Managing *Helicoverpa spp* on cotton with semiochemicals – The preliminary results.

R. K. Mensah[1] , H. T. Dang [1] C. Moore[2] and A. Singleton [1]

[1]/ NSW Agriculture, Australian Cotton CRC, ACRI, Myall Vale, Narrabri, NSW 2390. robert.mensah@nsw.agric.gov.au and ho.dang@agric.nsw.gov.au.
[2]/ QDPI, AFFS, locked bag 4, Moorooka, Qld 4105. chris.moore@dpi.qld.gov.au

Abstract :

Helicoverpa spp remain the most important pest in the Australian cotton industry. They are resistant to most of the insecticides used by the industry. The cotton industry is determined to reduce their independence against this pest. As a result there is a strong push by the industry in recent years to adopt a true IPM program in order to minimise insecticide use. Semio(signalling) chemical that may impact on pest behaviour are currently being studied to isolate potential chemicals for efficacy against cotton pests. Chemicals on the leaf surfaces of refuge crops, cotton cultivars and other plant species will be isolated , purified , formulated, bioassayed against cotton pests and the potential ones deployed in cotton IPM .Field and mesh house trials of different plant species and refuge crops have been screened for oviposition and feeding preference against *Helicoverpa spp.* adults and larvae. Results, so far showed that several less preferred crops and plant species deter *Helicoverpa spp.* adult oviposition and also cause mortalities in the larvae. An unidentified plant, code-named "Plant X" was found to reduce *Helicoverpa spp.* egg lay by 94 %. The top, middle and base leaves caused 89, 78 and 89 % mortalities respectively to *H. punctigera* second stage larvae. The seeds also caused 74% mortality to the larvae. Further studies are continuing to extract the toxic compounds in Plant X and bioassay it against *Helicoverpa spp.* If successful a new IPM tool will be developed for cotton growers for use in IPM programs.

INTRODUCTION:

Helicoverpa spp are considered the most economic insect pests of cotton and other field crops in Australia (Fitt , 1989, 1994) . Synthetic insecticides are mostly used to control these pests. Over-reliance on insecticides has generated problems such as insecticide resistance, disruption of natural enemies and environmental pollution.. The focus of the cotton industry now is to reduce the dependence on synthetic insecticides.

As a result, there is a strong push for adoption of integrated pest management (IPM) systems. The use of compounds from plants that can modify the behaviour of insects particularly *Helicoverpa spp.* is a step in the right direction to support IPM. Behaviour- modifying compounds such as antifeedants, oviposition deterrents, attractants , repellents and mating-disruptants can either reduce insect feeding or egg laying but not necessarily killing the pest can reduce pest populations to complement the activity of beneficial insects in cotton farms. The use of such compound are safer to the environment and can reduce synthetic insecticides use in cottons and other crops .

This paper reports of the preliminary studies on oviposition deterrent effect of some refuge crops and the mortalities caused by an unidentified plant when fed to *Helicoverpa spp.* larvae during the 2001-02 season.

MATERIALS AND METHODS:

Cotton, lucerne, pigeon pea , sorghum , sweet corn, faba bean chick pea and also a legumineous plant (referred hereunto as Plant X) were exposed to *Helicoverpa spp.* in the mesh house and also in the field to determine oviposition responses of *Helicoverpa spp.* to these crops . In addition the feeding responses of *Helicoverpa armigera* neonates to "Plant X" was also determined in the laboratory.

(1) Mesh house trial:

Plant X, faba bean, pigeon pea, chick pea, sorghum and cotton were planted in pots in the mesh house in January 2002. Plants were used for the studies 55 days after planting. The potted plants were arranged in a randomly complete block design with 8 replicates per plant. Fifty mated female moths were released in the mesh house to lay on the plants. The number of eggs laid on each plant were recorded every 3 days. Eggs were removed after counting and the experiment continued until all the moths died.

(2) Field trial:

In the field study, lucerne, pigeon pea, sorghum, sweet corn and Plant X were interplanted with irrigated commercial cotton in October, 2001. Each strip was replicated 4 times within the cotton crops. All the refuge crops were planted at the same time as cotton with the exception of lucerne which was planted earlier (September, 2001). The number of *Helicoverpa spp* eggs and larvae were recorded weekly from each refuge crop and cotton. Sampling started three weeks after planting, thus data for early-season were collected from November 11 to December 20, mid-season from December 28, 2001 to January 30, 2002 and late-season from February 8 to March 5 ,2002. Sweet-corn was sampled only during early-season, sampling at mid- and late-season was not possible because of its height.

(3) Efficacy of Plant X against Helicoverpa spp. larvae:

Following the results of the mesh house and field trials, Plant X was found to be toxic to *Helicoverpa spp* larvae in addition to being deterrent to adult moth oviposition. As a result, a separate experiment was conducted in the laboratory to investigate and quantify the toxic effect of Plant X to *Helicoverpa spp* larvae and to determine the plant structure or part which is most efficacious against larval stages of *H. armigera* and *H. punctigera* .

The leaves, pods and seeds of Plant X were fed to *Helicoverpa spp.* larvae in a petridish in the laboratory (one larvae per petridish to avoid cannibalism). Larval mortality was recorded daily until all the larvae died. Larvae fed on cotton leaves ,squares and seeds were used as control. Plant materials in each petridish were replaced every two days. Treated mortalities were corrected relative to the control mortalities using Abbott (1925) formula.

In testing the efficacy of leaves, new leaves (leaves at the two top nodes or terminals), middle leaves (leaves from the middle nodes) and old leaves (leaves from the two lowest nodes of the plant) were tested separately. The seeds were fed to the larvae in two forms (crushed and whole seed). Likewise, pods were fed to larvae as cut and whole pod.

All insects used in the mesh house and laboratory trials were from laboratory colonies that were refreshed with field collected insects every other generation. Bioassay was repeated three times from March to May, 2002.

RESULTS AND DISCUSSION:

(1) Oviposition trials:

Field Trials:

Number of eggs per metre recorded from the refuge and cotton crops in the field are given in Figure 1 . Plant X, Lucerne and sorghum were the less preferred plants for oviposition by *Helicoverpa spp.* moths. Cotton was the most preferred plant for oviposition.
Plant X had low number of eggs per metre and no larvae were recorded on the plant in the field. During early-season period, eggs were recorded on Plant X but no larva was found on it. During mid- and late season, small larvae were were recorded on Plant X but no medium and large larva were found indicating high larval mortality in Plant X.

Similarly, some eggs were recorded on lucerne, but no larvae were recorded indicating high mortality of larvae in lucerne.
Titmarsh(1992) highlights that in cotton cropping system natural enemies and weather factors were relatively unimportant and that host plant effects contributed to the major parts of the observed larval mortality. Thus, under unsprayed field condition, the number of eggs and larvae could be treated as an indicative measure for oviposition and non-preference/antibiotic factors.

Mesh house trials:

Under mesh house and choice condition, corn , chick pea and sorghum were more preferred for oviposition than cotton . Plant X was the least preferred for oviposition in the mesh house (Figures 2).

It is important to note that under field condition sorghum was among the less preferred crops than cotton (figure 1) and yet, in the mesh house it was more preferred than cotton (Figure 2). Therefore, in the context of oviposition preference, it is appropriate to assess refuge crops or any behaviour- modifying compounds under both mesh house and field conditions. This is because factors such as canopy size, plant height, plant leaf colour etc in confined space may affect oviposition preference or host selection .

(2) Determination of efficacy of Plant X:

In the mesh house studies, dead *Helicoverpa spp.* larvae were recorded on Plant X, therefore it may contain toxic compounds which can kill Helicoverpa larvae or prevent the larvae from feeding on the crop (antifeedant). In order to get enough toxin to extract from the plant, it is crucial to determine the location of the toxin(s) or the plant part which has a higher concentration of the toxin.

Tables 1 and 2 give the corrected mortality of the first and second larval stages on different plant parts. The results indicate that mortality of the second larval stage was higher than the first larval stage. This may be due to the ability of the second stage larvae to feed more than the first stage, thus second stage larvae ingest more toxic dose than first stage larvae in the course of their feeding.

H. punctigera was more susceptible to the toxin(s) than *H. armigera* , especially on leaves . The toxin(s) in the leaves caused 100% mortality in *H. punctigera* within 48 hours after feeding (77.8 to 88.9% of corrected mortality –on leaves) (Table 2).

Mortality caused by feeding on the other plant parts was slow to occur (from 4 to 9 days) and may be due to the combination of direct toxin effect at low concentration and reduced feeding by the larvae, particularly in the first stage larvae.

With regards to the second stage larval mortality of *H. punctigera* (Table 1), the results indicate that there is the different distribution of toxin's level within the plant. . Higher toxin's effect was found in the leaves compared to the other plant parts. Similarly Rhoads and Gates (1976) reported the variation of diethyl ether soluble phenolic resins and tannins on the leaf surface and in leaf tissue of Creosote bush , *Larrea tridentata* , that caused feeding deterrence to herbivores . The same authors reviewed the within plant distribution of toxins and digestibility reducing substances (DRS) in different plant species. A number of secondary plant products such as saponins and glucosides can cause dual functions as DRS as well as toxin.

In the pod, incidence of high mortality was suffered by *H. armigera* and *H. punctigera* larvae when they were fed with whole pods rather than on cut pods (Table 2) indicating that the toxin(s) may be present on the surface of the pod rather than the inside .

These results and observations will be confirmed by bioassay with leaf surface and homogenised extracts. The extracts from plant X's parts and from other refuge crops will be analysed by High Performance Liquid Chromatography. This will fractionate mixtures of compounds into individual components so that specific behaviour-modifying compounds can be isolated .The bioassay-directed fractionation will be further investigated by structure elucidation of purified compounds. Individual toxins, repellents, deterrents, or attractants etc. will be identified and used in a blend or alone to modify the behaviour of *Helicoverpa spp* or other pests on cotton.

The use of behaviour-modifying chemicals to protect a crop is not new but success rate has been low. There are three principal elements of behavioural manipulation method. They are (1) a behaviour of the pest, (2) a means by which the behaviour is appropriately manipulated and (3) a method that utilises the behaviour manipulation to protect the crop (Foster and Harris, 1997).

It is important to focus on the appropriate step of the pest's behaviour in host searching sequences whether for oviposition or for feeding. The manipulation of the pest feeding on the crop or finding of the crop or host plant is more useful for pest management than manipulation of insect or pest behaviour unrelated to the crop (for example, mating disruption) (Foster and Harris, 1997).If the feeding behaviour of the pest is manipulated successfully, it will ensure that the crop or resource is protected. However, successful manipulation of an unrelated behaviour may reduce the local population ,but still not protect the resource because of immigration of outside populations which may be already mated into the protected area , as can occur in moths(Carde and Minks,1995).

These concepts and principles are important guideline for future work as resource is limited, the choice in priority and considerations in selection of plant secondary products and their function in pest's behaviour manipulation should be weighed carefully, so that the product(s) can be effectively used in an IPM system that is feasible and sustainable.

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LITTERATURE CITED:

Adamson , D; Thomas ,G and Davis , E.(1997). An economic estimate of Helicoverpa 's effect on Australian agriculture production . Helicoverpa series , repport 1 . CRC for Tropical pest management , 23pp.

Carde , R. T. and Minks, A. K.(1995). Chemical ecology of insects 2, NewYork , Chapman and Hall, 433pp.

Fitt, G.P.(1989). The ecology of Helicoverpa in relation to agro-ecosystem. Annual review of Entomology 34:17-52.

Fitt, G.P (1994). Cotton pest management:Part 3. An Australian perspective. Annual review of Entomology 39: 543-562.

Foster ,S.P. and Harris ,M.O. (1997). Behavioural manipulation methods for insect pest management. Annual review of Entomology 42:123-146.

Hearn , A.B. and Fitt, G.P.(1992). Cotton cropping systems .In : Field crop ecosystems of the world 5, CJ Pearson(ed.) Amsterdam, Elsevier ,pp 85-142.

Rhoads, D.F. and R.G. Gates.(1976).Towards a general theory of plant antiherbivore chemistry . In: J.W.Wallace and L Mansell,eds. Biochemical interaction between Insects and Plants .pp 168-213. Plenum , Newyork.

Titmarsh, I.J.(1992). Mortality of immature Lepidoptera: a case study with *Helicoverpa species* (Lepidoptera:Noctuidae) in agriculture crops in the darling Downs . Ph.D. Thesis, University of Queensland , Australia .

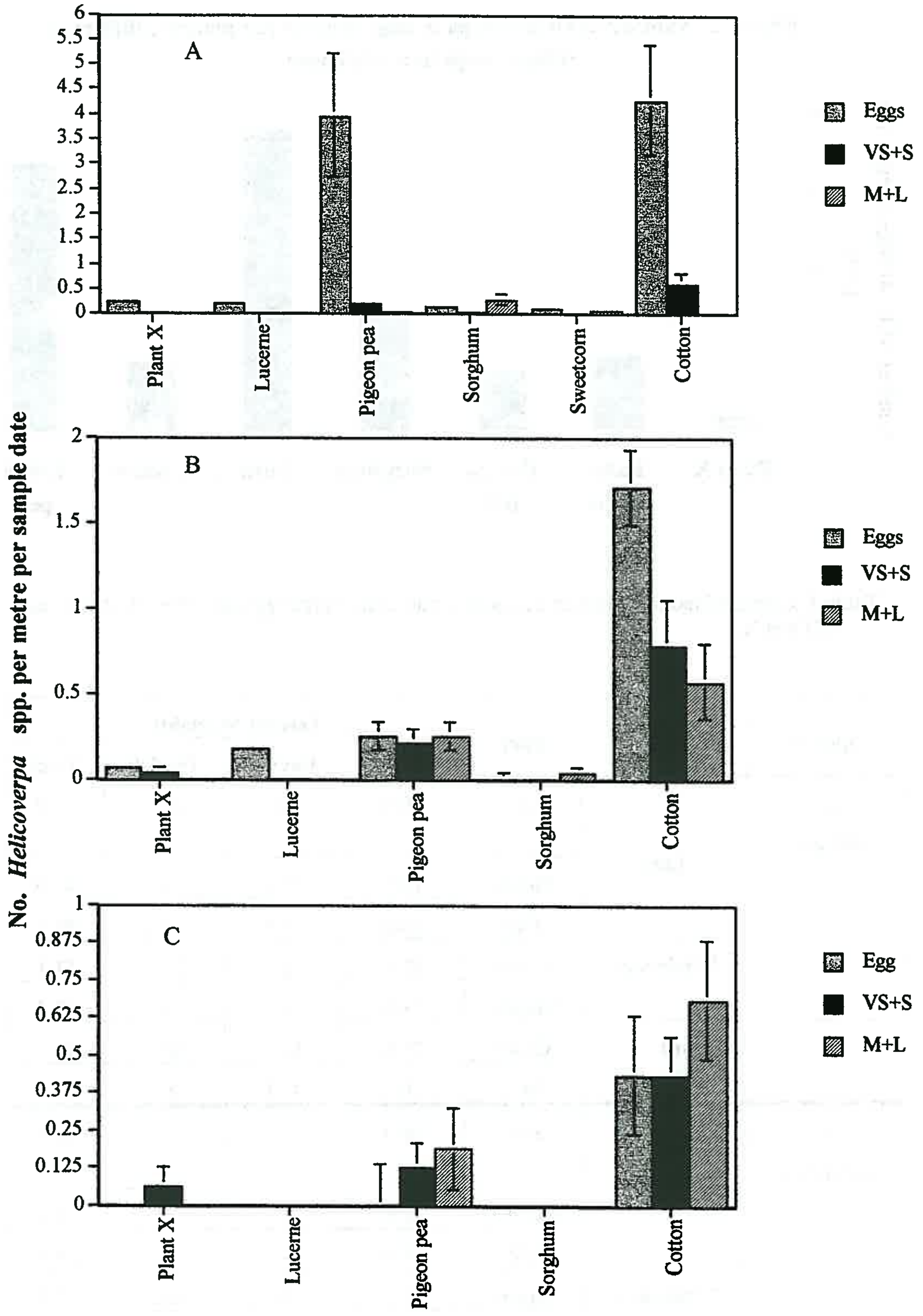

Figure 1. Number of *Helicoverpa* spp. recorded on refuge crops interplanted in cotton during early (A), middle (B) and late (C) seasons in commercial irrigated cotton farm at Norwood near Moree, 2001-2002.

Figure 2: Number of Helicoverpa armigera eggs per plant on different refuge crops in mesh house .

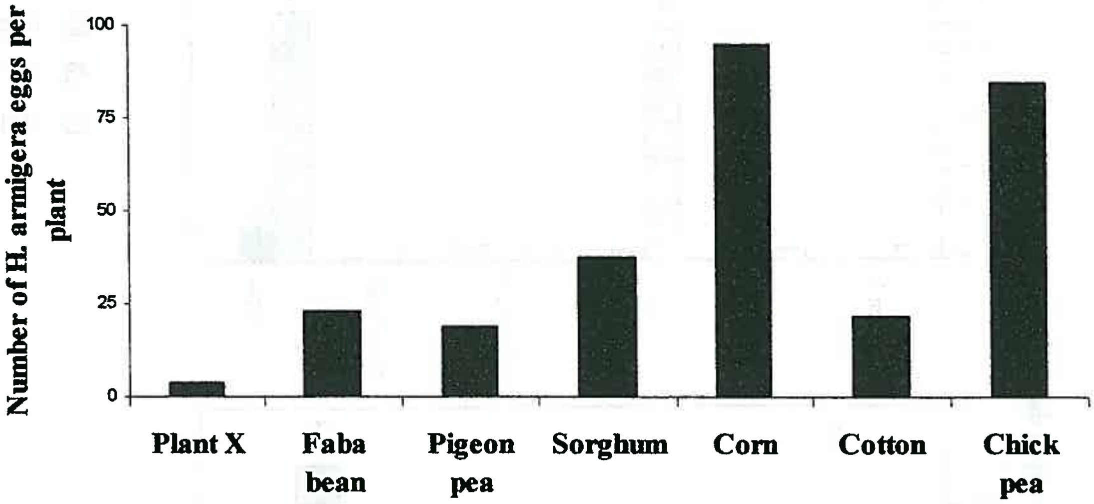

Table 1: Corrected mortality of second instar larvae *Helicoverpa spps* on different structures of Plant X.

Species	Plant Structure	Part	Percent Mortality			
			Day1-2	Day3-4	Day5-9	Total
H. armigera	Leaf	New	20.0	10.0	20.0	50.0
		Middle	10.0	30.0	0	40.0
		Old	20.0	30.0	31.1	81.1
	Whole seed	Green	31.1	0	20.0	51.1
		Mature	63.3	-	-	63.3
	Crushed seed	Green	20.0	11.1	40.0	71.1
		Mature	0	41.1	20.0	61.1
H. punctigera	Leaf	New	88.9	-	-	88.9
		Middle	77.8	-	-	77.8
		Old	88.9	-	-	88.9
	Whole seed	Green	12.5	0	10.0	22.5
		Mature	50.0	-	-	50.0
	Crushed seed	Green	44.4	0	10.0	54.4
		Mature	11.1	62.5	-	73.6

1/ Control treatment: Newly opened cotton leaf as compared to Plant X's leaf; Cotton square as compared to Plant X's pod and cotton seed as compared to plant X's seed. Larval mortality on cotton (control mortality) was used for calculation of corrected mortality.

Table 2: Corrected mortality of first instar *Helicoverpa spps* on different structures Of Plant X .

Species	Plant Structure	Part	Percent Mortality			
			Day1-2	Day3-4	Day5-9	Total
H. armigera	Leaf	New	21.2	17.8	20.0	59.0
		Middle	6.6	18.9	12.3	37.8
		Old	10.3	37.8	14.8	62.9
	Pod	Whole	47.2	28.0	0	75.2
		Cut	22.8	11.9	6.8	41.5
	Whole seed	Green	0	30.0	10.0	40.0
		Mature	22.2	42.8	0	65.0
	Crushed seed	Green	0	10.0	21.1	31.1
		Mature	0	20.0	40.0	60.0
H. punctigera	Leaf	New	47.8	28.6	-	76.4
		Middle	23.4	30.5	13.8	67.7
		Old	50.7	27.4	6.7	84.8
	Pod	Whole	35.0	20.0	-	55.0
		Cut	25.0	11.1	-	36.1
	Whole seed	Green	12.5	21.1	20.0	53.6
		Mature	25.0	0	-	25.0
	Crushed seed	Green	10.0	20.0	21.1	51.1
		Mature	30.0	10.0	10.0	50.0

1/ Control treatment: Newly opened cotton leaf as compared to Plant X's leaf ; Cotton square as compared to Plant X's pod and cotton seed as compared to plant X's seed. Larval mortality on cotton (control mortality) was used for calculation of corrected mortality.