Managing *Helicoverpa* spp. on cotton with semio (signalling) chemicals.

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1.0 Introduction

The over reliance on, and widespread use of, synthetic insecticides has led to resistance, increased concerns about the long term environmental impacts, and public health issues (Smart *et al* 1994). These issues of cost, efficacy, resistance and environmental impacts have led to the increased implementation of Integrated Pest Management (IPM) strategies. Crop plants including cotton can produce chemicals that can modify the behaviour of insects, particularly pests (Tinsworth 1990). The use of behaviour modifying compounds such as feeding deterrents, oviposition deterrents, attractants, repellents etc., which can reduce insect feeding or egg lay without killing pests, has intuitive appeal because such compounds can be used in IPM to reduce synthetic insecticide sprays. In addition they are safer to non-target organisms.

Recognition and selection of the host plant for egg lay by *Helicoverpa* spp. females after landing is determined by small quantities of many types of chemicals on the leaf surface. Detection of these chemicals on the leaf surfaces of the cotton plant provides specific information to the female moth on plant health, physiology and nutrition, which could be used to pre-empt the survival of offspring after egg lay (Rausher 1982; Thompson & Pellmyr 1991). The larvae hatching from Helicoverpa eggs also need to feed and survive. Subsequently, chemicals on the leaf surface, as well as organs in the leaf, provide information as to whether the insect should take a big bite of the leaf or square or to just scrape or take small bites to avoid ingesting a lethal dose of toxic compounds. Semiochemicals or behaviour modifying compounds used as stand alone products are seen as inefficient and ineffective for pest control, but in combination with existing tools in the context of IPM they may prove more effective (Pickett *et al* 1997).

The objective of this study was to isolate and identify, from several plant species, chemical compounds showing specific activity in relation to the feeding response and oviposition effects of *Helicoverpa armigera* spp. larvae and adult moths.

2.0 Materials and Method

2.1 Plant materials

Gossypium nelsonii, Lumein and MHR 11 are cotton plant types that are being studied. In addition, two leguminous plants from Australia and Africa, herein referred to as (Plant X) and (Plant Y) respectively, are being studied.

2.2 Extracts

Solid Phase extraction (SPE) procedures were employed by the Organic Chemistry team at QDPI in Brisbane to fractionate the crude homogenised solvent extract of Plant X and Y, *G. nelsonii* , Lumein and MHR 11. Six fractions were provided for bioassay and oviposition studies against *Helicoverpa armigera.* Out of the 6 fractions, fraction #1 was the water fraction and fractions # 2 – 6 were dissolved in methanol so that 1ml of methanol contained extractives equivalent to one gram of fresh leaf weight.

2.3 *No Choice Oviposition Trials*

One millilitre of each fraction, a methanol control and a water control was pipetted onto a 90mm filter paper and left to air dry in a fume hood for one hour. Four impregnated filter papers of each fraction were attached to the sides of an experimental oviposition chamber. A control filter paper treated with water was set up in another oviposition chamber. The oviposition chamber had a diameter of 25.5 cm and was 30 cm long. A total of eight oviposition chambers were used, one for each fraction and two controls. Five mated female *Helicoverpa armigera* were introduced into each chamber. A tray containing 10% honey solution was used as a food source in each chamber. The chambers were placed on a table and left for three days (72hours) in a temperature controlled room (temperature 24°C - 26°C and relative humidity 56%).

2.4 *Free choice Oviposition Trials*

In this study, the impregnated filter papers of each fraction were placed in the oviposition chambers. A 160mm hole was cut at the base of the chamber and connected to an exhaust pump to eliminate volatiles so as to avoid volatile contamination among fractions. In this way the female moths only detect the compound when it lands on the filter paper. Five mated *Helicoverpa armigera* females were placed in each chamber. The chambers were placed on a table with all fans operating at a similar speed. The chambers were left for 3 days (72hours) in a temperature controlled room (temperature 24°C - 26°C and relative humidity 56%).

2.5 *No choice feeding response*

A cotton leaf was treated with the equivalent of 1 ml of extract spread evenly on the lower and upper leaf surfaces and left to dry for one hour. Discs of 20mm in diameter were cut out of the leaf and placed into a 50mm Petrie dish with a moistened 47mm filter paper lining. One neonate larva (second instar) was placed in each Petrie dish. The initial weights of both the discs and the larvae were recorded prior to the experiment. Each treatment was placed in a Labec incubator with a temperature of 25°C (±2°C) for 48 hours. The final weights of the leaf discs and larvae in each treatment were recorded after 48 hours.

2.6 *Free choice feeding response*

A cotton leaf was treated with the equivalent of 1 ml of extract similar to the "no choice" trial. The treated leaf disc and the same size leaf disc treated with water were both placed in a 90 mm moist filter paper in a Petrie dish and one neonate larva was introduced into the Petrie dish as in the no choice trial. The Petrie dishes for each treatment were placed in a Labec incubator with a temperature of 25°C (±2°C). The initial and final weights of the larvae and leaf discs were taken similar to the no choice trial.

3.0 Results

Analytical representations of the various fractions derived from Plant X are presented in Figure 1. Some of the chemical compounds corresponding to these peaks on the high performance liquid chromatograms have been isolated on a scale sufficient to allow instrumental analysis by Nuclear Magnetic Resonance Spectrometry and High Resolution Mass Spectrometry in order to determine chemical structures. Larger scale purification of some of these compounds has commenced. Another plant, herein referred to as Plant Y, imported from Africa has also been reported to have behavioural influences on *Helicoverpa* spp. HPLC analyses of the C18 SPE fractions of Plant Y leaves are illustrated in Figure 2. Bioassay tests on Plant Y will be conducted before purification of the compounds commences. A similar process has been applied to the native cotton *G. nelsonii.*

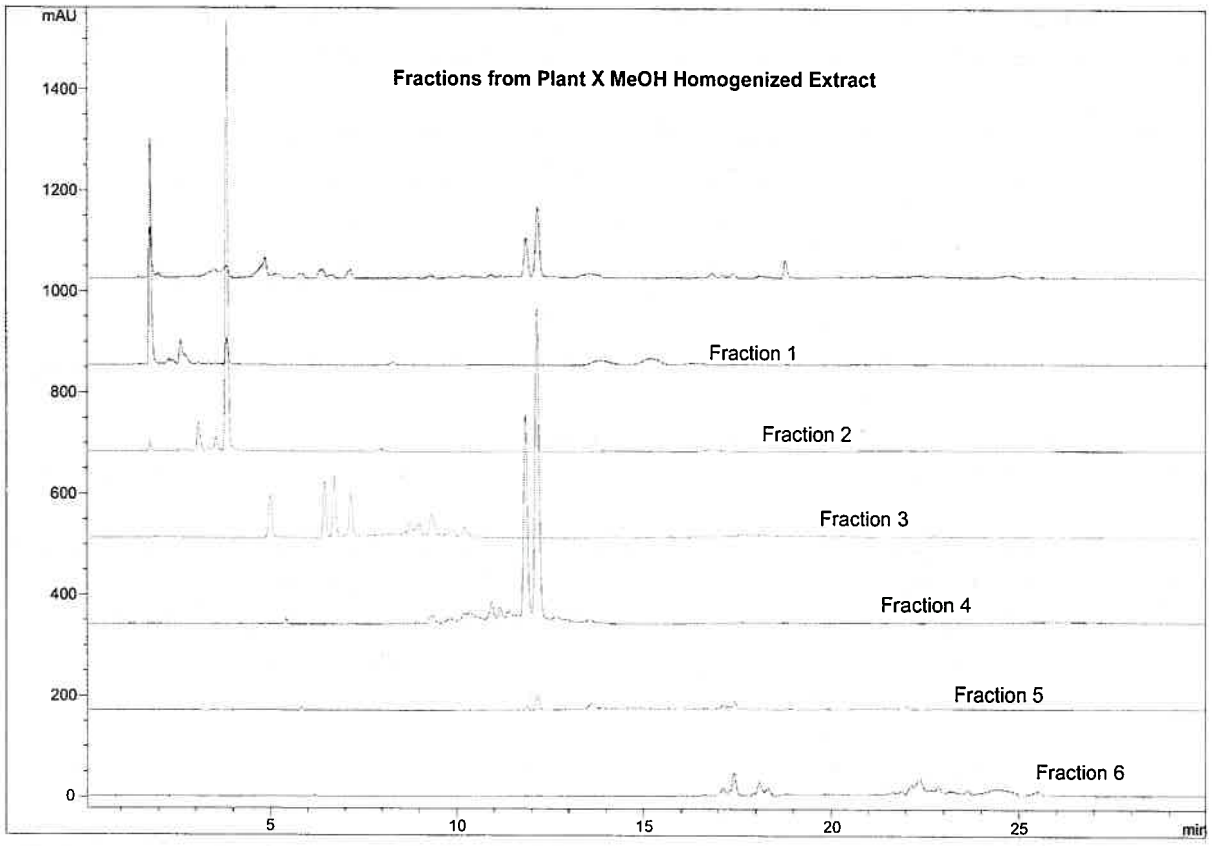

Figure 1. HPLC chromatograms of SPE fractions from Plant X

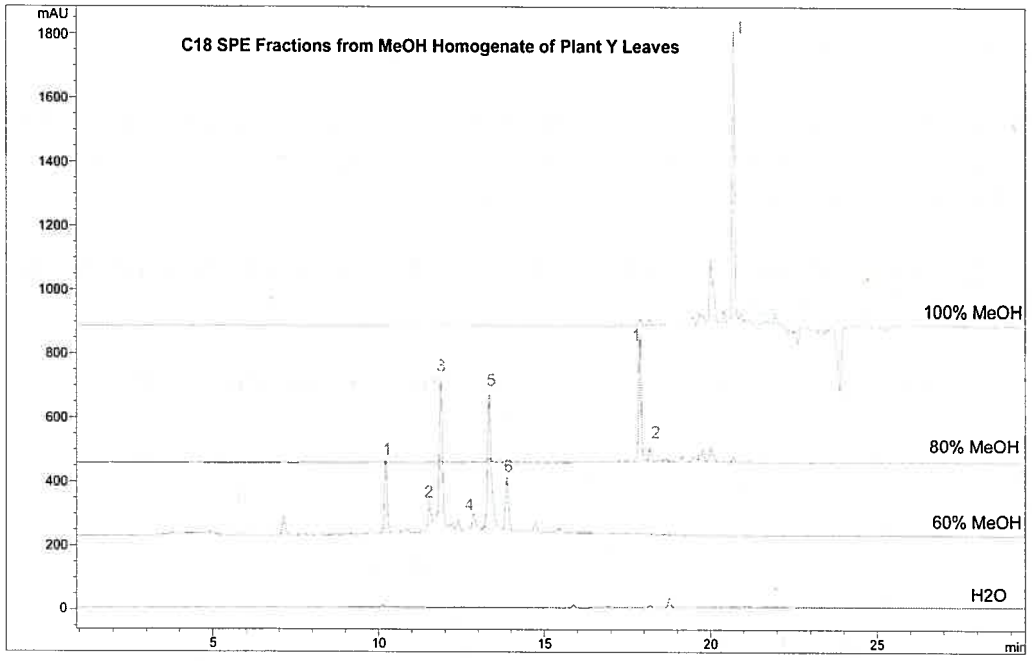

Figure 2. HPLC chromatograms of SPE fractions from Plant Y

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3.1 Oviposition response of H. armigera females on Plant X extracts

Oviposition-response tests showed that the oviposition deterrent index (ODI) recorded for fractions 3 and 4 was significantly higher ($P<0.01$) than for the other Plant X fractions tested, indicating that fractions 3 and 4 contain oviposition deterrent compounds (Tables 1 and 2).

Table 1. Free choice oviposition test of *Helicoverpa armigera* females on filter papers treated with Plant X fractions 1-3 at ACRI in Narrabri, 2003-04 (Summary of 2 trials)

Treatments	No. eggs/ filter paper± SE	[1] Oviposition Deterrent Index (ODI)
Fraction 1	32.75 ± 25.39 a	20.1 a
Fraction 2	41.00 ± 14.41 a	9.1 a
Fraction 3	17.50 ± 7.84 b	47.6 b (Oviposition deterrent)
Control (water)	49.25 ± 17.21 a	0.0 a

Means within columns followed by the same letter are not significantly different (P>0.05) (Tukey-Kramer Multiple comparison test).
[1]Oviposition Deterrent Index (ODI) was calculated as follows:
ODI= 100 x (C-T)/(C+T); where C = Total eggs laid in control; T=total eggs in treated filter paper.

Table 2. Free choice oviposition test for Helicoverpa armigera females on filter papers treated with Plant X fractions 4-6 at ACRI in Narrabri, 2003-04 (Summary of 2 trials)

Treatments	No. eggs/ filter paper± SE	[1] Oviposition Deterrent Index (ODI)
Fraction 4	6.50 ± 1.19 a	42.2 a (Oviposition deterrent)
Fraction 5	58.25 ± 22.96 b	-56.9 b
Fraction 6	35.00 ± 10.40 b	-37.3 b
Control (water)	16.00 ± 8.25 c	0.0 c

Means within columns followed by the same letter are not significantly different (P>0.05) (Tukey-Kramer Multiple comparison test).
[1]Oviposition Deterrent Index (ODI) was calculated as follows:
ODI= 100 x (C-T)/(C+T); where C = Total eggs laid in control; T=total eggs in treated filter paper.

3.2 Oviposition response of H. armigera females to G. nelsonii fractions

The results showed that fractions 1, 2, 4, and 5 may contain oviposition deterrent compounds (Table 3). The ODI for fractions 1 and 4 relative to the control was 100 indicating that these fractions contain a very strong oviposition deterrent compound (Table 3).

Table 3. No-choice oviposition test for on filter papers treated *G. nelsonii* fractions in the laboratory at ACRI in Narrabri, 2003-2004.

Treatments	No. eggs/ filter paper± SE	Oviposition Deterrent Index (ODI)
Fraction 1	0.00 a	100.0 a (Oviposition deterrent)
Fraction 2	7.75 ± 4.10 a	65.9 a (Oviposition deterrent)
Fraction 3	33.75 ± 4.11 b	5.6 b
Fraction 4	0.00 a	100.0 a (Oviposition deterrent)
Fraction 5	3.00 ± 1.08 a	85.3 a (Oviposition deterrent)
Fraction 6	53.00 ± 10.76 b	-17.4 c
Methanol (Control)	55.25 ± 16.65 b	-18.8 c
Water (control)	37.75 ± 20.9 b	0.0 b

Means within columns followed by the same letter are not significantly different (P>0.05) (Tukey-Kramer Multiple comparison test).
[1]Oviposition Deterrent Index (ODI) was calculated as follows:
ODI= 100 x (C-T)/(C+T); where C = Total eggs laid in control; T=total eggs in treated filter paper.

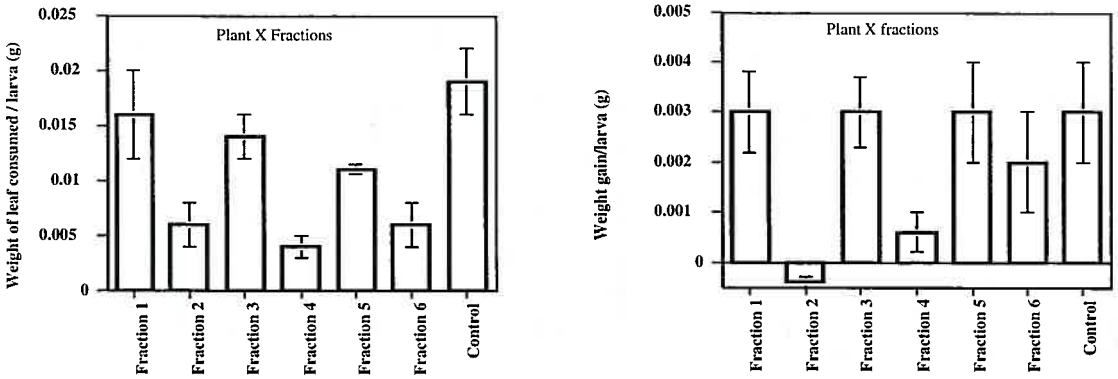

Figure 3. Feeding response of Helicoverpa armigera 3rd instar larvae on cotton leaves treated with Plant X fractions (no-choice tests) at ACRI in Narrabri, 2003-04 (Summary of 3 trials).

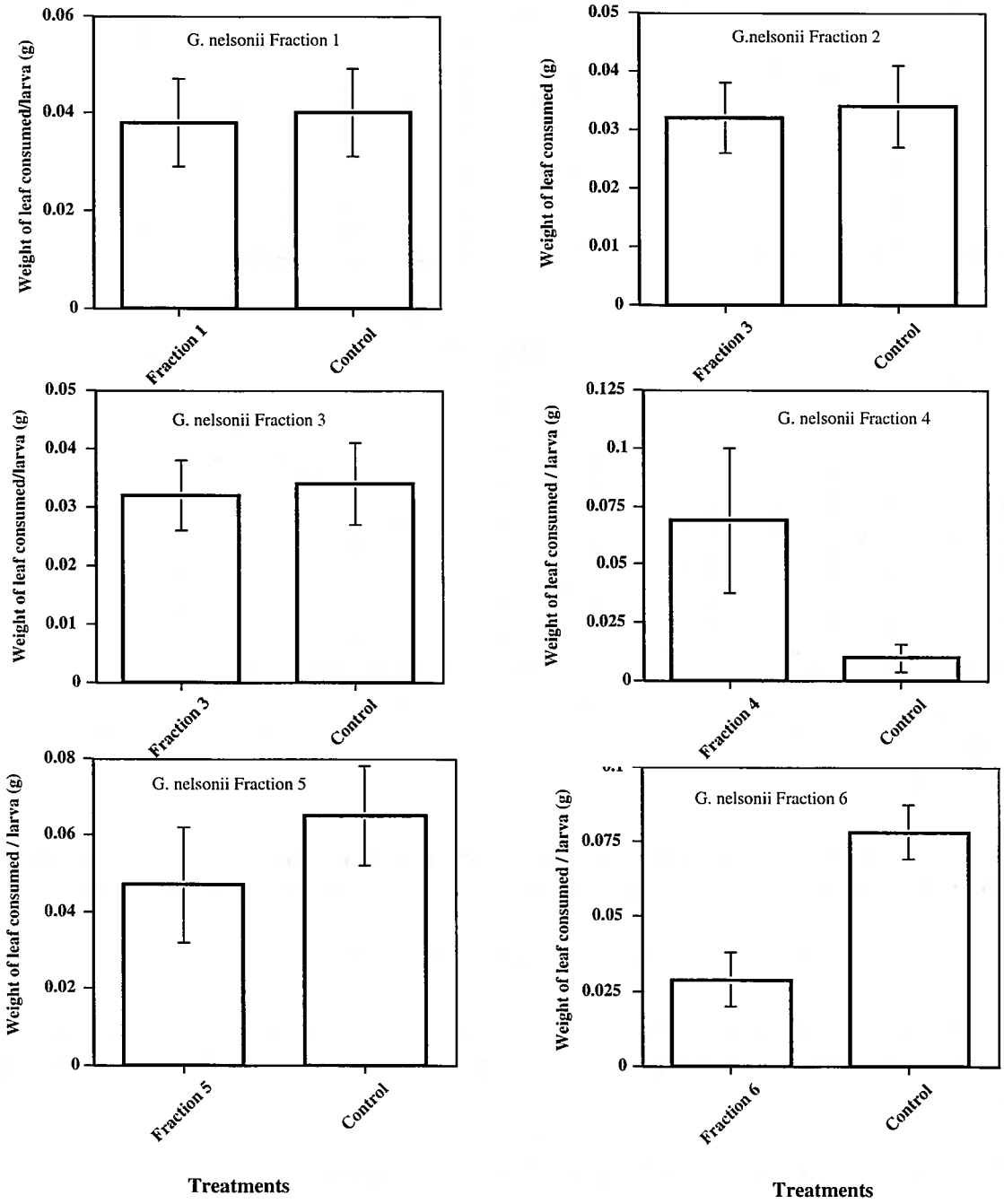

Figure 4. Effect of *G. nelsonii* fractions on the consumption of cotton leaves by Helicoverpa armigera 3rd instar larvae in the laboratory (choice tests) at ACRI in Narrabri, 2003-04 (Summary of 3 trials).

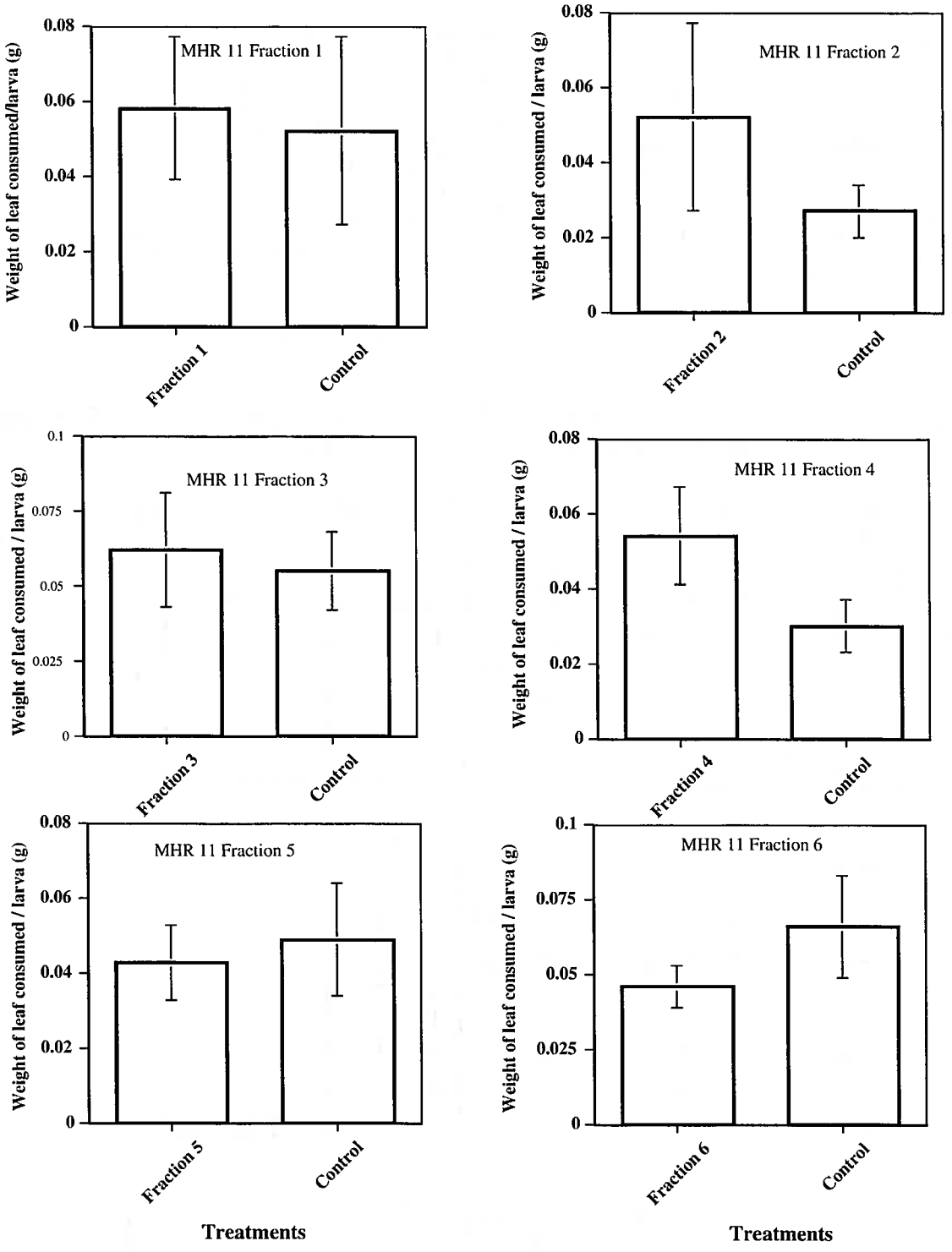

Figure 5. Effect of MHR 11 fractions on the consumption of cotton leaves by *Helicoverpa armigera* 3rd instar larvae in the laboratory (choice tests) at ACRI in Narrabri, 2003-04 (Summary of 3 trials).

3.4 Feeding responses of H. armigera larvae to Plant X fractions

The bioassay results of Plant X fractions showed that fractions 2 and 4 may contain chemical compounds which can deter the feeding of *H. armigera* larvae, based on the weight of leaf consumed per larva (Figure 3).

3.5 Feeding responses of H. armigera larvae to G. nelsonii fractions

The weight of leaves treated with fraction 4 and consumed by *H. armigera* larvae was significantly higher, and fraction 6 significantly lower, than the leaves treated with water (Figure 4) indicating fraction 4 may contain a feeding stimulant and fraction 6 feeding deterrent compounds.

3.6 *Feeding responses of H. armigera larvae to MHR 11 fractions*

Fractions extracted from MHR 11 has no effect on *H. armigera* larval feeding (Figure 5).

3.7 *Feeding responses of H. armigera larvae to Lumein fractions*

H. armigera larvae consumed significantly higher amounts of leaf treated with Lumein fractions 3 and 4 than the water-treated leaves (Figure 5) indicating that fractions 3 and 4 may contain a feeding stimulant.

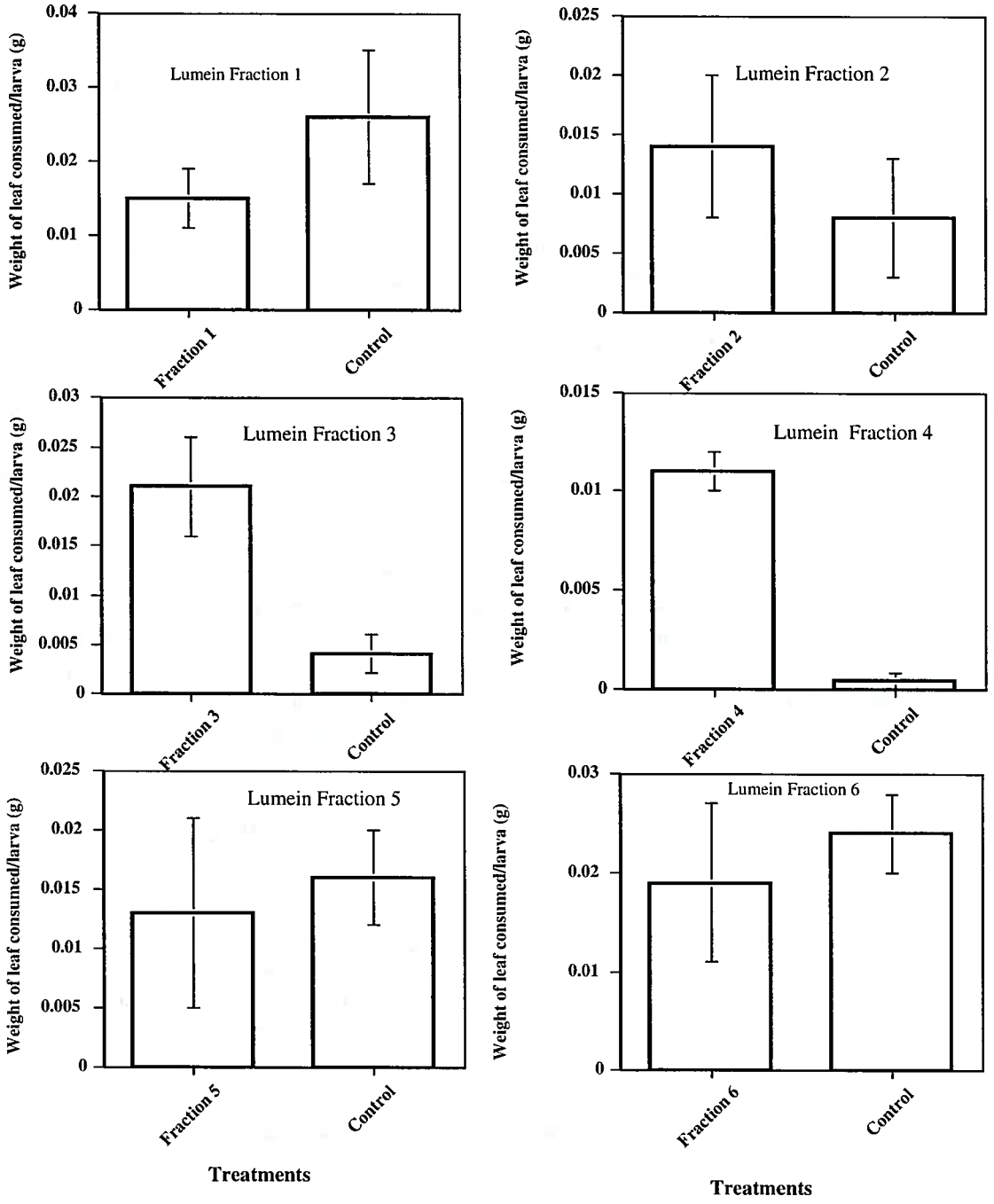

Figure 6. Effect of Lumein fractions on the consumption of cotton leaves by Helicoverpa armigera 3rd instar larvae in the laboratory (choice tests) at ACRI in Narrabri, 2003-04 (Summary of 3 trials).

4.0 Discussion

The study showed that fractions 3 and 4 of Plant X and fractions 1, 2, 4, and 5 of *G. nelsonii* may contain compounds that can be used to deter *H. armigera* egg lay. In addition to Plant X fractions 2 and 4, *G. nelsonii* fraction 6 may also contain feeding deterrent compounds, whereas *G. nelsonii* fraction 4 and Lumein fractions 3 and 4 may have feeding stimulant compounds. Oviposition deterrent compounds identified in Plant X and *G. nelsonii* are regarded as very important for the cotton industry in terms of pest management because oviposition or egg lay is an important step in an insect's reproductive process, particularly *Helicoverpa spp*. The oviposition step ensures the continuity of the species and any mistakes committed by the adult female in selecting an oviposition site will affect the offspring dearly. The application of an oviposition deterrent compound to the cotton leaf surface will make the plant seem a non-host for *Helicoverpa* females, hence attracting fewer egg lays. In addition, the application of a feeding deterrent chemical reduces insect feeding but a feeding stimulant chemical can increase larval feeding. Thus the presence of a feeding deterrent at the surface of leaves plays a major role in discriminatory feeding behaviour of the larvae of insects particularly *Helicoverpa* spp.

There is a general view that the efficacy of a deterrent based method may be increased if used in combination with another method that attracts the pest to a non-valued resource in a stimulo-deterrent diversion (Miller and Cowles, 1990) or push-pull (Pyke et al., 1987) strategy. In the cotton industry, any feeding or oviposition deterrent compound developed from plants can be used on conventional cotton crops to reduce the level of *Helicoverpa* spp. infestation. Furthermore, with respect to most of the contact/stomach poisons or biological pesticides where Helicoverpa larvae have to ingest a lethal dose to effect mortality, efficacy of the pesticide will increase if it is mixed with feeding stimulants to enhance the larval feeding. Feeding stimulants can increase contact and exposure to the pesticides that may be suppressed if the larvae respond to the pesticide by ceasing to feed thus avoiding a lethal dose.

Hedin *et al* (1988) stated that *H. virescens* larvae detect and avoid consuming areas of cotton plants that are high in gossypol. Instead, the larvae move to an area without or with less of this compound. They then feed until they moult and reach second stage at which time gossypol no longer affects them and they can feed and continue to develop. If *Helicoverpa armigera* behaves in similar ways to *H. virescens* then by exploring in more detail the effects of the 12 compounds focused on in this study it is quite possible that some may be incorporated into the Stimulo-Deterrent-Diversionary Strategy (SDDS) (Fig.6) (Pyke *et al* 1987; Miller & Cowles 1990; Pickett *et al* 1997).

"PUSH"	"PULL"
(away from the crop)	(into traps, trap crops, refuges)
Kairomone inhibitors	Kairomones
Repellents,anti-feedants,oviposition-deterrents	Aggregation, sex and oviposition pheromones
Attractants for parasitoids and predators	Selective control agents (e.g. pathogens)
	Visual Cues

Figure 7 – The push- pull or Stimulo–deterrent diversionary Strategy (Pickett et al 1997 p. 151).

By combining some of the compounds identified as being active and including them into the SDDS (Pyke *et al* 1987; Miller & Cowles 1990; Pickett *et al* 1997) and IPM strategy it may be possible to manipulate *H. armigera* to the point where the damage to crops is reduced and / or the population itself declines. For example, Plant X showed oviposition deterrent action and feeding deterrent qualities. The antifeedant effects were observed in second and third instar larvae, however neonate and first instar larvae may suffer toxic effects or starve to death if they select where they feed and avoid repellent areas (Hedin *et al* 1988). So by applying an oviposition deterrent to the desirable

crop (cotton) and / or an attractant to a "trap" crop area of Plant X, any oviposition occurring on Plant X will be followed by larvae facing the possibility of inhibited development, starvation or possible mortality from naturally occurring toxic compounds. Work on the toxicity effects of Plant X, *G. nelsonii,* MHR 11 and Lumein on neonates and first instar *H. armigera* larvae will be conducted in the near future.

Larval feeding stimulants have the potential to increase consumption of transgenic crops, should oviposition occur. The other benefit would be the combination of feeding stimulants with biopesticides, potentially resulting in increased efficacy of the biopesticide and / or increased mortality of pest species (Smart *et al* 1994). With the isolation of six potential oviposition deterrents, three larval feeding deterrents and three larval feeding stimulants, work must continue to determine the most effective ones. There may be several, each one acting differently. Factors such as concentration, application method, persistence and crop applied to must be investigated (Mensah & Moore 1999).

In conclusion, there is still much work to be done on the twelve compounds identified here as having bioactive effects. However, the potential benefits of adding these compounds as new tools to the IPM strategies is promising.

Acknowledgements

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