Kairomone Attractant for the Leafmining Fly, *Liriomyza bryoniae* (Diptera, Agromyzidae)

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A field test carried out in an industrial greenhouse in Lithuania revealed the attractiveness of synthetic methyl salicylate (MeSa) towards an economically important leafmining tomato pest, *Liriomyza bryoniae* (Kaltenbach) (Diptera, Agromyzidae). The behavioural reaction of the flies depended very much on the simultaneous presence of both olfactory and visual stimuli. The attractiveness depended on the colour of a sticky trap: MeSa attracted significantly more flies (ca. 2.2 times) when placed in yellow traps than in aluminium foil colour ones, when catches in such traps were compared to a corresponding control. *L. bryoniae* is the first species within the Agromyzidae family attracted by MeSa. The attractant was attributed to kairomones, as the compound is known as a plant-produced volatile. MeSa can be an effective extra-tool for increasing the attractiveness of traps. It should be evaluated in future whether such trap/bait combination is effective for the mass trapping of *L. bryoniae* leafminers in greenhouses (closed area).

Key words: Visual/Olfactory Stimulation, Trap Colour, Pest Monitoring

Introduction

Salicylic acid functions as a plant hormone. The biosynthesis of this hormone increases in response to herbivores rather than mechanical damage (Dicke and van Poecke, 2002). It is known that many herbivore-damaged plants release the methylated product of the hormone, methyl salicylate (MeSa), into the environment. The release of this volatile compound by plants can be induced following insect attack (*e.g.* bean leaves infested by the whitefly *Trialeurodes vaporariorum*, pear plants infested by *Psylla pyri* or *P. pyricola*, potato plants infested by the Colorado potato beetle), herbivorous mite attack (*e.g.* Lima beans infested by the spider-mite *Tetranychus urticae*) or virus infection (*e.g.* tobacco plants infested by tobacco mosaic virus) (Birkett *et al.*, 2003; Dicke *et al.*, 1999; Bolter *et al.*, 1997; Scutareanu *et al.*, 1997; Seskar *et al.*, 1998). There is an assumption that MeSa production is a way of disposing salicylic acid formed in infested plants (Dicke and van Poecke, 2002).

Like some other herbivore-induced plant volatiles MeSa attracts natural enemies of herbivores responsible for host-plant damage. The attraction of predatory species such as micro-hymenopterans ('parasitic wasps') and dipterans (Empididae) to MeSa was demonstrated in field-trapping studies (James and Price, 2004; James, 2005). MeSa was also reported as an attractant for some insect species from the orders Heteroptera, Homoptera, Coleoptera, Lepidoptera, Neuroptera and Thysanoptera (Scutareanu *et al.*, 1997; Raguso and Light, 1998; Ninkovic *et al.*, 2003; De Boer and Dicke, 2004; James and Price, 2004; Martel *et al.*, 2005; Wolde-Hawariat *et al.*, 2005; Tasin *et al.*, 2006). Among the dipterans, MeSa is known to be an attractant for three species only. All of them are from the Syrphidae family, with predatory larvae feeding on aphids. Recently it has been established that MeSa is attractive for unidentified species from the family Agromyzidae (James, 2005). All agromyzids are characteristic herbivorous insects (leafminers) with species known as economically important plant pests.

The aim of our study was to evaluate the attractiveness of MeSa towards one of the economically important plant pest species, *Liriomyza bryoniae* (Agromyzidae) under field conditions.

Materials and Methods

Behavioural test

A field test was conducted in an 1 ha greenhouse with tomato ('Barcelona RZ' variety ob-

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tained from Rijk Zwaan, De Lier, The Netherlands) plants infested by *Liriomyza bryoniae* in Kietaviškės, Kaišiadorys district, Lithuania, in August 2006.

Sticky traps of either yellow or aluminium foil colour were used. Both had the same size of a sticky area, although slightly different in proportions. Yellow sticky cards were obtained from Biobest N. V., Westerlo, Belgium (type "bug scan", 25×13 cm in size); those of aluminium foil colour were made from laminated paper (juice packs, Tetrapac, 20×16 cm in size) at the Institute of Ecology, Vilnius, Lithuania. The traps were tied vertically to wire poles at a height of ~30 cm above the plant's top. Cards were baited with 2 ml plastic vials supplied with 0.25, 0.5 or 1 ml of MeSa. Control cards were equipped with blank vials. Vials were slightly plugged with cotton wool and suspended by a wire at a height of ~2 cm above the centre of each card. MeSa of ≥99% purity was obtained from Carl Roth GmbH, Karlsruhe, Germany and used undiluted. The traps were placed in three rows with 12 m intervals between. Baits were randomized and 6 replicates of each treatment were used. After 10 days sticky traps were collected, catches recorded and analyzed.

Species identification

Abundant mines on tomato leaves corresponded to those made by *Liriomyza bryoniae* flies. The selected trapped specimens were subjected to genitalia analyses following Spencer (1976).

Statistical analysis

Catches were analyzed using the software Statistica 5.0; the test of Kruskal-Wallis ANOVA by ranks was applied (Sokal and Rohlf, 1995).

Results and Discussion

The field test revealed that the agromyzid flies *Liriomyza bryoniae* were attracted by methyl salicylate. However, the colour of the trap was critical for attraction.

In captures obtained by yellow traps baited with any MeSa dosage used (0.25, 0.5 or 1.0 ml MeSa/trap) there were no statistically significant differences. This allowed to pool the data and compare to those recorded in control traps (containing no bait). Statistically significant attraction to MeSa was revealed [(12.3 ± 0.7) flies/trap on average in MeSa-baited traps and (5.5 ± 1) flies/trap on average in control] (Fig. 1). MeSa increased the attractiveness of yellow traps by 2.2-times approximately.

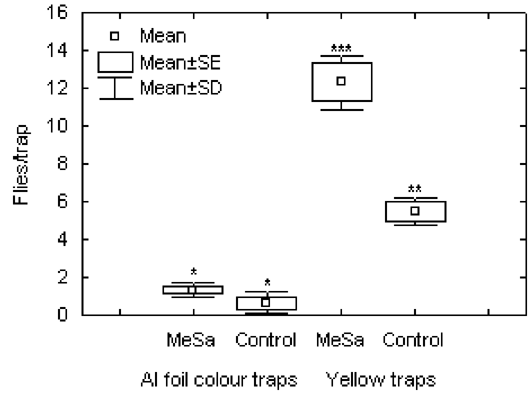

Fig. 1. Mean capture of *L. bryoniae* in sticky aluminium foil colour traps and sticky yellow traps, either baited with methyl salicylate (MeSa) or containing no bait (control), in the tomato greenhouse during a 10-day-period. Values marked by different numbers of asterisks differ statistically significant, $p < 0.05$.

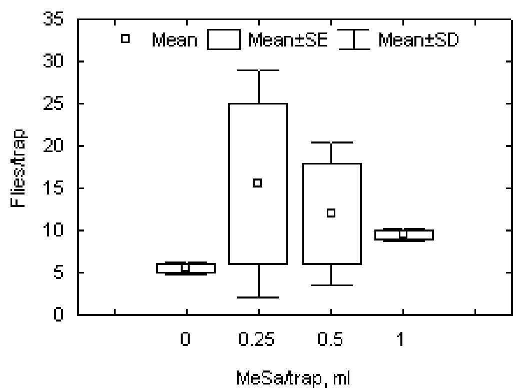

Fig. 2. Mean capture of *L. bryoniae* in sticky yellow traps baited with different amounts of methyl salicylate (MeSa) during a 10-day-period in the tomato greenhouse.

Among the dosages of MeSa tested, the lowest one (0.25 ml MeSa) tended to be even the most efficient (Fig. 2). It is well known, that the response to an attractant increases with increase of the attractant concentration (or dosage applied in a dispenser). However, this is true for low and medium, but not for very high concentrations (dosages). When concentrations are very high, the dose-response curve may differ. Trends towards a decrease in attractiveness with increasing MeSa dosage testify that too high dosages were applied in our test. This allows to assume even lower dosages compared to those we used; this should be sufficient and effective enough for *L. bryoniae* flies attraction.

In the number of flies captured by aluminium foil colour traps baited with any of the MeSa dosages we used, there were no statistically significant differences. Pooled data revealed no significant difference compared to the control as well [(1.33 ± 0.20) and (0.66 ± 0.33) flies/trap on average correspondingly] (Fig. 1). This indicates absence of attractiveness of MeSa for *L. bryoniae* flies when presented in aluminium foil colour traps.

Some difference in catches obtained by two control trap groups (Fig. 1) was obtained due to the difference in the trap colour: the 'yellow control' was more attractive compared to the 'aluminium foil colour control' ($p < 0.05$), as one could expect.

Basing on field-trapping data, we conclude that MeSa is attractive for *L. bryoniae* adult flies when presented alongside with yellow colour visual stimuli. When MeSa is presented alongside with aluminium foil colour stimuli, the compound is not attractive for the flies. It should be emphasized, that the same compound under the same pest population density gave very different results depending on trap colour, *i.e.* the behavioural reaction of *L. bryoniae* flies to MeSa depended very much on the simultaneous presence of both olfactory and visual stimuli.

The attractiveness of yellow colour objects is well known for some dipterans, including those from the Agromyzidae family. Namely, based on this phenomenon, commercial traps (sticky cards) were produced (Parella, 1987). As far as we know, there were no data on the combined effect of olfactory and visual stimuli on the behaviour of agromyzids. The phenomena when stimuli of different modality (both visual and olfactory) interact and predetermine the attractiveness of a bait/trap for insects are known in some other groups, *e.g.* in some syrphids (Diptera) (Laubertie *et al.*, 2006) and clearwing moths (Lepidoptera) (Būda and Karalius, 1993).

The data we obtained on the role of MeSa as an attractant of plant origin for herbivore insects allow to attribute this compound to the kairomones. *L. bryoniae* is among the phytophagous insect species attracted to herbivore-induced plant volatiles. The number of such species is increasing. This indicates that the well-known scheme of tritrophic interactions by means of semiochemicals presented by Dicke and Sabelis (1992) will need some additions making the scheme more complex in the nearest future.

MeSa is the first and the only attractant identified for the economically important leafminer *L. bryoniae*. Within the Agromyzidae family there were known attractants just for a single species (*L. sativae*). Those are 'green leaf volatiles', *i.e.* 2-hexenal, 3-hexen-1-ol, 2-hexen-1-ol, 3-hexenyl acetate, and plant volatiles, *i.e.* 1-octen-3-ol, (*E*)-4-(2,6,6-trimethylcyclohex-1-enyl)-3-buten-2-one, (*E*)-4-(2,6,6-trimethylcyclohex-2-enyl)-3-buten-2-one (Wei *et al.*, 2005).

Our results also confirm and extend those of James (2005), who found that MeSa is attractive for some unknown representative(s) of the Agromyzidae family.

The results we obtained are important in application aspects as well. *L. bryoniae* is one of the most widespread leafmining pest species in tomato greenhouses in Lithuania (Ostrauskas *et al.*, 2003) and in many other countries of Europe. In 2001–2003 ca. 30% of greenhouses were infested by *L. bryoniae* in Lithuania (Ostrauskas *et al.*, 2005). *Liriomyza* leafminers can impact crops by transmitting diseases, destroying young seedlings, causing reduction in crop yield, accelerating leaf drop, thus causing "sun burning" of tomato fruit, etc. (Parrella, 1987). At present yellow sticky cards are used as a monitoring tool for argromyzids both in smallholder farms and big vegetable industries. The data we obtained suggest that synthetic methyl salicylate can be an effective extra tool increasing the attractiveness of traps. This leads to a higher 'sensitivity' of a monitoring tool. The most efficient dosage of MeSa and dispenser needs to be established/elaborated. If such trap/bait combination is effective for the mass trapping of *L. bryoniae* leafminers in greenhouses (closed area) this system should be evaluated in future.

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