Progress towards identification of a pheromone of the asparagus beetle, *Crioceris asparagi* (Coleoptera; Chrysomelidae)

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Introduction

The asparagus beetle, *Crioceris asparagi* L. (Coleoptera; Chrysomelidae) originated in the Mediterranean region but now occurs wherever asparagus, *Asparagus officinalis,* is grown. Asparagus appears to be the sole source of food with infestations causing severe damage to both the foliage and the commercially valuable spears (LeSage *et al* 2008).

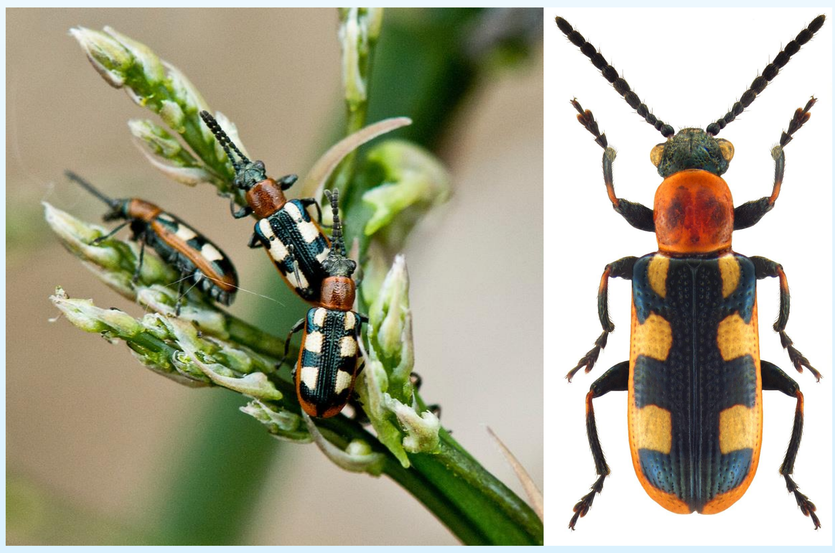

Figure 1. The asparagus beetle, *Crioceris asparagi* L. (Coleoptera; Chrysomelidae). CC Images courtesy of Udo Schmidt on Flickr and Alan Buckingham.

Currently, there are no known attractants for *C. asparagi* and much of its biology is still unknown, although a male-produced aggregation pheromone has been identified in the closely-related cereal leaf beetle, *Oulema melanopus* L. (Cossé *et al* 2002). An attractive pheromone for *C. asparagi* could be used in population monitoring and possibly control, and contribute towards a greater understanding of its biology.

Material and Methods

Insects

Crioceris asparagi beetles were collected as adults and as late larvae or pupae from asparagus fields in Cambridgeshire and Kent. Larvae and pupae were reared through to adults in individual containers in the laboratory at ADAS. Collections were made of beetles of the second generation in June-July 2017 and the overwintered generation in May-June 2018. Beetles were sexed by dissection and examination of the genitalia.

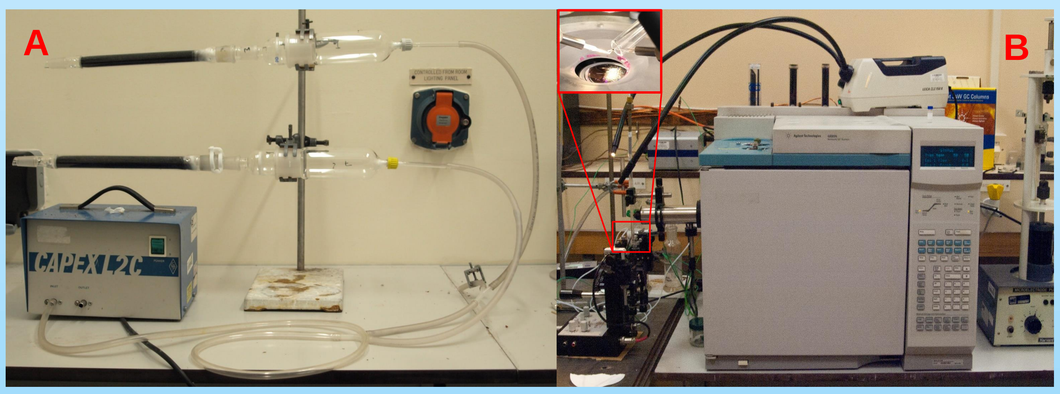

Figure 2. A: Entrainment apparatus for collecting volatiles from beetles, B: Gas Chromatography coupled Electroantennography (GC-EAG) apparatus

Collection and analysis of volatiles

Volatiles were collected from individual beetles on Porapak resin (Figure 2A) and eluted with dichloromethane. Collections were analysed by gas chromatography (GC) coupled to mass spectrometry (MS) on both polar (DBWax) and non-polar (VF5) columns. Collections were also analysed by GC coupled to electroantennographic (EAG) recording from a beetle antenna with intermittent, “puffed” delivery of the column effluent to the EAG preparation (Figure 2B).

Results and Discussion

A peak was observed in GC-MS analyses of 18 out of 66 collections from *C. asaparagi* beetles of the overwintered generation which elicited an EAG response from a beetle antenna (Figure 3), indicating it was a potential pheromone component. This compound was not detected in 30 collections from the second generation, adding further support to it being a component of a sex or aggregation pheromone, since only the overwintering beetles mate and those of the second generation overwinter as adults.

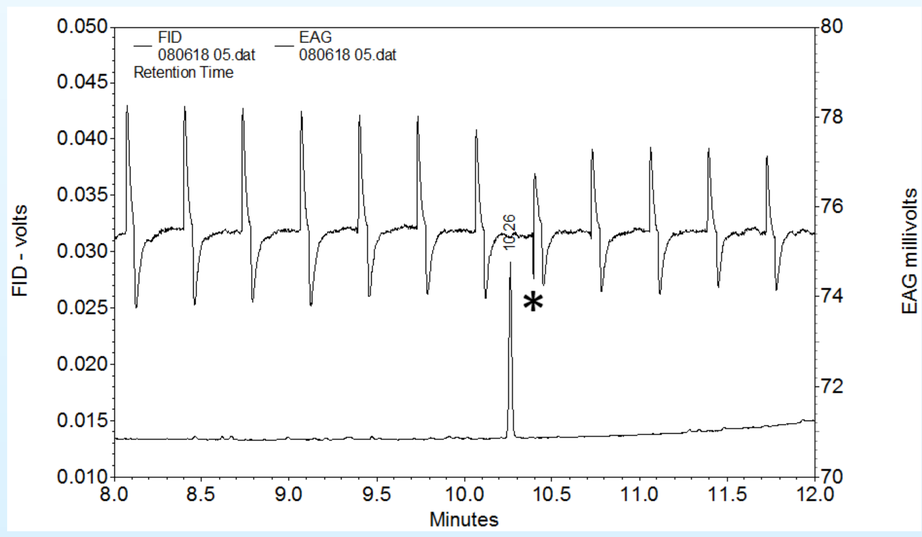

Figure 3. GC-EAG analysis of collection of volatiles from *Crioceris asparagi* (lower trace GC-FID, upper EAG response to intermittent delivery of column effluent; * EAG response).

The sex was determined of most of the beetles that produced this compound in significant quantities. The compound was apparently produced by both sexes (8:7 males:females). This was surprising as pheromones are almost exclusively emitted by one sex for any given species.

The mass spectrum had a molecular ion at *m/z* 260 and base peak at *m/z* 79 (Figure 4). The GC retention indices were consistent with it being a hydrocarbon with 19 carbon atoms and four non-conjugated double bonds. Hydrogenation produced nonadecane, confirming that it is a 19-carbon, straight chain hydrocarbon. Authentic (*Z,Z,Z*)-1,3,6,9- and (*Z,Z,Z,Z*)-3,6,9,12-nonadecatetraenes had similar but not identical mass spectra and retention times. This structure is very different from the hydroxyketone identified from *O. melanopus* L. by Cossé *et al* (2002), which was not detected in any collections from *C. asparagi* by comparison with an authentic standard. Complete identification and synthesis are in progress.

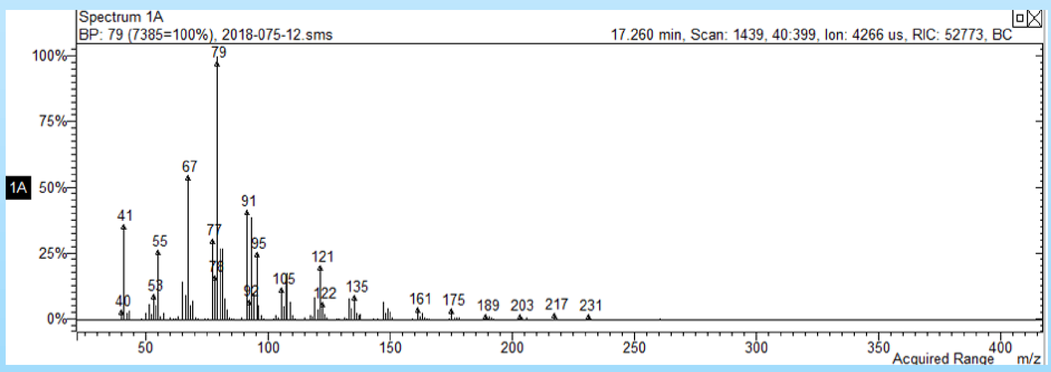

Figure 4. GC-MS spectrum of the potential pheromone component.

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LeSage, L., Dobesberger, E, J., & Majka, C. G. 2008: Introduced Leaf Beetles of the Maritime Provinces, 6: The common asparagus beetle, *Crioceris asparagi* (Linnaeus) and the twelve-Spotted asparagus beetle, *Crioceris duodecimpunctata* (Linnaeus). Proc. Entomol. Soc. Wash. 110(3) 602-621.

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