Post Release Monitoring of *Xubida infusella* (Lep.: Pyralidae): An Example of Using Pheromones for the Early Detection of Establishing Populations of Biological Control Agents

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Abstract

Synthetic pheromone trapping was used to detect early populations of *Xubida infusella* (Walker) (Lep.: Pyralidae), introduced for the classical biological control of the notorious aquatic weed, waterhyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach) in Australia and Papua New Guinea. This example demonstrates that for an agent that disperses readily, a pheromone of even modest attraction can provide evidence that an agent is present long before visual sampling methods. *Niphograpta albiguttalis* Warren (Lep.: Pyralidae) was also attracted to components intended to detect *X. infusella*. Pheromone trapping allowed continual monitoring in remote areas with a low requirement for technical expertise or equipment in the monitoring region or country. The benefits of early detection allow earlier assessment of release techniques and decisions concerning additional releases and allocation of resources. Early detection is especially important in projects of relatively short duration.

Keywords *Xubida infusella*; *Niphograpta albiguttalis*; pheromone; monitoring; biological control; establishment.

Introduction

If a biological control agent disperses widely upon release or emergence in the field it may be several years before even substantial populations are detected by commonly used sampling methods. Without sampling techniques that can detect early, low-densities populations, biological control practitioners may continue to mass-rear and release agents where they are possibly not needed because populations are already persisting, or where they are not suited because some unknown aspect of a particular site is deficient. Both represent an inefficient allocation of resources. Furthermore a prolonged period after release in which agents can not be recovered may have an adverse effect on the enthusiasm and support of practitioners, participating groups (e.g. councils and the public) and funding organisations.

One option for detecting early, low-density field populations is pheromone trapping. In most cases a protracted research effort is needed to produce a pheromone that performs well enough to provide estimates of population density, but only a moderately effective attractant is required to provide useful presence/absence information for initial biological control purposes.

The only published use of pheromones in weed biological control known to us was by Suckling *et al.* (1999) who used a codling moth *(Cydia pomonella* Lep.: Tortricidae)-derived pheromone to monitor *Cydia succedana* (Lep.: Tortricidae) released for the control of gorse (*Ulex europaeus* L.) in New Zealand. They concluded that pheromone mon-

itoring has excellent potential to provide information on the size of individual releases required for establishment, the suitability of habitats, and the phenology and efficacy of an agent. However this study was conducted four years after the release when populations were established.

Several unpublished attempts have been made to use pheromones to monitor biological control agents of weeds. J. Cullen (pers. comm. 1999) used virgin females caged in sticky delta traps to show that at least two generations of *Bradyrrhoa gilveolella* (Lep.: Pyralidae) had occurred after release in Australia on Skeleton weed *(Chondrilla juncea* L.). J. Hoffman (pers. comm. 1999) used virgin females caged over water baths to sample *Tucumania tapiacola* (Lep.: Pyralidae) released on tiger pear *(Opuntia aurantiaca* Lindley) in South Africa. He caught so many of a native species instead of *T. tapiacola* that he speculates that the failure of the agent to establish might have been due to a natural form of mating disruption. M. Steinbauer (pers. comm. 1999) tried a commercially available pheromone to monitor *Carmenta mimosa* (Lep.: Sesiidae) for the control of mimosa (*Mimosa pigra* L.) in the Northern Territory, Australia, but only a few individuals were recovered despite concurrently operated malase traps collecting substantially more.

This paper presents evidence of pheromone trapping, for appropriate insects, being an effective means of presence/absence monitoring of biological control agents within the establishment phase. We have used this approach to detect early field generations of *Xubida infusella* in Australia and Papua New Guinea, as part of a program for the biological control of waterhyacinth (*Eichhornia crassipes)*.

Materials and Methods

Pheromone Collection

Xubida infusella is a nocturnally active insect, which originates from South America. Newly emerged adults from laboratory cultures were paired and observed under red light to determine the time of female calling. Calling behaviour was not obvious, but all ten pairs set aside for observation began mating between 10 pm and midnight (~4 hours after sunset). Calling was assumed to begin shortly before this, a time when pheromone could be expected to be present in the largest quantities in the pheromone gland. Therefore, over several nights between 9.30 pm and 10.30 pm, newly emerged virgin females were anaesthetized with carbon dioxide and their ovipositors removed. Immediately after each dissection the ovipositor, containing the pheromone gland, was submerged in 5 µl of hexane in a 0.2 ml glass vial and stored in a freezer (*ca* -8°C) pending analysis. There were usually two ovipositors placed in each vial providing a sample for each analysis. The contents of *Niphograpta albiguttalis* pheromone glands were collected in the same way although mating time was not determined.

Gas Chromatography

Gas Chromatography was carried out in a Varian 3700 using a variety of capillary columns (Alltech® MC-1, MC-wax multibore, and SGE BP20), preceded by a guard column, a stainless steel large bore connector and a cold trap. Detection was by flame ionisation. The hexane extract was deposited on a sliver (1mm x 10mm) of glass fibre filter paper and the hexane was allowed to boil off. The filter paper was inserted into the large bore connector and the contents transferred to the cold trap (0°C) by running the GC oven at 150°C for 5 minutes. The oven was then rapidly cooled, the filter paper removed and

the trap warmed to room temperature. A GC run was then carried out using a temperature ramp of 10°C per minute to a maximum temperature of 200°C. The output from the detector was digitized and stored in a PC for later analysis and plotting.

Identification of pheromone components

Gas chromatagraphy (GC) was used to identify candidate pheromone components within the hexane extractions from the pheromone glands. The electroantennogram technique (EAG) was used to determine the position of double bonds using a reference series of 16-carbon acetate compounds. Mixtures of identified components were field tested in Australia at Loganholme (28 km southeast of Brisbane) and in Papua New Guinea (PNG) at Waigani Lake (approximately 10 km north of Port Moresby) in replicated trials (n = 4). The pheromone was presented in lures (rubber-septa) suspended within delta traps with sticky (Tanglefoot®) bases (Vickers *et al.* 1998). Traps were suspended within the upper third of the waterhyacinth canopy using tie-wire wrapped around several petioles of nearby plants. Leaves that might have blocked the opening of the traps were removed.

Field trial 1 included a trial in PNG and Australia, comparing four treatments: lures without components (controls), aldehyde alone or the aldehyde and alcohol in a ratio of 5:1 or 10:1 (aldehyde:alcohol, 1mg of Z11-16:aldehyde). Since insects were only collected in the 10:1 treatment, field trial 2 compared the 10:1 ratio at concentrations of zero (controls), 10µg, 100µg and 1mg per septum at Loganholme, Australia. Field trial 3 tested a third component (n16 Aldehyde), also detected in the GC traces, at the Loganholme site (n = 4). The treatments were controls (rubber septa without components), and 1 mg loading of 2-components (10:1) and 3-components (10:1:1). In each trial trap catches were recorded weekly for approximately one month after setting lures. All trials were conducted during the spring and summer between August 1997 and April 1999.

The presence of *X. infusella* at Loganholme was confirmed using a mercury vapour light trap at weekly intervals since October 1998. In field trial 4 we monitored this site using the 2-component (10:1) lure to determine whether the pheromone could detect the population. Furthermore, following observations that old lures (one month after setting) were capturing *X. infusella,* the low 10 µg concentration was re-tested. Samples of recovered insects were submitted to the Australian National Insect Collection to confirm the species caught in the traps were *X. infusella* and *N. albiguttalis*.

Visual and Light Trapping

Visual sampling involved searching an area (approximately 100 square meters) of waterhyacinth in the vicinity of the release site. Samplers searched for characteristic signs: the yellow subcutaneous mining of *X. infusella* larvae (often forming a ring around a petiole causing death of the leaf above), pupal emergence holes and pupal cases protruding from emergence holes. The petioles and rootstock of plants with any evidence of *X. infusella* damage were split and searched for larvae or pupae.

Light trapping was conducted once a week for two hours following dusk on the banks of the waterhyacinth infestation at Loganholme. A 400 watt mercury-vapour bulb was suspended at a height of 1.5 m and 0.5 m in front (waterhyacinth side) of a 2 m x 1.5 m white cotton sheet set up in a vertical plane, parallel to the bank. Power was supplied by a petrol driven, 240-volt generator operating 25m away, behind the sheet. The sheet was constantly surveyed for *X. infusella* which were stealthily captured in small plastic vials for counting and sexing.

Results

The combined results from the GC analysis (Fig. 1) and EAGs (Fig. 2) revealed that the sex attractant of *X. infusella* contained at least two components; Z11-16 aldehyde and Z11-16 alcohol in a ratio of approximately 5 to 10:1. Traces of n16 and n18 aldehydes were also detected in some but not all collections.

Catches of *X. infusella* in the field trials were small and they were only present in traps with both components (Z11-16 aldehyde and Z11-16 alcohol) at a ratio of 10:1 (Table 1).

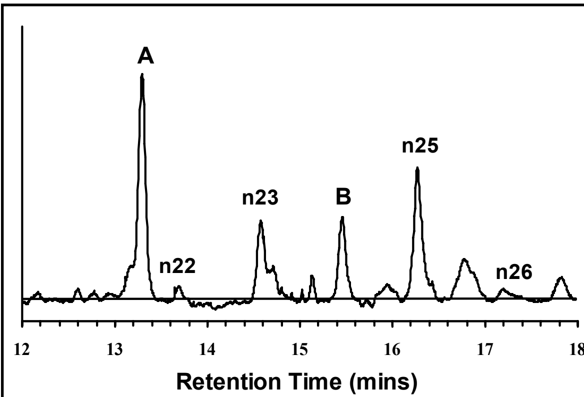

Fig. 1. Gas Chromatograph trace of the contents of the *Xubida infusella* female pheromone gland extract. 'A' and 'B' are the major components Z11-16 Aldehyde and Z11-16 Alcohol respectively. 'n23' and 'n25' were known standard saturated compounds added to identify, by their position, compounds represented by other peaks. 'n22' and 'n26' were naturally present in the sample.

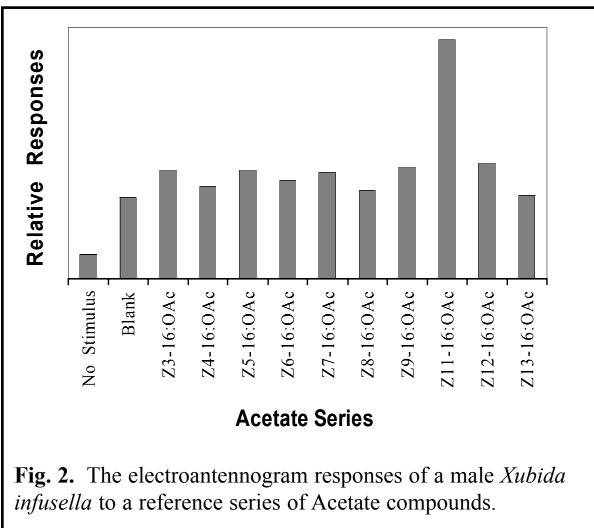

Fig. 2. The electroantennogram responses of a male *Xubida infusella* to a reference series of Acetate compounds.

Niphograpta albiguttalis, another pyralid moth species, was also captured. It was collected whether Z11-16 aldehyde was presented alone or in combination with Z11-16 alcohol but not in the 5:1 ratio (Table 1). GC analysis also identified Z11-16 aldehyde in extracts from *N. albiguttalis* pheromone glands but no other components were identified.

Catches of *X. infusella* were too small to determine any differences between the concentration treatments in the component concentration trials (Table 1, trial 2). However, during trial 2 a large number of *N. albiguttalis* were collected in one set of treatments (ie. only 3 traps at one location). These catches exhibited a positive linear relationship to the log of concentration (3, 17 and 26 insects/trap).

Xubida infusella has been released in two countries and subsequent field generated progeny recovered at one site in each using the 2-component lure in a concentration of 10:1. The first pheromone collections at Waigani Lake in PNG were made in August 1997 when three field gen-

Table 1.
Pheromone trap catches of X. infusella and N. albiguttalis in field trials in Australia (Loganholme) and PNG (Waigani Lake).

Site	Pheromone Components	Component Concentrations	Ratios	Number of Traps (n)	No. of each Species *X. infusella*	*N. albiguttalis*
Field Trial 1.	Which Component and Ratio?					
Loganholme	Control (only rubber septa)	na	na	4	0	0
Loganholme	Z11-16Ald	1mg	na	4	0	11
Loganholme	Z11-16Ald + Z11-16OH	1mg	(5:1)	4	0	0
Loganholme	Z11-16Ald + Z11-16OH	1mg	(10:1)	4	8	3
Waigani Lake	Control (only rubber septa)	na	na	3	0	0
Waigani Lake	Z11-16Ald	1mg	na	3	0	0
Waigani Lake	Z11-16Ald + Z11-16OH	1mg	(5:1)	3	0	0
Waigani Lake	Z11-16Ald + Z11-16OH	1mg	(10:1)	3	2	0
Field Trial 2.	Which Concentration?					
Loganholme	Control (only rubber septa)	na	na	4	0	0
Loganholme	Z11-16Ald + Z11-16OH	10μg	(10:1)	4	1	3
Loganholme	Z11-16Ald + Z11-16OH	100μg	(10:1)	4	0	19
Loganholme	Z11-16Ald + Z11-16OH	1mg	(10:1)	4	1	28
Loganholme	Z11-16Ald + Z11-16OH	10μg	(10:1)	4	9	0
Loganholme	Z11-16Ald + Z11-16OH	1mg	(10:1)	4	5	0
Field Trial 3.	Additional Component?					
Loganholme	Control (only rubber septa)	na	na	4	0	0
Loganholme	Z11-16Ald + Z11-16OH	1mg	(10:1)	4	11	2
Loganholme	Z11-16Ald + Z11-16OH + n16Ald	1mg	(10:1:1)	4	7	0
Field Trial 4.	Can the Pheromone Indicate Population Presence? (includes all 10:1 traps recorded during the light trapping period at Loganholme)					
Loganholme	Z11-16Ald + Z11-16OH	All	(10:1)	35	37	0
Overall Catch With 10:1 Lure						
Loganholme	Z11-16Ald + Z11-16OH	All	(10:1)	55	52	60
Waigani Lake	Z11-16Ald + Z11-16OH	1mg	(10:1)	3	4	0

erations of agent could be expected to have occurred since release and when visual sampling of the site failed to recover any evidence of the insect's presence. The first pheromone collections in Australia occurred at Loganholme in March 1998. This confirmed at least 3 field generations since the last release at this site in August 1997, however many releases were made here following the first in September 1996.

Discussion

We have identified two components of the pheromone of *X. infusella* as Z11-16 aldehyde and Z11-16 alcohol. In combination at a ratio of 10:1 they have proved a modest attractant, at least capable of detecting field populations in Papua New Guinea and Australia when visual sampling efforts detected nothing.

The single light trap collected, on average 8.5 and up to 43 *X. infusella* over the two-hour period it was operated each week. This was far more sensitive than the current pheromone technique for which the maximum catch for a single trap, operating continuously for a week, was two. Thus, light trapping would generally be preferred for detecting low density populations of *X. infusella* but pheromone trapping was more appropriate in situations, like PNG, where it was not feasible to station expensive equipment or personnel at field sites over night. A further benefit was the low cost of the pheromone traps which would allow very large areas to be surveyed constantly for several months. The pheromone method was obviously a marked improvement on visual searches, which absolutely failed to detect these populations.

The low pheromone catches suggest that improvements to the synthetic pheromone are possible, either through additional components, or by concentration and/or ratio manipulations. Our attempts to investigate this using a range of concentrations or by including n16 aldehyde did not appear to improve the catch of *X. infusella*, however the original aim of the study, which was to detect the presence of fledgling populations, was achieved.

Lures containing Z11-16 aldehyde also attracted *N. albiguttalis*, another waterhyacinth biological control agent. Z11-16 aldehyde alone and at the strongest concentration tested (1mg) appears, by the size of the catches to be a relatively effective attractant for this species. The question arises, because both agents originate from the same habitat in the same native range (Brazil and Argentina), how males of *N. albiguttalis* avoid females of *X. infusella.* This lack of apparent specificity could be solved by the presence of additional components in the pheromone of *X. infusella* which act as deterrents to males of *N. albiguttalis*. However, differences in mating behaviour might also explain this, perhaps the females call when the males of the other species are not responsive.

The early detection of field generations of *X. infusella* had several advantages. It allowed us to report to funding organizations that some degree of success had been achieved before the project funding had ceased. Practitioners who were carrying out fruitless visual searches were encouraged by the detection of field generations of the agent to persevere with laborious rearing and release schedules, possibly greatly improving the chances of establishment. Furthermore, such early information can be used to improve resource allocation within a biological control program. For example, *N. albiguttalis* was also released in PNG during this waterhyacinth program but no evidence of establishment was found during our pheromone monitoring. This and the fact that *X. infusella* appears to be establishing suggests that some of the resources to release *X. infusella* might best be diverted, or extra funding sought, to concentrate on establishing *N. albiguttalis*.

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References

Suckling, D.M., R.L. Hill, A.H. Gourlay, and P. Witzgall. 1999. Sex attractant-based monitoring of a biological control agent of gorse. Biol. Sci.Tech. 9: 99-104.

Vickers, R.A., W.G. Thwaite, D.G. Williams, and A.H. Nicholas. 1998. Control of codling moth in small plots by mating disruption: alone and with limited insecticide Entomol. Exp. Appl. 86: 229-239.