# Improving the efficacy of nuclear polyhedrosis virus (NPV) and *Bacillus thuringiensis* (Bt) against *Helicoverpa spp.* on cotton with petroleum spray oils

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## Introduction

Petroleum spray oils (PSOs) have been used for control of a wide range of pests and are now an essential part of many integrated pest management programs (IPM) for agricultural and horticultural crops (Simanton and Trammel 1966, Riehl 1981, Johnson 1985, Lee *et al.* 1991, Beattie 1995, 1997, Rae *et al.* 1996, 1997). PSOs have little impact on natural enemies of crop pests and therefore can complement the activity of beneficial insects in crop systems. However, the use of PSOs in the cotton industry is limited due to the risk of PSO induced phytotoxicity which is related to the high molecular weight of the oils (Riehl 1969). Recent research on citrus and a range of other horticultural crops has led to the development of new technology in UV light protectants, which reduce the risk of damage to plants.

*Helicoverpa spp.* is the key pest for the Australian cotton industry. To control this pest in conventional cotton, growers spray on average 11 times at a cost of approximately $528 per hectare (average between 1996/7 and 1998/9). There is an obvious need for the cotton industry to reduce these costs and as a result there has been an increase in grower adoption of IPM programs.

In the adoption of IPM programs, there is a need to increase the effectiveness of environmentally benign alternatives such as biological pesticides (Bt, NPV etc.). The use of Bt has increased significantly over the past 3 years from 0.2 in 1996-97 to 1.1 sprays in 1998-99. NPV is now registered in the cotton industry.

The performance of these biological pesticides (NPV and Bt) on cotton crop against *Helicoverpa spp.* can be highly variable. To increase the role of biopesticides as selective larvicides on cotton, it is necessary to improve their efficacy and persistence. Reduced persistence and efficacy of biopesticides may be due in part to the stability of the products under UV light. It may be possible that UV protected PSOs be used as adjuvants with the biopesticides to improve their efficacy and persistence against *Helicoverpa spp.* on cotton.

## Materials and methods

**Experiment 1**: This experiment was conducted on a 50 ha irrigated commercial cotton field at Moree on 20 January 2000. Caltex spray oils (Canopy™* and RD54) were

* Canopy is a registered trademark of Caltex Australia Pty Ltd.

evaluated at 1% and 2% v/v in mixture with 750 ml/ha NPV and 2.0 L/ha Bt. Treatments evaluated were: (1) 1% Canopy + NPV, (2) 2% Canopy + NPV, (3) 1% Canopy + Bt, (5) 1% RD54 + NPV, (6) 2% RD54 + NPV, (7) 1% RD54 + Bt, (8) 2% RD54 + Bt, (9) NPV alone and (10) Bt alone. Plots were arranged in a completely randomised design with 4 replicates for each treatment. Each replicate measured 16 metres (rows) wide and 170 metres long. An 8 metre-wide buffer separated the plots. Sprays were applied to cotton leaves at 5 pm from a ground rig using 100 litres of water per hectare. At the time the sprays were applied, the naturally occurring *Helicoverpa spp.* population was predominantly very small (first instar). Twenty four larvae from plots of each treatment were collected on 1, 2, 3, 4, 5, 6 and 7 days after spray application and placed into 24-well Limbro plates containing soybean-based artificial diet. A single larva was placed in every well to avoid cross infection of the virus. The number of dead larvae were counted and recorded daily until all the surviving larvae had pupated. The percent mortality for each treatment was calculated in relation to the control.

**Experiment 2**: This experiment was conducted at the Australian Cotton Research Institute in Narrabri from February to April 2001. The treatments were: (1) Control (sprayed with water), (2) 0.75% NPV, (3) 2% Bt, (4) 1% Canopy + 0.75% NPV, (5) 2% Canopy + 0.75% NPV, (6) 1% Canopy + 2% Bt, (7) 2% Canopy + 2% Bt, (8) 1% RD54 + 0.75% NPV, (9) 2% RD54 + 0.75% NPV, (10) 1% RD545 + 2% Bt and (11) 2% RD54 + 2% Bt. Each treatment was repeated 3 times. Four (4) pots of plants were used for each treatment. Sprays were applied using spray bottles. Plants were exposed to sunlight. Leaves were picked 1 hour, 24, 48, 72, 96 120 hours after spray. Leaf discs were cut using a cutter (1.5 cm diameter). Two leaf discs were placed in each Petri dish containing moisten filter paper. Five Helicoverpa neonates were released into the Petri dish. The Petri dishes were sealed with Parafilm and placed in a 25°C temperature room for 24 hours. Larvae after 24 hours were transferred into plastic tray wells with artificial diet. One larva was placed in each well. Wells were sealed with a wrapping film. Mortalities in each treatment and control were recorded until all the surviving larvae pupated.

**Experiment 3**: This experiment was conducted in the mesh house in the Australian Cotton Research Institute on 23 November 2001. Twelve (12) treatments plus a control (sprayed with water) was evaluated. Each treatment was replicated 4 times. The treatments evaluated were: (1) 0.75% NPV alone, (2) 2% Bt alone, (3) 2% Canopy alone, (4) 2% RD54 alone, (5) 2% Canopy + 0.75% NPV, (6) 5% Canopy + 0.75% NPV, (7) 2% RD54 + 0.75% NPV, (8) 5% RD54 + 0.75% NPV, (9) 2% Canopy + 2% Bt, (10) 5% Canopy + 2% Bt, (11) 2% RD54 + 2% Bt, (12) 5% RD54 + 2% Bt. Potted plants (4 plants/pot, plant height: 50cm) were used for the test. The total number of leaves in each replicated treatment was counted before spray application. Twenty (20) neonates of *H. armigera* were released onto the plants in each pot using a fine-hair brush. Sprays were applied using spray bottles. Immediately after spray, each pot was covered with a plastic cylinder with meshed opens to allow ventilation. Fourteen (14) days after the spray, the number of surviving Helicoverpa larvae from each treatment were collected and counted. Percent damage of each leaf was assessed. All leaves were scored for damage. Damage scores were represented as follows: 0 = no damage, 1= 0-12% damage, 2 = 13-25% damage, 3 = 26-50% damage and 4 = > 50% damage.

## Analysis of data

Data of the above experiments were analysed using repeated measure ANOVA (Graphpad Instat Software Inc., Version 2.03, San Diego, CA, USA). Turkey-Kramer multiple comparison tests were used to separate means. Arithmetric rather than transformed means are given in the results.

## Results

**Experiment 1**: Mortality of *Helicoverpa spp.* collected from the field plots was the highest in plots treated with 2% Canopy oil mixtures with NPV and Bt, followed by 1% and 2% RD54 oil mixtures (Figures 1.1 - 1.3). When either NPV or Bt was sprayed alone they both recorded the lowest mortality against *Helicoverpa spp.* larvae in 1 and 2 days after treatment (DAT). The mortality caused by mixture of 1% Canopy oil or RD 54 oil with either Bt or NPV was significantly lower ($P<0.05$) than mixture of 2% of either oils with NPV or Bt but was not different from the biopesticides sprayed alone at 1 DAT (Figures 1.2 and 1.3). Mixing the biopesticides with 2% Canopy oil resulted in a higher larval mortality than when the products were mixed with 2% RD 54 oil, indicating that 2% Canopy oil mixed with NPV or Bt increased the efficacy of the biopesticides higher than 2% RD 54 mixed with these biopesticides (Figure 1.1). At 7 DAT, the mortalities caused by mixture of 2% Canopy or RD 54 oils with NPV or Bt ranged from 20-22.5% compared with 15-16% in the biopesticides alone treatments (Figure 1.1), indicating a longer persistence of NPV and Bt when mixed with 2% oils.

Over the 7 days after spray, the average mortalities caused by mixture of 2% Canopy with NPV and Bt were 44.8 and 48.2% respectively compared to 25.9 and 31.5% when NPV and Bt were sprayed alone (Figure 1.2 and 1.3). When both NPV and Bt were mixed with 1% Canopy oil, the average mortalities over the 7-day period were 31.5 and 36.0% respectively (Figures 1.2 and 1.3). The average mortality caused by 2% RD54/NPV mixture was 40.7%, 2% RD54/Bt mixture was 41.1% (Figure 1.1). When NPV and Bt were mixed with 1% RD54, the average mortalities over the 7-day period were 32.4 and 36.4% respectively (Figures 1.2 and 1.3).

Overall, there was no significant differences between the average mortalities caused by mixture of 1% either Canopy or RD54 oils with NPV or Bt. The average mortality caused by 1% oil and NPV mixture was higher than NPV sprayed alone. However, 1% oil mixture with Bt was not significantly different from Bt sprayed alone. In contrast, the average mortalities caused by mixture of 2% either oils with NPV and Bt were significantly higher than both 1% oil mixtures and the biopesticides sprayed alone (Figures 1.2 and 1.3).

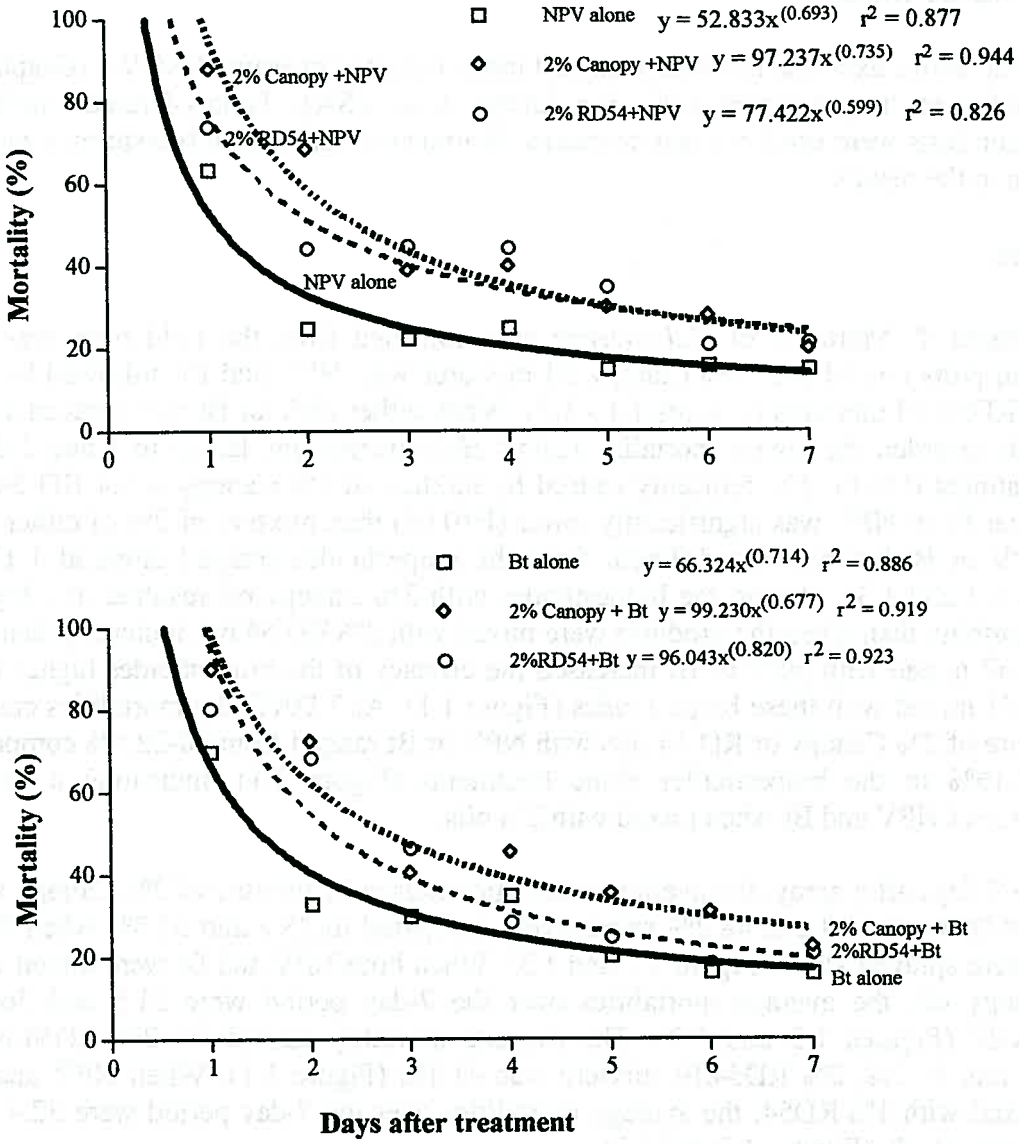


**Figure 1.1.** Efficacy of NPV and Bt mixed with 2% Canopy and RD54 oils on *Helicoverpa spp.* larvae in commercial cotton field at Norwood near Moree, 2000-2001.

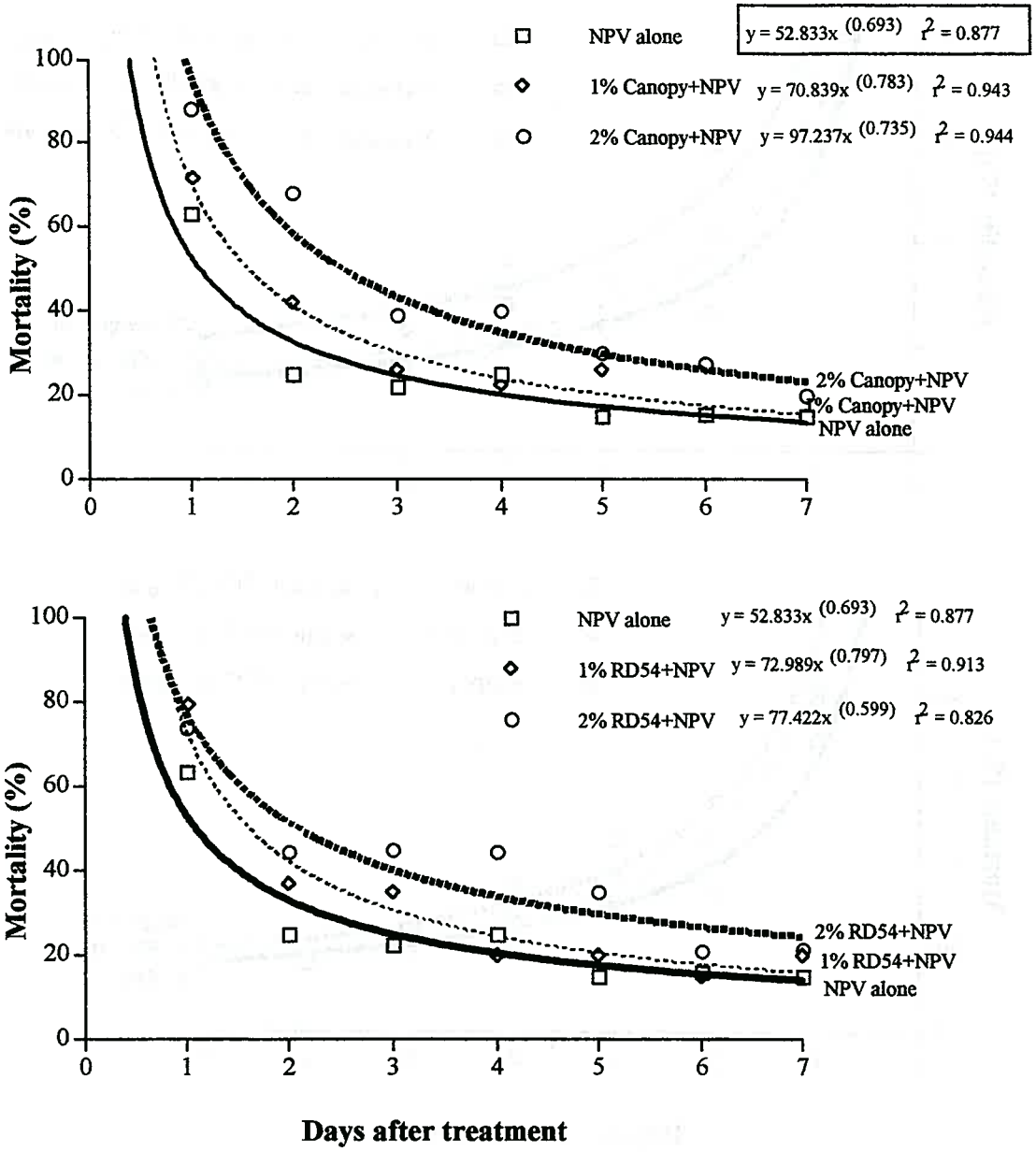


**Figure 1.2.** Efficacy of NPV with and without Canopy and RD54 oils on *Helicoverpa spp.* larvae in commercial cotton crop at Norwood, Moree, 2000-2001.

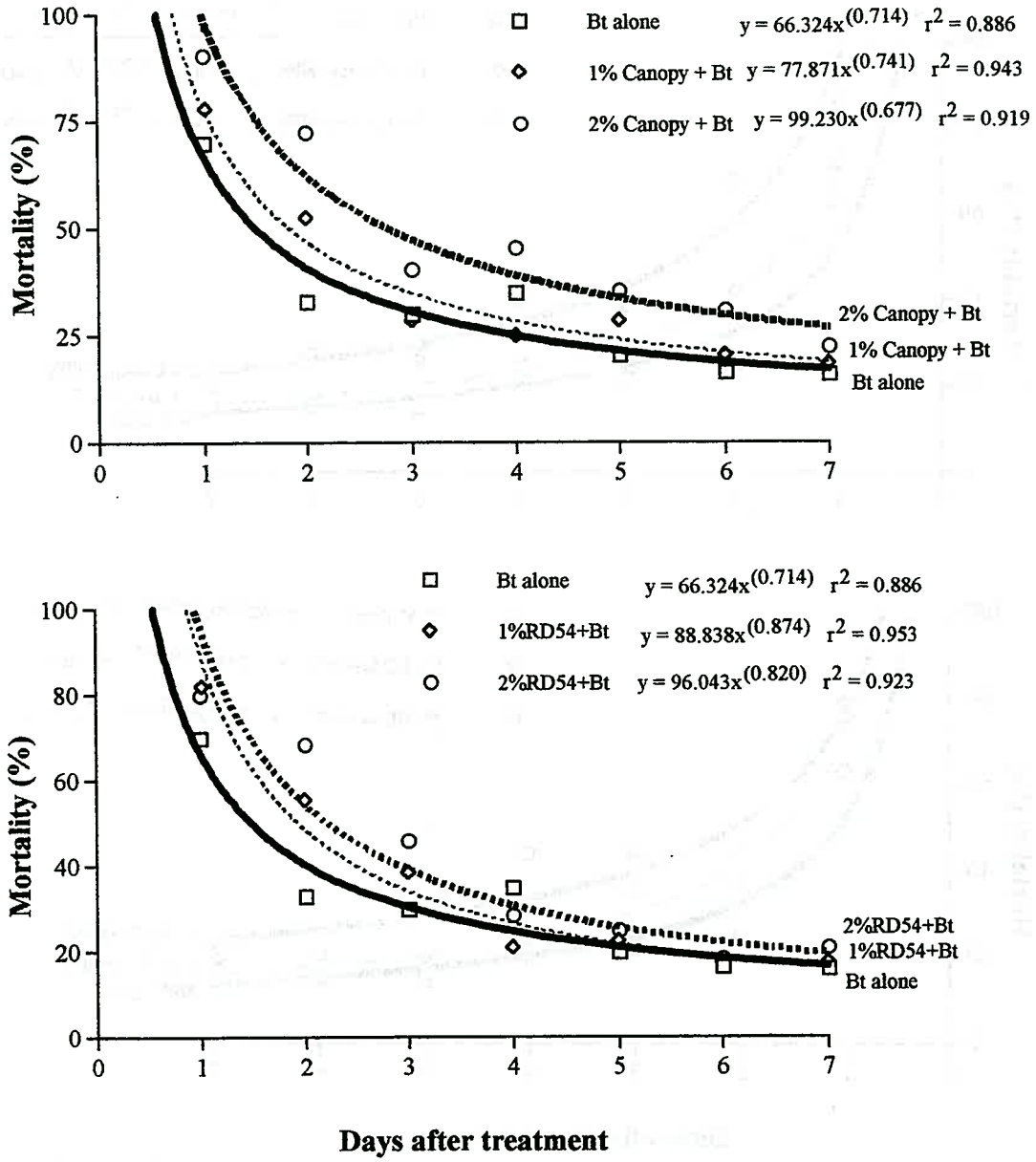


**Figure 1.3.** Efficacy of Bt with and without Canopy and RD54 oils on *Helicoverpa spp.* larvae in commercial cotton crop at Norwood, Moree, 2000-2001.

**Experiment 2:** Mixing 2% Canopy oil significantly increased the efficacy of NPV against first instar Helicoverpa larvae compared to NPV sprayed alone (Figure 2). The efficacy of 1% Canopy oil mixture with NPV was not significantly different to 2% Canopy oil + NPV (Figure 2). However, 2% Canopy oil/NPV mixture produced mortalities slightly higher than 1% Canopy mixed with NPV (Figure 2). In addition, the mortalities caused by mixing 1 and 2% Canopy oils with Bt was not significantly different ($P>0.05$) (Figure 2). Tests over the 6 days after the spray application indicated that addition of 1-2% Canopy oil to NPV and Bt significantly improved the persistence of the products.

The addition of 1 and 2% RD54 did not significantly increase the efficacy of NPV compared to the mixture with 1 and 2% Canopy oil. However, mixing RD54 oil to the biopesticides outperformed Canopy oil in increasing the persistence of the biopesticides.

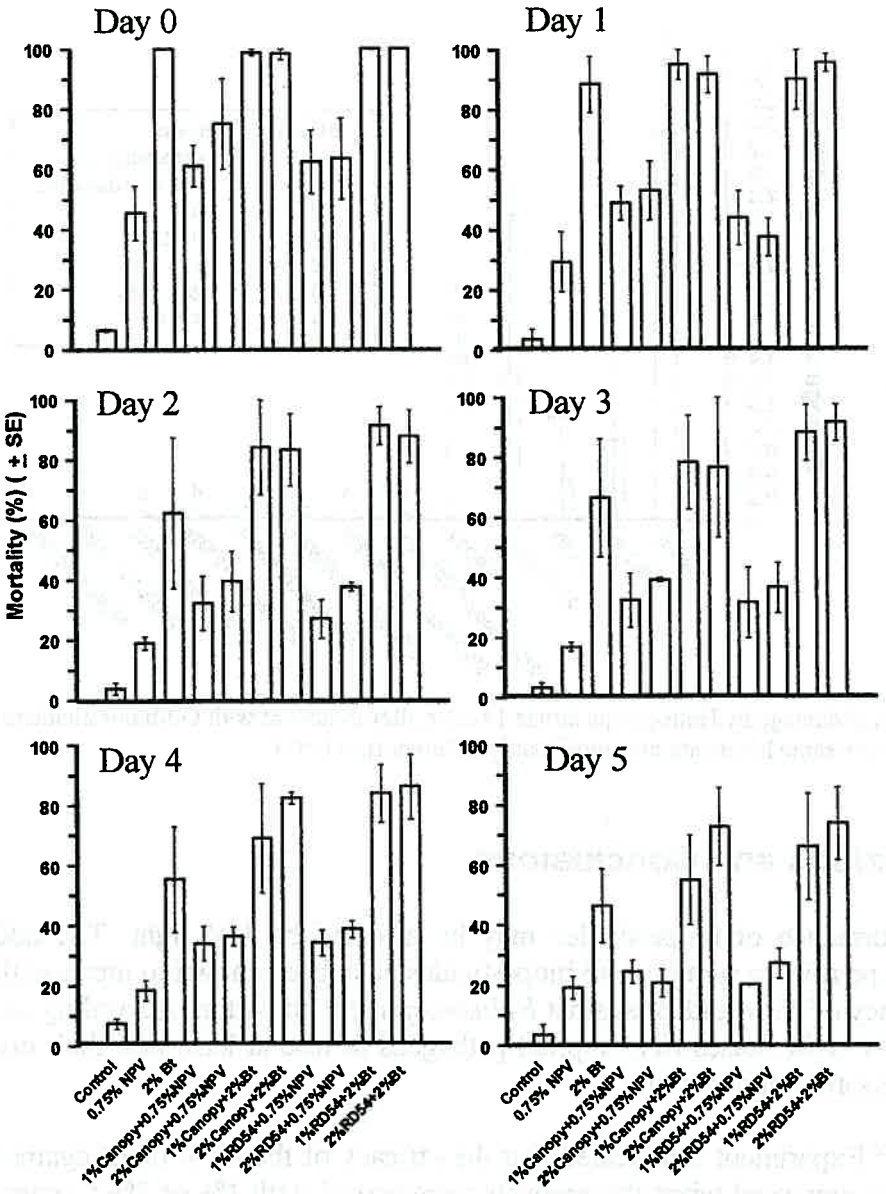


**Figure 2.** Mortalities of Helicoverpa larvae fed with leaves collected 1 (Day 0) hour, 24 (Day 1), 48 (Day 2), 72 (Day 3), 96 (Day 4) and 120 (Day 5) hours after treatment with oil/biopesticide mixture.

**Experiment 3**: When 2% or 5% Canopy oil or RD54 oil was mixed with NPV, the mixture significantly ($P<0.05$) reduced the leaf damage caused by Helicoverpa larvae compared to that when NPV was sprayed alone (Figure 3). Furthermore, the oils and NPV mixture produced a higher mortality of Helicoverpa larvae than the NPV alone. The mixture of both oils and Bt increased the mortality of larvae and reduce the leaf damage, but the difference was not significant ($P>0.05$) compared to the Bt alone treatment. Canopy and RD 54 alone caused mortalities higher than the control. Results also indicated that the

oils alone can significantly reduce the feeding damage of cotton plants caused by Helicoverpa larvae (Figure 3).

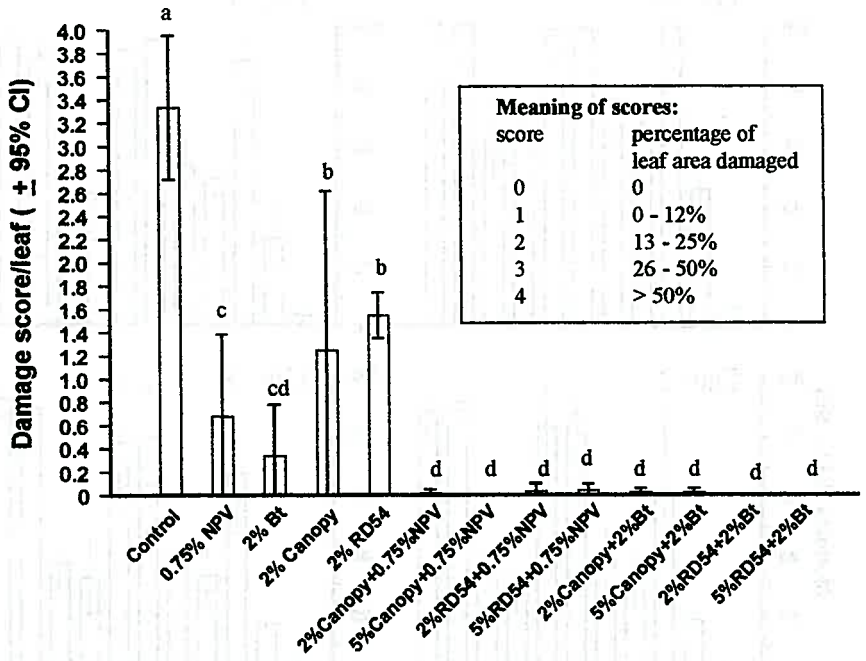


**Figure 3.** Leaf damage by Helicoverpa larvae 14 days after treatment with Oil/biopesticide mixture sprays. Means with the same letters are not significantly different ($p > 0.05$).

## Discussions and Conclusions

The performance of biopesticides may be affected by UV light. The addition of UV protected petroleum spray oils to biopesticides have been shown to increase the persistence and efficacy of NPV and Bt against *Helicoverpa spp.* in cotton. According to Ignoffo *et al.* (1977), UV light causes field applied pathogens to lose at least half their original activity within days after application.

Results of Experiment 1 indicated that the efficacy of the NPV or Bt against *Helicoverpa spp.* larvae increased when the products were mixed with 1% or 2% Canopy oil or RD54 oil. The mortality caused by mixing 2% Canopy oil or RD54 oil with NPV or Bt was significantly higher than that when mixing 1% of either Canopy oil or RD54 oil with the biopesticides. Canopy oil was found slightly better than RD54 oil for improving the efficacy and persistence of NPV and Bt.

Experiment 2 confirmed results in Experiment 1 under more uniformed conditions by using potted plants to ensure a precise comparison of the spray effects between treatments. Results of this experiment indicated that 1% or 2% Canopy and RD54 oils can increase the efficacy and persistence of NPV and Bt. No significant difference of efficacy was found between 1% and 2% Canopy or RD54 oils mixed with NPV or Bt. Canopy oil was found better than RD54 oil in improving the efficacy of the biopesticides.

In Experiment 3, under mesh house conditions, both Canopy oil and RD54 oil increased the efficacy of NPV and Bt against *Helicoverpa spp.* larvae. The mixture of 2% or 5% Canopy oil or RD54 oil with NPV or Bt significantly reduced the damage caused by Helicoverpa larvae compared to that when NPV or Bt was used alone. There was no significant difference between damages in treatments with 2% or 5% oils. This indicates that 2% oil is the optimum rate for mixed-use with biopesticides under mesh house conditions. In commercial field conditions, 2% or slightly higher concentration of oils are recommended for the control of *Helicoverpa spp.*.

The studies showed an increased efficacy of biopesticides when they were mixed with 2% of either Canopy oil or RD 54 oil compared to the using alone of these biopesticides. The increase of efficacy may have been due to the oils preventing the rapid breakdown of the biopesticides under UV light in the field, thus improving the persistence or stability of the biopesticides.

Mixing Canopy oil and RD 54 oils to NPV or Bt increased the persistence of these biopesticides. The increased persistence allows *Helicoverpa spp* larvae enough time to feed and ingest the quantity of toxins that could kill them, thus enhancing the efficacy of these biopesticides. Canopy oil performed better than RD 54 in increasing the efficacy of the biopesticides. This could be related to the different molecular weight, instead of sunscreen, of Canopy oil.

This study and other studies that we will publish later showed that, at low *Helicoverpa spp.* pressure (1.5 to 4 larvae per metre), oils mixed with biopesticide can control and reduce the numbers of *Helicoverpa spp.* larvae below the recommended threshold of 2 larvae per metre for up to 14 days. However, at high *Helicoverpa* pressure (above 5 per metre) the oil/biopesticide mixtures alone may not be able to reduce the number of larvae per metre below the recommended threshold. Commercially, the oil/biopesticide mixture treatments may have to be integrated with synthetic insecticides such as Tracer to be able to reduce the larvae numbers below the economic threshold and avoid crop damage and yield loss.

In Australia, cotton crops are planted under both irrigated and dryland conditions. Dryland cotton crops are usually more water stressed and take a longer time for the canopy to close compared to irrigated crops. As a result, groundrig can be used well into the late cotton season before canopy closure. Application volume of 100-120 litres per hectare can be used on dryland crops until the end of February. In this and other studies, the efficacy of oil/biopesticide mixtures against *Helicoverpa spp.* larvae on dryland cotton crops was similar to irrigated cotton crops even in the January - March period. As a result, the conditions and rates of application of oils and biopesticides should be the same for both dryland and irrigated cotton crops.

In conclusion, the results of this study suggest synergies for combined use of oils, particularly Canopy oil with NPV and Bt in cotton. The use of Canopy oil and biopesticides in cotton crops against *Helicoverpa spp.* is advantageous over conventional insecticides because they are less disruptive to natural enemies of pests. This combination can therefore be used as a useful tool or strategy to maintain beneficial insects in cotton systems thereby complementing existing IPM programs.

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