Project Title:	Feasibility of developing a Monitoring Trap for Detecting Pepper Weevil in the UK
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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.

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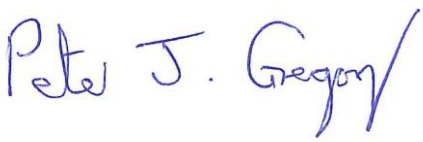

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GROWER SUMMARY

Headline

Laboratory work has begun to produce lures for pepper weevil, *Anthonomus eugenii*, based on the aggregation pheromone and host-plant volatiles, with the aim of creating a highly sensitive monitoring trap for this invasive pest.

Background

The pepper weevil, *Anthomonus eugenii,* originates from Mexico (Laborde and Pozo, 1984; Rodriguez-Leyva *et al*., 2007) and has spread throughout Central America (Andrews *et al*., 1986). Although not yet found in the UK, the first European finding in 2012 caused eradication measures in the Netherlands resulting in the destruction of four glasshouse crops of *Capsicum* (Baker *et al*., 2012). In addition, the pest was found for the first time attacking sweet pepper (*Capsicum annuum* L.; Solanaceae) in glasshouses and fields in the coastal area of the Lazio Region of Italy in October 2013 (Speranza *et al*., 2014).

Damage is caused by both adult and larval feeding, which results in bud drop and is the primary cause of yield loss. Adult *A. eugenii* also feed on leaves and blossoms and bore into fruits. Adult feeding punctures appear as dark specks or small holes in immature fruits, (Fig. 1) and small (2-5 mm) circular or oval holes in leaves. Damage is also caused by egg laying females who deposit a single egg within a cavity they excavate. Larval feeding on seeds and other tissue in the developing fruits (Costello and Gillespie, 1993) is very damaging, causing the core to become brown and often mouldy. The stem of pods infested by larvae turn yellow and the pod turns yellow or red prematurely.

Figure 1. Feeding hole caused by pepper weevil

The UK needs an effective monitoring trap for packhouses which import fruits from affected countries. These packhouses are often adjacent to glasshouse crops grown in the UK, placing crops at risk. Hence, growers would benefit from monitoring and early detection of the pest in pepper crops.

An aggregation pheromone produced by male pepper weevils was identified by Eller *et al*. (1994) and six components were identified. Traps with the pheromone captured more pepper weevils of both sexes than unbaited traps, but it is thought that this trap did not reach its full potential as one of the components, geranic acid, was not released properly.

In more recent work, Adesso *et al*. (2009, 2011) have shown that pepper weevils are attracted to volatiles from host plants. If the attractive components could be identified they might be used to increase the attractiveness of a lure containing the pheromone.

There is currently a lure commercially available from Trecé which is used in the USA. However, it is understood this does not contain all the pheromone components and certainly not host plant volatiles. Its effectiveness has not been reliably tested and there is obvious potential to increase its attractiveness by including all the pheromone components and attractive host plant volatiles.

Summary

In Year 1 (2014) a sample of infested peppers was sent by Esteban Rodríguez Leyva, SAGARPA, Mexico to establish a laboratory culture. These weevils were maintained at EMR under quarantine conditions (Licence No: 6996/211707/2). Pepper weevils were

grown on an artificial diet as documented in the scientific literature. However, the weevils did not like to lay eggs in the artificial substrate so they were switched to peppers grown in the glasshouse at EMR. Cultures were maintained for several weeks but gradually the numbers fell and the culture crashed.

Volatiles were collected from weevils in groups either with or without a food source. In analyses of these by gas chromatography coupled to mass spectrometry (GC-MS), no trace of the reported components of the aggregation pheromone could be detected. A paper published while this work was in progress suggested that weevils fed on an artificial diet produce much less pheromone than those fed on natural host material.

Volatiles were also collected from pepper fruits, flowers, leaves and buds. Analysis of these by GC-MS showed most material in collections from fruits. The major component was (*E*)-β-ocimene with much smaller amounts of some sesquiterpenes, and these may be responsible for the observed attraction of pepper weevils to their host plants.

In order to investigate these compounds further, electroantennographic (EAG) recordings were made from pepper weevil antennae to determine whether pepper weevils can detect the host plant volatiles and all of the proposed pheromone components. EAG responses were recorded to the synthetic pheromone when this was puffed over the antenna, but only very weak responses were recorded when the same material was delivered through the GC. This was probably because the insects were not in good condition by then and it was not possible to investigate the host plant volatiles.

Commercially-available lures from Trecé were shown to contain only five of the reported six pheromone components, lacking geranic acid which is reported to be essential for maximum attractiveness.

Financial Benefits

Economic damage by *A. eugenii* to peppers is reported to occur with adult populations of only 0.01 adult per plant. Current control measures in the Americas are based on broad spectrum insecticides which are extremely disruptive to IPM. This can lead to secondary problems with other pests and associated diseases, which must also be controlled with chemical insecticides. The disruption and termination of the IPM programme means that growers would lose an important marketing advantage over their overseas competitors.

The full economic implications of the arrival of *A. eugenii* have not yet been determined for UK growers. However, initial observations suggest that losses due to direct damage, secondary pest problems and the loss of goodwill with retail customers could be very substantial.

Action Points

- Monitoring in packhouses which import fruits from affected countries could intercept the pest arrival within infested crops. These packhouses are often adjacent to glasshouse crops grown in the UK, placing crops at risk
- As an interim measure adult *A. eugenii* are attracted to yellow sticky traps and can be placed through the packhouse and should be checked regularly
- See also AHDB Horticulture report by R J Jacobson 'Peppers and aubergines: A desk study to identify IPM compatible control measures for *Nezara viridula* and *Anthomonus eugenii* (2013) Final report PE 014

SCIENCE SECTION

Introduction

Anthomonus eugenii originates from Mexico (Laborde and Pozo, 1984; Rodriguez-Leyva *et al*., 2007) and has spread throughout Central America (Andrews *et al*., 1986), the Caribbean (Abreu and Cruz, 1985), southern USA (Elmore *et al*., 1934; Goff and Wilson, 1937; Riley and Schuster, 1992) and French Polynesia (CABI, 2012). *A. eugenii* has not yet been found in the UK but eradication measures were taken in four sweet pepper crops in the Netherlands during 2012 (Baker *et al*., 2012).

The main host plants are cultivated and wild species of *Capsicum* (Acosta *et al*., 1987). Oviposition and larval development occurs on plants in the genera *Capsicum* and *Solanum*. Feeding by adults (Fig. 2) extends to other Solanaceae, including tomatoes (*Lycopersicon* spp.), tomatillo (*Physalis philadelphica*), aubergine (*Solanum melongena*) and potatoes (*Solanum tuberosum*), as well as *Physalis, Datura, Petunia, and Nicotiana* (Elmore *et al*., 1934; Patrock and Schuster, 1992; Rodriguez-Del-Bosque and Reyes-Rosas, 2003; Diaz *et al*., 2004; Capinera, 2008; Addesso and McAuslane, 2009). Other wild *Solanum* species growing in the UK, such as nightshades, may potentially be alternative or intermediate hosts (Aguilar and Servin, 2000).

Figure 2. Weevil boring into pedicel

The most important damage and the main cause of yield loss (Segarra-Carmona and Pantoja, 1988a) is the destruction of blossom buds and immature fruits, which turn yellow and drop to the ground (Elmore *et al*., 1934). Both adult and larval feeding causes bud drop. Adult *A. eugenii* also feed on leaves and blossoms and bore into fruits. Adult feeding punctures appear as dark specks or small holes in immature fruits (Fig. 1) and small (2-5 mm) circular or oval holes in leaves. Sometimes the fruit is deformed. Females chew a small hole into the fruit, deposit a single egg within the cavity and seal the hole with a clear anal secretion that hardens into an 'oviposition plug'. Larval feeding on seeds and other tissue in the developing fruits (Costello and Gillespie, 1993) is very damaging, causing the core to become brown, and often mouldy. The stem of pods infested by larvae turn yellow and the pod turns yellow or red prematurely.

There is also a relationship between *A. eugenii* damage and internal mould due to *Alternaria alternata* (Bruton *et al*., 1989). Economic damage is reported to occur with adult populations of only 0.01 adult per plant (Segarra-Carmona and Pantoja, 1988b).

A. eugenii is not indigenous to the UK and Plant Health is currently reviewing the pest's status and issued a Rapid Pest Risk Analysis for industry consultation (Baker *et al*., 2013). In addition, The Food and Environment Research Agency (Fera) has recently produced a factsheet to increase UK growers' awareness of the pest and to aid recognition should it arrive in this country (Ostojá-Starzewski *et al*., 2012). The objectives of the project are to evaluate the effectiveness and optimise commercially-available pheromone traps and investigate the possibility of improving sensitivity of the trap by addition of host volatile compounds.

Materials and methods

Insect culture

The culture was established in the quarantine facility at East Malling Research. Infested peppers were provided by Esteban Rodríguez Leyva, SAGARPA, Mexico. Peppers were placed in 10 cm x 5 cm x 3.5 cm ventilated Perspex boxes and the lids were sealed on with electrical tape to prevent the weevils escaping. These were then stored within a larger 40x40x60cm Perspex box. As this pest is not currently not present in the UK every precaution was taken to prevent escapes. They were kept at 26°C at 16:8 h light: dark. The peppers were checked every other day for emergence and newly emerged weevils were moved into new ventilated boxes, 10 weevils per box. The weevils were grown on artificial

diet as documented in the scientific literature (Addesso *et al*., 2009) along with honey and water. However, the weevils did not like to lay eggs in the artificial substrate so we switched to various sweet and chilli pepper varieties grown in the glasshouse at EMR.

Collection of volatiles from peppers

Single fruits obtained from local shops were placed in a Kilner jar (5 litre) and air was drawn in through an activated charcoal filter (2 l/min) and out through a collection filter consisting of Porapak Q (50-80 mesh; 200 mg) held between plugs of silanised glass wool in a Pasteur pipette (4 mm i.d.). Collections were made from 0–6 hr and then from 6-24 h. Collections were also made after making 30 punctures in the surface of the peppers with a dissecting needle.

Collection of volatiles from growing plants

Volatiles were collected from pepper fruit, flowers and leaves at Valley Grown Nurseries, Essex EN9 2EX. A custom-made field entrainment kit was used and leaves/flowers were held within a polyethylene terephthalate oven bag (Figure 3). Charcoal filtered air was pumped into the bag and out through a Porapak collection filter, as above, at 500-600 ml/min, and collections were generally run for 24 h.

Figure 3. Entraining pepper flowers with field entrainment kit

Collection of volatiles from weevils

Entrainments of weevils were taken within the quarantine facility at East Malling Research, Room 3. 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.) (Figure 4). Six sets of entrainment apparatus were used simultaneously.

Figure 4. Apparatus for collection of volatiles from pepper weevils

The apparatus was cleaned by passing a continuous air flow through for 24 h before the collections began. Collections were made for 2 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 weevils in the chamber to give the insect time to settle. This was to reduce the likelihood of collection of any potential alarm compounds. At the end of volatile collection, all chambers were wiped clean with 100% ethanol and then by passing a continuous air flow through for 24 h before being stopped.

Analysis of volatile collections

Adsorbed volatiles were extracted from Porapak collection filters with dichloromethane (Pesticide Residue Grade; 1 ml). Extracts were concentrated approximately x 10 under a gentle stream of purified nitrogen.

Extracts were analysed by gas chromatography coupled to mass spectrometry (GC-MS) using a Varian 3500 GC coupled to a Saturn 2200 MS (Agilent) operated in electron impact mode. A polar GC column was used (30 m x 0.25 mm i.d. x 0.25 µ) coated with DBWax (Supelco), and the oven temperature was programmed from 40°C for 2 min then at 10°C/min to 240°C. Compounds were identified by their mass spectra, their GC retention indices relative to the retention times of *n*-alkanes and comparison with synthetic standards.

Extraction and analysis of lures

A rubber septum lure was supplied by Trécé. The septum was exposed in a wind tunnel at 27°C and 8 km/h windspeed for 24 h, and then volatiles were collected using a similar procedure as above for 3 h at 27°. The septum was then extracted in 5 ml hexane containing 1 mg decyl acetate (10Ac) for 24 h at RT.

The volatile collection and the extract were analysed by GC-MS as above and by GC with flame ionisation detection (FID) on polar and non-polar GC columns (30 m x 0.32 mm i.d. x 0.25 µ) coated with DBWax and HP5 respectively. The oven temperature was programmed from 50°c for 2 min then at 10°C/min to 250°. Carrier gas was helium (2.4 ml/min), injection was splitless (220°C) and the FID was at 250°C.

Electroantennography

It was planned to analyse collections of volatiles by GC coupled to electroantennographic (EAG) recording from male and female pepper weevils in April 2014 using insects fed on synthetic diet. In fact, work was only carried out with the synthetic pheromone as only weak responses were recorded to this.

EAG preparations were made by removing the head and inserting the ground electrode into the back of the head and the distal end of one antenna into the recording electrode. Electrodes were glass capillaries filled with electrolyte (0.1 M KCL + 1% polyvinylpyrrolidene) and connected via silver wire electrodes to a Syntech INR-2 integrated micromanipulators and amplifier. The latter was connected as a second detector to the GC.

The outlet of the GC was split 1:1 between the flame ionisation detector and a glass T-piece in the GC oven which was flushed continuously with humidified air (300 ml/min) over the EAG preparation. Analyses were carried out with a fused silica capillary column (30 m x 0.32 mm i.d. x 0.25 µ film thickness) with polar DBWax with helium as carrier gas (2.4 ml/min). Injection was splitless (220°C) and the oven temperature was programmed from 50°C for 2 min then at 10°C/min to 240°C.

EAG responses to collections were also measured by direct “puff” tests. The sample was deposited on a strip of filter paper in a Pasteur pipette and air blown over it (300 ml/min for 3 sec) to remove solvent. The end of the pipette was then inserted into the arm of a T-piece (4 mm i.d.) positioned over the EAG preparation and air blown over the sample and onto the preparation (300 ml/min for 3 sec). GC and EAG data were captured and processed with EZChrom Elite v6.

Results

Insect culture

Pepper weevils were fed an artificial diet but it became clear that they were not surviving very well and so were switched to a purely pepper diet but the culture had dwindled by this point and was unable to recover.

Collection of volatiles from peppers

Collections from fruits were dominated by the monoterpene (*E*)-β-ocimene with significant amounts of the sesquiterpene, α-copaene. Smaller amounts of limonene, linalool, β-elemene, longifolene and α-farnesene, were also detected along with (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, a compound produced on wounding. Wounding the fruit with 30 punctures with a dissecting needle did not greatly affect the quantity or composition of the volatiles collected.

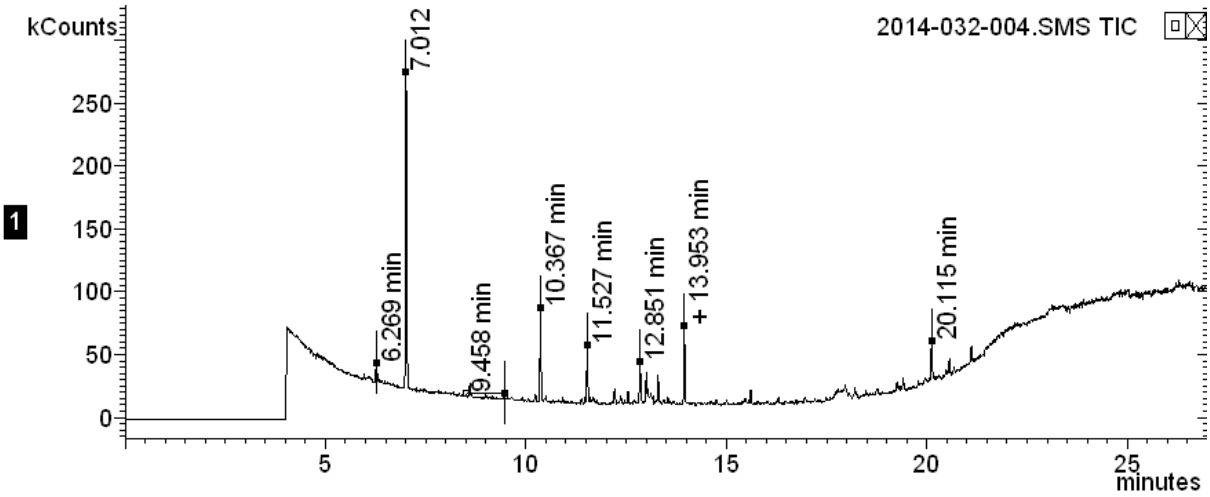

Figure 5. GC-MS analysis of volatiles from pepper fruit (limonene at 6.27 min, (*E*)-β-ocimene at 7.01 min, copaene at 10.37 min, β-elemene at 11.53 min, longifolene at 12.85 min, α-farnesene at 13.31 min; (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene at 13.95 min

Collection of volatiles from growing plants

Twenty-six collections were made from leaves, flower buds, developing fruits, flowers and whole fruits. Analyses of these collections by GC-MS showed significant amounts of material only in collections from fruits, even when these were quite tiny, the major component being (*E*)-β-ocimene. Only traces of material were detected in collections from leaves and flowers (Figure 6).

Collections from cut surfaces showed (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene as the major component (Figure 6).

2-*iso*Butyl-3-methoxypyrazine, a compound which has been reported previously from chillies and which was attractive to male and female pepper weevils in a laboratory bioassay (Merino, 2013) could not be detected reliably in any of the collections by

comparison with an authentic standard and single ion monitoring at *m/z* 124 (retention time 10.62 min; retention index 1517).

Collection of volatiles from weevils

Two collections each were made from 5 or 10 male or female pepper weevils for 48 h. Two collections were made from 3 males and 2 females together. No trace of the components of the male-produced aggregation pheromone could be detected in any of the collections (Figure 7).

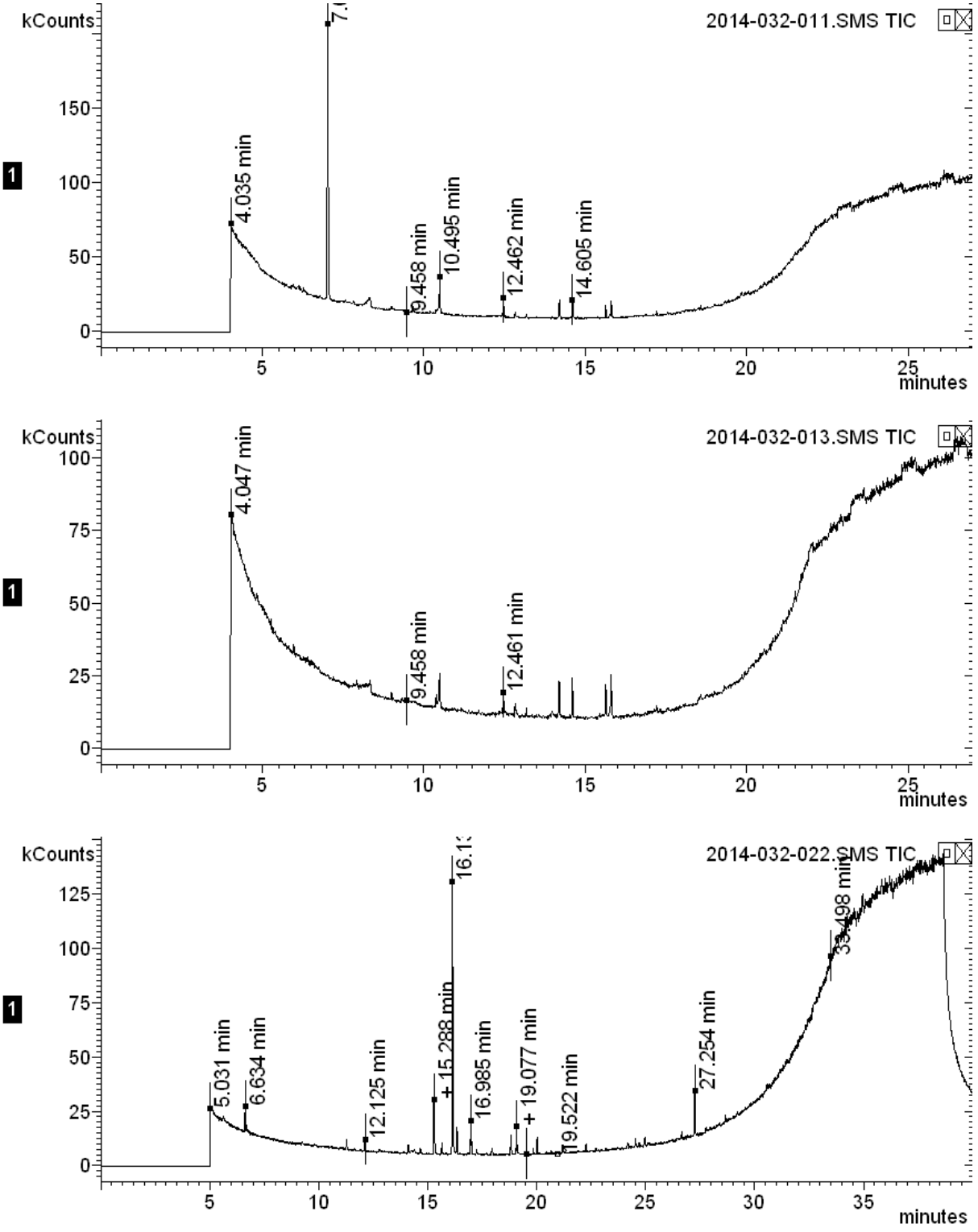

Figure 6. GC-MS analyses of collections of volatiles from whole plants (from top) developing fruits, leaves, cut stems ((*E*)-β-ocimene at 7.03 min; (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene at 16.13 min in lower trace at different temperature programme)

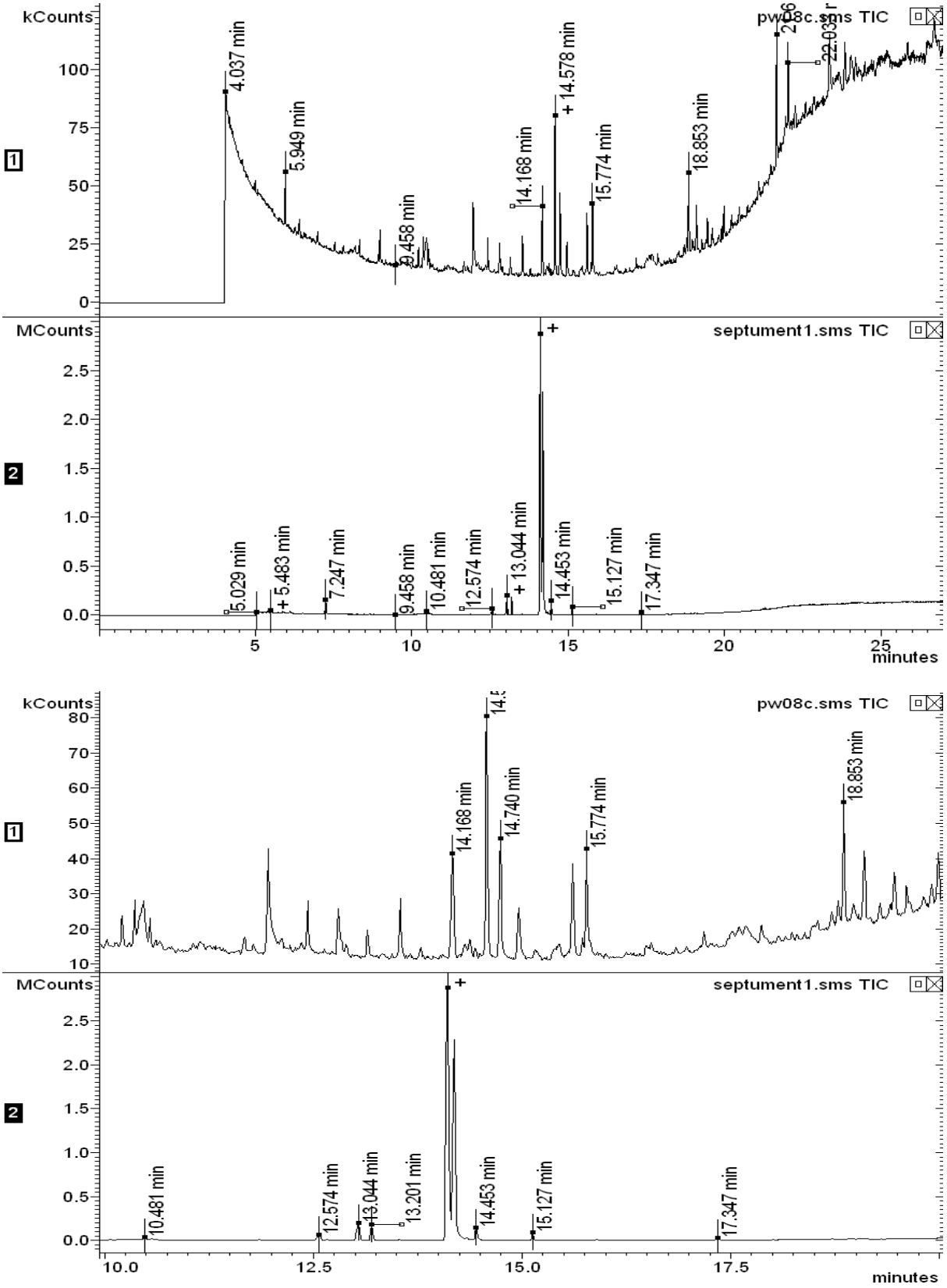

Figure 7. GC-MS Analyses of collection of volatiles from five male pepper weevils (upper) and from Trécé lure (lower); lower pair of traces is expansion of upper pair (Grandlures III and IV at 13.04 min and 13.20 min; (*Z*)- and (*E*)-Grandlure II at 14.10 min and 14.18 min; geraniol at 14.45 min)

Extraction and analysis of lures

Analyses of both the volatile collection and the extract of the Trécé lure showed the presence of Grandlures III ((*Z*)-2-(3,3-dimethylcyclohexylidene)-acetaldehyde) and IV ((*E*)-2-(3,3-dimethylcyclohexylidene)-acetaldehyde), II ((*Z*)-2-(3,3-dimethylcyclohexylidene)-ethanol), (E)-II ((*E*)-2-(3,3-dimethylcyclohexylidene)-ethanol) and geraniol (Figure 7 and Table 1). The relative amounts were similar to those reported by Eller *et al*. (1994) in the original pheromone identification (Table 1). The amount of the major component, Grandlure II, was 2.3 mg by comparison with decyl acetate as internal standard.

A commercial sample of geranic acid was found to contain two main isomers, presumably the minor (*Z*)- and major (*E*)-isomers. However, this could not be detected in either the volatiles or extract from the lure (Table 1).

Table 1. Compounds in Trécé pepper weevil lure, GC retention data and relative amounts

	Retention Index		**Relative amount**		**Eller *et al*.**
Compound	**Non-polar**	**Polar**	**Extract**	**Volatiles**	**(1994)**
Grandlure III	1259	1710	4.0	4.7	0.45
Grandlure IV	1268	1724	4.0	4.8	0.3
Grandlure II	1235	1804	100.0	100.0	7.2
(*E*)-Grandlure II	1237	1813	71.5	69.0	4.8
Geraniol	1176	1838	3.5	4.3	0.3
(*Z*)-Geranic acid	1324	2281			
(*E*)-Geranic acid	1356	2325			2.0

Electroantennography

Male and female pepper weevils showed responses to the synthetic pheromone extracted from the Trécé lure when this was delivered by “puffing” the whole extract (Figure 8). However, when the same material was delivered through the GC a weak response was observed on one occasion from a female antenna (Figure 9), but this could not be replicated in ten analyses with male antennae and five other analyses with female antennae.

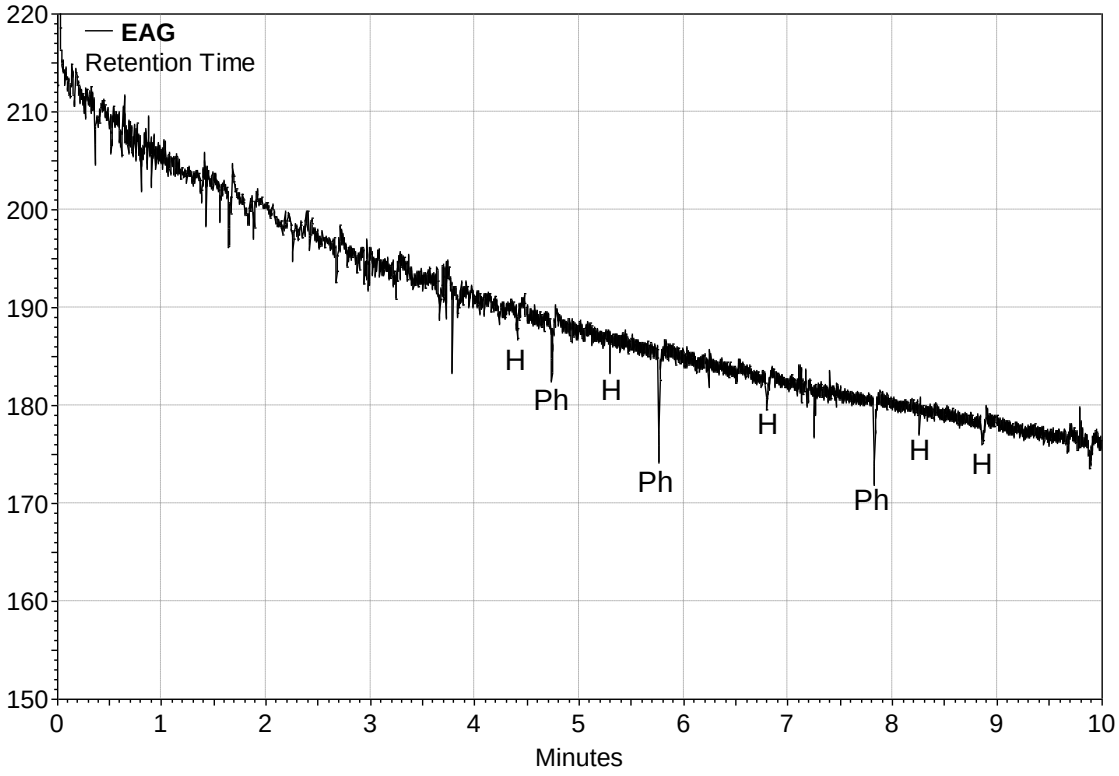

Figure 8. EAG responses of a male pepper weevil to synthetic pheromone extracted from the Trécé lure (approx. 100 ng): H response to hexane; Ph response to pheromone (response is mV x 10)

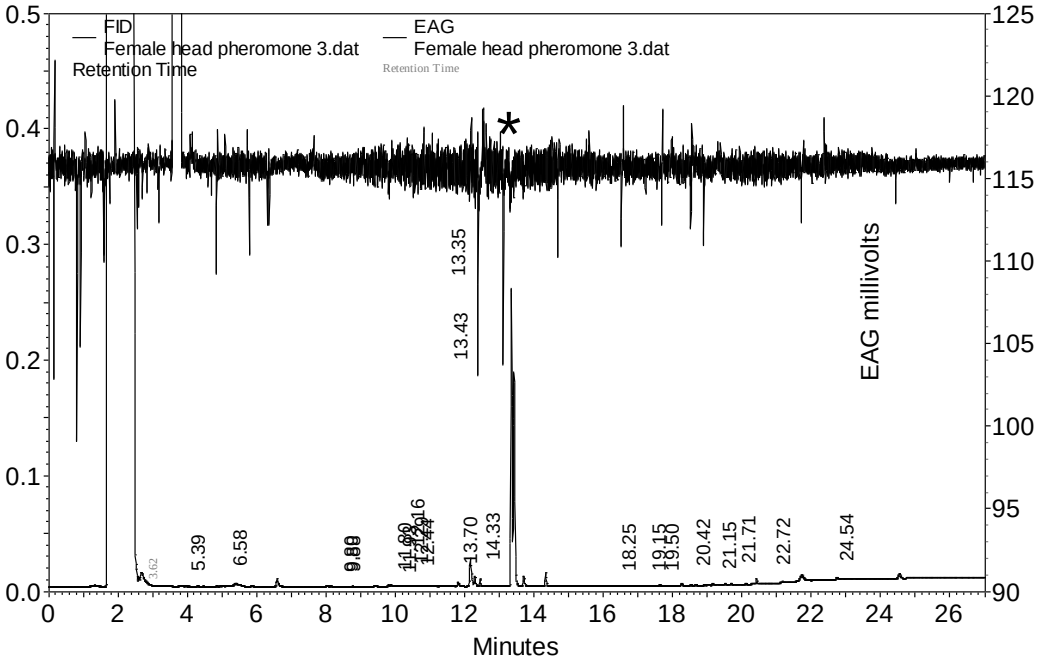

Figure 9. GC-EAG analysis of synthetic pheromone extract with female pepper weevil antenna: * small EAG response to major pheromone components

In view of the weak and irreproducible EAG response obtained to the synthetic pheromone delivered through the GC, it was not thought worthwhile analysing collections of volatiles from plants or insects by GC-EAG.

Discussion

Insect culture

The culturing of pepper weevil proved to be challenging, although we were sent a considerable amount of infested peppers from Mexico. Once we changed from the artificial diet to fresh pepper, weevils did survive longer but by this point the population had dwindled and we were unable to recover it. Caballeroa *et al*., (unpublished) also confirmed that cultured weevils performed better on a fresh pepper diet.

Host-plant volatiles

The entrainments of pepper fruits showed volatiles that were recently reported by Merino (2013), collected by solid-phase microextraction (SPME). The major component was the monoterpene (*E*)-β-ocimene with smaller amounts of limonene. The sesquiterpenes copaene, β-elemene, longifolene and α-farnesene were also detected along with (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, a compound typically produced by plants on wounding. Little volatile material was collected from intact leaves. 2-*iso*Butyl-3-methoxypyrazine was reported by Merino (2013) but this could not be detected in our collections.

Merino (2013) reported that both male and female pepper weevils were attracted to blends of limonene, (*Z*)-β-ocimene, 2-*iso*butyl-3-methoxypyrazine, (*Z*)-3-hexenyl acetate and terpinolene in a Y-tube bioassay. Replacement of the (*Z*)-β-ocimene by the (*E*)-β-ocimene found in peppers increased attractiveness. Addition of the same host-plant volatiles to the male-produced aggregation pheromone increased attractiveness of the pheromone to males but not to females.

Pheromone collection

Collection of volatiles from live pepper weevils showed no trace of the male-produced aggregation pheromone. The weevils used had been fed on artificial diet, and during the course of this work Eller and Palmquist (2014) reported that weevils fed on artificial diet

produced very low amounts of pheromone compared with those fed on fresh peppers. The latter produced up to 800 ng/h which would have been easily detected in our studies.

Electroantennography

Initial GC-EAG studies with pepper weevils at NRI showed weak, inconsistent responses to the synthetic pheromone components and so it was not thought worthwhile to investigate collections of volatiles from host plants or insects before more reliable responses could be obtained. The lack of response to the volatiles could also be explained by the weevils being fed on an artificial diet and not being in very good condition. These studies will be repeated when more weevils become available and will be cultures on a fresh pepper diet.

Commercial lures

Analysis of the contents and volatiles emitted from the commercial lures for pepper weevil produced by Trecé showed only five components, with geranic acid missing. The inclusion of this could increase the attractiveness of the lure and increase its effectiveness in trapping if a suitable dispenser can be found.

Conclusions

- A collaborator has been identified in Mexico who can provide peppers infested with pepper weevil larvae
- Pepper weevils have been reared to adults at EMR. Future work will use natural host material as food rather than an artificial diet
- Volatiles were collected from male and female weevils, but no aggregation pheromone was detected, probably because the weevils were fed on artificial diet. Future collections will use insects fed on natural diet
- Volatiles were collected from various parts of host plants. Collections from fruits showed (*E*)-β-ocimene as by far the major component. Other minor components were also identified
- Only weak EAG responses were recorded from pepper weevils to synthetic pheromone. Future work will use fresher weevils fed on natural diet in order to determine which components of host volatiles are detected by the weevils
- The commercially-available pheromone lure was found to contain only five of the six compounds reported to be components of the aggregation pheromone produced by

male pepper weevils. Geranic acid was missing and this has been reported to be essential for optimum attractiveness

- A reference was found referring to an outbreak of the weevil, in Italy, in 2013

Knowledge transfer

None so far.

References

Addesso, K.M., McAuslane, H.J. and Alborn, H.T. (2011). Attraction of pepper weevils to volatiles from damaged pepper plants. *Entomologia experimentalis et applicata* 138:1-11.

Addesso, K.M., McAuslane, H.J., Stansly, P.A., Slansky, F. and Schuster, D.J. (2009). Artificial Substrates for Oviposition and Larval Development of the Pepper Weevil (Coleoptera: Curculionidae). J. Econ. Entomol., 102(1), 257-264.

Addesso, K.N. and McAuslane, H.J. (2009). Pepper Weevil Attraction to Volatiles from Host and Nonhost Plants: Environmental Entomology, 38(1), 216-224.

Andrews, K.L., Rueda, A,, Gandini, G., Evans, S., Arango, A. and Avedillo, M. (1986). A supervised control program for the pepper weevil, Anthonomus eugenii in Honduras, Central America. *Tropical Pest Management*, 32:1-4.

Baker, R., Eyre, D., Matthews-Berry, S., Anderson, H. and MacLeod, A. (2012). Rapid Pest Risk Analysis for *Anthonomus eugenii* (the Pepper Weevil). *The Food and Environment Research Agency.* Ver. 4, 1-9.

Caballeroa, R., Schuster, D.J., Smith, H.A., Mangandi, J. and Portillo, H.E. (unpublished). A systemic bioassay to determine susceptibility of the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) to cyantraniliprole and thiamethoxam.

Costello, R.A. and Gillespie, D.R. (1993). The pepper weevil, Anthonomus eugenii as a greenhouse pest in Canada. *Bulletin SROP*, 16, 31-34.

Eller, F.J. and Palmquist, D.E. (2014). Factors affecting pheromone production by the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) and collection efficiency. *Insects*, 5:909-920.

Eller, F.J., Bartelt, R.J., Shasha, B.S., Schuster, D.J., Riley, D.G., Stansly, P.A., Mueller, T.F., Shuler, K.D., Johnson, B., Davis, J.H. and Sutherland, C.A. (1994). Aggregation

pheromone for the pepper weevil, Anthonomus eugenii Cano (Coleoptera: Curculionidae): identification and field activity. Journal of Chemical Ecology, 20, 1537-1555.

Laborde, J.A. and Pozo, A. (1984). Presente y pasado del chile en Mexico. Instituto Nacional de Investigaciones Agricolas. Publicacion Especial No. 85. Mexico. 80 pp.

Merino, M.M. (2013). Attracción del picudo del Chile a compuestos volátiles de sus hespuedos. Thesis submitted to the Institucion de Eseñanza y Investigación en Sciencias Agrícoles, Campus Montecillo, Texcoco, Mexico. 85pp.

Rodriguez-Leyva, E., Stansly, P.A., Schuster, D.J. and Bravo-Mosqueda, E. (2007). Diversity and distribution of parasitoids of Anthonomus eugenii (Coleoptera : Curculionidae) from Mexico and prospects for biological control, Florida Entomologist, 90(4), 693-702.

Speranza, S., Colonnelli, E., Garonna, A.P. and Laudonia, S. (2014). First record of Anthonomus eugenii (Coleoptera: Curculionidae) in Italy. Florida Entomologist, 97(2), 844-845.

