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Exploiting the aggregation pheromone of strawberry blossom weevil *Anthonomus rubi* Herbst (Coleoptera: Curculionidae): Part 1. Development of lure and trap

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Abstract

This study was the first part of a project to assess the applicability of the male-produced, aggregation pheromone of the strawberry blossom weevil, *Anthonomus rubi,* for commercial monitoring and control. An optimised pheromone lure and trap were developed. Low-cost, robust and reliable polyethylene sachet dispensers containing 100 μl of a blend of grandlures I, II and (±)-lavandulol in the naturally occurring 1:4:1 ratio were shown to have constant release rates of 0.64 and 0.96 mg/day at 20 and 27 °C, respectively and a life of over 8 weeks in the field. Field experiments showed that increasing the release rate by approximately five times marginally increased attractiveness but a four times reduction in the release rate significantly decreased attractiveness. It was concluded that the standard release rate was satisfactory. Male *A. rubi* weevils were shown to produce the *R* enantiomer of lavandulol, but it was also demonstrated that the *S* enantiomer is not repellent and that low-cost racemic (±)-lavandulol is equally attractive. Although (−)-germacrene-D showed a weak synergistic effect when added to the pheromone components, inclusion in a commercial lure was uneconomic. Two further experiments examined the effect of reducing the amount of grandlure I, a costly chemical, in the blend. Although the results of one of these experiments was inconclusive, in the other it was found that reducing the amount of grandlure I by a factor of four did not decrease attractiveness significantly, though the ratio of males to females decreased significantly. A four times reduction in grandlure I content gives a 40% reduction in the cost of the chemicals in the lures. Experiments were carried out to develop an effective and practical trap design. Various modifications of the sticky board trap used in the original work were compared with boll weevil, funnel, delta and sticky stake designs. Most weevils were caught with the sticky stake design, made from a pointed wooden stake inserted vertically into the ground with a band of polybutene sticker around the circumference and a plastic board fixed horizontally on top of the stake to provide protection of the sticky surface from rain. A lure was hung from one corner of the board.

Keywords: Strawberry blossom weevil; *Anthonomus rubi*; Aggregation pheromone; Germacrene-D; Grandlure I; Grandlure II; Lavandulol; Enantiomers; Release rate; Lure optimisation

1. Introduction

The strawberry blossom weevil, *Anthonomus rubi* Herbst, is an important pest of early season strawberries throughout Western and Central Europe. After laying an egg in an unopened flower bud, the female walks a few millimeters down the flower stalk and partially severs it with her rostrum (Jary, 1932; Alford, 1984). The flower bud withers and often falls from the plant. The larva develops within the damaged bud, pupates, and the adult emerges early in summer. The emerged

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adults, which are in reproductive diapause, feed in and around the crop for a few weeks before migrating to overwintering sites. Each female may sever large numbers of flower buds and damage can be severe.

A. rubi is controlled by the application of broad-spectrum insecticides, such as chlorpyrifos or synthetic pyrethroids, in spring to kill adults when the flower truss (inflorescence) petioles have partially grown, before flowering and before significant damage is done. Sprays are often applied routinely or when the first flower bud severing damage is seen, but it is difficult to quantify the likely severity of attack by brief visual crop inspection. Reduced insecticide application is environmentally desirable and alternative methods for monitoring and control are needed.

Innocenzi et al. (2001) identified grandlure I, grandlure II and lavandulol as components of the aggregation pheromone produced by male *A. rubi* (note: 'grandlure' is the the name given to four components of the aggregation pheromone lure of the cotton boll weevil, *Anthonomus grandis* Boh.). The components occurred naturally in the ratio 1:4:1, respectively, and the blend of synthetic compounds attracted both male and female weevils to traps in the field. Traces of grandlures III and IV were also detected, but these did not significantly increase trap catches when added to the other three components (Innocenzi et al., 2001). The grandlure I was the (+) enantiomer and the lavandulol was shown to be a single enantiomer but the absolute configuration was not determined. Germacrene-D, a known volatile from strawberry plants, was also collected in increased amounts in the presence of pheromone-producing weevils.

Innocenzi et al. (2001) used a simple prototype trap design for initial field evaluation and optimisation of the pheromone lure. This consisted of a horizontal white plastic board coated with adhesive on both sides and fixed to the top of a wooden stake. The lure was held on top in a small cage made from a hair curler. Although this first prototype proved satisfactory for the purposes of initial testing, no other work had been done to test alternative trap designs or improve on the prototype design. However, approximately 75% of the weevils were captured on the lower surface of the prototype trap.

Pheromone traps provide a potentially efficient and practical method for monitoring of *A. rubi*. Furthermore, the attractiveness of the strawberry blossom weevil pheromone to both males and females theoretically made it more likely that the pheromone could be exploited for control of the pest by mating disruption, mass trapping or lure and kill approaches. The pheromones of other Curculionid species have been identified, and several have found use in monitoring and control (Bartelt, 1999).

From the initial pheromone identification of an insect pest to the commercial availability of the pheromone system, there are numerous time-consuming and costly stages. One of these stages involved in this 'molecule to marketplace' process is the development of an efficacious and cost-effective formulation (e.g. Weatherston, 1990). Here we report the first part of a project to assess the applicability of the pheromone for monitoring and control of *A. rubi* in commercial strawberry crops. In order to provide an attractive and economical lure, studies were carried out of the effects on trap catches of the release rate of pheromone, the enantiomers of lavandulol, addition of germacrene-D and the relative amount of grandlure 1 in the blend. A range of alternative trap designs was also tested to develop an effective and practical trap for use in conjunction with the lure for pest monitoring. Use of the lure and trap for monitoring and control of *A. rubi* will be reported in the following paper.

2. Materials and methods

2.1. Chemicals

Grandlure I ((±)-*cis*-1-methyl-2-(1-methylethenyl)cyclobutaneethanol, purity 98%) and grandlure II ((*Z*)-2-(3,3,-dimethylcyclohexylidene)ethanol, purity 99.5%) were obtained from Bedoukian, Connecticut, USA. Grandlure III ((*Z*)-2-(3,3-dimethylcycloheylidene)-acetaldehyde) and grandlure IV ((*E*)-2-(3,3-dimethylcyclohexylidene)acetaldehyde were obtained as a mixture of grandlures I–IV in relative proportions of 3:3:1:1 from Plato Industries, Houston, TX, USA.

(−)-Germacrene-*D* ((−)-(7*S*)-1-(1-methylethyl)-4-methylene-8-methyl-2,7-cyclo-decadiene) was the major component (approximately 20%) in ylang ylang oil (Holland and Barrett, Nuneaton, Warwickshire, UK) and was purified to approximately 80% purity by flash chromatography on silica gel eluted with hexane. The main impurities were *β*-caryophyllene (14%) and *α*-farnesene (4%).

(±)-Lavandulol ((±)-2-(1-methylethenyl)-5-methyl-4-hexen-1-ol, 95% purity) was obtained from Fluka, UK. (*R*)-Lavandulol of known configuration was isolated from lavender oil (Koenig et al., 1992). Thus lavender oils of different origins were screened by GC for lavandulol and the acetate, the one with the highest levels (Goodebodies, Dublin, Ireland) containing 4.7%. A sample (6 g) was hydrolysed with potassium carbonate in methanol and the lavandulol isolated by column chromatography on silica gel eluted with 20% diethyl ether in hexane. Two fractionations gave material (0.18 g) containing 75% (*R*)-lavandulol, the major impurities being borneol (13%) and linalool (4%). For determination of enantiomeric composition of chiral materials, analyses were done using a Varian 3700 gas chromatograph (GC) on a cyclodextrin capillary column