ISOLATION, IDENTIFICATION AND POTENTIAL USES OF SEX PHEROMONES FOR THREE PESTS OF COTTON IN AUSTRALIA

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Preface

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

The findings in Chapter 6 of this thesis have been incorporated in Provisional Patent application 2004960490 filed on 12 November 2004 with the Australian Patent Office.

Samuel Tetteh Lowor

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ABSTRACT

The Australian cotton industry is heavily dependent on chemical insecticides for pest control. As a result, a number of environmental issues involving off-farm movement of these pesticides have been raised for the industry. One of the key issues in meeting the challenges of growing cotton in tomorrow's world involves pest management in a more environmentally friendly way. This involves reduction of pesticide use and adoption of integrated pest management (IPM) approaches. Introduction of transgenic cotton in recent years and application of insecticides targeting individual species has enabled a drastic reduction in pesticide use. This allows the numbers of important beneficial insects to build up, aiding in pest control. On the other hand, species like the green mirid (*Creontiades dilutus*), cotton tipworm (*Crocidosema plebejana*) and rough bollworm (*Earias huegeliana*), which were not major problems under intensive use of insecticides are forecast to be more significant pests, which will require a re-evaluation of IPM in cotton.

One potential component of IPM involves the use of insect sex pheromones in mating disruption, monitoring, attract-and-kill and mass trapping. Pheromones could be used to predict oviposition on a field by field basis and also give useful indications of the overall abundance of the pests mentioned above. Identified pheromones therefore could be used in area-wide pest management schemes. Pheromones can also be used in attract-and-kill strategies or for mating disruption. Although the Australian cotton industry has not previously made significant use of these techniques, there are ecological reasons for believing that they may be more applicable to some emerging pests than to the key pests of cotton under previous pest management regimes, *Helicoverpa* spp.

This project was carried out to identify the sex pheromones of three pests of cotton in Australia – the rough bollworm, cotton tipworm and green mirids, to test attractive blends and to investigate potential uses of their sex pheromones as part of the general IPM system of the Australian cotton industry.

The sex pheromone of the rough bollworm was identified using gas chromatography and mass spectrometry (GC-MS) from female gland extracts and air collections. Identified compounds were formulated into a blend and tested in the field for attractiveness to males. The GC-MS analysis revealed four compounds, (*E,E*)-10,12-hexadecadienal, (*E,E*)-10, 12-hexadecadienol, (Z)-11-hexadecenal, and (Z)-11-octadecenal in a ratio of 4:1:1:1 in the gland extracts. (*E,E*)-10, 12-hexadecadienol was not detected in the air collections. Field bioassay showed the two components, (*E,E*)-10,12-hexadecadienal and (Z)-hexadecenal to be essential for activity of the blend. This blend was highly attractive to males only. Two trap designs, the AgriSense® and Delta traps were tested, and the Delta trap was the better of the two. A weathering experiment to determine how long pheromone loaded septa would remain attractive in the field indicated that the lures could be used for a maximum of four weeks in the field. Male response to pheromone baited traps was found to be in the second half of the night, between 2 to 5 am. This was found to be synchronised to female calling time.

Sex pheromonal compounds from the glands of the cotton tipworm have been identified as a mixture of octadecanal, 2-nonadecanone, acetic acid octadecyl ester (18Ac) and octadecanol in a ratio of 2:2:1:2, respectively. Most lepidopteran sex pheromone systems are multi-component and the relative composition may be critical to be effective attractants. Preliminary field trials however indicated the possibility of using only 18Ac as an effective trap attractant.

The calling behaviour of the cotton tipworm was studied in the laboratory at 25°C and 16:8 light:dark condition. When calling, *C. plebejana* had wings slightly raised above the abdomen with full protrusion of the ovipositor. The age at which *C. plebejana* called for the first time was the third scotophase. The mean onset time of calling was found not to advance with age, and was about 5 hours into the scotophase. Duration of calling ranged from 6 minutes on the 3rd scotophase to a maximum of 77 minutes on the 7th scotophase before dropping gradually to 4 minutes on the 12th scotophase. There was a high correlation between the number of calling bouts and age. Generally, the number of calling bouts increased with age. Calling behaviour and pheromone production of females was synchronous. Female gland extracts generally contained about 10-12 ng/female as compared to 2 ng/female in the air collections.

The sex pheromone produced by adult females of the green mirid was identified as a blend of hexyl hexanoate and (*E*)-2-hexenyl hexanoate. The pheromone was found to be sex and species specific, attractive only to conspecific adult males. Hexyl hexanoate was identified in both sexes, while (*E*)-2-hexenyl hexanoate was produced only by the females. A blend in a ratio of 5:1 was estimated from field trapping experiments as optimal, though ratios of 3:1 to 7:1 were equally effective. Influences of pheromone septa loading on male attraction to traps were studied using loadings of 2, 20 and 40 mg. Results indicated blend attraction was generally not affected by the loading levels used. In the field, *C. dilutus* males were observed to respond to pheromone baited traps in the night, especially in the early part of the night, at least when the temperatures were high enough to permit night flight. Initial attempts at applying the pheromones in a sprayable formulation for mating disruption and attract-and-kill provided some encouraging results. The use of *C. dilutus* lures to provide an effective, economic, and environmentally sound monitoring tool for this pest is discussed.

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