The effect of semio (signalling) chemicals in Plant Y on the biological activity of *Helicoverpa armigera*.

Angela Singleton[1]; Heidi Rasikari[2]; Robert Mensah[1] & David Leach[2]

[1] NSW DPI and Cotton CRC, Australian Cotton Institute, Locked Bag 1000, Narrabri NSW 2390, Australia.
Email @ robert.mensah@dpi.nsw.gov.au

[2] Southern Cross University Centre of Phytochemistry, P.O. Box 157 Lismore 2480 NSW, Australia.
Email: dleach@scu.edu.au

Introduction

Cotton is a highly valuable crop and to obtain and maintain high yields, the industry has relied almost exclusively on the use of synthetic insecticides (Mensah 2002; Moore *et al* 2005). This over -use has resulted in negative impacts on the environment, beneficial and predatory insect communities and widespread resistance in pest species such as *Helicoverpa* to synthetic insecticides such as pyrethroids and endosulfan (Fitt 1994). Other issues include concerns about public health and the perception of the cotton industry in the wider community (Smart *et al* 1994).

In response to these problems, tools are being developed as part of IPM strategies that use less pesticides. These strategies use tools such as, but not restricted to, genetically modified crops, the effective use and preservation of pest predators, biological insecticides, trap crops, refuge crops and semio chemicals (Moore *et al* 2005).

Some semiochemicals are secondary plant compounds that were originally thought to have been of no benefit to plants at all (Gatehouse 2002). However it is now known they play a significant role in plant/insect interactions (Wittstock & Gershenzon 2002).

Secondary plant compounds (SPCs) modify the behaviour of other organisms (Tinsworth 1990) and this behavioural change provides the plant with a mechanism to protect itself against herbivory and predation or to assist in their reproduction (Rhoades & Coates 1976).

These substances can influence the egg laying behaviour of adult insects by attracting them to or repelling them from a surface. SPCs can stimulate or deter feeding in juvenile stages of pests and in some cases can prove fatal.

The objective of this study was to determine what if any biological activity occurred in *H. armigera* adults and larvae when exposed to extracts from a leguminous plant from Africa, herein referred to as Plant Y.

Materials and Method

Extracts

The methanol extract was partitioned using three solvents. The water partition was subjected to High Performance Liquid Chromatography (HPLC) and eight fractions were obtained.

Free choice Oviposition Trials

Filter papers impregnated with each fraction were placed in oviposition chambers. A 160 mm hole was cut at the base of the chamber and covered with nylon netting. 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 thus avoiding volatile contamination among fractions. The chambers were left for three days (72hours) in a temperature control room (24ºC - 26ºC and relative humidity of 56%).

No choice feeding response

There were a total of 7 fractions to be tested. Fractions f1 to f4 in one group and f5 to f8 in another. A cotton leaf disc (18mm in diameter) was treated with 200µl of one fraction pipetted and spread evenly on the lower and upper leaf disc surfaces and left to air dry for one hour. Each disc was placed into a 50mm petrie dish with a moistened 47mm filter paper lining. One second instar larva 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 25ºC (±2ºC) for 48 hours. The final weights of the leaf discs and larvae in each treatment were recorded after 48 hours.

The same procedure was used for f5, f6 and f8. However fractions 5, 6 and 8 were tested with a reduced volume of 100µl.

Unfortunately there was not enough of f7 to do more than one test and the result is not reported here because it could not be replicated.

Results

All fractions were characterised by LC-MS using a CI5-35 method. A number of compounds were detected in each fraction (Figure 1).

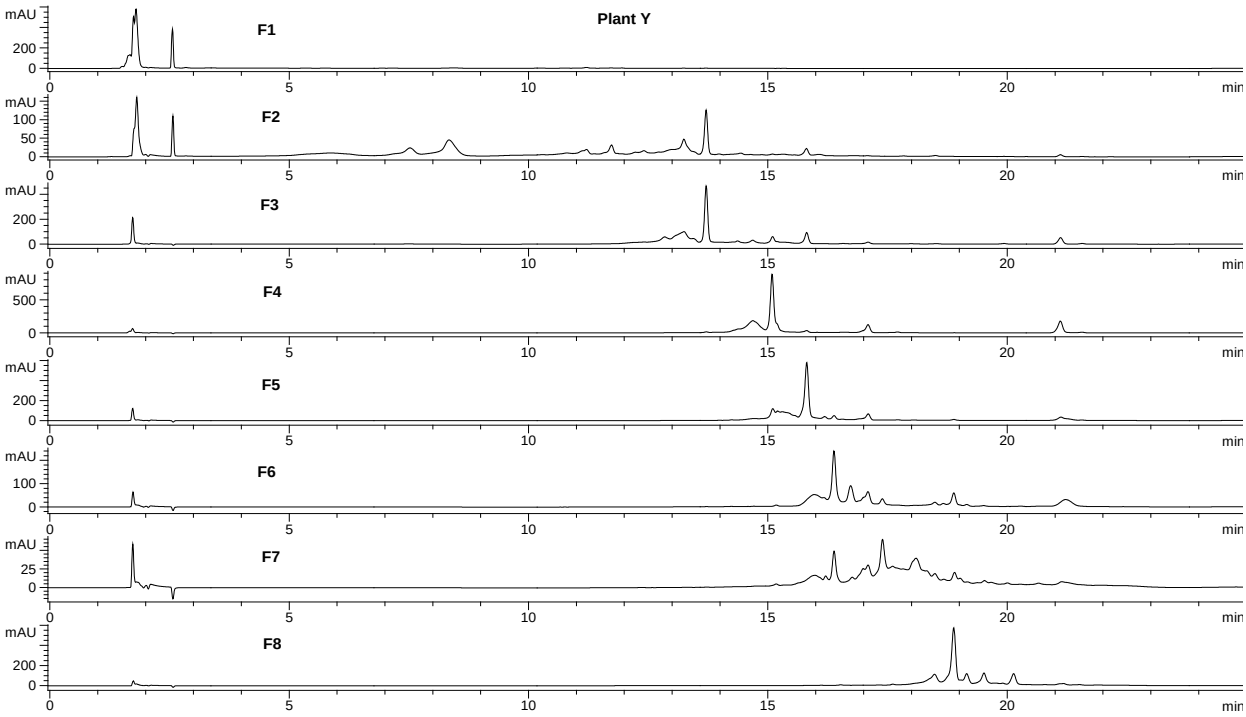

Figure 1. Comparison of HPLC chromatograms (280 nm) of fractions obtained for plant Y1.

Free choice oviposition

The oviposition activity of *H. armigera* was shown to be significantly stimulated when exposed to Plant Y crude extract as indicated by the higher egg numbers (Fig.2).

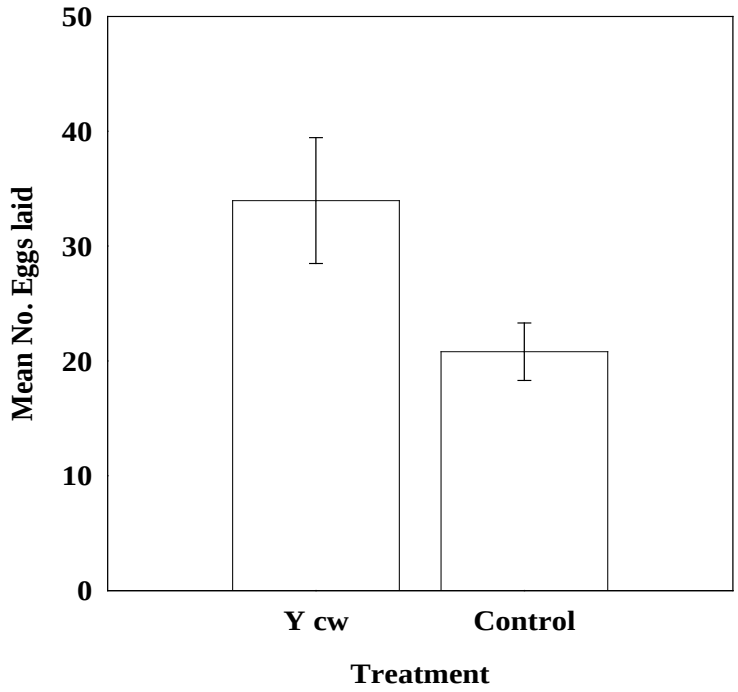

Figure 2- The stimulation of oviposition by *Helicoverpa armigera* when exposed to a crude extract of Plant Y, in the laboratory (free choice tests) at ACRI, Narrabri 2006.

Feeding Response

In no-choice feeding bioassays, significantly less leaf was consumed by 2nd instar larvae when treated with Plant Y fractions f3, f4, f5 and f8 (Fig.3 & 4). This resulted in a significantly lower weight gain in those larvae exposed to f3 & f4 (Figures 5 & 6). Fraction f4 had the greatest effect on consumption and larval weight which suggests it contains either stronger feeding deterrent compounds or a higher concentration of such compounds than the other fractions.

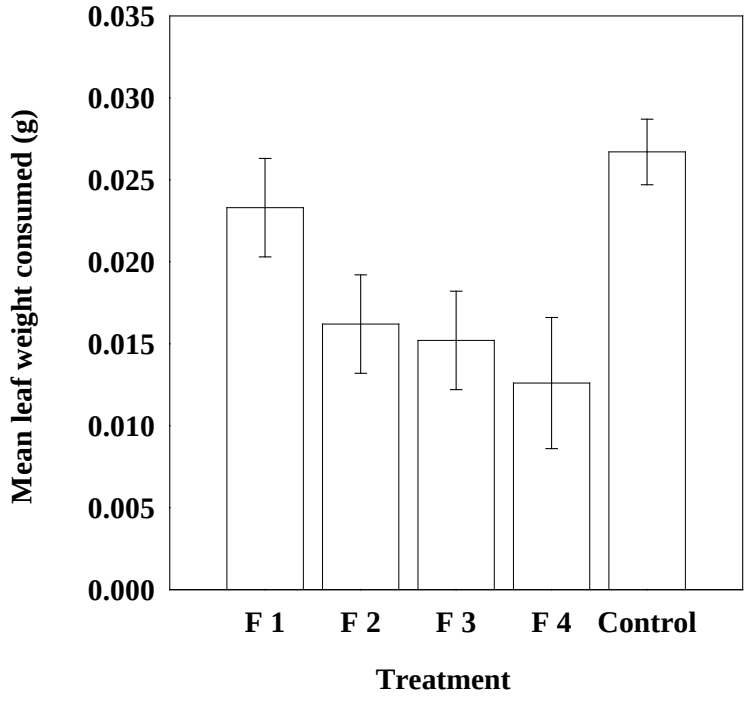

Figure 3- The effect of Plant Y fractions 1-4 on the leaf consumed by *Helicoverpa armigera* 2nd instar larvae in the laboratory (no choice tests) at ACRI, Narrabri 2006.

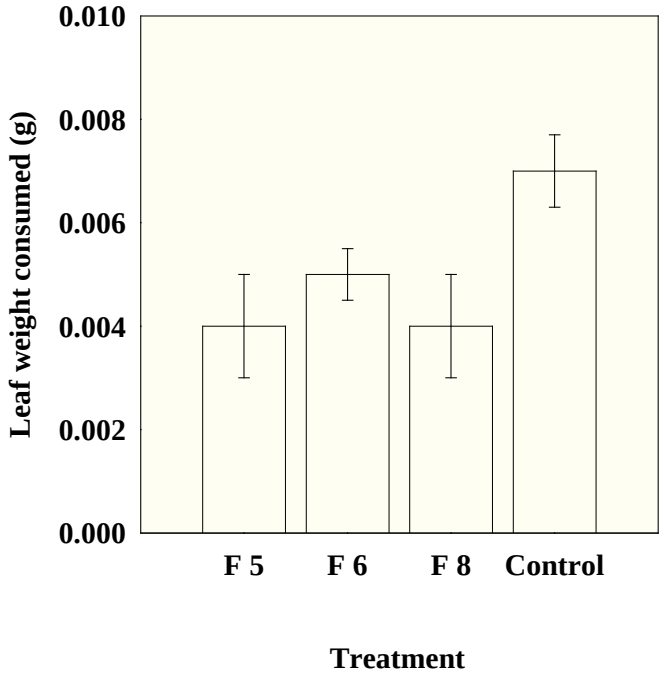

Figure 4- The effect of Plant Y fractions 5, 6 and 8 on the leaf consumed by *Helicoverpa armigera* 2nd instar larvae in the laboratory (no choice tests) at ACRI, Narrabri 2006.

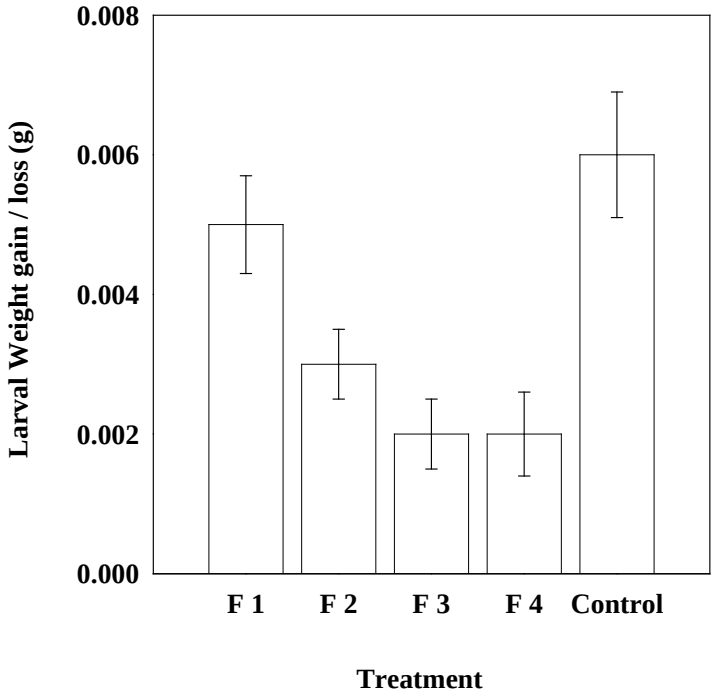

Figure 5- The effect of Plant Y fractions 1-4 on the weight gain or loss by *Helicoverpa armigera* 2nd instar larvae in the laboratory (no choice tests) at ACRI, Narrabri 2006.

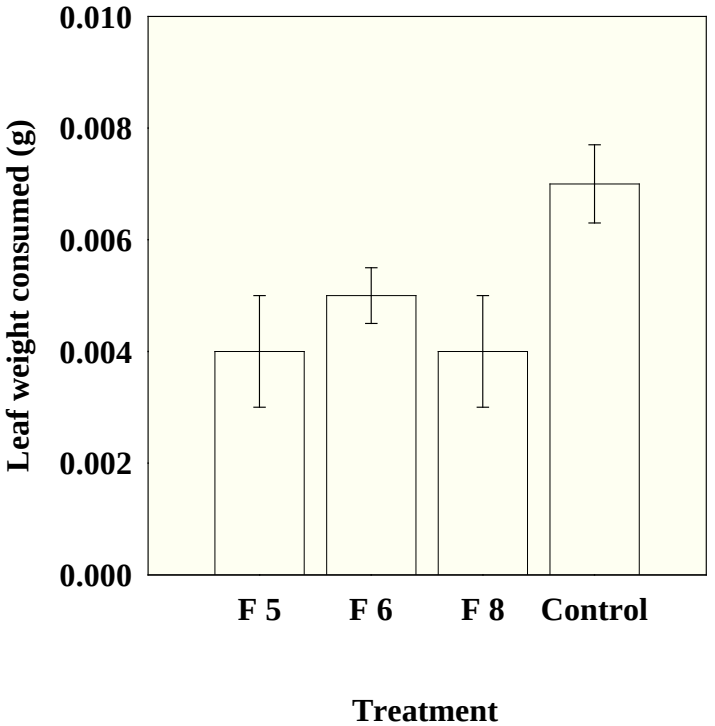

Figure 6- The effect of Plant Y fractions 5, 6 and 8 on the weight gain or loss by *Helicoverpa armigera* 2nd instar larvae in the laboratory (no choice tests) at ACRI, Narrabri 2006.

Discussion

The differing responses of the *H. armigera* adults and larvae to the secondary plant compounds present in Plant Y indicate the complexity of the insect-plant interaction. The presence of oviposition attracting compounds in Plant Y (Fig. 2) signal the plant as a suitable site for egg laying by *H. armigera* adults. This may reflect a need for assistance in reproductive processes (Rhoades & Coates 1976). The plant then protects itself with another suite of compounds that prove unpalatable to larvae (Fig. 3-6) thus protect it from herbivory (Rhoades & Coates 1976). It has been theorised that female moths select sites that are not only suitable for egg laying but increase the survivorship of the larvae (Rausher 1982; Thompson & Pellmyr 1991). However studies of egg laying preferences between transgenic and non-transgenic cotton showed no difference in egg numbers (Mensah *et al* unpubl.; Fitt & Wilson *et al* unpubl.), which suggests two things. First, moths cannot detect the presence of some toxins in the plant and thus no preference is shown (Moore *et al* 2005). Secondly *H. armigera* adults do not feed on the host plant and therefore rely on surface chemistry to identify suitable sites (Schultz 1988) and so have no knowledge of the internal chemistry which may be detrimental to grazing larvae.

This double interaction of the biologically active compounds in Plant Y (oviposition attractant and feeding deterrent) has potential to be included in IPM cotton systems. By manipulating the behaviour of pest species, it may be possible to reduce the number of pesticides used in the industry.

There is a widely held view that a stimulo-deterrent-diversionary strategy (SDDS) (Miller & Cowles 1990) or a push-pull strategy (Pyke et al 1987) will be more effective if the deterrent is teamed with an attractant. Thus, a trap crop (ie. Plant Y) that is attractive to ovipositing females could be used to draw the moths out of the economic crop. That same trap crop (Plant Y) would then have the potential to deter feeding, thus repressing larval development and increasing mortality through starvation. Another possibility is to utilize Plant X, another leguminous plant that has very strong oviposition and feeding deterrent compounds and has been shown to contain compounds that are toxic to 1st instar *H. armigera* larvae (Moore *et al* 2005). By treating Plant X with the oviposition attractant from Plant Y and drawing the moths into Plant X a significant decrease in the larval population can occur.

In conclusion, the combination of attracting and deterring compounds found in Plant Y show, on an isolated, scale the power of semio chemicals and the complex interactions between plants and insects. With the identification of the biologically active compounds of Plant Y and their inclusion with other semio chemicals from plants such as Plant X the realization of a potent stimulo-

deterrent-diversionary strategy (SDDS) (Miller & Cowles 1990) may lie in the not-too-distant future.

Acknowledgements

We would like to thank Cynthia Wilson for her technical assistance. This project is funded by the Australian Cotton CRC.

References

Fitt, G.P. (1994) – Cotton Pest Management: Part 3. An Australian perspective. *Ann. Rev. Entomol.* 39: 543-562.

Gatehouse, J.A. (2002) – Plant resistance towards insect herbivores: a dynamic interaction. *Tansley Review no. 40. New Phytologist* 156: 145-169.

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. *Intern. J. Pest Mgt.* 48(2): 95-105.

Miller, J.R. & Cowles, R.S. (1990) – Stimulo-deterrent- diversionary cropping: a concept and its possible application to onion maggot control. *J. Chem. Ecol*. in Singleton. A.; Wang, E.; Moore, C. & Mensah, R. (2004)–Managing *Helicoverpa* spp. using semio (signalling) chemicals. In the Proceedings of the 12th Australian Cotton Conference 8-12 August, 2004, Gold Coast Australia, pp.711-719.

Moore, C.; Mensah, R.; Wang, E.; Dang, H.; Cramp, A. & Singleton, A (2005) – *Managing Helicoverpa spp. on cotton using semio (signalling) chemicals.* Project CRC 36C incorporating 60C. A Final Report prepared for the Australian Cotton CRC.

Pyke, B.; Rice, M.; Sabine, B. & Zaluki, M. (1987) – The push-pull strategy – behavioural control of Heliothis. *Aust. Cotton Grower* May – July: p.7-9 in Singleton. A.; Wang, E.; Moore, C. & Mensah, R. (2004)–Managing *Helicoverpa* spp. using semio (signalling) chemicals. In the Proceedings of the 12th Australian Cotton Conference 8-12 August, 2004, Gold Coast Australia, pp.711-719.

Rausher, M.D. (1982) – Population differentiation in *Euphydryas editha* butterflies: larval adaptation to different hosts. *Evolution* 36: 581-590 in Mensah, R.K. & Moore, C. (unpubl.). A review of behaviour modifying chemicals in relation to pest host selection and management in

Australian cotton. Commissioned report prepared for the Cotton Research and Development Corporation.

Rhoades, D.F. & Coates, R.H. (1976) – Towards a general theory of plant anti-herbivore chemistry. In: J.W. Wallace and L. Mansell, eds. Biochemical Interactions between insects and d plants. Pp. 168-213, Plenum, New York in Moore, C.; Mensah, R.; Wang, E.; Dang, H.; Cramp, A. & Singleton, A (2005) – *Managing Helicoverpa spp. on cotton using semio (signalling) chemicals.* Project CRC 36C incorporating 60C. A Final Report prepared for the Australian Cotton CRC.

Schultz, J.C. (1988) – Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology* 69: 896-897 in Mensah, R.K. & Moore, C. (unpubl.). A review of behaviour modifying chemicals in relation to pest host selection and management in Australian cotton. Commissioned report prepared for the Cotton Research and Development Corporation.

Smart, L.E.; Blight, M.M.; Pickett, J.A. & Pye, B.J. (1994) – Development of field strategies incorporating semiochemicals for the control of pea and bean weevil, *Sitonia lineatus* L. *Crop Protection* 13 (2): 127-135 in Singleton. A.; Wang, E.; Moore, C. & Mensah, R. (2004)–Managing *Helicoverpa* spp. using semio (signalling) chemicals. In the Proceedings of the 12th Australian Cotton Conference 8-12 August, 2004, Gold Coast Australia, pp.711-719.

Thompson, J.N. & Pellmyr, O. (1991) – Evolution of oviposition behaviour and host preference in Lepidoptera. Annual Review of Entomology 36: 65-89 in Mensah, R.K. & Moore, C. (unpubl.). A review of behaviour modifying chemicals in relation to pest host selection and management in Australian cotton. Commissioned report prepared for the Cotton Research and Development Corporation.

Tinsworth, E.F. (1990) – Regulation of pheromones and other Semiochemicals in the United States of America. In Behaviour modifying chemicals for insect management, R.L. Ridgeway, R.M. Silverstein & M.N. Insloe, Marcel Dekker Inc., pp. 569-605 Mensah, R.K. & Moore, C. (unpubl.). A review of behaviour modifying chemicals in relation to pest host selection and management in Australian cotton. Commissioned report prepared for the Cotton Research and Development Corporation.

Wittstock, U. & Gershenzon, J. (2002) – Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current Opinion in Plant Biology* 5 (4): 300-307.