RESPONSES OF *DIABROTICA SPECIOSA* AND *CEROTOMA ARCUATA TINGOMARIANA* (COLEOPTERA: CHRYSOMELIDAE) TO VOLATILE ATTRACTANTS

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ABSTRACT

The relative responses of *Diabrotica speciosa* (Ger.) and *Cerotoma arcuata tingomariana* Bechyné (Coleoptera: Chrysomelidae) to traps baited with chemicals were studied. Volatile substances of *Curcubita maxima* Duchesne blossoms, other previously reported volatile attractants for Diabroticites and mixtures of chemicals were tested in common bean, *Phaseolus vulgaris* L., and soybean, *Glycine max* (L.) Mer., fields. Traps baited with 1,4-dimethoxybenzene caught 29.4 times more beetles than solvent controls in fields of soybeans, and 9.4 times more in common bean fields. Traps baited with VIP (veratrole + indole + phenylacetaldehyde) caught 6.5 times more beetles than solvent controls in soybean and 3.5 times more in common bean plots, whereas traps baited with TIC (1,2,4-trimethoxybenzene + indole + trans-cinnamaldehyde) caught 6.7 times more beetles in soybean and 3.5 times more in common bean plots. Volatile chemicals used in this study did not attract *C. a. tingomariana.* In a dose-response study, captures of *D. speciosa* increased significantly with increasing doses of 1,4-dimethoxybenzene.

Key Words: *Diabrotica speciosa, Cerotoma arcuata tingomariana, Phaseolus vulgaris, Glycine max,* semiochemical, kairomone

RESUMO

As respostas relativas de *Diabrotica speciosa* (Ger.) e *Cerotoma arcuata tingomariana* Bechyné (Coleoptera: Chrysomelidae) para armadilhas com substâncias químicas foram estudadas. Substâncias voláteis de flores de *Curcubita maxima* Duchesne, outros atraentes voláteis, previamente reportados para diabroticíneos, e misturas de substâncias foram testados em campos de feijão, *Phaseolus vulgaris* L., e soja, *Glycine max* (L.) Mer. Armadilhas com 1,4-dimethoxybenzene capturaram 29,4 vezes mais besouros do que a testemunha com solvente em campos de soja, e 9,4 vezes mais em feijão. Armadilhas com VIP (veratrole + indole + phenylacetaldehyde) capturaram 6,5 vezes mais besouros do que a testemunha com solvente em soja e 3,5 vezes mais em feijão, enquanto que armadilhas com TIC (1,2,4-trimethoxybenzene + indole + trans-cinnamaldehyde) capturaram 6,7 vezes mais besouros em soja e 3,5 vezes mais em feijão. As substâncias não atraíram *C. a. tingomariana.* Em estudo de dose-resposta, as capturas de *D. speciosa* aumentaram significativamente com doses crescentes de 1,4-dimethoxybenzene.

Relationships between Diabroticites and plants of the family Cucurbitaceae are mediated by kairomones. The extremely bitter cucurbitacins are arrestants and feeding stimulants for Diabroticite and Aulacophorite beetles (Luperini tribe) (e.g. Chamblis & Jones 1966, Ferguson et al. 1983, Howe et al. 1976). Adult *Diabrotica* also are attracted to *Cucurbita* spp. blossoms by volatile chemicals. These substances play an important role in cucurbit selection by Diabroticite beetles. Morgan & Crumb (1928) first reported the attraction of Diabroticites to volatile chemicals when they described the attraction of *D. undecimpunctata howardi* (Barber) to cinnamaldeyde and cinnamyl alcohol baits. Snapp & Swingle (1929) attracted the same species with benzyl alcohol. *D. barberi* Smith and Lawrence and *D. cristata* (Harris) were attracted to eugenol, which also attracts the Japanese beetle, *Popillia japonica* Newman (Ladd et al. 1983, Lampman & Metcalf 1988) and to eugenol analogs (Ladd 1984). The isolation and identification of several volatile compounds from *Cucurbita* spp. blossoms (Andersen & Metcalf 1986, Andersen 1987) contributed to the knowledge of *Diabrotica* spp. and related genera in their specific responses to chemicals. A series of *Cucurbita* blossom kairomones and closely related compounds (parakairomones) attract *Diabrotica* spp. and *Acallyma* spp. (e.g. Andersen & Metcalf 1986, Lampman & Metcalf 1987, 1988, Metcalf & Lampman 1989, Lewis et al. 1990, Lance et al. 1992, Deem-Dickson & Metcalf 1995, Petroski & Hammack 1998).

Diabrotica speciosa (Ger.) and *Cerotoma arcuata tingomariana* Bechyné were captured in traps baited with cucurbitacins (Roel & Zatarin 1989, Ventura et al. 1996) upon which these beetles feed compulsively and sequester and store 23,24-dihydrocucurbitacin D (Nishida et al. 1986, Nishida & Fukami 1990). This widespread similarity in behavioral response metabolism of cucurbitacins provides strong evidence for coevolution between these Chrysomelidae and Cucurbitaceae.

Although North American *Diabrotica* responses to volatile substances have been studied since the beginning of this century (Morgan & Crumb 1928), no reports are available on South American pests. We report here the results of field trials testing the relative attraction of *D. speciosa* and *C. a. tingomariana* to volatile kairomones and mixtures from *C. maxima* blossoms and some North American *Diabrotica* spp. parakairomones.

MATERIAL AND METHODS

Field experiments were carried out at the Universidade Estadual de Londrina School Farm, Londrina (latitude 23°19'S, longitude 51°12'W), Paraná State, Brazil. Soybean, *Glycine max* (L.) Mer., cv. Ocepar 14 (sown on December 19, 1996) and common beans, *Phaseolus vulgaris* L., cv. Iapar 59 (sown on February 26, 1997; February 10, 1998) fields (0.5-ha plots) were used as testing sites.

In 1997, beetle traps consisted of transparency film (15.2 × 27.9 cm) (3M do Brasil, Ribeirão Preto, SP, Brazil) painted with yellow gold Suvinil paint 2450-0103 (BASF S.A., São Bernardo do Campo, SP, Brazil) on the interior. Yellow traps previously were successful in capturing *D. speciosa* and *C. a. tingomariana* (Ventura et al. 1996). The film was clamped into a 15.2-cm tall cylinder and externally coated with the clear insect adhesive, Tangle Trap (Tangle Foot Co., Grand Rapids, MI, USA). In 1998, 750-ml plastic cups painted with the same paint replaced the transparency film traps. Dental wicks (40 mm long × 10 mm diameter) soaked with test chemicals were clamped (transparency film) or glued (on the bottom of the cups) to the traps. Solid chemicals were prepared as standard wt/vol. solutions in acetone. The baited traps were placed in the field at 3:00 P.M. and removed after 24 hours.

Traps with 100 μl or 100 mg of each chemical were fixed on a wooden stake above canopy height in soybeans on March 20, 1997 and 0.25 m height in common beans on March 22, 1997. Control traps received only acetone. The chemicals tested included the *C. maxima* blossom volatile substances 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene (nerolidol); benzyl alcohol; 2,3-benzopyrrole (indole); phenylacetaldehyde; 1,2-dimethoxybenzene (veratrole); 1,2,4-trimethoxybenzene; benzaldehyde; 4-[2,6,6-trimethyl-1-cyclohexen-1-yl]-3-buten-2-one (ß-ionone); benzyl acetone; α-ionone (Sigma Chemical Company, St Louis, MO); 1,4-dimethoxybenzene; 4-methoxyphenethyl alcohol; cinnamyl alcohol; trans-cinnamaldehyde (Aldrich Chemical Co., Milwaukee, WI) and the *Diabrotica* spp. parakairomone 2-methoxy-4-(-2-propenyl) phenol (eugenol), and eugenol-related 4-allyl-1,2-methylenedioxybenzene (safrole) (Sigma). SIC (safrole + indole + trans-cinnamaldehyde), TIC (1,2,4-trimethoxybenzene + indole + trans-cinnamaldehyde) and VIP (veratrole + indole + phenylacetaldehyde) mixtures were used at a dosage of 100 mg or 100 µl of each single chemical per trap. Traps were returned to the laboratory where the beetles were identified to species and sexed. Sex ratio of field populations of beetles was also determined from sweep net samples taken when the traps were removed.

The responses of *D. speciosa* and *C. a. tingomariana* to a range of dosages (1, 3, 10, 30, 100 or 300 mg per trap) of the compound most attractive to *D. speciosa* were evaluated on April 20, 1998. A regression analysis was performed to evaluate the relationship between lure concentration and trap effectiveness.

All experiments were conducted in a four replicate randomized complete block design. Distance between traps was 5 m within a block, and 10 m between blocks. Analysis of variance (ANOVA) was performed and Tukey's studentized range test (HSD) was used to compare individual means (SAS Institute 1989) on volatile chemicals screening. Data were transformed by log (x + 1) constant to normalize the data and reduce heterogeneity of variances. Means and standard errors of means are presented for untransformed data.

RESULTS AND DISCUSSION

Only traps baited with 1,4-dimethoxybenzene, VIP and TIC mixtures caught significantly more *D. speciosa* than the controls and this was true in both soybeans and common bean (Tables 1 and 2). 1,4-Dimethoxybenzene was the most attractive compound. Traps baited with the latter compound, VIP and TIC baited traps captured both males and females. The sex ratio of *D. speciosa* beetles determined with sweep net sampling was 1.1 (n = 100) in common beans and 1.0 (n = 100) in soybeans.

The TIC mixture is a strong attractant to North American *Diabrotica* spp. (Lampman & Metcalf 1987, 1988, Lance et al. 1992) and *Acalymma vittatum* (F.) (Lewis et al. 1990). Despite its geographic isolation from the North American inhabitants, *Diabrotica* spp. and *A. vittatum, D. speciosa* was also attracted to the simplified blend of *C. maxima* blossoms (Tables 1 and 2).

The *Diabrotica* genus has been grouped in three taxonomic units; two of which contain pest species (Branson & Krysan 1981). The *fucata* species group in which *D. speciosa* is included is multivoltine, polyphagous and overwinters as adults in regions where frost seldom occurs. The *virgifera* species group is univoltine, oligophagous, has an egg diapause and overwinters in soil at temperatures below zero (Branson & Krysan 1981, Krysan et al. 1989). *D. speciosa* shows responses similar to *D. u. howardi,* a species also belonging to the *fucata* group, in its responses to VIP and 1,4-dimethoxybenzene. *D. u. howardi* was attracted to other benzenoid compounds (Lampman et al. 1987). In contrast, VIP was reported to be largely non-attractive to the *virgifera* group species (Lampman & Metcalf 1987).

D. speciosa was not attracted to single-component lures that are known to attract other species of *Diabrotica* in either the *virgifera* or *fucata* groups [i.e. benzyl acetone, benzaldehyde, cinnamyl alcohol, eugenol, indole, ß-ionone, phenylacetaldehyde, cinnamaldehyde, and veratrole (Andersen & Metcalf 1986, Lampman et al. 1987, Lampman & Metcalf 1987, 1988, Metcalf & Lampman 1989, Lewis et al. 1990)] but

TABLE 1. MEAN NUMBER (±SE) OF ADULTS AND THE SEX RATIO (FEMALES PER MALE) OF *D. SPECIOSA* AND *C. A. TINGOMARIANA* CAUGHT PER YELLOW TRANSPARENCY FILM STICKY TRAPS IN A SOYBEAN CROP AFTER 24 H (MARCH 20, 1997).

	Beetles[1] (Sex ratio)	
Treatment[2]	D. *speciosa*	C. a. *tingomariana*
Benzyl acetone	5.0 ± 1.0bc (1.5)	4.2 ± 2.4ab (1.4)
Benzyl alcohol	8.7 ± 3.5bc (1.3)	6.0 ± 1.7ab (1.0)
Benzaldehyde	8.2 ± 3.2bc (0.9)	7.2 ± 0.9ab (0.9)
Cinnamyl alcohol	6.5 ± 1.1bc (1.0)	5.0 ± 3.3ab (0.7)
1,4-Dimethoxybenzene	108.7 ± 25.6 a (1.2)	5.7 ± 1.2ab (1.1)
Eugenol	4.7 ± 1.1bc (0.9)	5.7 ± 1.5ab (0.9)
Indole	10.2 ± 3.9bc (1.1)	5.5 ± 1.0ab (1.0)
α-ionone	2.5 ± 1.5c (1.0)	4.5 ± 1.0ab (0.8)
ß-ionone	7.5 ± 2.4bc (1.3)	7.7 ± 2.3ab (1.3)
4-Methoxyphenethyl alcohol	4.5 ± 2.2bc (1.2)	3.5 ± 1.0ab (1.8)
Nerolidol	20.7 ± 9.4bc (1.1)	13.2 ± 3.6a (0.9)
Phenylacetaldehyde	11.2 ± 1.1bc (1.2)	3.7 ± 1.3ab (0.7)
Safrole	5.0 ± 1.2bc (1.0)	4.5 ± 1.0ab (0.8)
Trans-cinnamaldehyde	3.7 ± 1.8c (2.0)	6.2 ± 2.4ab (1.5)
1,2,4-Trimethoxybenzene	10.0 ± 4.8bc (1.7)	4.0 ± 1.3ab (1.3)
Veratrole	7.7 ± 1.9bc (1.1)	5.2 ± 2.8ab (0.9)
SIC[3]	12.5 ± 1.4bc (1.5)	2.2 ± 1.3b (1.2)
TIC[4]	24.7 ± 2.2b (1.2)	4.0 ± 1.5ab (1.7)
VIP[5]	24.2 ± 5.4b (1.1)	3.0 ± 1.5ab (1.0)
Control	3.7 ± 0.9c (1.5)	6.2 ± 1.7ab (0.7)

[1] Means in the same column with different letter are significantly different based on Tukey's studentized range test ($P < 0.05$), $n = 4$.

[2] Single and mixed chemicals are dosed at 100 mg or 100 µl of each compound per trap.

[3] Safrole + indole + trans-cinnamaldehyde.

[4] 1,2,4-Trimethoxybenzene + indole + trans-cinnamaldehyde.

[5] Veratrole + indole + trans-cinnamaldehyde.

exhibited its own species-specific pattern of response. 1,4-Dimethoxybenzene is the major floral volatile component in *Curcubita maxima* Duchesne cv. True Hubbard and the 4th major one in cv. Blue Hubbard (Andersen 1987). *C. maxima* blossoms attract adults of *Diabrotica* species (Fischer et al. 1984, Andersen & Metcalf 1987). However, despite the great proportion of 1,4-dimethoxybenzene in the *C. maxima* floral odor, no records of its attractiveness to Luperini beetles have been reported. Metcalf & Metcalf (1992) reviewed the attractiveness of volatile chemicals from blossoms to Diabroticite beetles and attributed a >100-mg threshold of response by *D. barberi, D. cristata, D. u. howardii* and *D. v. virgifera* to 1,4-dimethoxybenzene.

D. speciosa apparently is attracted to methoxylated or methylene-bridged analogs, with or without an allyl or propenyl moiety, possibly with or without a free phenolic group. Further investigations concerning these structure-activity aspects might be achieved.

The response by *D. speciosa* appeared to vary in magnitude (only one comparison) depending on the host (differences in soybeans versus common beans). The common bean is recognized as a very attractive crop to this beetle, mainly early in its phenological cycle (Ventura et al. 1996). Similarly, *D. virgifera virgifera* LeConte was re-

Table 2. Mean number (±SE) of adults and the sex ratio (females per male) of *D. speciosa* and *C. a. tingomariana* caught per yellow transparency film sticky traps in common bean crop after 24 h (March 22, 1997).

	Beetles[1] (Sex ratio)	
Treatment[2]	D. *speciosa*	C. a. *tingomariana*
Benzyl acetone	8.5 ± 3.0c (1.4)	0.7 ± 0.2a(2.0)
Benzyl alcohol	17.2 ± 3.5bc (1.3)	2.0 ± 1.4a(1.0)
Benzaldehyde	10.7 ± 4.2bc (1.1)	1.2 ± 0.2a(1.5)
Cinnamyl alcohol	15.0 ± 7.4bc (1.0)	2.7 ± 0.5a (0.8)
1,4-Dimethoxybenzene	77.0 ± 32.0a (1.0)	1.0 ± 1.0a(3.0)
Eugenol	11.7 ± 2.8bc (0.7)	1.5 ± 0.3a(0.5)
Indole	16.0 ± 4.9bc (1.1)	1.0 ± 0.6a(0.3)
α-ionone	6.7 ± 2.4c (0.8)	0.7 ± 0.2a(0.5)
ß-ionone	8.5 ± 3.3c (1.0)	1.5 ± 0.9a(2.0)
4-Methoxyphenethyl alcohol	16.7 ± 3.9bc (0.8)	3.7 ± 2.1a(0.5)
Nerolidol	18.5 ± 2.8bc (1.0)	4.5 ± 2.9a(1.2)
Phenylacetaldehyde	15.5 ± 5.9bc (0.9)	0.7 ± 0.5a(0.5)
Safrole	9.5 ± 3.3bc (1.0)	4.0 ± 1.3a(1.5)
Trans-cinnamaldehyde	9.0 ± 1.1c (0.9)	2.2 ± 1.3a(1.2)
1,2,4-Trimethoxybenzene	11.2 ± 4.1bc (0.7)	1.5 ± 0.9a(2.0)
Veratrole	14.7 ± 3.6bc (1.0)	3.7 ± 1.9a(1.1)
SIC[3]	17.5 ± 6.8bc (0.7)	0.5 ± 0.3a(0.0)
TIC[4]	28.5 ± 13.5b (0.7)	4.0 ± 2.0a.(06)
VIP[5]	29.0 ± 13.0b (1.3)	3.7 ± 1.9a (0.5)
Control	8.2 ± 1.7c (1.1)	2.7 ± 1.5a (1.2)

[1] Means in the same column with different letter are significantly different based on Tukey's studentized range test ($P < 0.05$), $n = 4$.

[2] Single and mixed chemicals are dosed at 100 mg or 100 μl of each compound per trap.

[3] Safrole + indole + trans-cinnamaldehyde.

[4] 1,2,4-Trimethoxybenzene + indole + trans-cinnamaldehyde.

[5] Veratrole + indole + trans-cinnamaldehyde.

corded as responding differently to volatile attractants according to the host plant phenology (Andersen & Metcalf 1987, Lampman et al. 1987).

D. speciosa is a polyphagous beetle associated with numerous plant species and plant parts, but principally leaves and flowers (Lima 1952, Krysan 1986). Further investigation of the attraction and composition of volatile chemicals in flowers of species more frequented by *D. speciosa,* especially the wild South American Cucurbitaceae, may reveal more chemicals involved in insect-host interactions. The response of this pest to 1,4-dimethoxybenzene indicates that cucurbitacin-baited traps could be improved by adding this volatile chemical. This would be useful for crops in which *D. speciosa* is a rootworm pest, such as corn, *Zea mays* L.*;* wheat, *Triticum aestivum* L.; and potato, *Solanum tuberosum* L.; in which growers are not able to easily and quickly assess economic thresholds.

There were no significant differences between captures of *C. a. tingomariana* in traps baited with single test compounds or mixture of compounds and the controls (Tables 1 and 2). The sex ratio of *C. a. tingomariana* beetle samples with a sweep net was 1.1 (n = 100) when collected in soybean and 1.0 (n = 100) in common bean. Although *C. a. tingomariana* feeding is strongly stimulated by cucurbitacins (Nishida et al. 1986, Nishida &

Fukami 1990, Ventura et al. 1996), no records of this species infesting flowers of cucurbits exist. While the polyphagous *D. speciosa* is a pollen feeder that responds to volatile substances in *Cucurbita* spp., as well as a pest of corn, beans and cucurbits (among other hosts) (Krysan 1986), the oligophagous *C. a. tingomariana* is associated with beans (larvae and adults) and Cucurbitaceae (adults). It has been suggested that Luperine species, including Diabroticite and Aulacophorite, primarily coevolved with Cucurbitaceae, and their preference for other hosts is recent (Metcalf & Lampman 1989). These beetles retain an attraction to volatile substances in *Cucurbita* spp. blossoms and a feeding stimulation by cucurbitacins. It is possible that squash-bean-corn plantings in the pre-Columbian New World influenced host plant range (Metcalf & Lampman 1989). However the non-pollen feeder *C. a. tingomariana* might have a more recent relationship with wild Cucurbitaceae because the lack of response of *C.a. tingomariana* to attractants indicates the association with cucurbitacins is not as strong as with *Diabrotica* species. Feeding by another bean leaf beetle of the *Cerotoma* genus, *C. trifurcata* Forster, is deterred by cucurbitacins (Metcalf et al. 1980). *C. a. tingomariana* must have expanded its host range to tolerate the bitter cucurbitacins. This species sequesters 23,24-dihydrocucurbitacin D, as *D. speciosa* does, after which it gains bitterness in body tissue, and strongly deters feeding by predators (Nishida & Fukami 1990). The lack of attractiveness of volatile compounds to *C. a. tingomarina* is a limitation in the improvement of lures to be used in beans and cucurbits, crops in which *D. speciosa* and *C. a. tingomarina* are simultaneous pests.

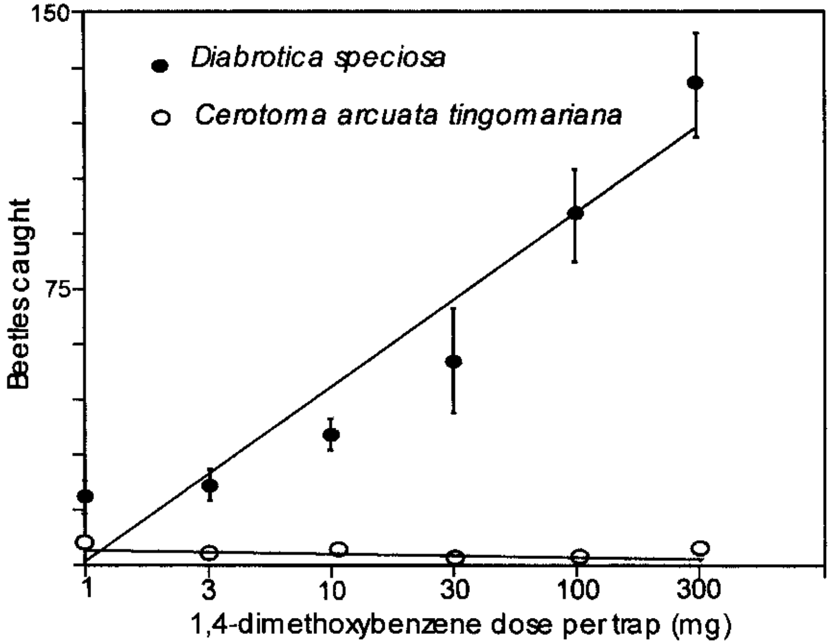

Fig. 1. Relationship between mean number of adults caught ±SE per yellow plastic cup sticky traps (n = 4) of *D. speciosa* and *C. a. tingomariana* after 24 h (on 20 April 1998) and dosage of 1,4-dimethoxybenzene. The linear regression equations were $y = 0.936242 + 47.084 \log x$ ($r^2 = 0.74$, $P < 0.0001$, $n = 6$) for *D. speciosa* and $y = 3.930574 - 0.2467 \log x$ ($r^2 = 0.0136$, $P < 0.5871$, $n = 6$) for *C. a. tingomariana*.

Captures of *D. speciosa* in traps increased significantly with rising doses of 1,4-dimethoxybenzene (Fig. 1) in a dose-dependent manner. The dose-dependent response pattern would be especially advantageous to a mass trapping concept (Hoffmann et al. 1996). *D. speciosa* is a very important pest in many species of vegetables and fruits cultivated in small field areas or in greenhouses in Latin America. In such crops, traps could be used baited with 1,4-dimethoxybenzene to reduce beetle populations.

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