Development of Trapping Tools for Detection and Monitoring of *Diabrotica* v. *virgifera* in Europe

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Trap designs baited with the synthetic sex pheromone have been optimized for trapping of the western corn rootworm *Diabrotica* v. *virgifera* LeConte (Coleoptera: Chrysomelidae) (WCR), which has recently been introduced into Europe. The best trap design proved to be the sticky “cloak” trap (code name “PAL”), which catches only males, and is being used in many countries of Europe for detection and monitoring the spread of the new pest. Preliminarily the range of attraction (as defined by Wall and Perry, 1987) of the pheromone traps was estimated to be <10 m. The performance of yellow sticky plates (used by others for monitoring of the pest) was insignificant as compared to the activity of the pheromone baited traps, and yellow colour had no discernible effect on catches in pheromone traps. The known floral lure of WCR containing 4-methoxy-cinnamaldehyde and indole proved to be active also towards the population in Europe, attracting both females and males. Yellow colour slightly increased catches by the floral lure, hence a yellow sticky “cloak” trap has been developed (code name PALs). Pheromone baited PAL traps caught a total of about 4 times more beetles than the floral baited PALs, which latter however appeared to be preferentially active for females. When placed into the same trap, the pheromonal and floral lures did not interfere with each other’s activity.

Keywords: *Diabrotica* v. *virgifera,* Coleoptera, Chrysomelidae, pheromone trap, floral lure.

The western corn rootworm (WCR) *Diabrotica* v. *virgifera* LeConte (Coleoptera: Chrysomelidae) was first discovered in Europe in 1993 near Belgrade Airport (Yugoslavia) (Camprag and Baca, 1995).

Since ecological conditions are favourable for the survival of this new pest in the maize-growing regions of Europe, a sensitive detection and monitoring tool was required by European agriculture to detect the first occurrence of the new pest in new regions and to monitor its spread all over Europe. Pheromone traps are used for similar purposes in many insect pests. In WCR a female produced sex pheromone has been identified (Guss et al., 1982). The first objective of this study was to develop a simple, effective trap which is baited with the synthetic sex pheromone and is suitable for detection and monitoring of the European population of WCR. For a pheromone bait, racemic 8-methyl-2-decyl propanoate was chosen as although the natural pheromone consists of one of the pure enantiomers (Guss et al., 1984), the presence of other enantiomers does not interfere with attractive activity.

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One drawback of pheromone-baited traps is that they will attract and catch only one sex (in case of WCR the males). However, the capture of females would have definite advantages in an agricultural monitoring system, so the development of another trap capable of catching also females was asked for by many agricultural experts.

In case of the WCR flower-derived attractants have been described which attract both females and males (see for a review Metcalf, 1994; Metcalf et al., 1998). A second objective of the present study was to investigate the attractive activity of such synthetic flower volatiles at European sites towards WCR and to try to develop a trap baited with these attractants which is active enough for agricultural use and is capable of catching both females and males of WCR. For testing of the female-attractive bait, 4-methoxy-cinnamaldehyde (MCA) and indole were chosen, as these compounds proved to be highly attractive for WCR in tests in the US (Metcalf et al., 1995). From these MCA has been found also to be an effective single component kairomone-type lure for adult WCR (Metcalf and Lampman, 1989).

Materials and Methods

Chemicals

Racemic 8-methyl-2-decyl propanoate was synthetized by a suitable modification of published procedures (see i.e. Naoshima et al., 1991; Keinan et al., 1992 and references therein). (±)-8-Methyl-1-decene was the key intermedier of the synthesis. This was obtained by coupling of the Grignard compound of 1-bromine-3-methyl pentane, easily available from the corresponding alcohol, and of 5-bromine-1-pentene catalized by Li_2CuCl_4. The resulting alkene was reacted by $Hg(OAc)_2$ (regioselective oxymercuration), and then reducing the resulting compound by $NaBH_4$ a diastereomeric mixture of 8-methyl-2-decanol was obtained. Acylation by propionic acid anhydride yielded the final product. Intermediates in the reaction series were purified by vacuum destillation, while the final product was purified by column chromatography [Kieselgel 60 (0.063–0.2 mm) column, eluent *n*-hexane]. Total yield of the 5-step reaction series was 20%. Structures of the compounds synthetized were verified by 1H-NMR (80 MHz) spectroscopy.

Indole (Sigma-Aldrich Kft, Budapest, Hungary) and 4-methoxy-cinnamaldehyde (Bedoukian Inc, Danbury, USA) were purchased commercially and were >95% pure as stated by the suppliers.

Bait dispensers

Rubber: baits for the tests were prepared by using pieces of rubber tubing (Taurus, Budapest, Hungary; No. MSZ 9691/6; extracted 3 times in boiling ethanol for 10 min, then also 3 times in methylene chloride overnight, prior to usage).

Rubber in PE vial: for making up the baits a 0.5 cm piece of rubber tubing (same quality as above) was placed inside a 0.7 ml polythene vial with lid (No. 730, Kartell Co., Italy). The lid of the vial was opened when the bait was set up into a trap in the field.

PE vial closed: 0.7 ml vials with lid (No. 730, Kartell Co., Italy) were used, the vial was kept closed when setting up the trap in the field.

PE bag: A 1 cm piece of dental roll (Celluron®, Paul Hartmann Ag. Heidenheim, Germany) was put into a tight polythene bag made of 0.02 mm linear polyethylene foil.

All types of baits were attached to 8 × 1 cm plastic handles for easy handling when assembling the traps.

For making up the baits the required amounts of compounds were administered to the surface (rubber), into (PE vial) or onto the dental roll (PE bag) dispensers in hexane or dichloromethane solutions. After having allowed the solvent to evaporate, the lid of the PE vial dispensers was closed, and PE bag dispensers were heat sealed. Dispensers were wrapped singly in pieces of alufoil and were stored at –65 °C until use.

Traps of different designs used

Diagrams of trap designs used are shown in *Fig. 1.*

The sticky traps of "delta" design (RAG) were basically the same as those generally used in Hungary in trapping tests of moth spp (Szőcs, 1993; Tóth and Szőcs, 1993). Trap bodies were prepared from transparent plastic sheets (23 × 36 cm), by folding the sheets into a triangular prism (length 23 cm, all three sides 12 cm; with the two ends open). Insects getting into the traps were captured on replaceable sticky inserts (16 × 10 cm) which were placed at the bottom side of the trap body.

The sticky panel (LEM) traps were prepared from transparent plastic sheets (23 × 18 cm), with a 3 cm diameter hole in the middle, and two 16 × 10 cm sticky plates were attached by clips at both sides of the sheet *(Fig. 1).* The bait dispenser was suspended from the top edge of the trap so that the bait was positioned into the middle hole, thus being easily reachable by air movements from both sides. The sticky sheets could be replaced in case of need by the user. The assembled trap was suspended vertically from a stick or the maize stem by a piece of wire.

The sticky "cloak" (PAL) traps were prepared from transparent plastic sheets (36 × 23 cm). One side was covered with sticky material. When setting up, the sticky sheet was placed around a maize stem in a "cloaklike" manner, with the sticky side facing outward, and the back corners were fastened together with clips. The trap was kept in place by a piece of wire, attached to the maize stem. The bait dispenser was fastened to the front top edge of the trap, with the bait hanging in front of the sticky surface.

In some tests PAL traps painted yellow were also tested. Of the two colours tested PALs yellow was a lemon-yellow hue similar by the human eye to the Pherocon AM® (Trécé, Salinas, USA) yellow sticky traps' colour hue, while the PALfl fluorescent yellow was similar to the colour of the Multigard® traps (Scentry, Buckeye, USA). *Figure 2* shows the reflectance spectra of the PALs and PALfl traps.

The RAGMOD trap was essentially the same as the RAG trap, but a 10 × 14 cm piece of transparent plastic was fastened horizontally in front of the bottom edge of the trap opening at both sides. This was thought to help the approaching beetles in alighting and making possible to apporach the attractant source by scrawling into the trap.

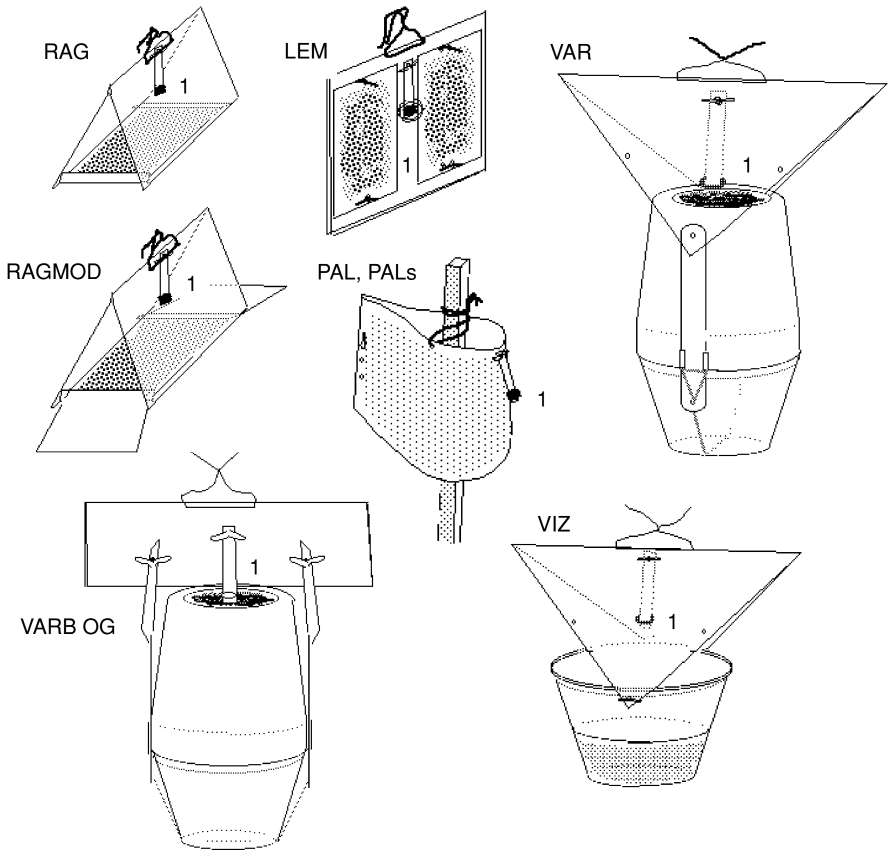

Fig. 1. Diagrams of trap designs used. 1 = bait dispenser

The VIZ trap was the standard water pan trap used by the Budapest laboratory for catching larger moth spp. like noctuids or geometrids. It consisted of a 1 litre round water pan (diameter of top opening: 14 cm; height 12 cm), above which a folded transparent plastic sheet (23 × 23 cm) is attached in a rooflike manner. The bait dispenser was attached under the roof, from the top middle portion. The bait hung in the middle of the trap, at ca 1 cm higher than the level of the upper opening of the water container. The killing liquid in the trap was ca 0.7 litre water with 1–2 ml household detergent added.

The VAR trap was the standard funnel trap used by the Budapest laboratory for catching larger moth spp. like noctuids or stored product phycitids. It consisted of a plastic funnel (top opening outer diameter: 13 cm, funnel hole diameter 3 cm, height of funnel 16 cm), under which a transparent plastic round catch container (same as the water container

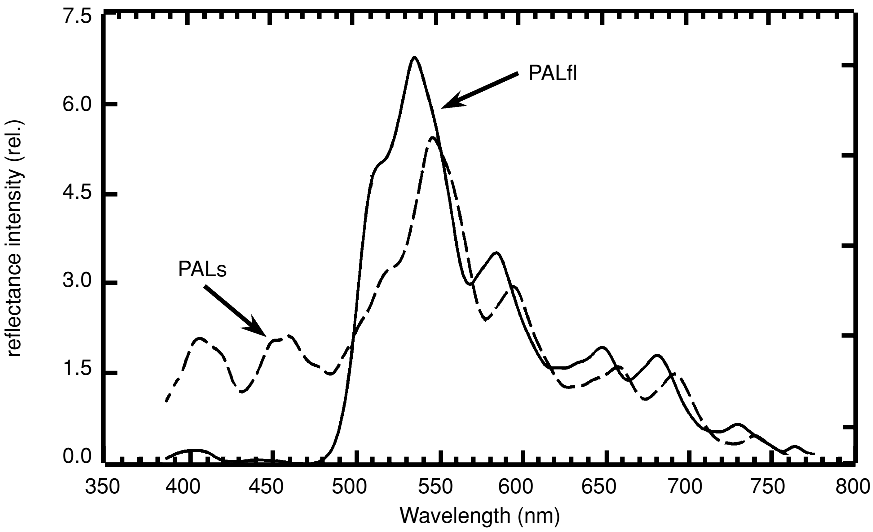

Fig. 2. Reflectance spectra of yellow coloured traps used in the tests

in the VIZ trap) was attached by a rubber band. On top of the funnel a folded transparent plastic sheet (23 × 23 cm) was attached in a rooflike manner. The bait dispenser was attached under the roof, from the top middle portion. The bait hung in the middle of the funnel opening, at ca 1 cm higher than the level of the upper edge of the funnel. A household insecticide strip (active ingredient 20% dichlorvos) was placed inside the catch container to kill the insects captured.

The VARBOG trap was the same as the VAR trap, except that instead of the plastic roof there was a piece of plastic sheet (10 × 16 cm) attached vertically reaching accross the opening of the funnel. The dispenser was suspended from the vertical plastic sheet, and the bait hung in the middle of the funnel opening, at ca 1 cm higher than the level of the upper edge of the funnel.

For preparing sticky traps in this study, TangleTrap® insect adhesive (TangleFoot Co., Grand Rapids, USA) was used.

Field testing procedures

During the tests different treatments (= trap/bait combinations) were set up in a block (one of each treatment). The distance of traps within a block was 8–15 m. The distance between blocks ranged between 100–1000 m. Traps were moved one position forward within a block at each occasion when the traps were inspected. At the same time, captured beetles were recorded and sticky inserts changed to new ones, if necessary. Inspections were performed at 3–4 day intervals.

Test sites

Tests were set up in maize fields where also maize was cultivated the previous year. Test sites included in Hungary Mezőhegyes (Békés county) and Szeged (Csongrád county), in Yugoslavia Surcin / Batajnica, Zemun Polje and Zemun (Beograd).

Statistics

In statistical analyses, catches recorded at an inspection were regarded as replicates. Capture data were transformed to $(x+0.5)^{1/2}$ and differences between means were tested for significance by ANOVA followed by Duncan's New Multiple Range Test (DNMRT). In tests where only two treatments were compared, the Student's *t*-test (unpaired) was used. Differences of single treatments from zero catch (unbaited) were tested by one-sample *t*-test or the non-parametric Mann-Whitney U test. Statistical analyses were performed by the softwares StatView™ v.4.01 and SuperANOVA™ v1.11 (Abacus Concepts, Inc., Berkeley, USA)

Preliminary trapping range study with pheromone baited traps

In the test pheromone-baited LEM traps were used. The test was performed at a site without maize in the vicinity of the Zemun Expt. Stn., Yugoslavia. Male WCR beetles collected from a distant maize field were released in the middle of the field from one point, and traps were set up at distances of 10, 30 and 100 m from the point of release. At each distances 4 traps were set up to the north, east, south and west from the point of release. On July 26, 29 and 31 300, 200 and 300 males were released, resp. Traps were inspected and captured beetles were recorded before the next release took place on July 29, 31 and August 2.

Measurement of reflectance spectra

Reflectance spectra were recorded with a Perkin Elmer MPF 44B spectrofluorometer. It was set to "ratio-mode", i.e. the wavelength-depended intensity variation of the xenon lamp light source was corrected. The excitation and emission monochromators were set into the same wavelength position and the spectra were scanned with dual drive operation, i.e. the excitation and emission monochromators were driven synchronously. The optical slits were 2 nm.

The colour samples were fixed on a solid sample holder in a 45 position to the direction of the excitation light and to the direction of the observation of the reflected light. The reflectance spectra were recorded in the 350–800 nm region. The spectra obtained were digitalised and stored in a PC. The averages of 5 spectra recorded from 5 different samples were calculated and processed with the SPSERV software (C. Bagyinka, Biophysical Institute of the Biological Research Center, Hung. Acad. Sci., Szeged, Hungary). The spectra were smoothed and their baselines were corrected with the reflectance spectrum of a ZnS plate.

Results and Discussion

Pheromone baited traps

Traps baited with racemic 8-methyl-2-decyl propanoate were clearly superior than unbaited traps already in the first preliminary tests *(Table 1),* demonstrating the activity of this compound for the first time towards a European WCR population. In these tests it was also clear that the RAG delta sticky traps routinely used for catching many moths (Szőcs, 1993) are not as effective towards WCR as the LEM sticky plates. A possible explanation may be that the WCR males were not able to home in so accurately to a pheromone point source as the moths which are far better fliers, and thus a smaller percentage of beetles could fly in through the relatively small triangular hole of the delta traps. This is supported by on-site visual observations (Sivcev and Tóth, unpubl.), as WCR beetles are very frequently observed to perform relatively short jump-like flights, alighting on a substrate, and running along its surface for several cm before starting on another "jump", instead of the characteristic orientated zigzagging flight of moths approaching a pheromone source.

Table 1

Catches of WCR in traps baited with synthetic racemic 8-methyl-2-decyl propanoate and unbaited traps of different design at sites near Belgrade Airport in Yugoslavia in 1995 (Site 1 = Surcin, July 21–August 28; Site 2: Zemun, July 24–August 15; Site 3: Zemun Polje / Batajnica, July 24–August 28)

Trap design	Bait	Mean catch/trap/inspection		
		Site 1	Site 2	Site 3
RAG	yes	16.5b	1.7a*	19.9b
RAG	no	0.1a	0.0*	0.1a
LEM	yes	55.3c	16.3b	129.6c
LEM	no	2.6a	0.0*	2.4a

Statistics: ANOVA followed by DNMRT on $(x+0.5)^{1/2}$ transformed data. Means with same letter within a column are not significantly different at P=5%. Means with asterisk do not differ significantly at P=5% from zero catch by Mann-Whitney U test. From each variation two replicates were set up at each site.

In the second test series RAG traps were again performing worse than LEM traps confirming the preliminary results *(Table 2).* Modifying the RAG design by supplying a horizontal plate as alighting place in front of the trap opening (RAGMOD) did not increase catches to the level of the LEM captures. The non-sticky trap designs (VIZ, VAR, VARBOG) all performed worse than LEM *(Table 2).*

Yellow sticky traps of LEM design, but with yellow (PALs hue) sticky plates and no chemical bait caught much less than the pheromone baited LEM traps with transparent plates *(Table 2).*

Table 2

Catches of WCR in traps baited with synthetic racemic 8-methyl-2-decyl propanoate and unbaited traps of different design in Yugoslavia (Zemun, July 11–29, 1996; August 4–15, 1997)

Trap design	Mean catch/trap/in spection	
	Zemun, 1996	Zemun, 1997
LEM	223.9b	77.8a
RAG	98.5a	not tested
VIZ	41.3a	not tested
VAR	46.8a	not tested
VARBOG	80.4a	not tested
PAL	not tested	94.0a
RAGMOD	not tested	24.1b
Yellow sticky (LEM, no bait)	not tested	14.8b

Statistics: ANOVA followed by DNMRT on $(x+0.5)^{1/2}$ transformed data. Means with same letter within a column are not significantly different at P=5%. From each variation 3 (in 1996) or 4 replicates (in 1997) were set up at each site.

The only trap type catching at the same level as LEM was the sticky PAL ("cloak") design. The PAL design offers a similar outside sticky surface directly reachable by the beetles as the LEM, and it can be set up much easier by a single sweeping movement around a maize stem and fastened at the back. This trap design was developed for easier assemblage and use as cooperators complained that through assembling the LEM traps their fingers got covered with glue.

Because of its ease of maintenance and high catching activity, the PAL trap design was selected as the standard sticky trap design for WCR in subsequent tests.

In a test comparing traps of different colour, traps baited with the pheromone caught similar numbers no matter whether they were transparent of yellow *(Table 3).* Unbaited traps caught significantly less than baited ones. Although the yellow traps caught numerically somewhat larger numbers, there were no significant differences between catches of unbaited transparent, yellow, or fluorescent yellow traps *(Table 3).*

The inactivity of the yellow traps was a bit surprising, as different hues of yellow have been described as being attractive towards WCR (Tollefson et al., 1975; Ball, 1982; Ladd et al., 1984; Karr and Tollefson, 1987; Hesler and Sutter, 1993; Youngman et al., 1996) and yellow sticky traps have been suggested for agricultural use (Tollefson, 1986; Karr and Tollefson, 1987; Kuhar and Youngman, 1998). In the present tests the expected attractive activity of the yellow colour was insignificant as compared to the activity of the pheromone bait.

Preliminary range of attraction study with pheromone baited traps

In this test we recorded no significant difference in captures in traps set up at 10, 30 or 100 m from the point of release *(Table 4).* The recapture rates ranged from 2.09 to

Table 3

Catches of WCR in traps baited with synthetic sex pheromone in different trap designs in Yugoslavia in 1998 (Zemun, August 7–12)

Trap design	Bait	Mean catch/trap/inspection
PAL	pheromone	319.0b
PAL	unbaited	46.0a
PALs	unbaited	59.0a
PALfl	unbaited	69.2a
PALs	pheromone	258.7b

Statistics: ANOVA followed by DNMRT on $(x+0.5)^{1/2}$ transformed data. Means with same letter are not significantly different at P=5%. From each variation 6 replicates were set up

2.97% with no significant difference between the three distances. Although to exactly determine the range of attraction of pheromone-baited traps more detailed release-recapture studies will be necessary, data obtained in our preliminary study already suggest that the range of attraction (as defined by Wall and Perry, 1987) of a pheromone baited trap is below 10 m for WCR, since there was no sign of interference (significant decrease in catches) even at the most closely spaced traps as compared to more distantly spaced ones.

Table 4

Catches of released male WCR in traps baited with the pheromone at 10, 30 and 100 m distance from the point of release (Zemun, Yugoslavia, July 26–August 2, 1996)

Distance from point of release	Mean catch/trap	Mean % recaptured/trap
10 m	5.6a	2.09a
30 m	7.0a	2.65a
100 m	8.6a	2.97a

Statistics: ANOVA followed by DNMRT on $(x+0.5)^{1/2}$ transformed data. Means with same letter within one column are not significantly different at P=5%. At each distance 4 traps were set up. Male beetles were released on three occasions

The recapture percentages were considerably low in this study. The similar level of catches and recapture percentages in the traps spaced from 10 to 100 m in this study suggests that WCR males had a high potential for migration away from the point of release, capable of covering several hundred m or even more within a couple of days. Probably only beetles coming into the closer vicinity (<10 m) of the traps were attracted and captured. These data are supplemented by direct visual observations mentioned earlier on the behavior of beetles around a pheromone source.

Flower derived attractants

When comparing the performance of synthetic floral baits formulated in different ways, all three bait types caught significanltly more WCR than the unbaited traps did *(Table 5),* clearly showing that the mixture of MCA and indole was attractive also towards the European population of WCR. Pheromone baited traps caught ca 3–5 times more beetles, than the floral baits. The attractive activity of the floral baits was clear for both males and females, while the catch of the pheromone baited traps consisted of almost exclusively males (*Table 5,* Test 1). Among the bait formulations, the closed PE vial caught slightly less that the other variations. For later tests the sealed PE bag formulation was selected because this dispenser type caught numerically the most beetles and also because it was easier to use than the "rubber in PE vial" variation. In a subsequent test traps baited with the floral attractant in PE bag formulation caught far more than unbaited traps (both males and females; *Table 5,* Test 2), confirming results of the first test (*Table 5,* Test 1).

Table 5

Catches of WCR in traps baited with synthetic sex pheromone or a 1:1 mixture of indole and 4-methoxycinnamaldehyde (IND-MCA) in PAL traps baited with different dispensers in Yugoslavia in 1997 (Zemun, test 1: August 4–15; test 2: September 23–October 6)

Dispenser	Bait	Mean catch/trap/inspection					
		Test 1			Test 2		
		males	females	both sexes	males	females	both sexes
Rubber	pheromone	140.2d	0.3a	140.5d		not tested	
Rubber in PE vial	IND-MCA	19.8c	37.7b	57.5c		not tested	
PE vial closed	IND-MCA	9.4b	22.9c	32.2b		not tested	
PE BAG sealed	IND-MCA	21.1c	47.6b	68.6c	82.3	129.5	220.3
Rubber	unbaited	1.4a	1.2a	2.6a	0.5	1.2	1.7
P value (Student's *t*-test)					<.0001	<.0001	<.0001

Statistics: Test 1: ANOVA followed by DNMRT on $(x+0.5)^{1/2}$ transformed data. Test 2: Student's *t*-test. Means with same letter within a column are not significantly different at P=5%. From each variation 3 replicates were set up at each site.

When testing the longevity of floral baits in the field, PE bag formulated baits aged for 10 or 24 days were catching similar numbers as fresh baits, and all were significantly different from the catch of unbaited traps *(Table 6).*

When testing the importance of indole in the floral bait mixture, at the site with high populations there was a significant increase in catches (males, females, and total) when indole was added to MCA in the bait (*Table 7,* Zemun Polje). Both floral-baited variations caught significantly more than the unbaited traps. At the site with low populations, there was no significant difference between MCA-baited traps and those baited with the indole-containing mixture (*Table 7,* Szeged). Traps baited with the indole plus MCA mixture caught always significantly more than unbaited ones. There was no significant difference

Table 6

Catches of WCR in PAL traps baited with a 1:1 mixture of indole and 4-methoxycinnamaldehyde (IND-MCA) with different field pre-exposure (Zemun, Yugoslavia, August 7–12, 1998)

Field pre-exposure time of bait	Mean catch/trap/inspection
0 days	368.8bc
10 days	393.8c
24 days	263.7b
unbaited	46.0a

Statistics: ANOVA followed by DNMRT on $(x+0.5)^{1/2}$ transformed data. Means with same letter are not significantly different at P=5%. From each variation 6 replicates were set up.

between yellow and transparent traps no matter whether they were baited or unbaited, with the exception of female catches, where yellow traps baited with the indole plus MCA mixture caught significantly more than transparent baited ones (*Table 7,* Szeged).

Table 7

Catches of WCR in traps of different design baited with 4-methoxycinnamaldehyde (MCA) or its 1:1 mixture with indole (IND-MCA) (Zemun Polje, Yugoslavia, August 6–9, 1999; Szeged, Hungary, August 3–September 1, 1999)

Trap	Bait	Mean catch/trap/inspection					
		Zemun Polje			Szeged		
		males	females	both sexes	males	females	both sexes
PAL	MCA	36.4b	18.8b	55.2b	0.6ab	3.4bc	3.9b
PAL	IND-MCA	62.4c	43.3c	105.7c	0.8b	2.3b	3.1b
PAL	unbaited	11.6a	3.4a	14.9a	0.1a	0.1a	0.2a
PALs	IND-MCA		not tested		0.9b	3.7c	4.6b
PALs	unbaited		not tested		0.1a	0.2a	0.3a

Statistics: ANOVA followed by DNMRT on $(x+0.5)^{1/2}$ transformed data. Means with same letter within one column are not significantly different at P=5%. From each variation 10 replicates were set up at each site.

The above data support earlier reports on the synergistic activity of indole when added to MCA (Metcalf et al., 1995). At the low population density site probably the number of beetles captured was too low to allow for a valid comparison. In this latter case there is some indication in the case of female catches that the presence of yellow colour on baited traps might be advantageous. When insects are presented simultaneously with visual and olfactory stimuli, their response to the combination may differ from their response to any other stimulus by itself (Finch, 1986). In an earlier report on WCR, unbaited yellow sticky Multigard traps captured the greatest number of males while most females were caught on yellow traps baited with MCA (Hesler and Sutter, 1993), suggesting that colour plus chemical stimuli had greater impact on female beetles. The trend of captures in our tests also supports this hypothesis, and we concluded that when baited with the floral bait it is advantageous to paint the trap in yellow for best performance.

When comparing traps baited with the pheromone or with a dual combination of pheromone plus floral bait, traps baited with both baits caught significantly, albeit slightly more WCR than traps baited with the pheromone only at the low population site (*Table 8,* Mezőhegyes). This slight increase was due to the catch of females in the traps containing the floral bait, as there was no significant difference between the two traps in the catches of the males (*Table 8,* Mezőhegyes). At the high population site the difference between the traps with the dual bait and with pheromone alone was not significant, although slightly more beetles were caught numerically in the traps with the dual bait (*Table 8,* Zemun). Unfortunately the catches at this site were not sexed, so further analysis was not possible.

Table 8

Catches of WCR in traps baited with synthetic sex pheromone and/or a 1:1 mixture of indole and 4-methoxycinnamaldehyde (IND-MCA) in PAL traps (Zemun, Yugoslavia, August 9–17, 1998; Mezőhegyes, Hungary, July 17–September 10, 1998)

Bait	Mean catch/trap/inspection			
	Zemun	Mezőhegyes		
	both sexes	males	females	both sexes
Pheromone	269.6	4.05	0.00	4.05
Pheromone & IND-MCA	312.5	5.23	0.88*	6.03
P value (Student's *t*-test)	0.2812	0.0908	<0.0001*	0.0035

* One sample *t*-test
Statistics: Student's *t*-test. From each variation 4 replicates were set up at each site.

It can be concluded that the two types of baits did not interfere with each other when placed in the same trap. The lack of significant increase in the dual-baited traps in case of male captures may be attributed to the fact that the high attractive activity of the pheromone bait largely overwhelms the relatively moderate activity of the floral bait. Similarly to results in the present study in WCR, no dramatic increase in captures was observed in *D. barberi* Smith and Lawrence when pheromonal and floral baits were presented together (Ladd et al., 1985).

In a direct comparison of the performance of pheromone-baited PAL traps and floral baited PALs traps, pheromone baited traps caught significantly more males, than floral baited traps, and floral baited traps caught significantly more females than pheromone baited traps *(Table 9).* The pheromone baited PAL traps caught a total of about 4 times more beetles than the floral baited PALs traps at both the high and low populations sites.

Table 9

Catches of WCR in PAL and PALs traps baited with a 1:1 mixture of indole and 4-methoxycinnamaldehyde (IND-MCA) (Zemun Polje, Yugoslavia, August 4–16, 1999; Szeged, Hungary, August 3–September 14, 1999)

Trap	Bait	Mean catch/trap/inspection					
		Zemun Polje			Szeged		
		males	females	both sexes	males	females	both sexes
PAL	pheromone	131.0	4.1	135.1	3.3	0.0	3.9
PALs	IND-MCA	12.1	28.4	40.5	0.2	1.3	1.6
P value (Student t test)		<.0001	<.0001	<.0001	<.0001	.0002*	<.0001

*One-sample *t*-test
Statistics: Student's *t*-test on $(x+0.5)^{1/2}$ transformed data. From each variation 5 replicates were set up at each site.

Pheromone baits have been observed to be more attractive than floral baits also in other *Diabrotica* spp.: i.e. in tests on *Diabrotica undecimpuncatata howardi* Barber pheromone traps caught more beetles than floral baited ones (Herbert et al., 1996).

The ratio of females in floral baited PAL or PALs traps tended to be higher than in unbaited traps, although the difference was significant only at the high population site *(Fig. 3),* suggesting that our bait influenced females more than males. This supports earlier reports in the literature where synthetic floral attractants for *Diabrotica* spp. appeared to be preferentially active for females (Ladd et al., 1985; Andersen and Metcalf, 1986; Lampman et al., 1987; Yaro et al., 1987).

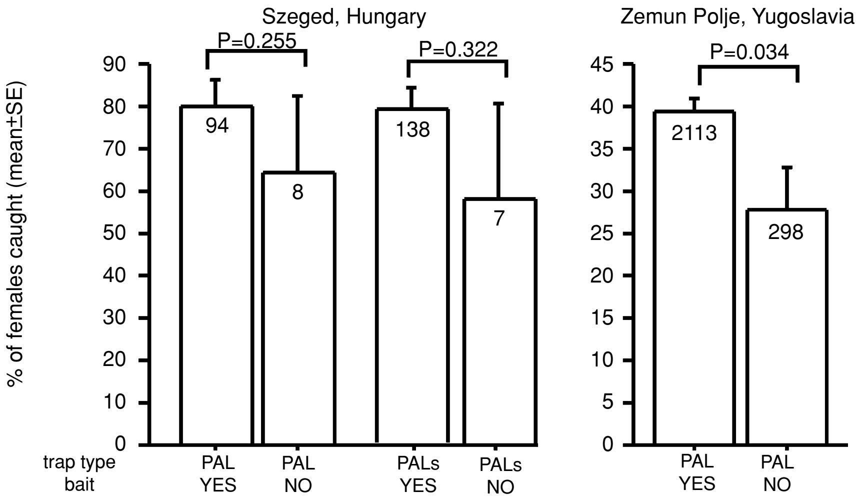

Fig. 3. Ratio of females captured in PAL and PALs traps baited with a 1:1 mixture of indole and 4-methoxycinnamaldehyde (IND-MCA) (Zemun Polje, Yugoslavia, August 4–16, 1999; Szeged, Hungary, August 3–September 14, 1999). Statistics: Student's *t*-test. From each variation 5 replicates were set up at each site. Numbers within columns show total number of beetles captured

Conclusions

Based on the above results we recommend the transparent PAL sticky trap desing baited with the synthetic sex pheromone as especially suitable for low population situations, or when the detection of occurrence is the main question. Data on the spread of *Diabrotica* in Europe came mostly from such traps in recent years. Starting from its original site of introduction in Yugoslavia, by 2000 the pest has been well established in Hungary (Ripka and Princzinger, 2001), Croatia (Igrc-Barcic et al., 2001) and Romania (Florica, 2001), and has been detected to be present in Bulgaria (Ivanova, 2000, 2001), Slovakia (Sivicek, 2000, 2001), Bosnia-Hercegovina (Festic et al., 2000), the Ukraine (Movchan et al., 2001), Italy (Furlan et al., 2000, 2001; Boriani and Gervasini, 2001) and Switzerland (Bertossa et al., 2001).

The PALs yellow sticky traps baited with the mixture of indole + MCA are more suitable in areas where the pest has already been established, and especially in situations where the capture of females is needed. When used for monitoring population changes, such traps can be influenced by seasonal presence of other food sources emitting semiochemicals; for example the silking and tasseling periods of maize are known to affect trap captures in traps baited with plant derived compounds in *Diabrotica* spp. (Andersen and Metcalf, 1986; Lampman et al., 1987; Hesler et al., 1994; Hammack, 1996). This phenomenon requires more thorough study in European agrobiotops.

It is general experience that the application of sticky traps – although very advantageous for detection purposes – is not the optimal choice for quantitative monitoring. Traps in the field may become covered with debris, captured beetles of the target pest, other insects, and other material in a relatively short time (Karr and Tollefson, 1987). In addition, assembling and handling the sticky trap types may be difficult and messy for the inexperienced user. When testing traps for cucumber beetles (*Acalymma vittatum* F.) and *Diabrotica* spp. Hoffmann et al. (1996) suggested that to overcome these problems a non-sticky, non-saturating trap design is needed. At present research is underway in the Budapest laboratory to develop a non-sticky, non-saturating trap type for WCR.

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