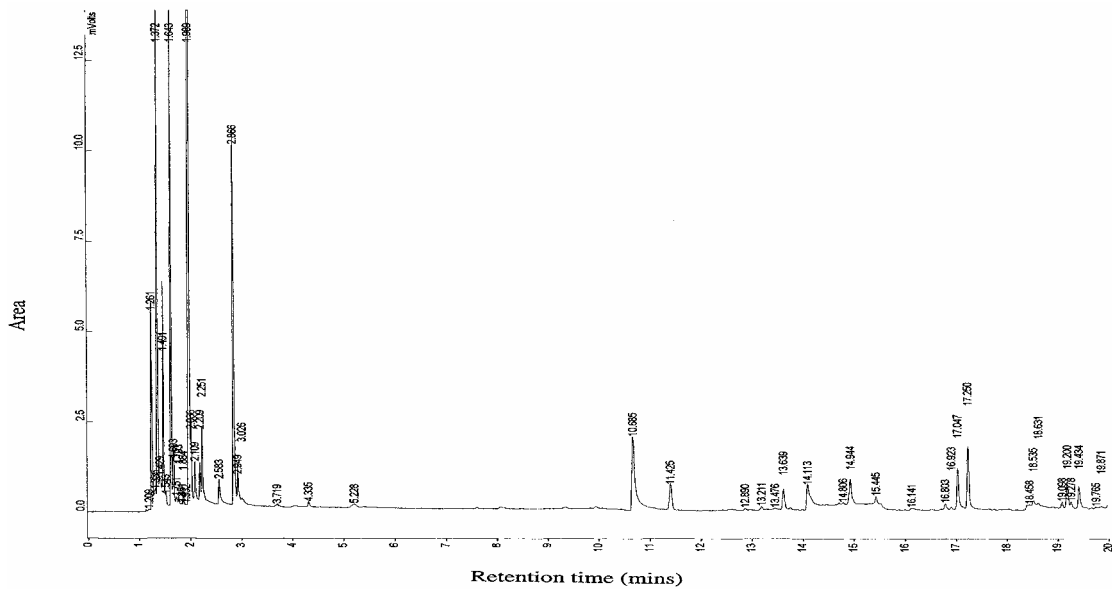
Table 5. Wind tunnel bioassay of H. armigera females in relation to days after treatment of cotton plants with 2% v/v Canopy oil (PSO) in the laboratory at INRA, Versailles, France, July 2003 (Total of 10 females).

Treatments	No. of female landings on PSO treated and untreated (control) plants	No. of female landings on treated and untreated (control) plants (as %)
Control (water)	10.00 ± 0.00 a	100% a
0 Day after treatment (DAT)	0.00 ± 0.00 b	0 b
Control (water)	10.00 ± 0.00 a	100% a
1 Day after treatment (DAT)	0.00 ± 0.00 b	0 b
Control (water)	10.00 ± 0.00 a	100% a
2 Day after treatment (DAT)	0.00 ± 0.00 b	0 b
Control (water)	9.00 ± 0.10 a	90% a
3 Day after treatment (DAT)	1.00 ± 0.10 b	10% b
Control (water)	6.00 ± 0.16 a	60% a
4 Day after treatment (DAT)	4.00 ± 0.16 a	40% a
Control (water)	4.00 ± 0.16 a	40% a
5 Day after treatment (DAT)	6.00 ± 0.16 a	60% a

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

5.1.5 Are there any differences in the quantity of volatiles released by PSO treated and untreated cotton plants? and if yes how long after PSO treatment will these differences last? (SPME Tests)

Differences in the quantity of volatiles released by leaves from 0 DAT, 4 DAT and control (water) treated plants were detected among the treatments (Figures 1-3). The quantity of volatiles released by 0 DAT plants (Figure 1) was lower than the 4 DAT (Figure 2) and control plants (Figures 3). In contrast, the quantity of volatiles particularly the lower molecular weight compounds released by the 4 DAT plants (Figure 2) increased to the same level as the control (water) treated plants (Figure 3) indicating low



5.2 Canopy oil (PSO) and *O. nubilalis* oviposition on maize crops

5.2.1. Can PSO deter oviposition of *O. nubilalis* on maize and what is the optimum oviposition deterrent rate of PSO against *O. nubilalis* (Free and no-choice tests)

Significant differences ($P < 0.0001$) in oviposition-deterrence activity of PSO against *O. nubilalis* were found among the different concentrations of the oil tested (table 6). The oviposition deterring activity of the PSO sprayed at 3, 5 and 10% v/v was significantly higher ($P < 0.001$) than when sprayed at 1 and 2% v/v but the latter was significantly better than the control (water-treated) plants (table 6). The ODI calculated for 1 and 2% v/v was significantly lower ($P < 0.01$) than 3, 5 and 10% v/v ($P < 0.0001$) (table 6). However, the ODI of all the concentrations tested were significantly higher ($P < 0.001$) than zero indicating that all the concentrations had oviposition deterrence activity against *O. nubilalis* but the deterring activity was strongest in 3, 5 and 10% (table 6).

In the no-choice tests, no significant differences were found in the number of eggs per plant and ODI between plants treated at 1 and 2% v/v PSO and control plants (table 7). In contrast, plants treated with 5 and 10% v/v PSO recorded the lowest number of eggs per plant compared to plants treated with 3% v/v PSO (table 7). The ODI calculated for 3, 5 and 10% v/v was significantly higher than zero but the 5 and 10% rates recorded the strongest deterrence to *O. nubilalis* oviposition (table 7). The optimum rate of application of PSO on maize to deter *O. nubilalis* oviposition was determined as 5% v/v (table 7).

Table 6. Ovipositional response of *O. nubilalis* to different concentrations of Canopy oil® (PSO) sprayed on maize plants under free choice conditions in the laboratory at INRA, Versailles, France, June 2003.

Treatment	No. egg masses/plant	No. of eggs/plant	Mean Ovipositional Deterrent Index (ODI)
Control (water)	3.80 ± 0.58 a	68.80 ± 15.51 a	0 a
1% v/v	2.40 ± 0.98 ab	45.40 ± 4.02 b	20.5 b
2% v/v	1.60 ± 0.51 b	23.20 ± 4.47 c	49.6 c
3% v/v	0.60 ± 0.25 b	11.80 ± 5.85 d	70.7 d
5% v/v	1.00 ± 0.45 b	9.00 ± 3.77 d	76.9 d
10% v/v	0.40 ± 0.25 b	5.00 ± 3.52 d	86.5 d

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

Table 7. Ovipositional response of *O. nubilalis* to different concentrations of Canopy oil® (PSO) sprayed on maize plants under no-choice conditions in the laboratory at INRA, Versailles, France, June 2003 (Mean of 3 experiments).

Treatment	No. egg masses/plant	No. of eggs/plant	Mean Ovipositional Deterrent Index (ODI)
Control (water)	3.87 ± 0.59 a	124.07 ± 14.96 a	0.00 a
1% v/v	2.73 ± 0.30 b	121.73 ± 19.05 a	0.95 a
2% v/v	2.13 ± 0.26 bc	106.67 ± 17.13 a	7.54 a
3% v/v	1.53 ± 0.19 cd	63.33 ± 5.87 b	32.41 b
5% v/v	0.93 ± 0.12 d	36.40 ± 3.46 c	54.63 c
10% v/v	0.40 ± 0.13 d	21.67 ± 2.76 c	70.26 c

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

2.2.3 Is PSO efficacious against *O. nubilalis* larvae?

The mortality of *O. nubilalis* larvae increased with oil concentration with 5 and 10% v/v recording the highest mortalities against the three stages of larvae tested (table 8).

Table 8. Effect of Canopy oil® (PSO) on *O. nubilalis* 1st, 2nd and 3rd instar larvae in the laboratory at INRA, Versailles, France, June 2003 (Mean of 4 replicates).

Treatment	1 st stage larvae			2 nd stage larvae			3 rd stage larvae		
	No. of larvae used	No. dead	% Kill	No. of larvae used	No. dead	% Kill	No. of larvae used	No. dead	% Kill
0 (water)	25.0	2.0	8.0 a	25.0	1.0	4.0 a	25.0	2.0	8.0 a
1% v/v	25.0	14.0	56.0 b	25.0	12.0	48.0 b	25.0	8.0	32.0 b
2% v/v	25.0	21.0	84.0 c	25.0	18.0	72.0 c	25.0	16.0	64.0 c
3% v/v	25.0	24.0	96.0 c	25.0	24.0	96.0 c	25.0	22.0	88.0 cd
5% v/v	25.0	24.0	96.0 c	25.0	25.0	100.0 c	25.0	25.0	100.0 d
10% v/v	25.0	25.0	100.0c	25.0	25.0	100.0 c	25.0	25.0	100.0 d

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

6.0 Discussion

6.1 PSO against *H. armigera* on cotton plants

The results demonstrate the effectiveness of PSOs sprays to deter oviposition of *H. armigera* on cotton plants. Under free choice and no choice tests in the laboratory, cotton plants treated with different concentrations of PSO suppressed *H. armigera* egg lay. Application of 2% v/v of the oil to the cotton plants deters oviposition similar to 5% v/v rate. Thus the optimum rate of the oil was determined as 2% v/v. This supports the findings of the Australian study reported by Mensah *et al.* (2001 and 2002).

The results also showed that the oviposition deterrent activity of PSO against *H. armigera* females can last for only 4-5 days. Thus, for a grower to take advantage of the oviposition deterrent activity of the PSO, it is crucial that the oil be applied at 4-5 days interval. Application of the oil at an interval of 4-5 days, may not be cost effective and economical. However, for the economic use of the PSO, growers should mix reduced rates (i.e. 1% v/v rate) of the oil in every spray (insecticides, growth regulators, foliar fertilizers etc) they may apply to their cotton crops. In this way, the cumulative effect of the oil residues on the leaves will enhance or allow the oviposition deterrence activity persist longer making it cost effective.

6.2 PSO against *O. nubilalis*

The results showed that PSO sprays can deter oviposition of *O. nubilalis* adult females on maize plants. Under free and no-choice tests in the laboratory, maize plants treated with different concentrations of the PSO (Canopy® oil) suppressed the oviposition of *O. nubilalis*. The optimum rate at which the oil could deter the insect's oviposition was determined at 5% v/v. Increasing this rate to 10% v/v did not significantly increase the oviposition deterrence activity of the product. However, decreasing the rate to 3% v/v resulted in a 73.9 per cent increase in the number of eggs laid on the maize plant and this resulted in a reduction of the ODI from 54.6 to 32.4.

In addition, the PSO was found to be efficacious against *O. nubilalis* 1st, 2nd and 3rd stage larvae when applied at 2, 3, 5 and 10% v/v rates. The problem of applying oil sprays to maize plants was the architecture of the maize leaves. The architecture of the maize leaves in contrast to the cotton plant leaves, does not permit the sprays to stay on the leaves long enough to accumulate the required oviposition deterrent PSO residues on the leaf. The sprays run off quickly from the leaves compared to cotton. As a result, the PSO residues left on the leaves after the spray has dried off are not enough to deter oviposition particularly when lower PSO concentrations such as 1, 2 and 3% v/v were used. Contrarily, higher PSO rates (5 and 10% v/v) after run-off leaves enough residues which can deter egg lay. This explains any differences in the optimum PSO rates determined on maize and cotton in this study.

6.3 The underlying mechanisms of oviposition deterrence activity of PSO against *H. armigera*

The results of the study showed that airborne volatiles released by the cotton plants play an important 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 enabled the female moths to select an appropriate oviposition site. In a wind tunnel tests, mated *H. armigera* females took 26.8 seconds to perceive the volatiles emitted from the cotton plants sprayed with water and then took off towards the plant. It took the female moths only 12.8 seconds to locate and land on the water-treated plant. 100 per cent of the female moths tested in the wind tunnels against the water-treated plants landed on the plant. In contrast, when the same insects were exposed to cotton plants which has been

treated with the PSO on the same day (0 DAT), the insects took 88.5 seconds to respond and took-off. After the moths took off they flew for 58 seconds searching in the wind tunnel but did not locate and land on the plant. Similar results were obtained when 1 and 2 DAT plants were used. However, when 4 and 5 DAT plants were exposed to the insects, the insects took similar time as in the water treated (control) plants to take off and land on the plants. However, only 40-60% of the adult moths landed on the 4 and 5 DAT plants. This could be explained on the basis of the quantity of airborne volatiles released by the PSO treated and untreated plants.

In the SPME tests, it was found that the quantity of airborne volatiles released by 0 DAT plants were far less than those released by the 4 DAT and control (water) plants indicating that the PSO might have suppressed the quantity of volatiles released by the 0 DAT plants. The low quantity of volatiles emitted by the 0 DAT plants might have resulted in a longer time taken for the moths to take off, search and locate the plants. 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* might be due to the suppression of the quantity of airborne volatiles released by the PSO treated plants. The effect of this suppression activity lasted for 4 to 5 days after treatment. The results of this study supports the findings of Mitchell *et al.* (1991) who 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. Since *H. armigera* female adults migrate and lay most of their eggs during night time (Fitt, 1989), 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* will lay on the selected host plant.

In conclusion, this study has demonstrated that PSOs can deter oviposition of *H. armigera* and *O. nubilalis* on cotton and maize plants respectively. The underlying mechanism of the ovipositional deterrence activity of PSOs against moths may be due to the PSO suppressing the quantity of volatiles released by the plants. This suppression activity of the PSO lasts for only 4 to 5 days. Therefore, it is crucial for cotton growers to mix lower rates (at least 1% v/v) to every spray (either insecticides, growth regulators or foliar fertilizers etc) they apply to their cotton crops to maintain the oviposition deterrence activity and reduction of egg lay on their crops.

7.0 Conclusion and Recommendations

There are great benefits for the use of UV-protected petroleum spray oils (PSOs) in controlling pests in agricultural crops. Potential benefits are;

- Petroleum spray oils are safe to use
- PSOs are environmentally friendly. They breakdown easily in the environment
- Pest resistance to PSOs are non-existent
- PSOs are compatible with natural enemies of crop pests because they have only a minimal effect on their populations particularly predators
- PSOs are cost effective. Thus its use in the context of IPM will improve profitability and international competitiveness of the agricultural industries
- PSO use will complement the development and adoption of IPM programs in many agricultural industries particularly cotton and maize industries

To maximise the benefits from the use of UV-protected PSOs, it is recommended that:

- PSOs be used in high volume sprays to achieve run-off for capillary movement to suffocate pests and formation of films on plant surfaces to reduce egg lay on the crops. PSOs should be used as mixtures every spray applied to the cotton crops so as to maintain the residual activity and subsequently reduce egg lay.
- PSOs be used as adjuvant to biological insecticides such as nuclear polyhedrosis virus (NPV) and *Bacillus thuringiensis* (Bt) to improve the efficacy of these biological products against bollworms on agricultural crops.
- Growers continue to update their knowledge and understanding of the effective use of PSO. In particular, growers should be aware that PSOs alone may not provide adequate control of pests to achieve their high yield expectations, but can be used to integrate other control methods to achieve their required yield and more importantly a sustainable agricultural production.

An extension effort has commenced in the Australian cotton industry to improve grower knowledge about the effective use of PSOs.

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