Petroleum spray oils - Lubricating the path to IPM : Part 3. Use of biological insecticides with Petroleum spray oil to improve persistence and efficacy against *Helicoverpa* spp. on cotton crops

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1.0 Introduction

Nuclear polyhedrosis virus (NPV) and *Bacillus thuringiensis* (Bt) are the most commonly used biopesticides for the control of *Helicoverpa* spp. larvae on cotton crops in Australia. These naturally occurring entomopathogens can regulate populations in agricultural and forestry ecosystems. However, in many instances, entomopathogens have not provided consistent control of pests to an acceptable level (Benz 1987) and in some cases yield loss has occurred (Mensah 2002).

In Australia, NPV and foliar Bt are used to control *Helicoverpa spp.* on conventional cotton crops particularly early in the cotton season. The efficacy of NPV and Bt against *Helicoverpa spp.* larvae is often found to be inconsistent and can be inadequate when population pressure is high (Mensah 2002). This may be due to the narrow host range, retarded response and/or poor residual activity of biopesticides after application (McGuire 2000). Ultra-violet light (UV) is known to cause pathogens such as NPV and Bt to lose at least half their original activity within days of being applied in the field (Bull *et al.* 1976; Krieg *et al.* 1980; Jeyakumar and Gupta, 1999). For these biopesticides to fulfil their role as effective, selective larvicides in cotton, it is essential that their persistence and efficacy be improved.

Most studies aimed at overcoming the constraints of short persistence and low efficacy of entomopathogens, have focussed on the formulation of the pathogens, *viz.* fungi virus and Bt in oils within a biologically based framework (Inglis et al 2000). Oil based formulations has been reported to increase the adhesion of propagules to the insect integument, enhance spread of inoculum over the insect body, enhance penetration of the insect cuticle, protect propagules from ultra-violet radiation and enhance infection under low humidity (Ingris et al. 2000).

Recent research on citrus and a range of other horticultural crops led to the development of a PSO formulation which incorporated heavy base oil for maximum efficacy (Beattie *et al.* 1995, Beattie and Smith 1997) and UV light absorbing compounds to reduce the detrimental effects of UV light on unstable oil molecules. Minimising UV induced breakdown of the petroleum base oil can in turn reduce the potential of the PSO to damage plants (Hodgkinson *et al* 2002a; Hodgkinson *et al* 2002b). Further PSOs were developed based on this premise, to improve the effectiveness of UV labile biopesticides against cotton pests.

The aim of this study was to determine the effect on persistence and efficacy of NPV and Bt of a UV protected PSO. The effect was measured by the control of *Helicoverpa spp* larvae, in relation to days after treatment application.

2.0 Methodology

2.1 Plant and materials

The PSO containing UV absorbing compounds used in these studies was Canopy® (Caltex Australia Pty Ltd). Canopy® is an emulsified, nC27 PSO currently being commercialised in Australia for use as a bulking agent, carrier and insecticide. The base oil is paraffinic with a typical unsulfonatable residue of 92%. The biopesticides used in these studies were commercially available nuclear polyhedrosis virus (NPV) (Gemstar® supplied by Bayer CropSciences Pty Ltd) and *Bacillus thuringiensis* (Bt) (Dipel® SC supplied by Sumitomo Chemicals Australia P/L). The buffer row treatment Tracer® is an insecticide product sold commercially by Dow Chemicals Australia P/L.

2.2 Effect of PSO/biopesticide mixtures on mortality rates of Helicoverpa spp. larvae in commercial cotton crops.

The experiment was conducted on a 50 ha irrigated conventional commercial cotton (Sicala V2) field at Norwood near Moree in New South Wales in January 2000. The treatments evaluated were: (1) 1% PSO + NPV, (2) 2% PSO + NPV, (3) 1% PSO + Bt, (4) 2% PSO + Bt, (5) NPV alone (6) Bt alone and (7) unsprayed (control). Plots were arranged in a randomised complete block design with four replicates, each treatment replicate measuring 16 metres (rows) wide and 170 metres long. An 8 metre-wide buffer separated each treated and control plot.

Treatments were applied during the flowering to boll setting stage when the naturally occurring *Helicoverpa* spp. population consisted of predominantly very small larvae (1^{st}-3^{rd} instar). Treatments were applied from a ground rig using 100 L/ha of water, at 5 p.m. on 20 January, 2000. Twenty-four larvae were collected from each treated plot 1, 2, 3, 4, 5, 6 and 7 days after treatment application and placed into 35 ml clear plastic containers (P101M; Solo, Urbana, Illinois, USA) containing a soybean-based artificial diet. Sampling from each treatment plot was replicated 5 times. The number of dead larvae was then counted daily until all the live larvae had pupated. The percentage mortality was calculated relative to the control.

2.3 Effect of PSO on the efficacy and persistence of the biopesticides against Helicoverpa spp. on sun-exposed, potted cotton plants.

The study was conducted at the Australian Cotton Research Institute in Narrabri in New South Wales from February to April 2001. Plants used for the study were potted cotton plants in the early squaring stages of growth. All the treated plants were left outdoors (*i.e.* in the sun) throughout the study. The treatments evaluated were: (1) Control (water), (2) 0.75% NPV, (3) 2% Bt, (4) 1% PSO + 0.75% NPV, (5) 2% PSO + 0.75% NPV, (6) 1% Canopy® + 2% Bt, (7) 2% PSO + 2% Bt. A 200 mL emulsion of each treatment was applied using hand-held spray bottles to four pots of cotton plants (2 plants per pot). The experiment was repeated 3 times. The plants were left outdoors and leaves were picked 1 hour, 24, 48, 72, 96 and 120 hours after spray. 2 x 1.5 cm diameter leaf discs were then cut from each picked treated leaf and placed in a petri dish containing moist filter paper and one *H. armigera* second instar larvae. The petri dishes were sealed with parafilm and placed in a 25°C temperature room for 24 hours. Thereafter, each larva was then transferred into 35 ml clear

plastic containers containing artificial diet. The containers were sealed with a wrapping film. Mortalities in each treatment and control were recorded until all the surviving larvae had pupated. Data was analysed using an SPSS general linear regression model to test the significance of difference between treatments. Half-lives of the treatments were calculated from the linear regression model at the point at which 50% mortality was reached.

3.0 Results

3.1 Effect of PSO/biopesticide mixtures on mortality rates of Helicoverpa spp. larvae in commercial cotton crops.

Helicoverpa spp. larvae with the highest rate of mortality were sampled from plots treated with 2% v/v PSO/biopesticide mixtures (Table 1 and Figure 1). NPV and Bt sprayed alone recorded the lowest mortality 1 and 2 DAT. 1 DAT, the mortalities of larvae from plots treated with a 1% PSO/biopesticide mixture were significantly higher ($P<0.05$) than that of plots treated only with the corresponding biopesticide. The plots treated with a 2% PSO/biopesticide mixture showed significantly higher ($P<0.05$) mortality 1 DAT than plots treated with the corresponding 1% PSO/Biopesticide mixture. The performance of both NPV and Bt was enhanced by 2% PSO on every individual DAT.

Table 1. The mortality of *Helicoverpa spp.* larvae sampled from plots treated with biopesticides and biopesticide/PSO mixtures in commercial dryland cotton at Elroi Downs, Gunnedah, 1999-2000.

Treatment (%v/v)	Days after treatment (Corrected mortality %)							7 day Average	$t_{1/2}$# (days)
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT		
NPV alone	63.2 a	25.0 a	22.2 a	25.0a	15.0 d	15.8 a	15.0 a	25.9 a	1.08
1% PSO + NPV	72.2 b	42.1 b	26.3ab	22.7a	26.3a	15.8 a	15.0 a	31.5 a	1.56
2% PSO + NPV	88.2 c	68.4 c	38.9 c	40.0 c	30.0 b	27.8 b	20.0bc	44.8 b	2.47
Bt alone	70.0 b	32.8 a	30.0 b	35.0 b	20.2 e	16.5 a	16.0ab	31.5 a	1.49
1% PSO + Bt	78.5 d	52.5 d	28.5 b	25.0a	28.2ab	20.5 c	18.5ab	36.0 a	1.82
2% PSO + Bt	90.5 c	72.5 c	40.5 c	45.5 d	35.5 c	30.8 b	22.5 c	48.2 b	2.75
Control	0	0	0	0	0	0	0	0	0

Means between treatments followed by the same letter are not significantly different ($P>0.05$), Tukey-Kramer Multiple Comparison Test.
half-life – days to 50% mortality according to fitted model

Overall, the 7-day average mortality from plots treated with 2% PSO/biopesticide mixtures was significantly higher ($P<0.01$) than mortality from plots treated with 1% PSO/biopesticide mixture or the biopesticides alone. There were no significant differences ($P>0.05$) among plots treated with a 1% PSO/biopesticide mixture. Although on certain individual days the PSO at 1% v/v significantly ($P<0.05$) enhanced the biopesticides' efficacy, overall no significant enhancement was evident. Calculations (Table 3), based on the fitted models described in Figure 1, indicated that the half-life of NPV was increased from 1.08 days to 1.56 and 2.47 days when mixed with 1 and 2% PSO respectively, whilst the half-life of Bt was increased from 1.49 days to 1.82 days and 2.75 days respectively.

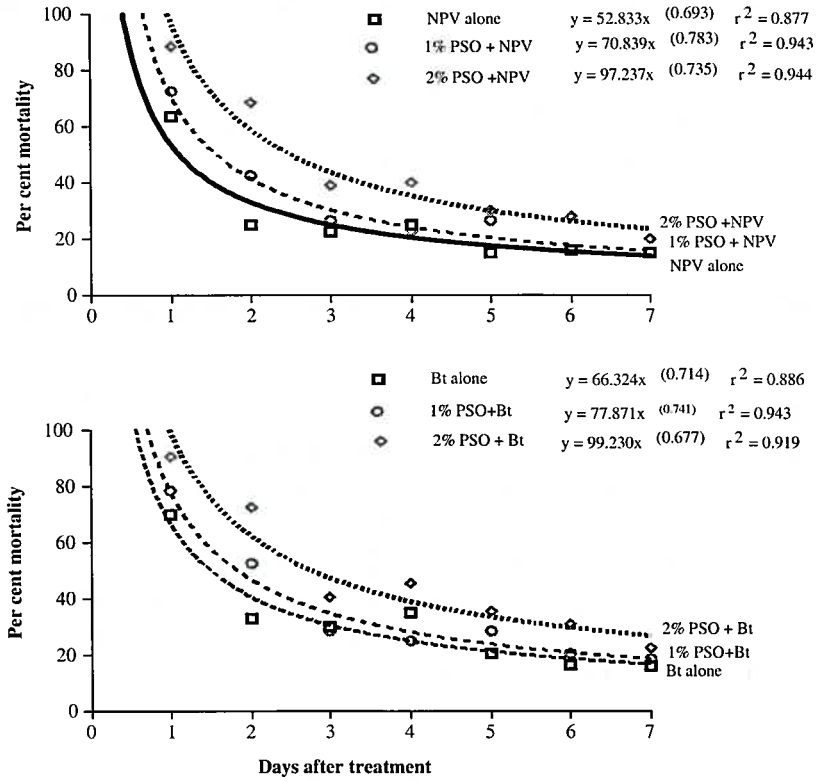

Figure 1. The effect of different rates of PSO (Canopy®) on the efficacy of NPV and Bt against *Helicoverpa spp.* larvae in commercial cotton crops at Norwood near Moree, 2000-2001.

3.2 Effect of PSO on the efficacy and persistence of NPV and Bt against Helicoverpa spp. on sun-exposed, potted cotton plants.

The average mortality of first instar *Helicoverpa spp.* larvae exposed to NPV treated leaves was significantly increased ($P<0.01$) when it was mixed with either 1 or 2% v/v PSO (Table 4). However increasing the PSO rate from 1 to 2% v/v did not increase the efficacy of the NPV. The efficacy of Bt against first instar *Helicoverpa spp.* larvae was only significantly increased when it was mixed with 2% v/v PSO (Table 4). The persistence of NPV, as measured by the half-life, increased from 0.68 d to 1.43 and 2.10 d when it was mixed with 1 and 2% v/v PSO, respectively (Table 5). Similarly, addition of either 1 or 2% v/v PSO to Bt substantially increased its persistence (half-life) from 5.2 d to 6.6 and 10.2 d, respectively.

Table 2. Corrected mortality of Helicoverpa larvae fed with leaves collected at different days after treatment with PSO/biopesticide mixture.

Treatment (%v/v)	Days after treatment (Corrected mortality %)						5 day Average
	1 hr AT	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	
NPV alone	41.71 a	26.09 a	15.68 a	13.96 a	11.87 a	15.82 a	20.86 a
1% PSO + NPV	58.45 a	46.88 a	29.75 ab	29.87 ab	28.45 ab	21.77 a	35.86 b
2% PSO + NPV	73.35 ab	50.59 a	44.12 ab	37.21 ab	31.18 ab	24.87 a	43.55 b
Bt alone	100.0 b	87.24 b	60.55 bc	64.91 bc	51.99 bc	43.61 ab	68.05 c
1% PSO + Bt	98.81 b	94.41 b	83.38 c	77.37 c	66.66 c	52.06 b	78.78 cd
2% PSO + Bt	98.24 b	90.88 b	82.63 c	75.44 c	81.07 c	70.82 b	83.18 d
Control	0	0	0	0	0	0	0

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

Table 3. Half-lives of NPV and Bt and their mixtures with different concentrations of PSO (Canopy®) against Helicoverpa larvae, ACRI, 2001.

Treatment	Model (Y = corrected mortality, X = days	$t_{1/2}$# (days)
Control	---	
NPV	$Y = 38.841X^{-0.660}$, $r^2 = 0.865$	0.68
1% PSO + NPV	$Y = 60.531X^{-0.530}$, $r^2 = 0.931$	1.43
2% PSO + NPV	$Y = 76.057X^{-0.565}$, $r^2 = 0.968$	2.10
Bt	$Y = -10.953X+106.384$, $r^2 = 0.909$	5.18
1% PSO + Bt	$Y = -9.229X+111.083$, $r^2 = 0.975$	6.62
2% PSO + Bt	$Y = -4.963X+100.552$, $r^2 = 0.856$	10.19

*Abbott (1925) formula was used to correct mortalities.
half-life – days to 50% mortality according to fitted model

Discussion

The study showed that NPV, when mixed with 1 and 2% v/v of UV protected PSO, had increased *Helicoverpa* larval mortality from 25.9% to 31.5 and 44.8 %, respectively (Table 1 and Figure 1). Similarly, Bt related mortality increased from 31.5% to 36.0 and 48.2%, respectively. The persistence as measured by half-life increased from 1.08 days to 1.56 and 2.47 days when NPV was mixed with 1 and 2% v/v PSO. The half-life persistence of Bt increased from 1.49 days to 1.82 and 2.75 days, respectively (Table 1 and Figure 1). Furthermore, this experiment showed that the UV protected PSO slowed down the decay rates of both NPV and Bt (see Table 1 and Figure 1) and did not just increase the initial knockdown result (0 DAT). This provides evidence of an inhibitory effect from the PSO on the destruction of the active ingredients of the biopesticides.

In the more controlled trial in which treated potted cotton plants were exposed to sunlight, both the average mortality of *Helicoverpa spp.* first instar larvae, and the persistence of insecticidal activity, were enhanced over the biopesticides sprayed alone, by the addition of PSO to the tank mix (Tables 2 and 3).

Generally PSOs can cause direct mortality to *Helicoverpa* spp. larvae themselves, particularly to the 1^{st} -3^{rd} instar stages, they require direct droplet contact to achieve this ((Mensah et al. 2001). The direct knockdown efficacy of PSOs arises through a suffocation mode-of-action. This means that the oil has to come into direct contact with the insect to block their spiracles and cause mortalities of the larvae. Since PSOs have little residual effect on larval mortality, unlike synthetic insecticides, there is unlikely to be any direct contribution from the PSO itself to the larval mortality collected after 1 day after treatment (DAT) in these experiments. Therefore, their outcome cannot only be explained through a direct additive contribution from the PSO to mortalities, but must largely have arisen through the enhanced efficacy of the biopesticides due to a synergism with the PSO. The PSO used in this study has a high molecular weight (nC27) and this is likely to reduce the rate of dissipation of the oil, and its UV protection, from the leaf surface and biopesticide. Thus not all oils are likely to enhance the efficacy of biopesticides to the level achieved in this study, and that UV absorbing

compounds in spray oil formulations may be essential for improving the efficacy of PSO/biopesticide mixtures.

The efficacy of NPV and Bt are highest against very small and small larvae. Thus, application of PSO/biopesticide mixtures should coincide with periods when the *Helicoverpa spp.* larval population is predominantly at the first and second instar stage. In addition the volume of application required for effective performance of PSOs as adjuvants of biopesticides is important particularly during the mid and late cotton season when cotton plants are bigger. In this study, the spray application volume used was 100L/ha. Ground rigs were used for all the spray treatments in the field. The normal commercial aerial spray application volume used for NPV and Bt is 30 L/ha. In practice, this low volume of spray does not diminish the field efficacy of NPV and Bt. Therefore a biopesticide mixed with a PSO offering UV protection rather than direct kill should show improvement in efficacy similar to that demonstrated by the ground rig applied sprays used in this current study. Indeed, since aerially applied sprays will tend to concentrate in the top of the cotton canopy which is exposed to more sunlight, the improvement might be even more marked. Since biopesticides are known to be sensitive to UV-light, logic dictates that the optimum time to apply PSO/biopesticide sprays would be after sunset in order to further extend the persistence of the biopesticides offered by PSO. Thus, evening sprays will significantly increase the persistence of the biopesticides longer than that achieved in this study. This will allow *Helicoverpa spp.* larvae greater opportunity to ingest a lethal dose of pathogens before the toxins are destroyed by UV light.

In conclusion this study showed clear synergies from the combined use of a UV protected PSO, with NPV or Bt for the control of *Helicoverpa* spp. larvae in cotton and therefore form a useful tool to complement existing IPM programs in cotton.

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References

BEATTIE, G. A. C., LIU, Z. M., Watson, DM, CLIFT, AD and JIANG, L., 1995. Evaluation of petroleum spray oils and polysaccharides for control of *Phylllocnistis citrella. Journal of Australian Entomological Society* **34,** 349-53.

BEATTIE, G. A. C. and SMITH D., 1997. Integrated pest management:sustainable pest control for the future based on the past? *Proceedings of the International Society of Citriculture, Southern Africa* **1**, 51-58.

BENZ, G., 1987. Environment. In: Fuxa J. R., Tanada, Y., eds. Epizootiology of insect diseases. New York, John Wiley and Sons, 177-214.

BULL, D. L., RDGWAY, R. L., HOUSE, V. S. and PRYOR, N. W., 1976. Improved formulations of the Heliothis nuclear polyhedrosis virus. *Journal of Economic Entomology.* **69** (6), 731-736.

HODGKINSON, M. C., JOHNSON, D. and SMITH G., 2002a. Using FTIR to predict the potential for petroleum derived spray oils to photograde. In: Beattie, G. A. C., Watson, D.

M., Stevens, M.L., Rae, D. J., Spooner-Hart, R. N. (eds.) Spray oils-Beyond 2000, University of Western Sydney, pp. 72-76.

HODGKINSON, M. C., JOHNSON, D. and SMITH G., 2002b. Causes of phytotoxicity induced by petroleum spray oils. In: Beattie, G. A. C., Watson, D. M., Stevens, M.L., Rae, D. J., Spooner-Hart, R. N. (eds.) Spray oils-Beyond 2000, University of Western Sydney, pp. 170-178.

INGLIS, G. D., JARONSKI, S. T. and WRAIGHT, S. P., 2000. Use of spray oils with entomopathogens. In: Beattie A, Watson D, Stevens M, Rae D and Spooner-Hart R, eds. Spray oils – Beyond 2000: Sustainable and Disease Management. University of Western Sydney, Australia Publishers, 302-320.

JEYAKUMA, P., GUPTA, G. P., 1999. Impact of UV and white lights on the bio-potency of *Bacillus thuringiensis* against *Helicoverpa armigera* Hubner. *Annals of Plant Protection Sciences*. **7 (2),** 121-124.

KRIEG, A., GRONER, A., HUBER, J., and MATTER, M. 1980. The effect of medium- and long-wave ultraviolet rays (UV-B and UV-A) on insect-pathogenic bacteria and viruses and their influence by UV-protectants. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes*. 32 (7), 100-106.

McGUIRE, M. R., 2000. Formulation and application of biopesticides. In: *Proceedings of 21st International Congress of Entomology 2000,* Brazil.

MENSAH, R. K., Harris, W. E., BEATTIE, G. A. C., 1995. Response of *Helicoverpa* spp. and their natural enemies to petroleum spray oil in cotton in Australia. *Entomophaga* **40**, 263-272.

MENSAH, R. K ., 2002. Development of an integrated pest management programme for cotton. Part 2: Integration of a lucerne/cotton interplant system, food supplement sprays with biological and synthetic insecticides. *International Journal of Pest Management* **48 (2)**, 95-105.