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Project leader:	Professor Jerry Cross East Malling Research
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Location of project:	East Malling Research Natural Resources Institute
Industry Representatives:	Mark Holden, Adrian Scripps Ltd. John Evans, J A Colthup & Partners
Date project commenced:	1 April 2013
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Professor Jerry Cross

Research Programme Leader

East Malling Research

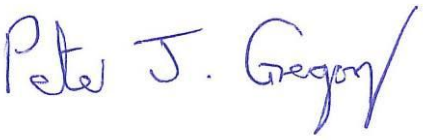
Signature ..... Date .....

Report authorised by:

Professor Peter Gregory

Chief Executive

East Malling Research



Signature ..... Date: 17 March 2014

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GROWER SUMMARY

Headline

Field and laboratory work have been started to determine whether apple fruit rhynchites produce pheromones.

Background and expected deliverables

Damage by apple fruit rhynchites (AFR), *Rhynchites aequatus,* (Fig. 1) has been increasing in UK apple orchards and sometimes pear orchards in recent years, probably due to changing patterns of insecticide use. Losses of 1% of fruit are common and losses >5% are not unusual. Hawthorn is the pest's normal host. Damage to apple is caused by feeding punctures in young developing fruitlets during and after blossom. However, egg laying and larval development inside apples (Massee 1954; Alford 1984) must be rare because it is never seen or reported by growers or advisors. The pest causes damage at low population densities and the weevils are difficult to spot whilst they are feeding or egg laying. The extent of damage only becomes apparent when the characteristic corky scars develop when it is too late to take action.

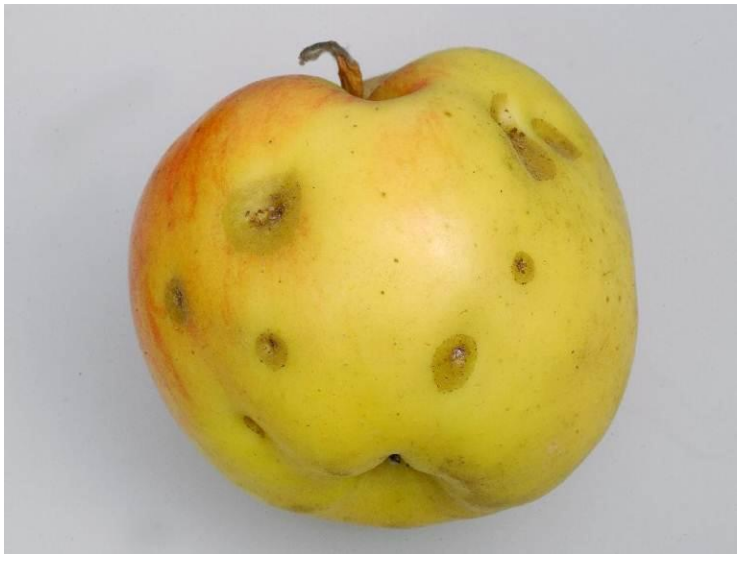

Figure 1. Damage by apple fruit weevil, *Rhynchites aequatus,* on apple

The weevil can be controlled by sprays of chlorpyrifos or thiacloprid (Calypso) but the former cannot be used during blossom because of its risk to bees and growers are reluctant to use thiacloprid during flowering for the same reason, even though the label indicates it can safely be applied during bloom. Chlorpyrifos is also broad spectrum and can damage

other beneficial insects in the orchard and both chlorpyrifos and thiacloprid are damaging to earwigs.

It would be beneficial to develop a sensitive, species-specific semiochemical based monitoring trap for this pest. However, it is not known whether *R. aequatus* produces a sex or aggregation pheromone, when it is produced or which sex produces it. Many weevils are known to produce sex or aggregation pheromones, e.g. strawberry blossom weevil and pepper weevil, but in others, pheromones do not seem to be so important, e.g. apple blossom weevil. Nothing is known about pheromones of Rhynchitidae and so it is important to demonstrate in preliminary studies whether or not semiochemical-mediated sexual attraction occurs before embarking on a major project to identify, synthesise and exploit it for pest monitoring or control.

Summary of the project and main conclusions

In Year 1 (2013) we aimed to determine whether sexual attraction occurs between male and female apple fruit rhynchites (AFR), which sex is attractive and the time that attraction occurs. We also conducted preliminary collections and analyses of volatiles from AFR to determine whether any candidate pheromone components can be readily detected and identified.

Live AFR were collected in the spring from several apple orchards and hawthorn trees and a method to determine the sex of the weevils was identified (previously alluded to by M. G. Morris, 1990). In the laboratory, males and females were able to identify each other and successfully mate resulting in eggs being laid. This indicated that the weevils were suitable for use in trapping experiments and for collection of volatiles to identify potential pheromone components.

These weevils were used as bait in trapping experiments deployed in an unsprayed apple orchard with a known AFR population. Traps were checked for weevils and trees were tap sampled over a tray. Data was collected weekly and the sex of the weevils was identified.

More AFR weevils were obtained by tap sampling than in the traps used in this trial which were white cross vane bucket traps and red delta traps with sticky bases. There was some evidence that male AFR maybe repelled by other male AFR and they were potentially attracted to female AFR. However, the method for sexing AFR was not developed until mid-way during this trial so some weevils could not be sexed initially. The trial should be repeated with known sexes of all weevils using the tap sampling method with weevils of

known mated status. This will provide more robust data on the interactions between the sexes.

Volatiles were collected from weevils of each sex with or without a food source as individuals or in groups of 2-8. Sixteen collections were made, but analyses of these collections by gas chromatography coupled to mass spectrometry (GC-MS) were unsuccessful and no sex specific compounds were identified. These collections and analyses will be repeated.

Financial benefits

This project could eventually lead to the development of a sensitive, specific, semiochemical-based monitoring trap for apple fruit rhynchites. This will enable growers to minimise losses due to the pest, which probably average >1% in apple, and direct sprays against it only when they are needed. This project is thus consistent with the industry's need to minimise and rationalise the use of pesticides.

Action points for growers

- Growers should monitor for AFR by tap sampling trees from early April.
- Orchards with high fruit damage the previous year should be treated in the spring avoiding bloom.

SCIENCE SECTION

Introduction

Apple fruit rhynchites weevil (AFR), *Rhynchites aequatus,* is a common pest of apple and pear. It damages fruit directly by inserting its rostrum into developing fruitlets and feeding. Females have been observed laying eggs in fruitlets and severing stems, causing them to drop. Although hawthorn is AFR's normal host, it has been increasing in numbers in commercial orchards, causing losses of between 1% and 5%. Chlorpyrifos and thiacloprid are capable of controlling the weevil. However, to be effective they should be applied during blossom, and this is not approved as they can cause harm to pollinating bees.

In recent years growers have reported problems in controlling AFR. This because damage is only noticed when the fruit develop the characteristic corky scars and by this time it is too late to take action.

Damage is caused by adult weevils feeding on the fruit or females laying eggs in the fruit. The rostrum is inserted into the fruit by both males and females for feeding. The females make a large hole in the fruitlet in which to lay a single egg.

Figure 2. Female *R. aequatus* partially severing the fruitlet stem after egg laying

R. aequatus is a host alternating species, successfully breeding on hawthorn, blackthorn, apple and pear. Single eggs are laid within each fruitlet within a cell that the female excavates with her rostrum. The female then partially severs the peduncle which causes the fruitlet to drop later on (pers. obs. in this study) (Fig. 2). The larvae develop within the fruitlet through the summer, migrating to the soil to pupate after the fruitlet drops. Adults emerge from the soil in the early spring. Only one generation a year is produced. Some adults are said to overwinter under bark but this has not been observed directly. Adults feed on buds, shoots and flowers, but primarily young fruitlets. It is not certain when mating occurs in the field.

There are currently no known biological controls for the AFR. It is possible to control AFR with insecticide sprays, although timing of sprays can be damaging to bees.

Chlorpyrifos is a moderately persistent OP insecticide which is fairly effective against apple fruit weevil. In order for chlorpyrifos to be effective against AFR it must be applied to orchards throughout all stages from bud burst to fruit set development. For apple orchards, one spray can be applied pre blossom and three sprays can be applied post blossom with a 14-day harvest period. However, it cannot be applied during blossom because of its risk to bees.

Thiacloprid (Calypso) is a neonicotinoid that targets the central nervous system of the insect. Two applications can be applied each year. Although there is no evidence that thiacloprid has any adverse effects on bees and other pollinators, growers are reluctant to spray during blossom. It has been shown that thiacloprid is harmful to earwigs.

Materials and methods

Field trapping tests

Site

All facilities and testing were at East Malling Research, New Road, East Malling, Kent ME19 6BJ by kind permission of the farm manager Graham Caspell. *R. aequatus* were collected from hawthorn (Aylesford Rugby Club, Kent), Apple (Wiseman Orchard, East Malling Research and Gaskains Farms, Selling Court Farm, Selling, Faversham, Kent, ME13 9RL). The trial was done in Wiseman Orchard at East Malling Research. Trees were a mixture of

Discovery, Fiesta, Ahra (scab resistant) and CPRO 80015-25 and were part of an unsprayed apple orchard which was planted in spring 1994 (Fig. 3).

Figure 3. Location of Wiseman orchard

Experimental design and layout

The trial was a randomised block experiment with eight replicates of four treatments, including an untreated control divided between blocks in Wiseman Orchard. Each plot consisted of an individual apple tree. The plots in each block were arranged end to end in a row; with one tree spacing between each plot both running down the row and across (Fig. 4).

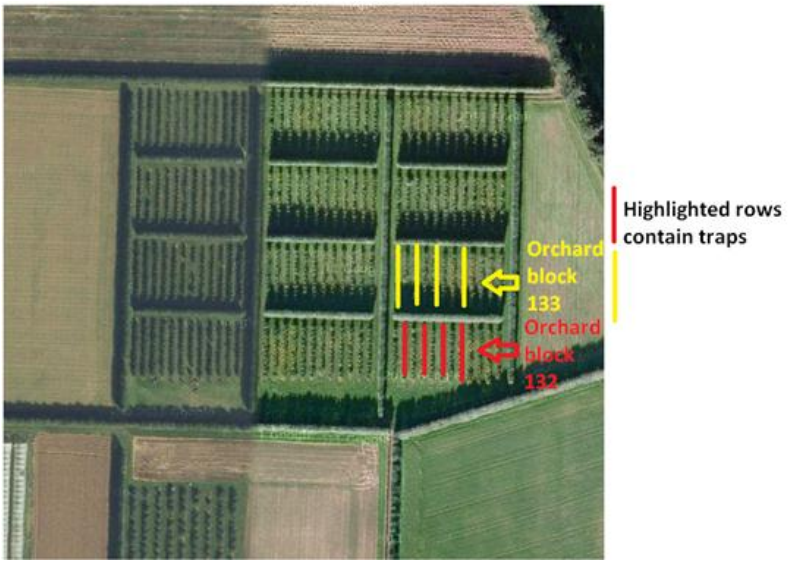

Figure 4. Location of traps in Wiseman Orchard

Treatments

Live AFR were used as the bait and were held in a mesh ball (tea strainer 5 cm circumference) suspended from either the trap or the tree. Weevils were provided with a food source of either a fruitlet or flower bud depending on growth stage of the tree. A sugar solution feeder, consisting of a cotton ball soaked in 15% sugar solution, was also provided. The baits contained two males, two females or mixed with one male and one female (Table 1).

Traps were either a white cross-vane bucket trap, as used for trapping strawberry blossom weevil, *Anthonomus rubi,* with bait, or a red delta sticky base trap with bait and tap sampling with or without bait (control). There was variation in the number of repetitions per treatment depending on the availability of AFR (Table 2).

Table 1. Sex of *R. aequatus* lures and number of repetitions

Sex of lure	No. reps
Male	18
Female	8
Male/Female	6
Unbaited	8

Table 2. Treatments and number of repetitions

Treatment	No. reps
Tap sample without bait	8
Tap sample with bait	16
Bucket bait	8
Delta bait	8

Assessments

Assessments were undertaken every week. Traps were checked and weevils collected and the sex determined in the laboratory.

Plot maintenance

All AFR used as bait were checked weekly at the same time as the assessment. Each was given a new flower bud and sugar feeder. Any dead weevils were replaced.

Meteorological records

Records were taken from the East Malling Research meteorological station (Fig. 5).

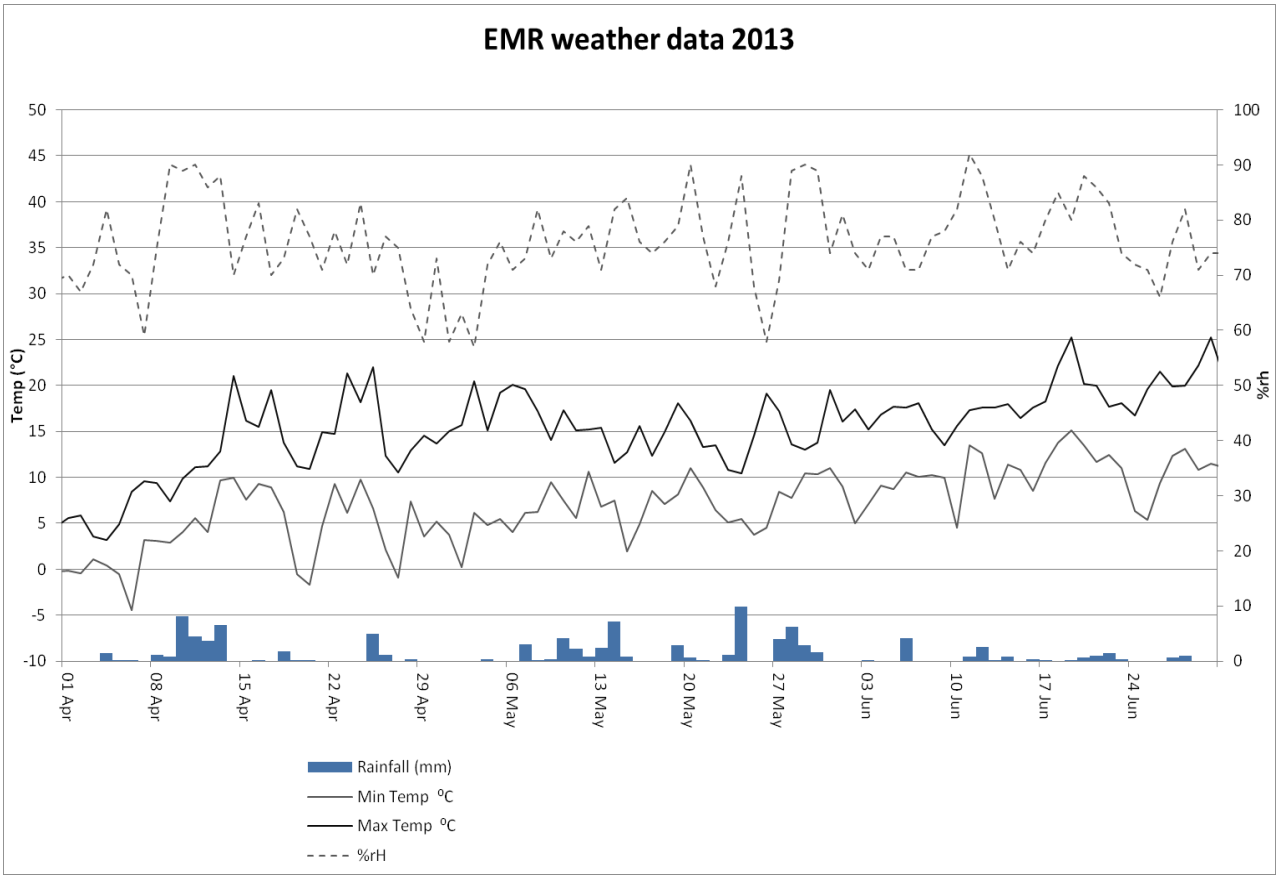

Figure 5. Meteorological records from 1 April to 30 June 2013

Statistical analysis

Repeated measures ANOVA were done where applicable and analyses were conducted on untransformed and SQRT transformed data.

Collection of volatiles

Collection of volatiles from *R. aequatus* was carried out in Controlled Temperature Room 2 at East Malling Research. Lighting was on between 0900 and 0130 h and off between 0130 and 0900 h.

Insects were contained in silanised glass vessels (12 cm x 5 cm) and air was drawn in (200 ml/min) through an activated charcoal filter (20 cm x 2 cm; 8-10 mesh) and out through a collection filter consisting of Porapak Q (200 mg; 50/80 mesh) held between glass wool plugs in a Pasteur pipette (4 mm i.d.). Six sets of entrainment apparatus were used simultaneously.

The apparatus was cleaned by passing a continuous air flow through for 24 h before the collections began. Collections were made for three-four days using the same filter for the whole period. The filters were connected and the pump was switched on for 30 min after placing the AFR in the chamber to give the insect time to settle. This was to reduce the likelihood of collection of any potential alarm compounds.

It was not possible to determine if AFR were mated or unmated. The five treatments consisted of: 1, 2, 6 or 8 males; 1, 2, 6 or 8 males with an apple bud as food source; 1, 2, 6 or 8 females; 1, 2, 6 or 8 females with an apple bud or an apple bud only. The sixth collection vessel was run empty as a system blank.

Analysis of collections of volatiles

Collections of volatiles were analysed by gas chromatography coupled to mass spectrometry (GC-MS) at NRI.

The Porapak filters were eluted with dichloromethane (1 ml; Pesticide Residue Grade). Samples were analysed by GC/MS using a Varian 3800 GC coupled to a Saturn 2200 ion trap MS. A fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µ film thickness) was used, coated with polar DBWax with helium as carrier gas (1ml/min). Injection was splitless (220°C) and the oven temperature was programmed from 50°C for 2 min then at 6°C/min to 240°C. Data were captured and processed with Varian MS workstation v6.8.

Collection and analysis of volatiles

A total of 16 collections were made, including two blank runs.

GC-MS analyses showed that all the samples were contaminated to a greater or lesser extent. Figure 7 shows the analysis of one of the blank entrainments.

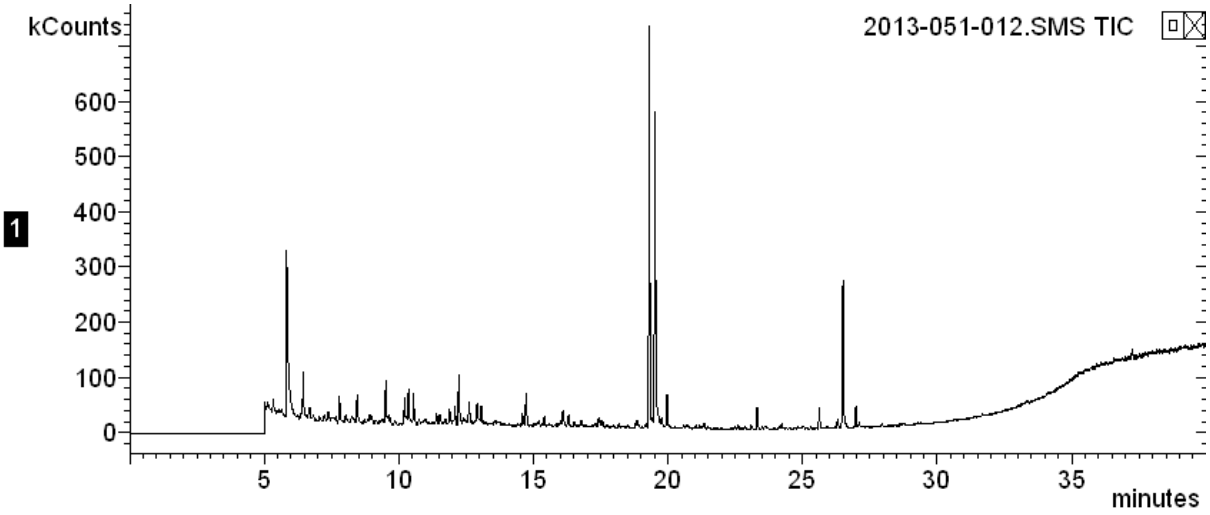

Figure 7. GC-MS Analysis of blank entrainment.

The two main peaks at 19.31 min and 19.52 min (Fig. 7) were identified as the 3-hydroxy-2,4,4-trimethylpentyl and the 2,2-dimethyl-1-(2-hydroxy-1-methylethyl) esters of 2-methylpropanoic acid respectively. These are known components of acrylic paint and it was realised that the room had been painted recently and there was a strong smell of paint.

The other two significant peaks at 5.84 min and 26.50 min (Fig. 7) were siloxane impurities.

There were numerous other smaller impuritiy peaks associated with these that made it impossible to detect any significantly different peaks associated with either sex that might be candidate pheromone components.

Results

Sexing and reproduction of AFR

It was observed that the male rostrum is as long as the head and pronotum together. The female is larger in size and the rostrum is 1.3-1.4 times longer than the head and pronotum together (Fig. 6).

Figure 6. Female *R. aequatus* (left) with larger rostrum and male (right)

It was subsequently found that similar observations were documented by Morris (1990).

In the laboratory males and females were able to identify each other and successfully mate, resulting in eggs being laid. This indicated that the weevils were suitable for use in trapping experiments and for collection of volatiles to identify potential pheromone components.

Field trapping tests

More AFR weevils were obtained by tap sampling than were caught in traps (Table 3). Although significantly more males were obtained from trees that had traps baited with weevils (male, female or male+female), total numbers obtained were greater from trees without traps (Table 3). There was no significant difference between numbers of females from baited and unbaited trees.

Table 3. Mean numbers of AFR in each treatment (totals include indeterminate sex)

Sampling method (No. reps)	Actual Means				SQRT Means		
	Male	**Female**	**Total**		**Male**	**Female**	**Total**
Tap no bait (8)	0.25	0.50	2.13		0.25 b	0.34	1.31 a
Tap with bait (16)	0.75	0.38	1.56		0.68 a	0.30	1.04 b
Bucket Bait (8)	0.00	0.00	0.13		0.00 c	0.00	0.13 c
Delta Bait (8)	0.00	0.00	0.00		0.00 c	0.00	0.00 c
				Trap			
				F pr.	<.001	NSD	<.001
				s.e.d.	0.207	0.230	0.287
				l.s.d.	0.420	0.466	0.524

There were no significant differences ($P > 0.05$) between the numbers of males or females caught in traps in relation to the sex of the bait weevil (Table 4). However, the total numbers of AFR were significantly lower in traps baited with male+female or male only than in the unbaited traps.

Table 4. Mean numbers of AFR caught in traps baited with male and/or female AFR weevils. (Total includes those of undetermined sex)

Sex (rep)	Actual means				(SQRT)means		
	Male	Female	Total		Male	Female	Total
Male (18)	0.22	0.06	0.50		0.22	0.06	0.44 b
Female (8)	0.75	0.38	1.63		0.67	0.30	0.97 ab
Male/Female (6)	0.33	0.33	0.67		0.24	0.24	0.33 b
Un-baited (8)	0.25	0.50	2.13		0.25	0.34	1.31 a
				Sex			
				F pr.	>0.05	>0.05	0.017
				s.e.d.	0.264	0.254	0.378
				l.s.d.	0.536	0.515	0.766

Discussion

It was possible to collect *R. aequatus* from hawthorn or apple in sufficient numbers for experiments. A method for sexing the weevils was developed, based on the relative length of the rostrum, although this was subsequently found to have been reported previously in the literature.

In the laboratory, males and females were able to identify each other and successfully mate, resulting in eggs being laid. This indicated that the weevils were suitable for use in trapping experiments and for collection of volatiles to identify potential pheromone components. There were problems with rearing as although eggs and larvae were all observed in the laboratory, none survived through to adult stages. This will also be refined and repeated and repetitions increased in future experiments.

In the field trapping tests there was no evidence for the existence of a pheromone in either sex which attracted either sex. In fact total numbers of weevils obtained by tap sampling were greater from trees without traps than from trees with traps, and numbers of weevils caught in traps baited with either a male alone or a male+female were lower than those caught in unbaited traps.

Volatiles were collected from individual *R. aequatus* of each sex and from groups of two-eight individuals, with and without an apple bud as a food source. However, all the collections were contaminated to a greater or lesser extent by paint volatiles and it was impossible to detect any clear differences between collections from males and females

Conclusions

Tap sampling is a more effective method of sampling AFR than the traps used in this trial (white cross vane bucket traps and red delta traps with sticky bases).

No clear evidence for the existence of a pheromone in AFR was obtained from trapping trials and these trials need to be repeated with careful sexing of the insects using the procedure developed here.

It was possible to collect volatiles from individual or groups of AFR weevils, but these were contaminated and need to be repeated.

Knowledge and technology transfer

None to date.

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