COTTON RESEARCH COMPENDIUM

Leaders in spray oil and adjuvant technology for the cotton industry

2003 - 2010

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Background to this Report

BIOPEST's Cotton Registration

BIOPEST is currently registered for use in cotton for the control of cotton aphid, as a carrier for certain ULV insecticides and as a defoliation aid.

The focus of SACOA's Cotton Research Program is to extend the potential of BIOPEST as a pest management tool and this document aims to provide detail on recent results from that program.

The content of this document does not in any way constitute a recommendation by SACOA to use BIOPEST outside of its registered use.

BIOPEST Paraffinic Oil

The BIOPEST oil used in the trials reported here is an emulsified nC24 petroleum spray oil (PSO) with a 50% distillation temperature and an unsulphonated residue (UR) of >98% and was supplied by SACOA Pty Ltd to Dr Robert Mensah, NSW Department of Agriculture, Narrabri and Ms Adriana Najar, The University of Queensland, Brisbane.

BIOPEST's formulation utilises a pharmaceutical grade iso-paraffinic base oil that provides an excellent balance of efficacy and safety. BIOPEST's base oil is supported by a surfactant package that delivers reliable mixing stability in the tank and excellent coverage enhancement on the crop.

Several years of in-field research in cotton and a range of commercial food crops have highlighted BIOPEST's ability to deliver a unique combination of crop and grower safety and efficacy against a range of pests and diseases.

Cotton Research Program

The primary goal of SACOA's Cotton Research Program is to test, validate and extend the use of spray oils in cotton IPM programs. SACOA is currently spearheading this goal via research into their BIOPEST formulation.

The premise underlying this research commitment is relatively simple – that BIOPEST's unique formulation can deliver improved pest control, reduce the negative effect of traditional chemistry on pest resistance and beneficial insects, and provide economic benefits in the form of reduced spray costs without any negative effect on yields.

But talk is cheap, and this belief is founded on precedent and evidence. Precedent from other commercial crops such as bananas and citrus and evidence from previous season's research in cotton.

SACOA's Cotton Research Program is driven via partnerships with leading independent research groups who design and conduct the extensive trial, mesh house and laboratory programs. SACOA then disseminate key findings to the industry via publications such as this one, online at www.sacoa.com.au and via regular discussions with consultants, resellers and growers.

This approach not only ensures that SACOA share their knowledge with the industry but also that BIOPEST continues to deliver on the current and future needs of the cotton industry.

SACOA's Cotton Research Program evidences a long term commitment by a company that is dedicated to developing their position as leaders in spray oil and adjuvant technology.

PSOs in Cotton

Generally, the concept and principles of Integrated Pest Management (IPM) do not exclude the use of synthetic insecticides as a control measure per se, instead they aim to reduce sole dependence upon synthetic insecticides for pest control. This is because IPM is a containment strategy, not an eradication strategy.

As a logical part of research into IPM strategies, the question should be asked: "Can other IPM tools such as petroleum spray oils (PSOs) be used as adjuvants of synthetic insecticides to reduce the rate of insecticide sprays without affecting insecticide efficacy and cotton yields?"

PSOs have been used as stand-alone insecticides, or adjuvants of other insecticides, in the control of many pests of agricultural crops in many parts of the world. Within the cotton industry, until recently, PSOs have been regarded only as ULV bulking agents and carriers, rather than as adjuvants of the synthetic insecticides they carry.

Recently, the insecticidal properties of PSOs themselves have been used to deter Heliothis egg lay, cause mortality in Helicoverpa spp. neonate larvae, and to improve the efficacy and persistence of biological insecticides against Helicoverpa spp. larvae (Mensah et al. 2004). They are also less toxic to predators (Mensah et al. 2001; Mensah et al, 1995) and parasitoids (Trichogramma spp.) (Parker et al., 2004; Al Dabel et al 2004).

Abstract

Aphid Trials

NOTE: BIOPEST is registered for use in the control of aphids in cotton.

A series of six field, mesh-house and laboratory experiments were conducted to assess the efficacy of BIOPEST oil against the cotton aphid, (Aphis gossypii Glover) on cotton to provide the data necessary for the incorporation of Petroleum Spray Oils (PSOs) into cotton integrated pest management systems.

BIOPEST oil at concentrations between 1% and 10% at a spray volume of 82 L/ha produced high levels on mortality of aphids on cotton. Aphid death was rapid, with most mortality occurring within 15 minutes of spraying. In only one trial were there differences between application concentrations, with 1% oil being less efficacious than rates between 2% and 10% for some developmental stages. Rates of 5% and 10% did not produce higher levels of mortality than 2% in any experiment.

Aphid mortality occurs from the direct interaction of the oil with the aphid; there was no evidence for mortality caused by vapours from the oil, or trans-laminar or systematic actions. Mortality was higher on young than old leaves. In the field, aphids occur most commonly on young leaves. Oil residues on leaves produced aphid mortality for up to 9 days after spraying, with mortality at Day 8 being approximately 50% of Day 1 mortality.

BIOPEST sprayed plants did not repel aphids from landing, but survival of alates was reduced once on the sprayed plants, as was the subsequent production of nymphs. Prophylactic application of BIOPEST oil (two sprays 9 days apart) controlled aphids on cotton as well as Regent insecticide at low aphid-infestation pressures. At high aphid-infestation pressures, BIOPEST oil reduced aphid numbers to approximately 15% of the untreated control, compared to 5% for conventional insecticides.

These experiments indicate that at low infestation aphid rates, BIOPEST oil alone at a concentration of 2% at a spray volume of 82 L/ha can control of cotton aphid on cotton as well as chemical insecticides such as Regent. At high aphid infestation rates, the level of control is moderate (approximately 85%) compared to that achieved by chemical insecticides (approximately 95%).

These results indicate that BIOPEST oil can have a valuable role in integrated pest management systems for cotton in Australia, removing the need for chemical insecticide sprays that have the potential to disrupt the control of other pests by natural enemies. Additionally, BIOPEST oil could provide an alternative to standard chemical aphicides on cotton, thus increasing the scope for effective resistance management programs for cotton aphids.

Green Mirid and Helicoverpa

Trials

Two season-long field experiments were conducted at ACRI, Narrabri, and Greenbah, Moree, during the 2003-04 season to evaluate the impact of adding BIOPEST oil to standard chemical insecticide regimes. These experiments showed that a half-rate insecticide regime plus 2% BIOPEST oil can produce control of green mirids equivalent to a full-rate insecticide regime.

Neither experiment detected any significant change in Helicoverpa egg densities in treatments including BIOPEST oil, compared to the full-rate insecticide regime. Both experiments showed significant reductions in VS+S larval densities from the addition of BIOPEST oil, compared to the equivalent insecticide-only regime. That is, the addition of BIOPEST oil to insecticide treatments regimes produces significantly greater mortality of Helicoverpa larvae than occurs in the equivalent insecticide-only regimes.

These two experiments also demonstrated that a 2% BIOPEST oil + half-rate insecticide regime produces control equivalent of Helicoverpa VS+S larvae to a full-rate insecticide regime.

Laboratory Research

Mode of Action

The primary mode of action for a spray oil has traditionally been recognised as suffocation and drowning, However recent research on BIOPEST's proprietary formulation highlighted three new modes of action occurring.

The following treatments were recorded via a confocal microscope with fluorescent light and a contrast phase microscope.:

- BIOPEST oil+ Fluorescent dye (Red Nile)
- nC24 without emulsifier + Red Nile
- Emulsifier alone + Red Nile

Through the use of these fluorescent dyes researchers at the University of Queensland have observed that BIOPEST's unique formulation, by penetrating the insect cuticle and accumulating in the nerve and fat-containing tissues, is providing at least three new modes of mortality:

- A nervous system effect
- A desiccation effect
- A storage tissue effect

Importantly, the requirement for cuticle penetration to occur would appear to limit the potential for nC27 oils to provide these additional modes of action.

research results

New Modes of Action Identified

The primary mode of action for a spray oil has traditionally been recognised as suffocation and drowning, However recent research on BIOPEST's proprietary formulation has highlighted three new modes of action occurring.

METHODOLOGY

Treatments:

- BIOPEST oil+ Fluorescent dye (Red Nile)
- nC24 without emulsifier + Red Nile
- Emulsifier alone + Red Nile

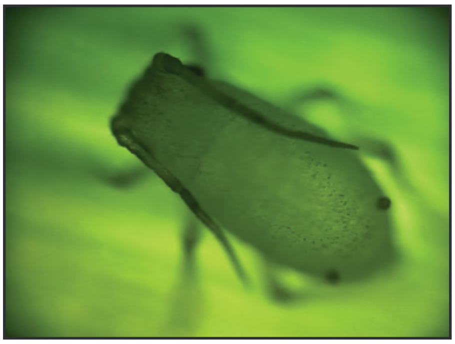
Observations recorded via a confocal microscope with fluorescent light and a contrast phase microscope.

RESULTS

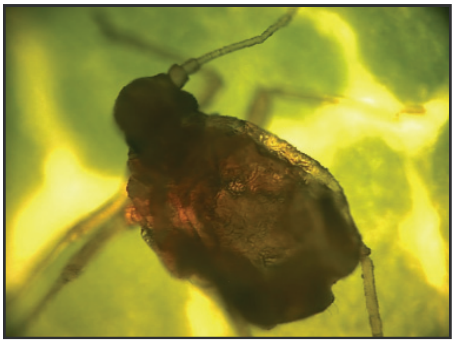
Through the use of fluorescent dyes researchers at the University of Queensland have observed that BIOPEST's unique formulation, by penetrating the insect cuticle and accumulating in the nerve and fat-containing tissues, is providing at least three new modes of mortality. Importantly, the requirement for cuticle penetration to occur would appear to limit the potential for nC27 oils to provide these additional modes of action.

MODE ONE: NERVOUS SYSTEM EFFECT

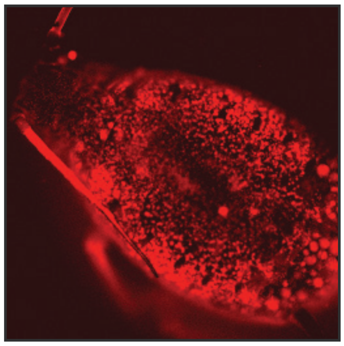
BIOPEST is affecting the motor neurons that control the activity of the muscles operating the spiracles, legs, antennae, mouthparts and last abdominal segment of the insects.



Aphid before BIOPEST



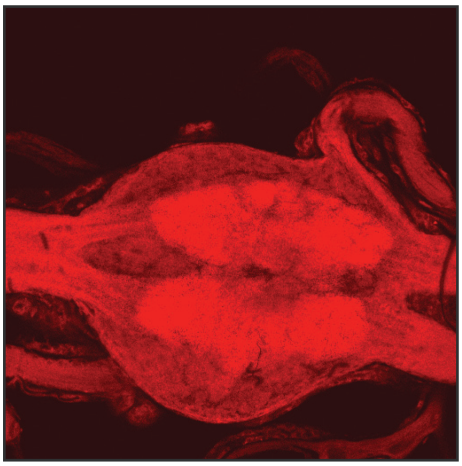
Aphid after BIOPEST



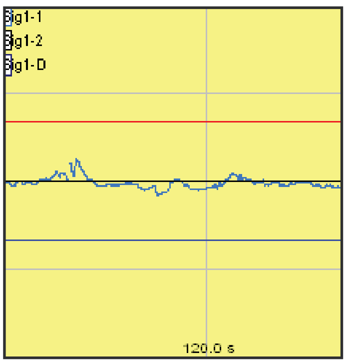
Aphid 2 min after BIOPEST with dye showing penetration of cuticle

Because the nerves of one ganglion are connected to the nerves of the next and of the previous ganglion there is a signal induced by the oil which moves along the ventral cord of the insect. This probably explains the wave-like contractions observed on dying caterpillars.

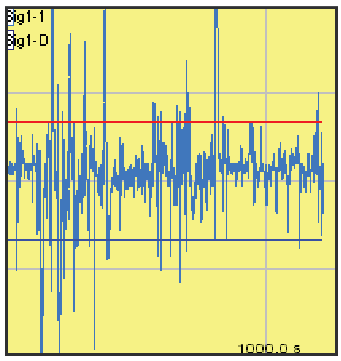
Death of the insect appears to occur when the accumulation of the oil in the nerves of the insect reaches a lethal or toxic level. Thus, time to kill depends on the volume and concentration of oil applied to the insect.



Dye shows BIOPEST penetrating into nerve ganglia



Nerve activity before BIOPEST

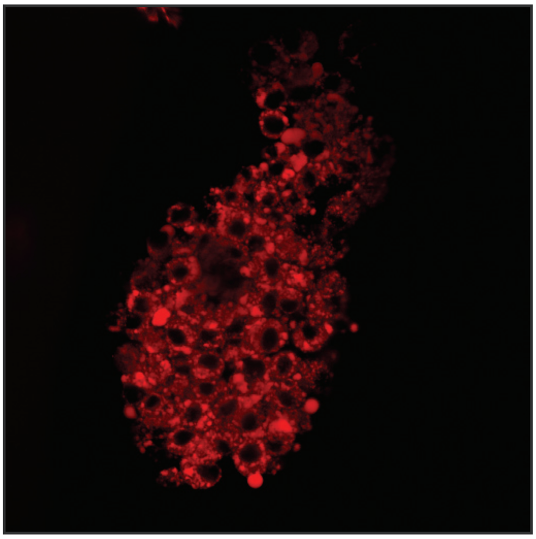


Nerve activity after BIOPEST

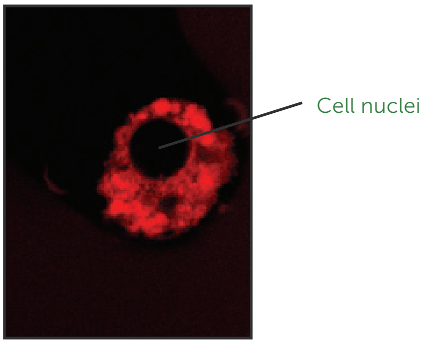
New Modes of Action Identified

EFFECT OF BIOPEST ON INSECT CELL MEMBRANE PERMEABILITY

BIOPEST appears to penetrate the cell membrane but not the nuclear membrane indicating that the accumulation of oil into the insect nerve cells could be the main mechanism underlying the efficacy of BIOPEST against insects.



Insect cells stained with BIOPEST



Isolated insect cell

MODE TWO: DESICCATION EFFECT

The desiccation of the insect bodies observed after death indicates BIOPEST is affecting the permeability of the insect cuticle.

Even if the oils were not accumulating in the nerves of the insects, desiccation will ultimately lead to the death of the insects.

MODE THREE: STORAGE TISSUE EFFECT

Fat bodies are storage tissues that supply energy for metabolic activities within the insect body.

So, the accumulation of the oils into the fat bodies of the insects will also lead to insect death.

Aphid Trials

Effect of nC24 v nC27 on toxicity and persistence of oil deposits

METHODOLOGY

MESH-HOUSE TESTS

Six groups of potted cotton plants (n=10 plants/group), at the flowering stage, with 10 leaves each, were sprayed with one of the following five treatments:

- BIOPEST at 2% (v/v).
- nC27 (Caltex Canopy®) at 2% oil (v/v).
- Imidacloprid (treated control 1).
- Fipronil (treated control 2).
- Water (untreated control).

Every 24h after spraying, for 11 days, a new leaf on each plant was randomly selected for infestation with a new cohort of 25 aphids (5 adults and 20 nymphs). Each infested leaf was subsequently caged and aphid mortality and numbers (surviving aphids and newly born ones) were counted every 24h for seven days after initial exposure of the aphid cohorts (5 aphid cohorts in total) to the oil deposits.

RESULTS

Deposits of BIOPEST are as toxic to aphids, or even more toxic than those of an nC27 (Caltex Canopy®) up to 5 day after treatment. However, the deposits of an nC27 oil persist longer on/in the cotton leaves.

Indeed, cumulative mortality percentages inflicted on those aphid cohorts exposed to the heavier oil deposits were lower and statistically different (P=0.05) from those recorded for aphids exposed to the deposits of BIOPEST oil, at least on days 1 and 3 after treatment.

The nC27 oil did have a longer toxic life than BIOPEST, though. The deposits of BIOPEST oil remained effective against aphids up to 8 days after spraying whereas those of the nC27 continued to affect aphids, though at very low levels (≈10% mortality), up to 11 days after spraying.

The "behaviour" of the deposits otherwise proved to be the same for the two oils. Older deposits inflicted lower mortalities in all cases.

None of the oils induced phytotoxicity symptoms, and this is probably related to the oil quality and the presence of ultra-violet light protective compounds in the oil formulations.

Results presented in Fig 1 below.

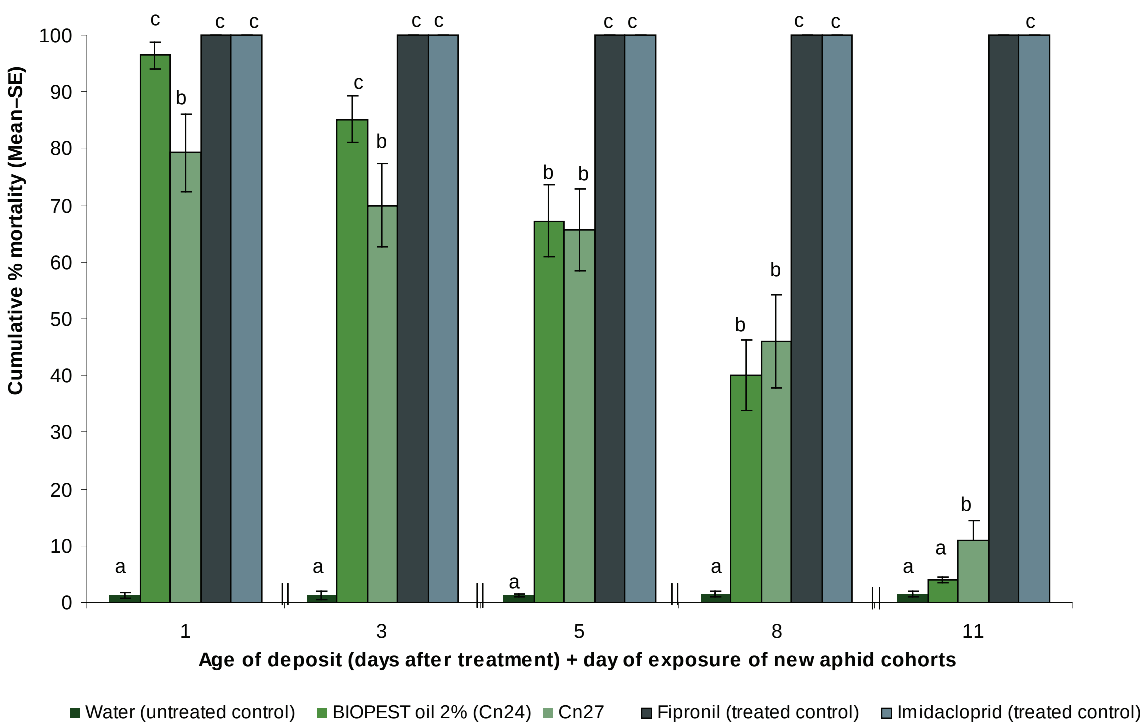


FIG 1: Cumulative mortalities of Aphis gossypii cohorts (one cohort/day) at day 7 after exposure of each cohort to oil deposits of different ages (i.e. days after spraying). Means within a day followed by the same letters are not significantly different from one another (p>0.05) (Scheffeé Multiple Comparison Test).

Aphid Trials

Effect of oil concentration on aphid mortality

METHODOLOGY

MESH-HOUSE TESTS

Six groups of flowering, 5-leaf-stage, aphid-infested potted cotton plants (10 plants/group) were sprayed to run-off on both surfaces, with one of the following six treatments:

- BIOPEST oil at 1%, 2%, 5% and 10% v/v (at a spray volume equivalent to 82L of water/ha),
- Distilled water (at a rate equivalent to 82L of water/ha) (untreated control) and
- Regent® (Fipronil 200g/L at a field rate of 64.5 mL/ha in 82L of water/ha) (treated control).

FIELD TESTS

Three groups of flowering, aphid-infested plants (24 plants/group) were randomly selected at three sites within a conventional cotton field at ACRI. Each site was considered a separated block. Four plants per group (block) were treated as per the mesh house trials and individually caged soon after.

RESULTS

Results were consistent for both mesh house and field trials, supporting the value of mesh house trials for the evaluation of BIOPEST.

- Regent, the treated control, again reduced aphid numbers by the 1-day post-treatment count, but took several more days to reach final mortality.
- All rates of BIOPEST oil killed aphids rapidly, reaching final mortality within 1 day of spraying, with no recovery of populations within 14 days of spraying.
- All BIOPEST rates from 1% to 10% were equally effective in controlling aphids on cotton leaves in the field.
- While BIOPEST oil significantly reduced survival of all life stages, alates showed somewhat lower mortality from BIOPEST oil than apterae.

Results presented in Fig 2 below.

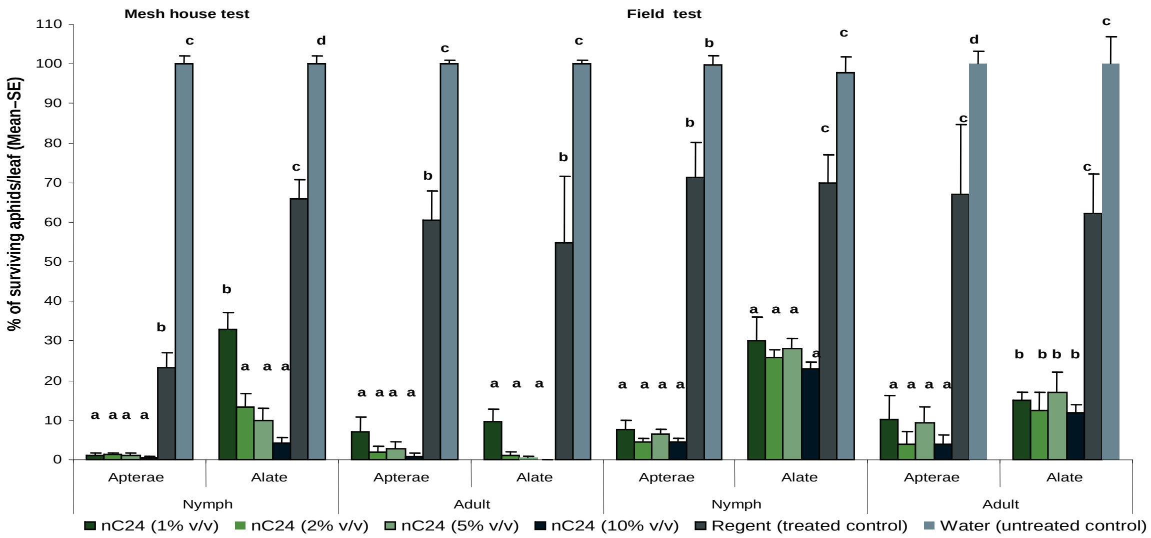


FIG 2: Effect of BIOPEST nC24 oil concentration on survival of four aphid life stages on cotton plants in the mesh-house and in the field 1-day after spraying. Within each trial and insect life stage, treatments followed by the same letter are not significantly different (Scheffé Multiple Comparison Test, P = 0.05).

Aphid Trials

Rate of action of the BIOPEST oil

METHODOLOGY

Three groups of equal-sized aphid-infested leaves (20 leaves/ group) were selected from cotton plants in a conventional field and sprayed in situ on both surfaces to run-off with a hand-held pressure sprayer with the BIOPEST oil at 2% or 5% v/v, or with distilled water at a spray volume of 82L of water/ha.

The number of aphids per leaf was counted prior to treatment application. Immediately after spraying, leaves were detached from their plants and taken back to the laboratory. To test for any effect of vapours on increasing the killing efficacy of the oils, each treatment of 20 leaves was then divided into two subgroups of ten leaves each, and placed individually into Petri dishes that were either covered with a lid (to retain fumes) or left uncovered.

Mortality was assessed at 5, 10, 15 and 30 minutes after treatment and thereafter at 1, 2, 3, 4, 5, 6, 12 and 24 h after spraying. Aphids were considered dead if they did not move when dislodged gently with a fine brush from their feeding sites.

To test whether the volume of applied oil influences its killing efficacy, three groups of aphid-infested cotton leaves (10 leaves/ group), were treated with the BIOPEST oil at 2% or 5% v/v or with distilled water (as above), but the volume of spray was reduced to half the run-off volume.

RESULTS

This experiment showed that BIOPEST oil kills cotton aphids quickly, with almost all mortality occurring within the first 10 minutes of spraying.

Additionally, the experiment demonstrated that the other factors examined in this experiment had no significant effect on final mortality. BIOPEST oil rate (2% vs 5%) and spray volume (run-off vs ½ run-off) did not affect final mortality.

Additionally, the vapours from BIOPEST oil did not contribute to the mortality because there were no differences between covered and uncovered leaves in this experiment, pointing to the mode of action of BIOPEST oil being by direct contact.

Results presented in Fig 3 below.

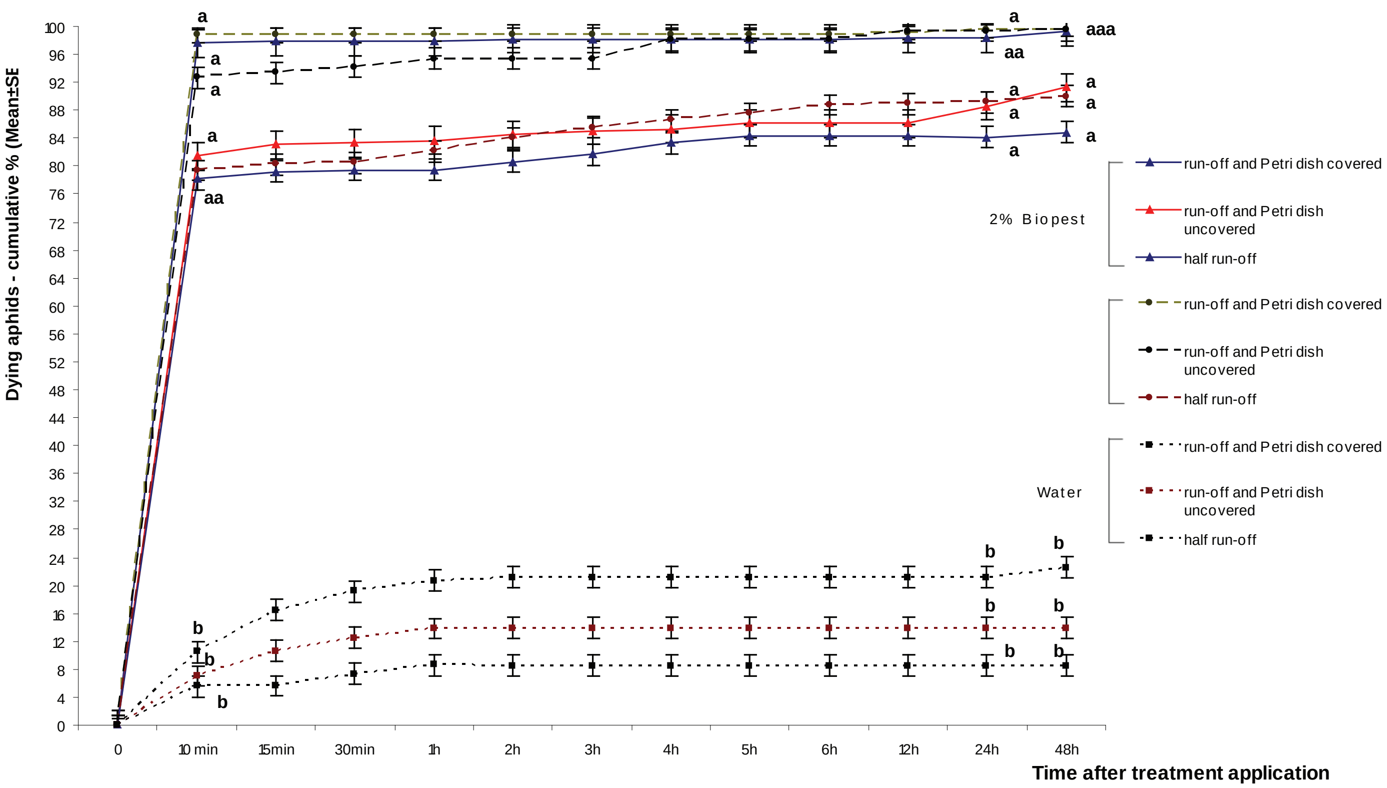


FIG 3: Effect of BIOPEST oil and water sprays on aphid survival in the laboratory. Within each time interval, means followed by the same letters are not significantly different (Scheffé Multiple Comparison Test, P = 0.05).

Aphid Trials

Effect of leaf surface sprayed on aphid mortality

On cotton, aphids feed predominantly on the lower surface of leaves (Gonzaga et al., 1991; Singh et al., 1993; Saha & Raychaudhuri, 1995). For some pesticides, application to the upper leaf surface can kill aphids feeding on the lower leaf surface via trans-laminar movement. Additionally, some pesticides can be distributed to other plant parts, killing aphids via systemic action.

METHODOLOGY

Two groups of 40 equal-sized cotton leaves were sprayed on plants in the field with either the BIOPEST oil (2%) or distilled water (control) on the adaxial or the abaxial surface (10 leaves per treatment and leaf surface) at a spray volume equivalent to 82L/ha.

After spraying, the first group of leaves were detached and taken back to the laboratory for periodic mortality assessments. Aphids, regardless of treatment, no longer accept leaves detached for more than 48h as hosts because of desiccation. The second group of leaves were left attached to the plants in the field for 72h after spraying and caged after spraying to exclude predators and prevent migration.

RESULTS

- BIOPEST applied to the lower leaf surface is highly effective at controlling the aphids feeding there.
- BIOPEST applied to the upper leaf surface has no effect on the survival of aphids feeding on the lower leaf surface. This indicates that BIOPEST oil has no systemic or trans-laminar effect on cotton aphids, only direct contact action.
- These results emphasises the importance of appropriate application of BIOPEST oil for aphid control on cotton.
- Mortality recorded in the field and the laboratory from field-sprayed plants was virtually identical. This result extends the capacity to extrapolate from laboratory-generated data to field performance.

Results presented in Fig 4A and 4B below.

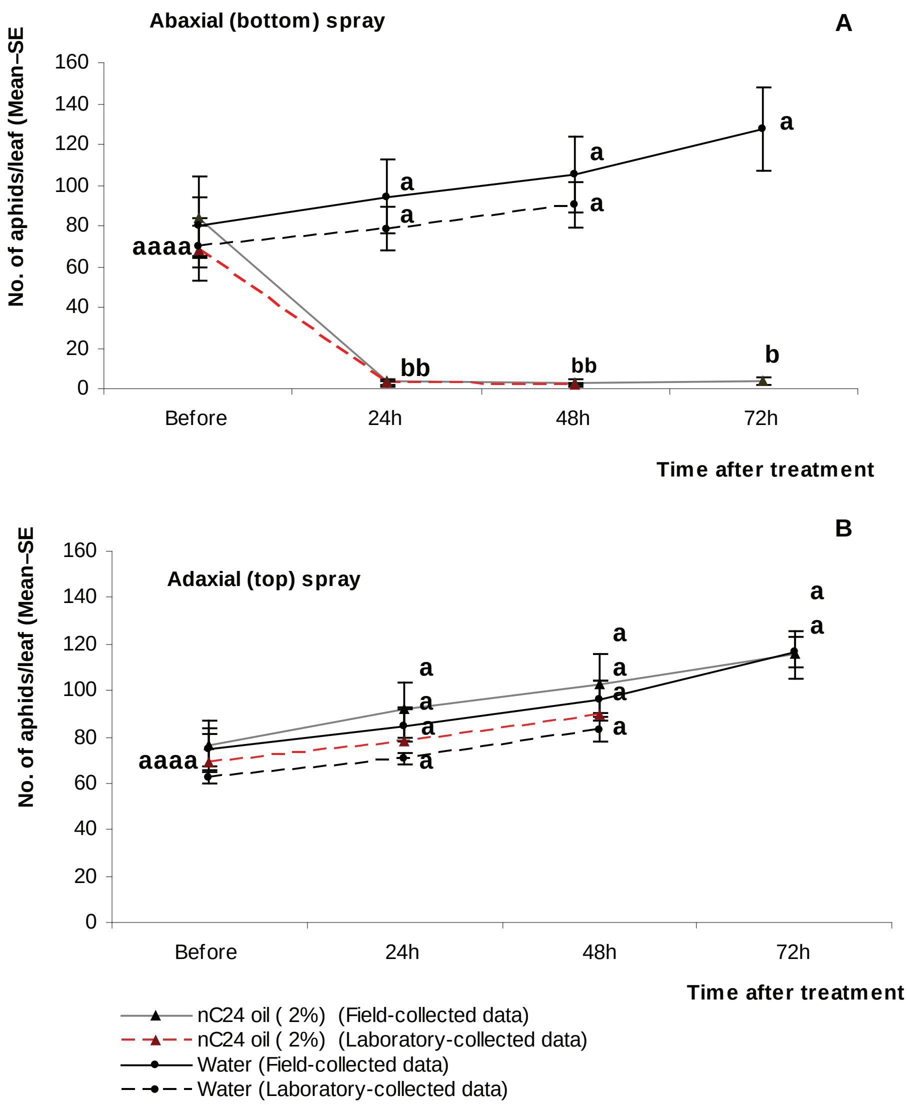


FIG 4A and B: Effect of leaf surface of spraying on aphid mortality. Within each graph and time, means followed by the same letters are not significantly different (p>0.05) (Scheffés Multiple Comparison Test).

Aphid Trials

Duration of activity

METHODOLOGY

In a mesh-house, a group of flowering, 10-leaf-stage, aphid-infested potted cotton plants (6 groups; 10 plants/group) were sprayed with one of the six following treatments:

- BIOPEST oil at 1%, 2%, 5% and 10%v/v (at a spray volume equivalent to 82L of water/ha),
- Distilled water (at a rate equivalent to 82L of water/ha) (untreated control), or
- Regent® (Fipronil 200g/L at a field rate of 64.5 mL/ha in 82L of water/ha) (treated control).

Every 24 hours after spraying for nine days, two unused leaves (one new, one old) on each treated plant was randomly selected for infestation with a new cohort of 25 aphids (5 adults and 20 nymphs representing all nymphal instars). All aphids were manipulated with a fine camel hair brush. Each infested leaf was subsequently caged and aphid mortality and numbers (surviving aphids and newly born ones) were assessed every 24 h for seven days after initial exposure of the aphid cohorts to the oil deposits.

RESULTS

In this experiment, the treated and untreated controls performed as expected from previous experiments (Figures 1 - 5). Table 1 presents the results for BIOPEST oil from this mesh-house experiment. It is clear that BIOPEST oil deposits reduce aphid survival, and remain at least partially effective against the aphids, up to eight days after spraying. Deposits of BIOPEST oils are equally effective across a range of concentrations between 1% and 10%. Mortality from residues after 6 days is approximately half that of 1-day-old residues.

Leaf age was a significant factor in the efficacy of the deposits, with significantly greater mortalities on young leaves than mature ones. In the field, aphids are mostly found on young leaves towards the top of the plant, rather than old leaves. On the basis of this data and the demonstration that laboratory and field assessments produce equivalent results (Figure 5), the residues from BIOPEST oil applications in the field can be expected to cause mortality of aphids they contact for up to 8-days after spraying.

Results presented in Table 1 below.

Age of deposit (DAT)	Leaf age	Percentage mortality after 24 hours exposure (Mean ± SE)					
		Water (untreated control)	Regent ® (treated control)	BIOPEST oil concentration			
				1%	2%	5%	10%
1	Young	0.0±0.00 a	0.0±0.00a	88.3±9.53 b	84.8±10.25 b	71.6±6.98 b	76.4±12.32 b
	Mature	0.0±0.00a	0.0±0.00a	12.1±3.16 c	14.6±5.81 c	16.3±5.52 c	14.3±4.10 c
2	Young	0.0±0.00a	1.6±4.00 a	78.4±7.54 b	70.7±5.29 b	71.2±10.81 b	75.3±12.95 b
	Mature	0.0±0.00a	0.0±0.00a	8.3±4.20 c	12.5±3.09 c	16.7±10.24 c	11.3±2.39 c
3	Young	0.0±0.00a	0.0±0.00a	56.8±9.14 b	50.5±10.80 b	69.7±10.5 b	54.4±10.61 b
	Mature	0.0±0.00a	3.2±1.20 a	12.3±6.50 c	10.8±6.23 c	8.8±6.25 c	6.5±2.74 c
4	Young	0.0±0.00a	0.0±0.00a	57.6±9.77 b	57.1±5.72 b	68.2±7.89 b	69.4±7.43 b
	Mature	0.0±0.00a	3.2±2.01 a	8.4±3.92 c	13.3±7.06 c	11.3±4.45 c	11.2±3. 45 c
5	Young	0.0±0.00a	0.6±0.94 a	58.2±9.70 b	56.3±9.76 b	52.6±13.72 b	62.2±18.21 b
	Mature	0.0±0.00a	0.4±0.20 a	3.2±1.20 a	3.3±3.28 a	12.1±3.12 c	13.5±9.33 c
6	Young	0.0±0.00a	0.8±0.20 a	44.5±19.00 b	43.3±6.7 b	49.3±3.57 b	47.3±10.21 b
	Mature	0.0±0.00a	0.0±0.00 a	13.5±5.22 c	3.43±0.98 a	10.0±2.40 c	3.43±1. 23 a
7	Young	0.0±0.00a	0.0±0.00a	26.4±2.20 b	28.4±5.27 b	35.4±127 b	30.7±17.25 b
	Mature	0.0±0.00a	0.8±0.10 a	9.33±2.43 c	4.48±1. 09 a	6.4±2. 09 a	8.7±2.45 c
8	Young	0.0±0.00a	2.4±1.25 a	16.2±1.65 b	22.5±2.66 b	32.2±2.28 b	35.8±2.32 b
	Mature	0.0±0.00a	0.0±0.00a	5.5±1.09 a	1.70±0. 87 a	7.0±2. 09 a	3.5±1.23 a
9	Young	0.0±0.00a	0.0±0.00a	7.2±1.54 b	4.0±1. 09 a	3.3±1. 21 a	0.0±0. 00 a
	Mature	0.0±0.00a	0.0±0.00a	0.0±00.0 a	1.0±0. 67 a	1.0±0. 56 a	0.0±0. 00 a

TABLE 1: Effect of oil concentration and deposit age on cotton aphid mortality on mature leaves and young leaves after 24 hours exposure. Means within each deposit age followed by the same letter are not significantly different (p>0.05) (Scheffé Multiple Comparison Test).

Aphid Trials

BIOPEST oil and host plant attraction, acceptance and suitability for alate aphids

METHODOLOGY

This experiment examined the propensity of alate aphids flying out of an infested cotton field to land on cotton plants treated with BIOPEST oil.

6 groups of potted cotton plants (10 plants/group) were sprayed to run off by hand-sprayer with one of the six following treatments:

- BIOPEST oil at 1%, 2%, 5% and 10%v/v (at a spray volume equivalent to 82L of water/ha),
- Distilled water (at a rate equivalent to 82L of water/ha) (untreated control), or
- Regent® (Fipronil 200g/L at a field rate of 64.5 mL/ha in 82L of water/ha) (treated control).

One hour after spraying, all plants were placed at one of the edges of an aphid-infested cotton field (with a high incidence of emigrating alates) for 48 hours. Pest counts were then done periodically.

Those test plants that became infested with alates were caged and placed under mesh-house conditions where further pest counts were conducted periodically to test whether these nymphs could feed and reproduce successfully on the sprayed plants with BIOPEST oil residues.

RESULTS

- The colony numbers on oil-treated plants 9 days after spraying were half that on the water-treated plants, demonstrating clearly the impact of 2% BIOPEST oil applications on the longer-term development of aphid populations on cotton.
- All aphids landing on BIOPEST-treated plants were dead by 4 DAT, i.e. within 2 -3 days of alighting, compared to more than 80% survival on untreated plants at the same time. This result indicates that while infestation rates of BIOPEST-treated plants would be the same as untreated plants, the reduced alate survival on BIOPEST-treated plants would be a key component of achieving lower aphid populations over longer periods.
- The rate of nymphal production by the founding group of alates was reduced. Consequently, the production of adults by these nymphs and the growth of a subsequent generation of nymphs by the new adults were also affected.
- All BIOPEST treatments had produced the same number of nymphs after 48-hours as the water-sprayed plants, indicating that there was no immediate effect on rate of reproduction once on the treated plants.

Results presented in Table 2 and Fig 5 below.

Treatment	Cumulative % of aphids dying /plant (Mean ± SE)					
	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
Water (untreated control)	0.93 ±0.41 d	5.1±0.08 d	19.6±0.11 cd	61.7±0.09 b	96.3±8.06 a	100.0±0.00 a
Regent (treated control)	99.8±10.00 a	100.0± 0.00 a	100.0±0.00 b	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
BIOPEST oil (1%)	22.9±3.14 c	59.7±9.11 b	100.0±0.00 b	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
BIOPEST oil (2%)	23.4±6.14 c	61.6±11.09 b	100.0±0.00 b	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
BIOPEST oil (5%)	28.3±3.09 c	75.5±15.05 b	100.0±0.00 b	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
BIOPEST oil (10%)	32.3±4.12 c	72.9±9.09 b	100.0±0.00 b	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a

TABLE 2: Effect of BIOPEST oil ® deposits on the mortality of cotton aphid alates attracted to oil-sprayed cotton plants. Mean ± SE shown from Day 2 to Day 7 after treatment (DAT= Days after oil treatment). Means within a column followed by the same letter are not significantly different (p>0.05) (Scheffé Multiple Comparison Test).

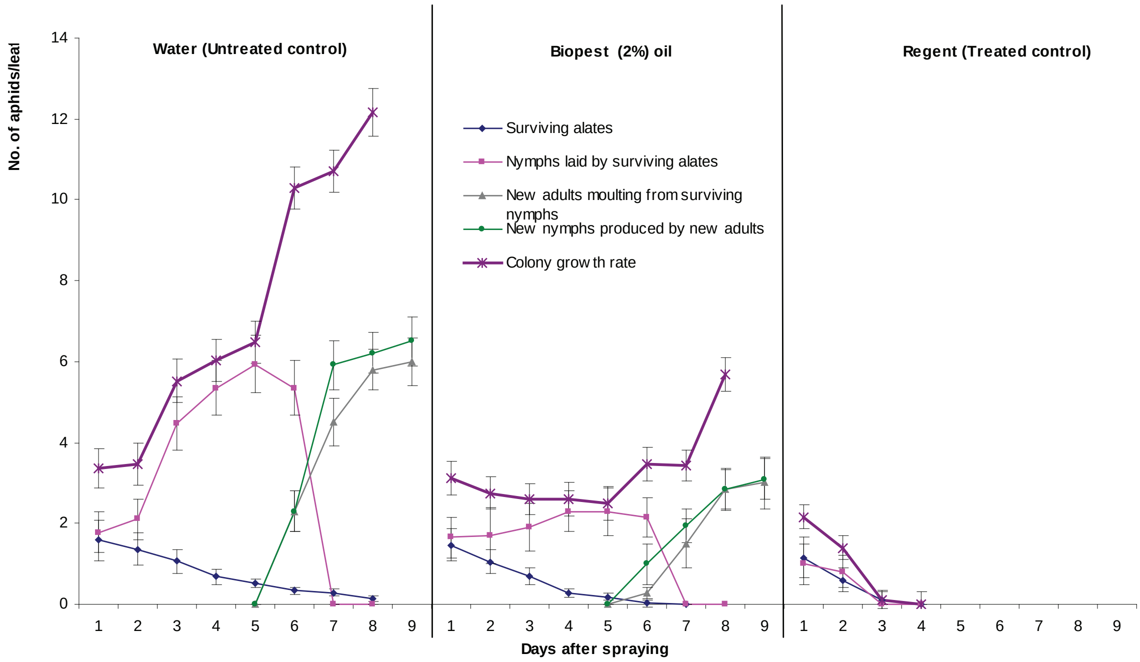


FIG 5: Aphid population growth on BIOPEST and Regent sprayed cotton plants where alates landed on sprayed potted plants adjacent to an infested field. Plants moved to the mesh-house 48-hours after spraying and populations counted daily until Day 9.

Aphid Trials

Effect of prophylactic applications of BIOPEST oil on Aphis gossypii mortality

METHODOLOGY

This experiment provides an estimate of the degree of direct and cumulative impact that could be expected in the field from a series of BIOPEST oil sprays.

Five groups (blocks) of twelve-week-old cotton plants (8 plants/group) were randomly selected within a conventional cotton field. At flowering stage (with no aphids present), two plants within each group were sprayed with one of the following four treatments:

- BIOPEST oil 2% v/v (in 82L of water/ha),
- Water (at 82L of water/ha) (untreated control),
- Confidor (treated control 1) (imidacloprid 200g/l at a field rate of 250 mL/ha in 82L of water/ha)) and
- Regent (Fipronil 200g/L at a field rate of 64.5 mL/ha in 82L of water/ha) (treated control 2).

Following spraying, two leaves (1 leaf/plant) per treatment in each group were infested with one of two aphid densities: 10 aphids (3 adults and 7 nymphs of mixed stages) or 60 aphids (18 adults and 42 nymphs of mixed stages). The same procedure was repeated across the remaining four plant groups.

The number of surviving aphids was assessed daily. To simulate arrival of new aphids, a new group of 10 leaves per treatment (across all 5 groups) was infested every three days, as above. The new aphids were not added to the existing colonies, to keep them differentiated and to allow assessment of the residual activity of the oils on the new arrivals every few days.

All plants were re-sprayed on day nine. Again, every three days after spraying, a new group of 10 leaves per treatment was randomly selected, infested with one of the two aphid cohorts, and evaluated as previously described.

RESULTS

- Prophylactic applications of BIOPEST oil (2%) prevented the development of aphid populations compared to the untreated control (Figures 9 and 10).
- At high aphid infestation rates (Figure 9), aphid populations on BIOPEST-treated plants were on average approximately 15% of those on the untreated control.
- While this population level was higher than on the Regent and Confidor treatments (approximately 5% of untreated control), this reduction represented a considerable degree of aphid population suppression.
- At low infestation rates (Figure 10), populations on BIOPEST-sprayed plants remained close to zero for the entire period of the experiment, compared to a population explosion on the untreated plants.
- At low infestation rates, BIOPEST oil provided control almost identical to the conventional insecticides Regent and Confidor.

Aphid mortality in the individual cohorts of this experiment, showed the same relationships with age of oil deposit as documented in Figure 6. That is, mortality from BIOPEST oil soon after spray application was high (70 – 90%), falling as the spray deposit aged, to be 5 – 20% after 6- 8 days after a spray. This is reflected in the population change on BIOPEST oil treatments between Days 8 – 10 and after Day 16 (Figures 6 and 7).

This experiment indicates that at low aphid infestation rates, BIOPEST oil alone can control aphid populations. Where infestation rates are high, use of registered aphicide in conjunction with BIOPEST oil may be required to keep aphids at low levels, but testing such combinations was beyond the scope of this series of experiments.

Results presented in Fig 6 and Fig 7 at right.

Aphid Trials

Effect of prophylactic applications of BIOPEST oil on Aphis gossypii mortality

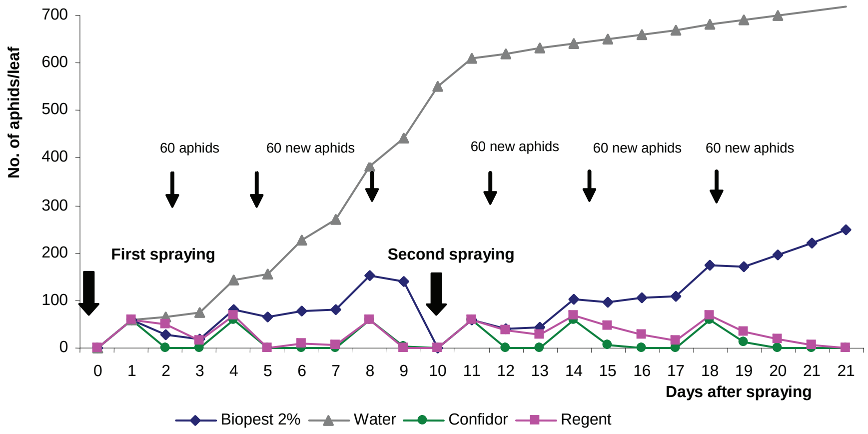


FIG 6: Effect of prophylactic applications of BIOPEST oil (2%) on aphid population growth at high aphid infestation rates.

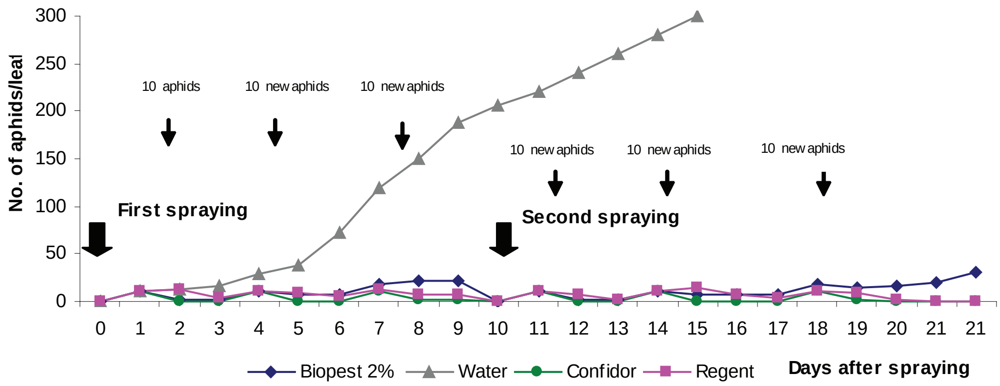


FIG 7: Effect of prophylactic applications of BIOPEST oil (2%) on aphid population growth at low aphid infestation rates.

Mirid and Helicoverpa Trials

Australian Cotton Research Institute, Narrabri (2003/04)

METHODOLOGY

This season-long experiment, conducted at the Australian Cotton Research Institute (ACRI), Narrabri during the 2003 – 2004 season, compared four treatments:

- Standard insecticide treatments at full rates,
- Standard insecticide treatments at half rates
- Full rate insecticides + 1% BIOPEST oil, and
- Half-rate insecticide treatments + 2% BIOPEST oil. Prior to 20 January, sprays were applied with a ground rig at 100 L/ha.

After that date application was via fixed wing aircraft at 30L/ha (R. Mensah, personal communication, 16/2/2006).

The trial was laid out in a randomised block design with 3 replications and 2 insect samples were taken per plot on each of 7 sampling dates between 12 and 69 days after the first treatment. During the season, the range of insecticides included in the chemical treatment regime included imidacloprid (Confidor), emamectin (Affirm) (both once each), plus indoxacarb (Steward) three times.

A total of five foliar applications of each treatment were made from 10 December 2003 until 4 February 2004. The decision to apply these treatments was based on a predator-to-Helicoverpa spp. (pest) ratio of 0.5 (Mensah 2002a; Mensah 2002b). The decision to spray against green mirids was made based on the IPM Guidelines and CottonLogic recommended economic threshold of 0.5 green mirids per metre (CottonLogic 1999).

Visual counts of Helicoverpa spp. on cotton plants in each treatment were made at approximately weekly intervals in two randomly selected 1 metre lengths of row of each treatment replicate, i.e. a total of 8 metres were examined per treatment. Counts were separated into Helicoverpa spp. eggs, very small and small (VS+S) larvae and medium and large (M+L) larvae. Insufficient numbers of M+L larvae were collected for statistical analysis. Green mirids were sampled weekly using a D-vac suction tube (Mensah 1997, Mensah and Khan, 1997; CottonLogic 1999). On each sampling occasion, a 10-metres of row was sampled as a single pass of the vacuum tube along the tops of the plants. After each sampling, the contents of the sampler were transferred to separate plastic bags, taken to the laboratory and counted.

RESULTS

GREEN MIRIDS

Treatment effects were very highly significant (P < 0.001). All treatment comparisons were of interest from the perspective of determining BIOPEST's efficacy, and were tested using the Fisher's Protected LSD test with a sequential Bonferroni correction (to achieve a family-wise probability level of P = 0.05. These multiple-comparison tests showed that a half-rate chemical regime gave inferior control of mirids compared to the full-rate regime and both the treatments with BIOPEST oil.

Additionally, the half-rate chemical + 2% BIOPEST regime was superior to half-rate-alone chemical regime, with an additional 33% mortality being recorded. The full-rate + 1% BIOPEST regime was superior to the full-rate regime, with the addition of 1% BIOPEST producing an extra 30% mortality.

HELICOVERPA EGGS

Treatment effects were highly significant (P < 0.001) (Table 3). All treatment comparisons were of interest from the perspective of determining BIOPEST's efficacy, and were tested using the Fisher's Protected LSD test with a sequential Bonferroni correction (to achieve a family-wise probability level of P = 0.05).

These multiple-comparison tests (Table 3) showed that a half-rate chemical regime had higher Helicoverpa egg densities than the full-rate regime and both the treatments with BIOPEST oil.

Additionally, the half-rate chemical + 2% BIOPEST regime was superior to half-rate-alone chemical regime, with an additional 37% mortality being recorded. The addition of 1% BIOPEST oil to the full-rate chemical regime did not significantly increase mortality over that for the full-rate chemical regime alone.

HELICOVERPA VS+S LARVAE

Treatment effects were highly significant (P < 0.001) (Table 6). All treatment comparisons were of interest from the perspective of determining BIOPEST efficacy, and were tested using the Fisher's Protected LSD test with a sequential Bonferroni correction (to achieve a family-wise probability level of P = 0.05).

These multiple-comparison tests (Table 4) showed that a half-rate chemical regime gave inferior control of VS+S larvae compared to the full-rate regime and both the treatments with BIOPEST oil.

The addition of 2% BIOPEST oil to the half-rate chemical regime significantly reduced populations compared to half-rate alone, with an additional 38% mortality being recorded. The addition of 1% BIOPEST oil to the full-rate chemical regime significantly increased mortality over that for the full-rate chemical regime, with an additionally 29% mortality recorded.

Results presented in Table 3 and Fig 8 at right.

TABLE 3: Season-long efficacy of insecticide and insecticide+ BIOPEST mixtures, ACRI 2003-2004.

Season-long treatment regime	Mirids/m [1]	Helicoverpa eggs/ m[1,2]	Helicoverpa VS+S larvae/m[1]
Insecticides (full-rate)	0.1325 b	1.579 (1.9932) a	1.467 b
Insecticides (full-rate) + BIOPEST 1%	0.0942 a	1.498 (1.7440) a	1.042 a
Insecticides (half-rate)	0.2100 c	1.776 (2.6542) b	2.242 c
Insecticides (half-rate) + BIOPEST 2%	0.1417 b	1.470 (1.6609) a	1.383 b
LSD (P = 0.05)	0.03151	0.1304	0.2596

1 Means followed by the same letter are not significantly different (Fisher's Protected LSD test with a sequential Bonferroni correction to achieve a family-wise probability level of P = 0.05).

2 Data transformed by SQRT(X+0.5) prior to analysis. Equivalent means presented in brackets.

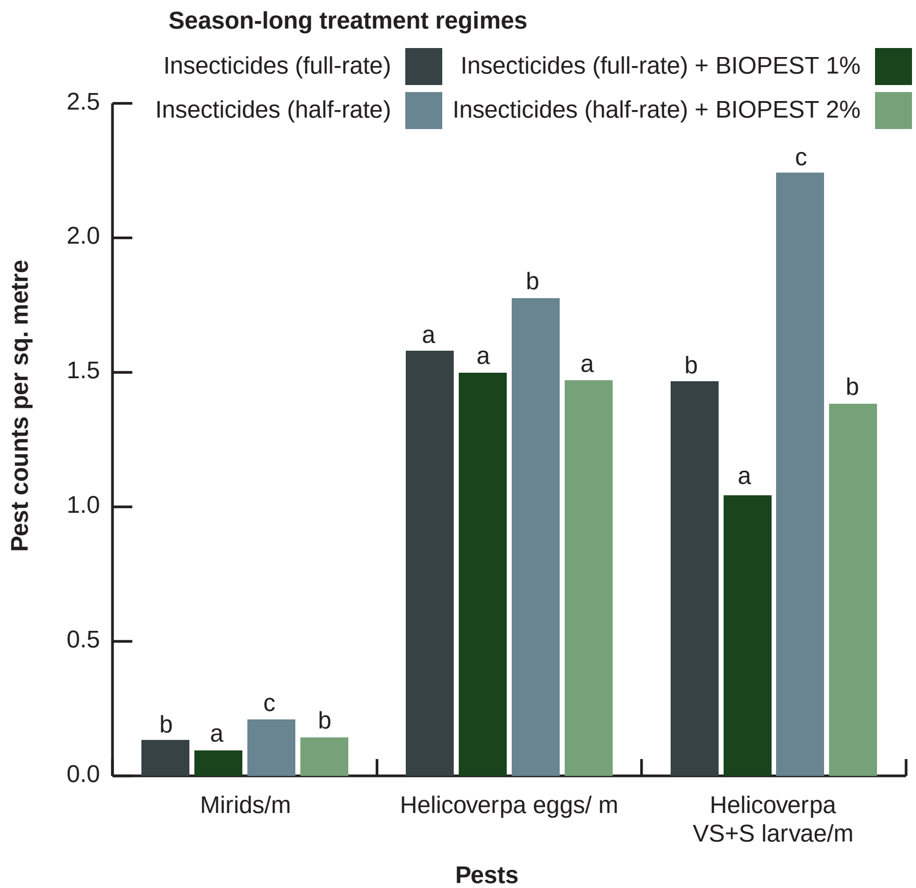


FIG 8: Season-long efficacy of insecticide and insecticide+ BIOPEST mixtures, ACRI 2003-2004.

Mirid and Helicoverpa Trials

Greenbah, Wee Waa (2003/04)

METHODOLOGY

This season-long experiment, conducted at Greenbah, Wee Waa during the 2003 – 2004 season, compared three treatments: (1) standard insecticide treatments at full rates, (2) full rate insecticides + 1% BIOPEST oil, and (3) half-rate insecticide treatments + 2% BIOPEST oil. Prior to 20 January, sprays were applied with a ground rig at 100 L/ha. After that date application was via fixed wing aircraft at 30L/ha (R. Mensah, personal communication, 16/2/2006).

The trial was laid out in a randomised block design with 3 replications and 2 insect samples were taken per plot on each of 7 sampling dates between 12 and 65 days after the first treatment. During the season, the range of insecticides included in the chemical treatment regime included endosulfan, chlorpyrifos (Predator), indoxacarb (Steward) + methyl parathion, and emamectin (Affirm) (all once each), plus indoxacarb (Steward) twice.

The decision to apply these treatments was based on a predator-to-Helicoverpa spp. (pest) ratio of 0.5. The decision to spray against green mirids was made based on the IPM Guidelines and CottonLogic recommended economic threshold of 0.5 mirids/metre.

Helicoverpa spp. were counted using a similar method to experiment 1.2.1. Green mirids were sampled weekly (except rainy days) using D-vac suction tube using a similar method to experiment 1.2.1.

At this site, pest numbers were variable during the season, with Helicoverpa eggs, and very small+ small larvae present at all sampling dates while mirid populations were significant on four of the 7 sampling dates. Only 5 medium+ large Helicoverpa larvae recorded during the whole trial, so it was not possible to conduct for any statistical analysis for this parameter. There was no pre-treatment data for this trial, because treatments started prior to the first insect sampling.

RESULTS

GREEN MIRIDS

There were no significant treatment effects (P = 0.597) (Table 4). From this, it can be concluded that the half-rate chemical + 2% BIOPEST regime provided equivalent control to a full-rate chemical regime. However, the addition of 1% BIOPEST to the full-rate chemical regime did not significantly improve control of green mirids.

HELICOVERPA EGGS

There were no significant treatment effects (P = 0.054) in the ANOVA table , indicating that all three treatments has equivalent Helicoverpa egg densities over the course of the season.

VERY SMALL + SMALL HELICOVERPA LARVAE

There were significant differences between sampling dates (range 0.67 – 3.06 larvae/m) with a gradual increase in larval densities as the season progressed. However, the absence of a Time x Treatment interaction indicates that the effects of the treatments were consistent over the range of larval densities recorded.

There were significant treatment effects, with 1% BIOPEST oil + full-rate insecticide being superior to the other two treatments. That is, the addition of 1% BIOPEST oil to standard-rate insecticide treatments significantly improved Helicoverpa control over chemical insecticide alone. Additionally, 2% BIOPEST oil + half-rate chemical gave equivalent control to the full-rate chemical-only treatment.

Results presented in Table 4 and Fig 9 at right.

TABLE 4: Treatment means (insects/m) for the Greenbah 2003/04 experiment.

Treatment	Green mirids [1]	Helicoverpa eggs [1,2]	Very small + small Helicoverpa larvae [3]
BIOPEST 1% + Chemical (full-rate)	0.111 a	1.877 (3.023) a	1.33 a
BIOPEST 2% + Chemical (half-rate)	0.128 a	1.916 (3.171) a	2.38 b
Chemical (full-rate)	0.089 a	2.139 (4.075) a	2.14 b
LSD (p = 0.05)	0.0835	0.2180	0.526

1 Within this column, means followed by the same letter are not significantly different (P > 0.05 in ANOVA table), so no pair-wise testing done.

2 Data transformed by SQRT(X + 0.5) prior to analysis, equivalent (back-transformed) means are presented in brackets.

3 Means followed by the same letter are not significantly different (Fisher's Protected LSD test with a sequential Bonferroni correction to achieve a family-wise probability level of P = 0.05).

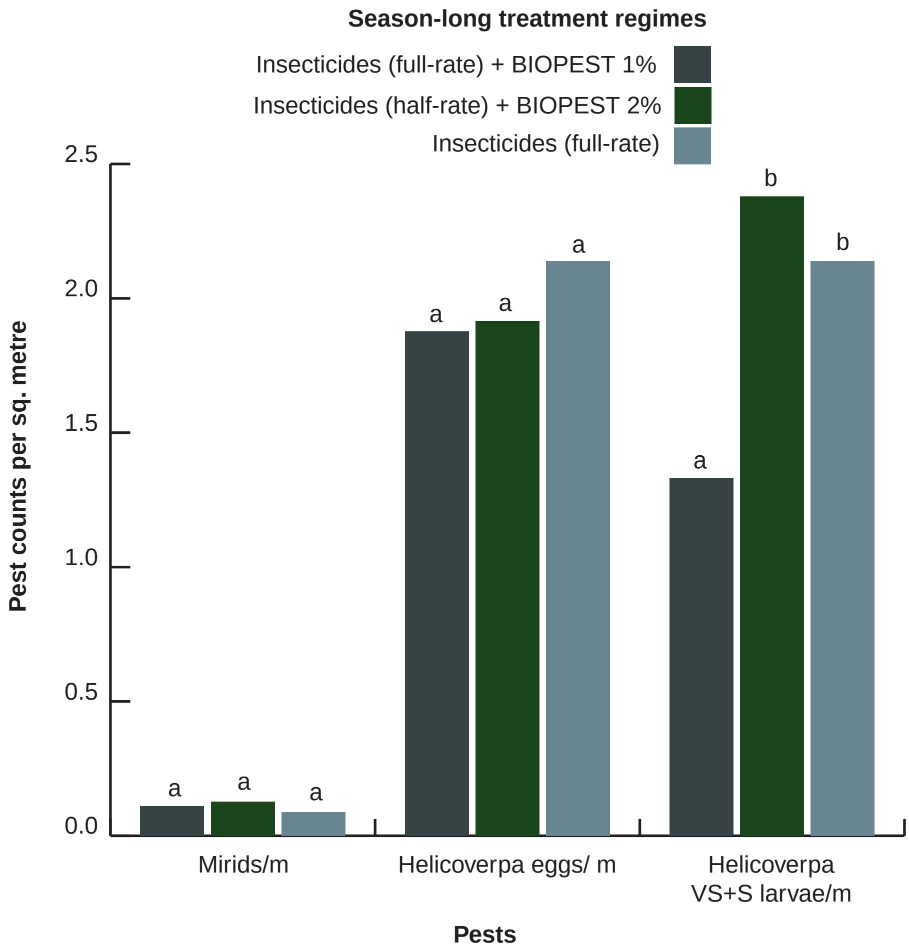


Fig 9: Treatment means (insects/m) for the Greenbah 2003/04 experiment.

Discussion and Conclusions

Aphid Management

BIOPEST's registration for the control of aphids in cotton recommends a rate of between 2L - 5L/Ha with a minimum of 80L/Ha of water. Download a copy of the product label at www.sacoa.com.au.

BIOPEST oil at concentrations between 1% and 10% (at a spray volume equivalent to 82 L/ha) produced high levels of mortality of aphids on cotton in the experiments reported here.

Aphid death was rapid, with most mortality occurring within 15 minutes of spraying. This rapid killing action is much more rapid than that for standard chemical insecticides such as Regent, which in these trials took several days to produce final mortality.

In only one trial were there differences between application concentrations, with 1% oil being less efficacious than rates between 2% and 10% for some developmental stages. Rates of 5% and 10% did not produce higher levels of mortality that 2% in any experiment, indicating that rates higher then 2% are unnecessary against aphids on cotton. On the basis of these experiments, the 2% rate is indicated as being providing the best balance between cost and efficacy.

The series of experiments reported here show that aphid mortality occurs from the direct interaction of the oil with the aphid;

There was no evidence for mortality caused by vapours from the oil, or trans-laminar or systematic actions.

For this reason, good spray application is critical to achieving effective control.

The BIOPEST oil spray should be directed at the undersides of leaves where the aphids normally live; application of BIOPEST oil mostly to upper leaf surfaces would produce less satisfactory results. Mortality was higher on young than old leaves.

However in the field, aphids occur most commonly on young leaves towards the top of the plant. These two aspects of BIOPEST oil performance point to key aspects of effective application;

sprays should be directed preferentially towards the undersides of the upper leaves to achieve the highest levels of aphid control on cotton.

Oil residues on leaves produced aphid mortality for up to 9 days after spraying, with mortality at Day 8 being approximately 50% of Day 1 mortality.

This means that the population suppression effect of BIOPEST oil sprays occurs for quite some time after the direct mortality effects.

BIOPEST sprayed plants did not repel aphids from landing, but survival of alates was reduced once on the sprayed plants, as was the subsequent production of nymphs. Prophylactic application of BIOPEST oil (two sprays 9 days apart) controlled aphids on cotton as well as Regent insecticide at low aphid infestation pressures. At high aphid infestation pressures, BIOPEST oil reduced aphid numbers to approximately 15% of the untreated control, compared to 5% for conventional insecticides.

These experiments indicate that at low aphid infestation rates BIOPEST oil alone at a concentration of 2% at a spray volume of 82L/ha can control of cotton aphid on cotton as well as chemical insecticides such as Regent.

At high aphid infestation rates, the level of control is moderate (approximately 85%) compared to that achieved by chemical insecticides (approximately 95%).

These results indicate that BIOPEST oil can have a valuable role in integrated pest management systems for cotton in Australia, removing the need for some chemical insecticide sprays that have the potential to disrupt the control of other pests by natural enemies.

BIOPEST oil now provides an alternative to standard chemical aphicides on cotton, thus increasing the scope for effective resistance management programs for cotton aphids.

Mirids and Helicoverpa

The two whole-of-season trials demonstrated that a half-rate insecticide regime + 2% BIOPEST oil can produce control of green mirids equivalent to a full-rate insecticide regime.

In one of the experiments, the addition of 1% BIOPEST oil to the full-rate regime improved mirid control over that achieved by the full rate regime alone. Neither experiment detected any significant change in Helicoverpa egg densities in treatments including BIOPEST oil, compared to the full rate insecticide regime.

At ACRI in 2003-04, the half-rate insecticide regime had higher Helicoverpa egg densities than the other treatments, including the half-rate insecticide + 2% BIOPEST oil treatment. At best, this is weak evidence of oviposition deterrence of Helicoverpa from BIOPEST oil applications.

In terms of control of VS+S Helicoverpa larvae, both experiments showed significant reductions in VS+S larval densities from the addition of BIOPEST oil, compared to the equivalent insecticide-only regime.

That is, the addition of BIOPEST oil to insecticide treatments regimes produces significantly greater mortality of Helicoverpa larvae than occurs in the equivalent insecticide-only regimes.

These two experiments also demonstrated that a 2% BIOPEST oil + half-rate insecticide regime produces control equivalent to a full-rate insecticide regime.

New Modes of Action

Through the use of fluorescent dyes researchers at the University of Queensland have observed that *BIOPEST's unique formulation, by penetrating the insect cuticle and accumulating in the nerve and fat-containing tissues, is providing at least three new modes of mortality on aphids.*

The primary new mode is nervous system effect where death of the insect appears to occur when the accumulation of the oil in the nerves of the insect reaches a lethal or toxic level. Thus, time to kill depends on the volume and concentration of oil applied to the insect.

This new mode appears to be supported by another two: (1) a desiccation effect and (2) an accumulation of oil in the fat bodies of the pest. Both providing supporting mortality effect.

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