The Aleochara (Coleoptera: Staphylinidae) attractant in mustard seed meal is not dimethyl disulphide

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Abstract — The cabbage maggot, *Delia radicum* (Diptera: Anthomyiidae), is an important pest of brassicaceous vegetables and field crops. In canola, management options for root maggots are restricted to cultural and biological methods. A European natural enemy of *D. radicum* is the staphylinid beetle *Aleochara bipustulata*, larvae of which parasitize *D. radicum* pupae, and adults eat eggs and larvae of the pest; *A. bipustulata* is being assessed for introduction to Canada as a classical biological control agent of *D. radicum*. Volatiles given off by mustard seed meal attract *A. bipustulata*, and field applications of meal have successfully enhanced mortality of *D. radicum*. Dimethyl disulphide (DMDS) also attracts *A. bipustulata*, as well as its Holarctic congener, *A. bilineata*. Solid phase microextraction of mustard seed meal volatiles, followed by gas chromatography-mass spectrometry, reveals that the attractive volatile from the meal is not dimethyl disulphide. Implications for the chemical bases of host- and prey-finding, and for chemical manipulation of *D. radicum* mortality due to *Aleochara* species, are discussed.

Introduction

The cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae) is an important pest of brassicaceous vegetables in Europe and North America (Finch 1989), and of canola (*B. napus* L. and *B. rapaoleifera* (DeCandolle) Metzger (Brassicaceae)) in prairie Canada (Soroka and Dosdall 2011). Female *D. radicum* oviposit near the base of host plants, and the larvae feed externally on the roots causing direct injury and facilitating root rot infections; pupation occurs in the root or in nearby soil (Griffiths 1986). In canola, management of root maggots currently relies on cultural and biological approaches (Soroka and Dosdall 2011). In canola in the Prairie Provinces and in Europe, *Aleochara bilineata* Gyllenhall (Coleoptera: Staphylinidae) is a major pupal parasitoid of *D. radicum* (Hemachandra *et al.* 2007). In Europe,

but not in North America, a second staphylinid, *A. bipustulata* (L.), also attacks *D. radicum* (Hemachandra *et al.* 2005, 2007); it is being evaluated for introduction for classical biological control in North America (Andreassen *et al.* 2009).

Females of both *A. bilineata* and *A. bipustulata* lay eggs near dipterous host puparia, which the newly-hatched larvae seek and then enter (Fuldner 1960). Larval and pupal development occurs within host puparia, where the *Aleochara* larva is ectoparasitic on the host pupa; adults are active predators on eggs and larvae of *D. radicum* (Fuldner 1960). Chemical cues play a considerable role in the biology of the two *Aleochara*. Plants attacked by *D. radicum* give off volatiles that are attractive to adults of *A. bilineata* and *A. bipustulata* (Ferry *et al.* 2007). In addition to volatiles from infested plants, adult *A. bilineata* are also attracted to odours from *D. radicum* larvae and their frass (Royer and Boivin 1999). Larvae of *A. bilineata* use chemical cues during assessment of the suitability of host puparia (Royer *et al.* 1999; Lizé *et al.* 2010).

The chemical ecology of the two *Aleochara* species may be manipulated to enhance biological control of *D. radicum*. Mustard seed meal, the defatted residue left after crushing white mustard (*Sinapis alba* L. (Brassicaceae)) seed for oil extraction (Jonasson 1995), has been used for this purpose; the meal is applied as a thin layer (20 g/m^2) to the soil surface. Early in the growing season, mustard seed meal attracts adult *A. bipustulata* in brassica vegetables in southern Sweden; in one of four trials, *A. bilineata* was also attracted (Ahlström-Olsson and Jonasson 1992). Mustard seed meal applications reduce *D. radicum* damage to swedes (Ahlström-Olsson and Jonasson 1992), possibly because of enhanced predation of immature *D. radicum* by *Aleochara* spp., although other mechanisms may be involved (Jonasson 1995). In field plots of canola in Switzerland, later season applications of mustard seed meal attract adult *A. bipustulata* and significantly increase levels of its parasitism of *D. radicum* (Riley *et al.* 2007). In a Y-tube olfactometer, adult *A. bipustulata* are attracted to volatiles released by wet or dry mustard seed meal (Riley *et al.* 2007). Riley *et al.* (2007) found no response to mustard seed meal by *A. bilineata*, either in the field or in the laboratory. An alternative attractant is dimethyl disulphide (DMDS), a volatile compound produced by *D. radicum*-infested roots of *Brassica napus* L. (Ferry *et al.* 2007). Pitfall traps baited with DMDS catch significantly more adult *A. bilineata* and *A. bipustulata* than do unbaited control traps (Ferry *et al.* 2007). However, attracting the two *Aleochara* species to broccoli plots early in the growing season by enhancing the levels of DMDS does not increase predation of *D. radicum* eggs or reduce crop damage (Ferry *et al.* 2009).

It might be inferred that DMDS is the active ingredient in mustard seed meal and, as the meal is a health food of poorly defined chemical composition, that future research efforts should focus on DMDS. However, the responses of the *Aleochara* species to DMDS and mustard seed meal are not identical. Thus either the attractive volatile in mustard seed meal is not DMDS or, if DMDS is a component of mustard seed meal volatiles, other components affect the responses of the *Aleochara* species. Therefore, the objective of this study was to determine whether DMDS is a component of the volatile mixture given off by mustard seed meal.

Methods

Two batches of mustard seed meal were tested. Both were white defatted mustard seed meal purchased from Kräuterpflug, Kiel, Germany. One was an aliquot of the meal that attracted *A. bipustulata* in the study of Riley *et al.* (2007); the meal had been stored in a sealed glass jar at room temperature until our analysis in 2011. The second batch was newly purchased in 2011. Headspace solid phase microextraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS) was used to detect volatile species. The headspace of 1 g of sample was exposed to a 1 cm SPME fibre with a 100 µm nonbonded PDMS coating (Supelco, Bellefonte, PA) for 15 minutes at room temperature in a sealed 10 mL vial. Quantitative standards were prepared by diluting DMDS (Sigma-Aldrich, St. Louis, MO) in methanol; the headspace of 10 µL of solution was extracted under identical conditions to those for meal samples. A Varian (Walnut Creek, CA) Saturn 2100T GC-MS, equipped with a FactorFour VF-5ms column (30 m, 0.25 mm i.d., 0.25-µm, Varian, Walnut Creek, CA) was used for the analysis. Fibres were desorbed using a 2 minute splitless injection at 200 °C. GC programming for samples (modified from Meija *et al.* 2002) was: hold at 35 °C for 4 minutes, ramp to 125 °C at 15 °C/minute, hold for 5 minutes, then ramp to 300 °C at 25 °C/minute and hold for 2 minutes. Standard analysis was abridged at 125 °C. Column flow was 1.2 mL/minute. Using separate aliquots on each occasion, the entire procedure of sampling and analysis was replicated at least three times for the standards and twice for each batch of mustard seed meal. Fibres were cleaned in the GC injector port at 260 °C for 30 minutes before initial use and for 15 minutes between uses.

Results and Discussion

For each batch of mustard seed meal, and for the standards, results from each of the replicates were extremely similar. SPME extraction has previously been used to detect DMDS from a variety of sources (e.g. Ferry *et al*., 2007; Pelusio *et al.* 1995), and the effectiveness of our methodology for DMDS detection was demonstrated by the major peak at a retention time of 4.6 minutes for the DMDS standard (Fig. 1A). Although run under identical conditions, samples of mustard seed meal from Riley *et al.* (2007) (Fig. 1B), and those from meal purchased in 2011, had no major peaks with retention times less than 7 min. We therefore conclude that DMDS was not present at detectable levels in either batch of mustard seed meal. Using integration at *m/z* 94, the method detection limit of the analysis was determined to be 14 ng of DMDS. Ferry *et al.* (2007) successfully attracted adults of the two *Aleochara* species to pitfall traps that they estimated released about 2 mg (2 µL) of DMDS per 24 hours.

Peaks we saw in the mustard meal samples appear to match 10 of the 14 peaks seen by Riley *et al.* (2007), although with the exception of peak 2 (limonene) in Riley *et al*., these peaks were identified by matches with library data, rather than by comparison with standards. Differences in the peaks for mustard seed meal between the two studies are likely methodological: Riley *et al.* (2007) used a different method to extract volatiles and a different GC temperature program that would have precluded detection of DMDS. There were differences in signal intensities of some peaks be-

tween the two batches of mustard seed meal that we tested, but the principle chemical components remained the same.

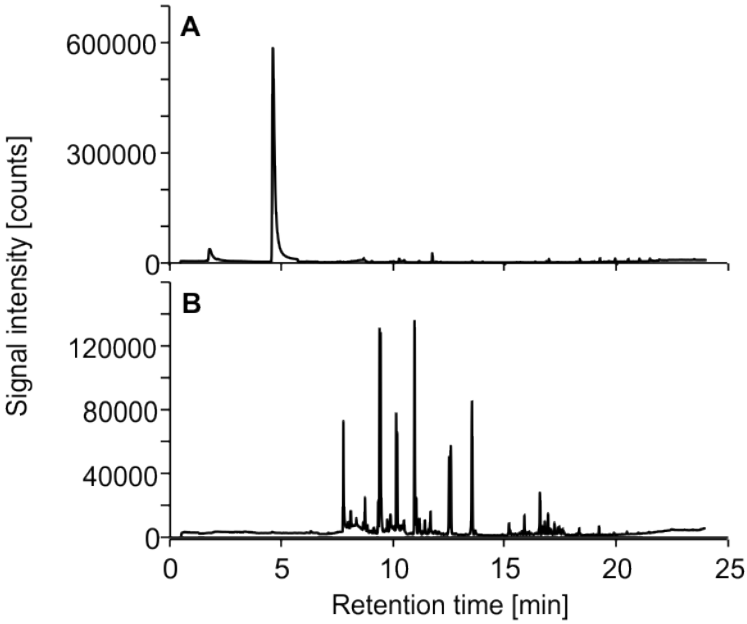

Fig. 1. Chromatograms from SPME samples of the headspace of A. DMDS standard, B. Mustard seed meal from the batch used by Riley *et al.* (2007).

DMDS is known to be released by decomposing brassica plants (Lewis and Papavizas 1970), including buried plant residues of *S. alba* (Wang *et al.* 2009), and by rotting roots of *B. napus* infested with *D. radicum* (Ferry *et al.* 2007). However, Jonasson (1995) showed that mustard seed meal is most attractive to *A. bipustulata* when it is freshly applied, and Riley *et al.* (2007) demonstrated that fresh, dry mustard seed meal attracts *A. bipustulata* adults. DMDS may be released during subsequent decomposition of the mustard seed meal, but the successful enhancement of biological control by fresh mustard seed meal (Ahlström-Olsson and Jonasson 1992; Jonasson 1995; Riley *et al.* 2007) is not caused by DMDS.

Attraction of *A. bipustulata* adults to mustard seed meal that lacks DMDS suggests that diverse chemical cues are involved in host and prey finding in this species. The failure of enhancement of *D. radicum* mortality with DMDS (Ferry *et al.* 2009) and successes with mustard seed meal (Ahlström-Olsson and Jonasson 1992; Riley *et al.* 2007) suggest that further work with mustard seed meal would be useful. In particular, elucidation of *A. bipustulata* responses to each of the volatile compounds given off by the meal might provide a chemically-defined tool for manipulation of this important

natural enemy of *D. radicum*. In addition to enhancing *D. radicum* mortality, such a tool might inhibit initial dispersal from release sites and so reduce the potential for Allee effects to interfere with establishment (Hopper and Raush 1993), in the event that *A. bipustulata* is released in a classical biological control program.

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