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KLP+ ("hat") Trap with Semiochemical Lures Suitable for Trapping Two *Diabrotica* spp. Exotic to Europe

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The KLP+ ("hat") trap baited with pheromone or floral lures is a highly efficient non-sticky trap for the western corn rootworm, *Diabrotica v. virgifera*. We tested the suitability of this trap design for the related species, *D. speciosa* and *D. barberi*, baited with their respective lures. Both species are exotic to Europe: the former inhabits South America, and the latter occurs in some parts of North America.

In screening tests performed in Brazil, several synthetic floral compounds and their combinations were found to be attractive to *D. speciosa*. However, the greatest effect was recorded for the previously described attractant 1,4-dimethoxybenzene. When the most active compounds in the preliminary test, 2-phenylethanol, methyl anthranilate, eugenol or benzaldehyde were added to 1,4-dimethoxybenzene, no synergistic effects were observed. When 1,4-dimethoxybenzene was formulated in three types of polyethylene (PE) dispensers in KLP+ traps, PE bag dispensers were superior to two types of PE vial dispenser, and caught hundreds of *D. speciosa*. Unbaited traps caught only negligible numbers. There was an interesting non-target effect. KLP+ traps with 1,4-dimethoxybenzene caught large numbers of the cornsilk fly, *Euxesta eluta*, which is known as a maize pest.

For *D. barberi*, both a pheromone and a potent floral lure are already known. In tests with KLP+ traps, we found that the pheromone and floral lures can be applied together in the same trap to maximize both male and female catches.

In conclusion, for early detection programs in Europe, the application of KLP+ traps baited with 1,4-dimethoxybenzene in PE bag dispensers could be recommended for *D. speciosa*, and KLP+ traps with dual (pheromone and floral) lures for *D. barberi*. In the case of *D. barberi,* one should note that the lures also show some attraction for *D. v. virgifera*, and the ratio of *D. barberi* vs. *D. v. virgifera* in the catch will be predominantly determined by the relative population densities at the given site.

Keywords: *Diabrotica speciosa, D. barberi*, *Euxesta eluta*, semiochemical trapping, detection.

The KLP+ ("hat") trap baited with pheromone or floral lures is a highly efficient non-sticky trap for the western corn rootworm, *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae) (Tóth et al., 2006; Tóth, 2011).

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The objective of our research was to test the suitability of the KLP+ trap design for two related species, *D. speciosa* Germar and *D. barberi* Smith and Lawrence, baited with their respective lures.

Both *D. barberi* and *D. speciosa* are exotic to Europe and are on the EPPO A1 list. Sensitive detection tools are sought for in case any of them is accidentally introduced into the EU.

D. speciosa inhabits mostly temperate regions of South America. Its host plants include maize, wheat, groundnuts, soybeans, potatoes, but it also feeds on many other vegetables and ornamental plants (Gassen, 1989; Gallo et al., 2002; Viana, 2010).

D. barberi occurs in North America, where its range partly overlaps with that of *D. v. virgifera* (Branson and Krysan, 1981). *D. barberi* is more cold-resistant than *D. v. virgifera,* so its potential introduction into Europe cannot be precluded. Climatic conditions in most maize-growing areas of Europe could be suitable for its survival. Damage occurs on maize and resembles that caused by *D. v. virgifera.*

Materials and Methods

Field tests

Tests were conducted at sites in Brazil, the U.S. and Hungary, using accepted methods for trapping experiments of similar nature (Roelofs and Cardé, 1977). Traps were arranged in blocks so that each block contained one trap of each treatment. Traps within blocks were separated by 8–10 m, and blocks were sited at least 30 m apart. Traps were inspected at fixed intervals (preferably twice weekly), when captured insects were recorded and removed.

Traps

In the tests, CSALOMON® KLP+ "hat" traps (produced by Plant Prot. Inst., CAR HAS, Budapest, Hungary) were used (Tóth et al, 2006). This trap type is routinely used for the trapping of *D. v. virgifera* and *Phyllotreta* spp. (Coleoptera: Chrysomelidae) in Europe (Csonka and Tóth, 2006; Tóth, 2011); photos of the trap can be viewed at *www.csalomontraps.com.* In the preliminary Experiment 1, sticky yellow traps (CSALOMON® PALs, produced by Plant Prot. Inst., CAR HAS, Budapest, Hungary – for reflectance spectrum of the colour hue used refer to Tóth et al., 2004) were used.

Chemicals

Synthetic floral compounds 1,4-dimethoxybenzene, 1-phenethyl alcohol, methyl eugenol, *(E)*-anethol, *(E)*-cinnamyl alcohol, *(E)*-cinnamyl acetate, *(E)*-cinnamaldehyde, 2-phenylethanol, methyl anthranilate, eugenol, benzaldehyde, *ß*-ionone, methyl salicylate, phenylacetaldehyde, 4-methoxyphenethyl alcohol, indole and 4-methoxy cinnamaldehyde were purchased from Sigma-Aldrich Kft. (Budapest, Hungary). All compounds were >95% pure as stated by the supplier. Pure 8*(R)*,2*(R)*-8-methyl-2-decyl propanoate was a generous gift from Kamlesh R. Chauhan (Beltsville, USA).

Attractant dispensers

Polyethylene bag (PE bag) dispensers for synthetic floral compounds were prepared by placing a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) into a tight polyethylene bag made of 0.02 mm linear polyethylene foil. Size of the polyethylene sachets was ca. 1.5 × 1.5 cm. The dispenser was attached to a plastic strip (8 × 1 cm) for easy handling when assembling the traps.

Polyethylene vial (PE vial) dispensers (for 1,4-dimethoxybenzene in Exp. 3): 0.7 ml vials with lid (No. 730, Kartell Co., Italy) were used either as they were, or with a 0.5 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) placed inside. Bait components were released by penetration through the vial walls.

Rubber dispensers [for 8*(R)*,2*(R)*-8-methyl-2-decyl propanoate] were prepared by using pieces of rubber tubing (Taurus, Budapest, HG; No. MSZ 9691/6; extracted 3 times in boiling ethanol for 10 min, then also 3 times in methylene chloride overnight).

When making the lures, the required amounts of compounds in hexane solutions were administered onto the surface (rubber) or into the PE bag or PE vial dispensers. After having allowed the solvent to evaporate, the PE bag dispensers were heat sealed and the lids of the PE vial dispensers were closed.

All types of dispensers were wrapped singly in pieces of aluminum foil and were stored at –30 °C until use in the field trials. Lures were replaced with new ones after 4 weeks of exposure.

Experimental details

Experiment 1. This preliminary test was aimed at screening blends of synthetic floral compounds (common attractants of a number of insects) to see whether any of them attracted *D. speciosa.* Composition of lures (dose of single compounds = 100 mg): treatment A = 1,4-dimethoxybenzene; treatment B = 1-phenethyl alcohol + methyl eugenol + *(E)*-anethol; treatment C = *(E)*-anethol + *(E)*-cinnamyl alcohol + *(E)*-cinnamyl acetate + *(E)*-cinnamaldehyde; treatment D = 2-phenylethanol + methyl anthranilate + eugenol + benzaldehyde; treatment E = *ß*-ionone + methyl salicylate + phenylacetaldehyde; treatment F = 4-methoxyphenethyl alcohol + *(E)*-cinnamyl alcohol + *(E)*-anethol + indole; treatment G = 4-methoxy cinnamaldehyde + indole; treatment Unb = unbaited control. Exp. 1 was run in a maize field at Sete Lagoas, Brazil, February 27–April 9, 2007, with 3 blocks of traps. In this test yellow sticky traps were used.

Experiment 2. The objective of this test was to check whether compounds from blends found attractive in the preliminary Exp. 1 increase catches of *D. speciosa* when added to 1,4-methoxybenzene. Compounds were tested at the dose of 100 mg each. Treatments included 1,4-dimethoxybenzene alone, its ternary blends with 2-phenylethanol plus methyl anthranilate, its ternary blend with eugenol plus benzaldehyde, the 5-component blend containing all compounds, and unbaited traps. The experiment was conducted in a maize field at Sete Lagoas, Brazil, March 5–April 25, 2008, with 5 blocks of KLP+ traps.

Experiment 3. This test was aimed at comparing the activity of 1,4-dimethoxybenzene, formulated in 3 different dispenser types, on *D. speciosa*. Treatments included 1,4-dimethoxybenzene in a PE bag, a PE vial or a PE vial with dental roll dispensers (100 mg dose each), and unbaited traps. The experiment was run in a maize field at Sete Lagoas, Brazil, March 28–May 2, 2010, with 5 blocks of KLP+ traps.

Experiment 4. This test was aimed at studying interactions between the synthetic floral and pheromone lures of *D. barberi*. Treatments included the synthetic pheromone lure [2 μg of (2*R*,8*R*)-8-methyl-2-decyl propanoate on a rubber dispenser] (Guss et al., 1984), the floral lure (200 mg each of 4-methoxyphenethyl alcohol and indole in one PE bag dispenser) (Metcalf et al., 1995, 1998), the floral and pheromone lures together in the same trap, and unbaited traps. The experiment was run in a maize field at Pine Grove Mills, PA, USA, July 22– August 15, 2011, with 10 blocks of KLP+ traps.

Experiment 5. This test was aimed at comparing the performance of the floral lure of *D. barberi* with that of *D. v. virgifera*, at a European site where only *D. v. virgifera* is present. Treatments included the *barberi* floral lure (200 mg each of 4-methoxyphenethyl alcohol and indole in one PE bag dispenser) (Metcalf et al., 1995, 1998), the *virgifera* floral lure (200 mg each of 4-methoxy cinnamaldehyde and indole in one PE bag dispenser) (Metcalf et al., 1995, 1998), and unbaited traps. The experiment was run in a maize field at Pusztazámor, Pest county, Hungary, August 1–September 6, 2005, with 5 blocks of KLP+ traps.

Statistical analyses

Catches from field trapping tests were transformed using (x + 0.5)1/2 (Roelofs and Cardé, 1977) and analyzed by Student *t* test or ANOVA, as appropriate. If the ANOVA yielded significance, then treatment means were separated by Games–Howell test (Games and Howell, 1976; Jaccard et al., 1984). Where one of the treatments caught no insects, the Bonferroni–Dunn test (Dunn, 1961) was used to check if mean catches in other treatments were not significantly different from zero catch.

In case capture data did not fulfil requirements for a parametric analysis, the non-parametric Kruskal–Wallis test was performed, followed by pairwise comparisons by Mann–Whitney U test (see also Figure legends).

All statistical procedures were conducted using the software packages StatView® v4.01 and SuperANOVA® v1.11 (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

D. speciosa

In Exp. 1, only traps with 1,4-dimethoxybenzene or with the blend of 2-phenylethanol + methyl anthranilate + eugenol + benzaldehyde caught significantly more *D. speciosa* beetles than unbaited traps (Fig. 1). There was no significant difference between these two treatments, although 1,4-dimethoxybenzene-baited traps caught ca. twice as many beetles as those with the 4-component blend. As for other beetle species, a total of

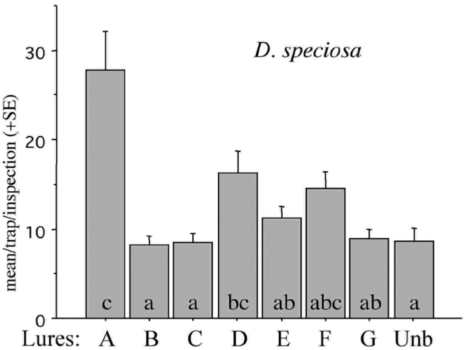

Fig. 1. Mean catches of *D. speciosa* in traps baited with blends of candidate attractants in a preliminary test. Composition of lures (dose of single compounds 100 mg): A = 1,4-dimethoxybenzene; B = 1-phenethyl alcohol + methyl eugenol + *(E)*-anethol; C = *(E)*-anethol + *(E)*-cinnamyl alcohol + *(E)*-cinnamyl acetate + *(E)*-cinnamaldehyde; D = 2-phenylethanol + methyl anthranilate + eugenol + benzaldehyde; E = *ß*-ionone + methyl salicylate + phenylacetaldehyde; F = 4-methoxyphenethyl alcohol + *(E)*-cinnamyl alcohol + *(E)*-anethol + indole; G = 4-methoxy cinnamaldehyde + indole; Unb = unbaited control. (Exp. 1: Total caught in test 3753 beetles. Columns with the same letter within one diagram are not significantly different at P = 5% by ANOVA, Games–Howell)

1157 *Cerotoma* sp. (Coleoptera, Chrysomelidae) were recorded (not shown in Fig.). However, there was no significant difference in mean catches of *Cerotoma* sp. between any of the treatments, including unbaited traps ($P = 0.42503$; $F = 1.016$; $DF = 7$, by ANOVA).

In Exp. 2, all treatments containing 1,4-dimethoxybenzene caught significantly more male *D. speciosa* than unbaited traps (Fig. 2). Addition of combinations of the 4-components of the blend found active in Exp. 1 to 1,4-dimethoxybenzene did not increase catches, no matter whether pairs or all four of the compounds were added. Female *D. speciosa* catches showed the same trend as male catches (Fig. 2).

When different dispensers, loaded with 1,4-dimethoxybenzene, were compared (Exp. 3), all baited traps caught significantly more *D. speciosa* than unbaited ones (Fig. 3). Although in absolute terms, traps with the PE bag dispensers caught approximately three times as many beetles as either treatments with the PE vial, the difference was not significant.

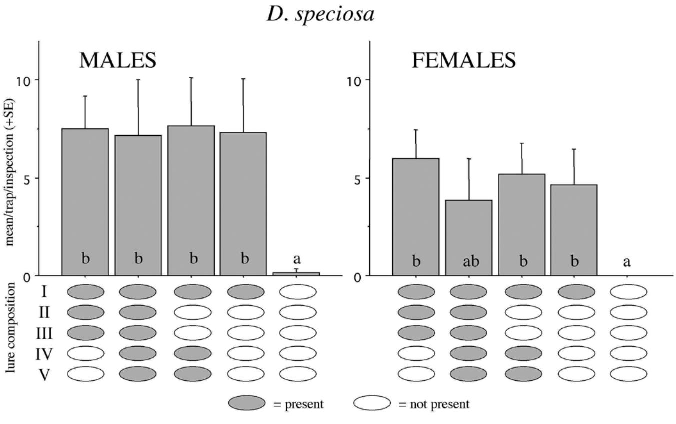

Fig. 2. Mean catches of *D. speciosa* in traps baited with 1,4-dimethoxybenzene and its blends with candidate co-attractants. Compounds tested (dose 100 mg each): I = 1,4-dimethoxybenzene; II = 2-phenylethanol; III = methyl anthranilate; IV = eugenol; V = benzaldehyde. (Exp. 2: Total caught in test 179 males and 118 females. Columns with the same letter within one diagram are not significantly different at P = 5% by ANOVA, Games–Howell)

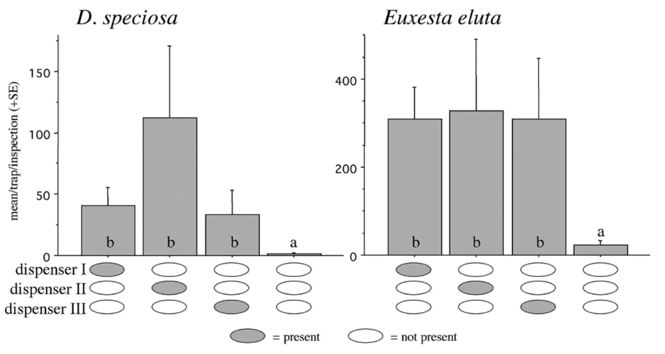

Fig. 3. Mean catches of *D. speciosa* and *Euxesta eluta* in traps baited with 1,4-dimethoxybenzene formulated in three dispenser types. Dispenser types: I = PE vial; II = PE bag; III = PE vial with dental roll. (Exp. 3: Total caught in test 751 *D. speciosa*, and 3861 *E. eluta*. Columns with the same letter within one diagram are not significantly different at P = 5% by Kruskal–Wallis, Mann–Whitney)

In this test, a total of 3861 specimens of the cornsilk fly, *Euxesta eluta* Loew. (Diptera, Ulidiidae) were caught (Fig. 3). All treatments with 1,4-dimethoxybenzene caught far more than unbaited traps. There was no significant difference between treatments with different dispensers.

D. barberi

In Exp. 4, no male *D. barberi* were recorded in traps baited with the *barberi* floral lure or in unbaited traps (Fig. 4). Treatments with the *barberi* pheromone alone or in combination with the floral lure caught significantly more beetles than unbaited ones, but there was no difference between them. As for female *D. barberi*, no beetles were caught in traps with the pheromone or in unbaited traps (Fig. 4). Similar mean catches were recorded in traps baited with the floral lure or in those baited with the combination of the floral lure and the pheromone, and catches in traps with the floral lure were significantly higher than those in unbaited traps.

In this experiment, the trend of catches of male *D. v. virgifera* was the same as for *D. barberi* (Fig. 4). As for female *D. v. virgifera*, the highest catches were recorded in

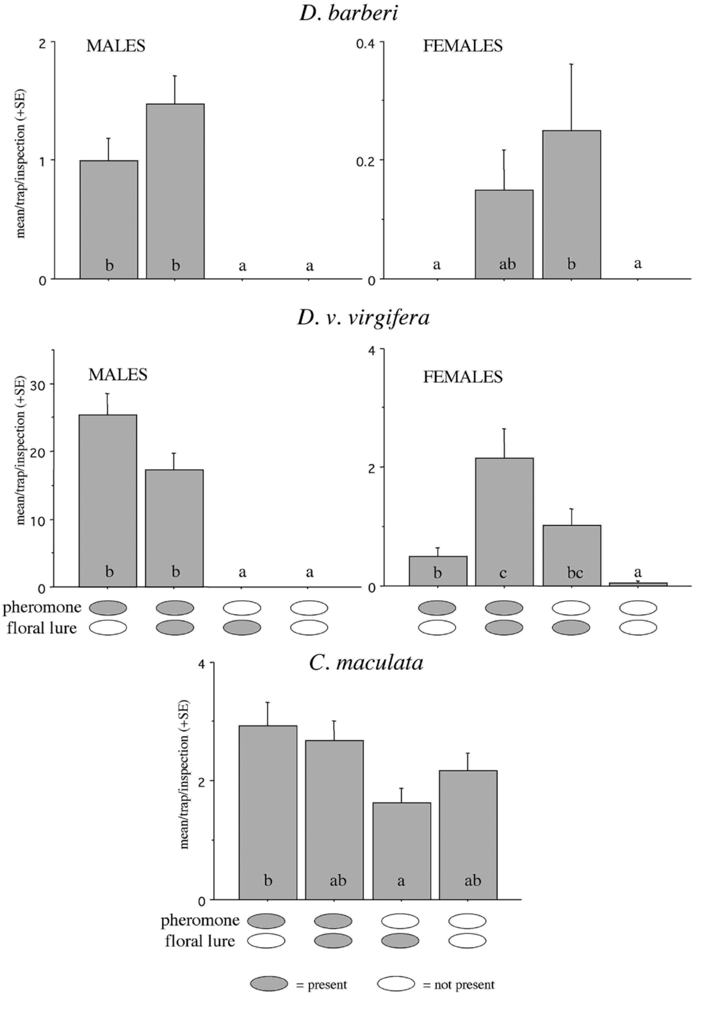

Fig. 4. Mean catches of *D. barberi* and *D. v. virgifera* in traps baited with the *barberi* pheromone, the *barberi* floral lure, and with the two lures together. (Exp. 4: Total caught in test 99 and 1712 males and 16 and 149 females of *D. barberi* and *D. v. virgifera*, resp. (Columns with the same letter within one diagram are not significantly different at P = 5% by Kruskal–Wallis, Mann–Whitney)

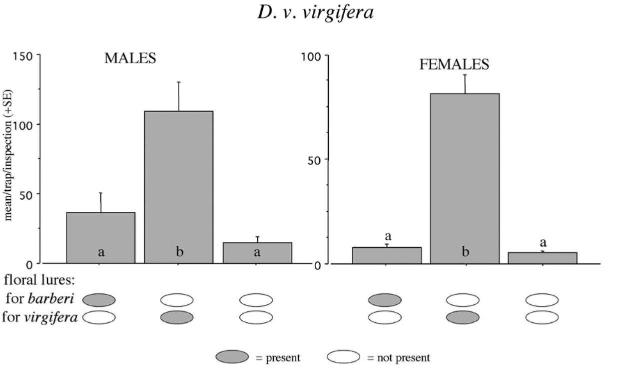

Fig. 5. Mean catches of *D. v. virgifera* in traps baited with the *barberi* floral lure, the *virgifera* floral lure, and in unbaited traps. (Exp. 5: Total caught in test 2576 males and 1513 females. Columns with the same letter within one diagram are not significantly different at P = 5% by ANOVA, Games–Howell)

treatments with the *barberi* floral lure alone or in combination with the pheromone, which did not differ from each other (Fig. 4). The pheromone alone caught very few beetles (but more than unbaited traps).

In addition, a total of 376 specimens of *Coleomegilla maculata* De Geer (Coleoptera, Coccinellidae) were also captured in Exp. 4 (Fig. 4). Traps with the pheromone alone caught more of these lady beetles than those with the floral lure alone (which caught the lowest mean number). There were no significant differences between catches of the other treatments.

In Exp. 5, traps with the *barberi* floral lure did not catch more female or male *D. v. virgifera* than unbaited traps (Fig. 5). Traps with the *virgifera* floral lure caught significantly more *D. v. virgifera* than unbaited traps or traps with the *barberi* lure. The *virgifera* floral lure caught ca. 20 and 9 times more females and males than the *barberi* floral lure, respectively.

Discussion

D. speciosa

To the best of our knowledge, there is no information in the literature on the pheromone composition of *D. speciosa*. On the other hand, 1,4-dimethoxybenzene has previously been described as a floral attractant for *D. speciosa* adult beetles (Ventura et al., 2000). In screening tests of this study, a 4-component blend, comprising 2-phenylethanol, methyl anthranilate, eugenol and benzaldehyde also elicited attraction. However, addition of these compounds to 1,4-dimethoxybenzene in two- or four-component blends did not increase the activity of the known attractant. 1,4-Dimethoxybenzene worked well in this study in all three types of dispensers for catching *D. speciosa*. Consequently, for detection surveys of *D. speciosa* in Europe, KLP+ traps baited with 1,4-dimethoxybenzene formulated in PE bag dispensers (which caught numerically the highest number of *D. speciosa* in this study) can be recommended.

To our knowledge, this is the first report on the attraction of the cornsilk fly, *E. eluta,* to a synthetic chemical attractant. Since the species is regarded as a pest of maize, it should be investigated whether 1,4-dimethoxybenzene yields any advantages over protein-based natural attractants used at present for trapping this pest (Cruz et al., 2011).

Cerotoma sp. in this study did not show preference towards any of the floral compounds and combinations tested. We suggest that the catches are either chance captures of a locally high population, or may reflect a low-intensity response of these beetles to yellow colour.

D. barberi

As for *D. barberi*, the pheromone [as (2*R*,8*R*)-8-methyl-2-decyl propanoate] has been identified by Guss et al. (1985). KLP+ traps baited with this compound caught sizeable numbers of *D. barberi* in this study, confirming the previous report. When pure enantiomers of 8-methyl-2-decyl propanoate were compared, *D. v. virgifera* (although responding well to the racemic compound) also preferred the (2*R*,8*R*) enantiomer (Guss et al., 1984). This explains why in this study males of *D. v. virgifera* were also attracted to the traps. In areas where *barberi* and *virgifera* co-occur, the ratio of *D. barberi* vs. *D. v. virgifera* in the catch will be predominantly determined by the relative population densities at the given site. This phenomenon is a definite drawback in detection surveys for *D. barberi* in Europe, where the previously introduced *D. v. virgifera* can be present at high densities in some countries. Consequently, all captured specimens should be determined to species (i.e. based on morphological characters).

A potent floral lure (4-methoxyphenethanol + indole), that attracts both female and male *D. barberi,* is already known (Ladd et al., 1985; Metcalf et al., 1995, 1998). In the present study, *D. barberi* females were recorded only in treatments containing the floral lure, confirming literature data. Low catches of *D. v. virgifera* females in the same treatments suggested that the *barberi* floral lure showed some attraction also for this species. However, it appears that the specificity of the *barberi* floral lure with respect to *D. v. virgifera* catches is less of a problem than it is for the pheromone. When directly comparing the *virgifera* floral lure (4-methoxy cinnamaldehyde + indole) as described by Metcalf et al. (1995, 1998) with the *barberi* floral lure (Exp. 5 of this study), the former caught 20 times more female *D. v. virgifera* than the latter. Also, in this test, no attraction of *D. v. virgifera* females by the *barberi* floral lure was recorded.

Thus, in theory, the KLP+ traps with the *barberi* floral lure could be a more selective trapping tool in detection programs of *D. barberi* in Europe. However, their sensitivity lags behind that of pheromone traps. In this study, *barberi* pheromone traps were sufficiently attractive to catch ca. four times more beetles than traps with the *barberi* floral lure. Pheromone baits have been observed to be more attractive than respective floral baits also in other *Diabrotica* spp., i.e. in *D. undecimpunctata howardi* Barrer (Herbert et al., 1996) or *D. v. virgifera* in Europe (Tóth et al., 2003).

For detection purposes it is advantageous if the trap captures individuals of both sexes. In the present study, we found that pheromone and floral lures can be applied together in the same KLP+ trap to maximize both male and female *D. barberi* catches, confirming an earlier report by Ladd et al. (1985). In this respect, *D. barberi* is similar to *D. v. virgifera*, in which latter species the respective pheromone and floral lures can also be applied together in the same trap (Tóth et al., 2003; Tóth, 2005).

Consequently, for first detection programs of *D. barberi* in Europe, the KLP+ trap baited with both the pheromone and floral lures will likely be most effective.

Captures of the coccinellid *C. maculata* in Exp. 4 of this study did not show clear preferences for any of the treatments (including unbaited traps), and catches probably were due to some physical characteristics of the KLP+ trap design. These insects can easily be told apart (based on size and morphology) from *Diabrotica* spp., and so do not detract from the usefulness of the KLP+ trap for *Diabrotica* spp. detection.

Acknowledgements

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