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The chemical ecology of Diabroticites and Cucurbitaceae

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Summary. The coevolutionary association between plants of the family Cucurbitaceae and beetles of the tribe Luperini (Coleoptera: Chrysomelidae; Galerucinae) is mediated to a large extent by chemicals of the host plants. The kairomones involved in host selction by the rootworm beetles are the oxygenated tetracyclic triterpenoid cucurbitacins that act as arrestants and feeding stimulants and a number of volatile cyclic plant blossom components that act as long-range orientation cues. The complex ecological and evolutionary factors regulating the chemical ecology of host-plant selection are discussed.

Key words. Chrysomelidae; Diabroticites; attractants; cucurbitacins; host-plant selection.

Introduction

Utilization of suitable hosts by phytophagous insects is considered to involve a chain of internally programmed behaviors that are initiated, maintained and modified by the interaction of excitatory and inhibitory inputs from the host, internal state of the herbivore, and the environment[13]. Although insect-plant relationships can not be fully understood if one sensory modality is divorced from the others, isolating and mimicking the chemical, tactile, and visual cues is the only practical way of determining their singular and interactive effects on insects. Chemical cues that convey information concerning the location and suitability of a host at a distance, upon contact, and after the onset of feeding are particularly appropriate starting places for investigating the interactions of insects and plants. The chemical ecology of diabroticite beetles and Cucurbitaceae plants is a uniquely attractive area for study because of the large number of plant and insect species involved and the amount of detailed chemical information available.

This review describes 1) the bionomics and evolutionary association of Diabroticites and Cucurbitaceae, 2) the role of cucurbitacins as allomones for Cucurbitaceae, 3) the distribution of cucurbitacins in the Cucurbitaceae, 4) the chemical identity of cucurbitacins, 5) the behavioral response of Diabroticites to cucurbitacins, and 6) the sensitivity of Diabroticites to pure cucurbitacins. The coevolutionary interrelationship between Diabroticites and Cucurbitaceae is further substantiated by the sections describing 7) the attraction of beetles to *Cucurbita* blossoms and 8) the recent isolation, identification, and bioassay of individual blossom odor components.

Apart from the evolutionary, ecological, and ethological insights arising from the study of Diabroticite-Cucurbitaceae interactions, many of the feeding stimulants and attractants isolated from the Cucurbitaceae have been used successfully as lures and baits to monitor and control economically important species of Diabroticites[30, 33, 35].

Bionomics and evolutionary overview of Diabroticites and Cucurbitaceae

The phytophagous Old World Aulacophorites and New World Diabroticites (Chrysomelidae: Galerucinae: Luperini: Aulacophorina and Diabroticina) comprise approximately 480 and 900 species and subspecies, respectively[25, 53, 60]. Maulik[32] cites the remarkable resemblance between major genera of the two groups: "In the old world, *Aulacophora* represents *Diabrotica* ... In larval, pupal, and adult structures, in breeding habits, and in food plants there is a remarkable resemblance between these two genera." The Aulacophorites and Diabroticites include some of the world's most destructive insects, e.g. the plain pumpkin beetle *Aulacophora abdominalis* (Fab.), the red pumpkin beetle *A. foveicollis* (Lucas), the pumpkin beetle *A. hilaris* (Boisduval), the cucurbit leaf beetle *A. femoralis* (Motschulsky), the banded cucumber beetle *Diabrotica balteata* (LaConte), the spotted cucumber beetle *D. undecimpunctata howardi* (Barber), the cucurbit beetle *D. speciosa* (Germar), and the striped cucumber beetles *Acalymma trivittatum* (Mannerheim) and *A. vittatum* (Fab.)[6, 33]. As the common names suggest, these luperine species are closely associated with the host plants of the Cucurbitaceae[33]. Several important economic pests, such as the northern corn rootworm *D. barberi* (Smith and Lawrence) and *D. virgifera virgifera* (Leconte), feed on the roots of Poaceae (grasses) and are primarily pests of corn *Zea mays* L.; however, these species are often collected from the blossoms of wild and domesticated cucurbits[3, 20, 21]. Although host plant records are regrettably sparse, at least 50 species of Aulacophorites and Diabroticites have been collected from Cucurbitaceae and these represent more than 80% of the published host records[33]. A more careful search could greatly expand this number. For example, 12 species of Aulacophorina were collected from wild and cultivated Cucurbitaceae in Taiwan[55] and 16 species of *Diabrotica* from the flowers or foliage of Cucurbitaceae in Peru[24].

The most extensive studies of life history and host range of Diabroticites are derived from the pest species of the largely neotropical genus *Diabrotica* which consists of 338 valid species, approximately one-third of all the Diabroticites[25]. The majority of the species (ca 300) are in the *fucata* species group and primarily occur in Central and South America; the *virgifera* species group (18–35 species) exhibit an opposite hemispheric distribution with more than half of the species found in North America[25, 60]. Members of the *fucata* species group are polyphagous as adults and larvae, feed on the plants of up to eight families, and are typically multivoltine and overwinter as adults in the southern areas of the United States. The *virgifera* species group are usually univoltine and overwinter as eggs in the northern hemisphere. A review of the host affiliations of this subgroup[25] suggests the majority of the species feed as larvae on grasses and as adults are polyphagous pollen feeders.

Apparently the proclivity of *Diabrotica* for grasses (e.g. corn) and other hosts does not affect the adult preference for cucurbits as most species in the tropics from the *virgifera* group are collected from the flowers of cucurbits[24, 25].

The Cucurbitaceae is a moderately large family of plants with 900 species and about 130 genera, many familiar as the cucumbers, gourds, melons, and squash from the genera *Cucurbita, Cucumis, Citrullus, Lagenaria, Marah, Sicyos, Echinocystis, Ecballium,* and *Bryonia.* Cultivated *Cucurbita* species were an important part of the squash-bean-corn cultures in the pre-Columbian New World[59] and the association of these three crops may have influenced the extant host plant relationships of several *Diabrotica* species. The family is phytochemically characterized by the presence of more than 20 extremely bitter and toxic oxygenated tetracyclic triterpenoids, the cucurbitacins (Cucs) (fig. 1).

A remarkable co-evolutionary association between many Luperini beetles and plants of the Cucurbitaceae has been established in the literature[11, 33, 34, 37]. The evolutionary scenario for the behavioral and ecological interactions between Cucurbitaceae and herbivores is portrayed as[34]: 1) ancestral Cucurbitaceae with *Bi* genes for

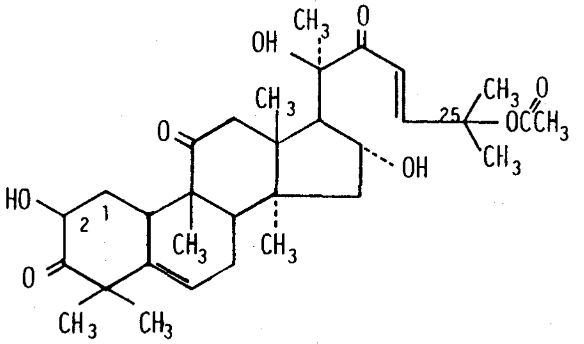

Figure 1. Cucurbitacin B. D is $C_{25}-OH$; E is $C_1=C_2$; F is C_2-OH, C_3-OH, $C_{25}-OH$; G is $C_{24}-OH$, $C_{25}-OH$; I is $C_1=C_2$, $C_{25}-OH$; L is $C_1=C_2$, $C_{23}-C_{24}$; $C_{25}-OH$.

Cucs are heavily preyed upon by herbivores, 2) mutation in Cucurbitaceae to *Bi* forms bitter and toxic Cucs that deter herbivore attack, 3) strong selection pressure spreads *Bi* genes throughout evolving Cucurbitaceae species, 4) mutant Cucurbitaceae flourish in absence of herbivore attacks, 5) mutant ancestral Luperini rootworm beetle develops detoxication and excretion pathways to neutralize harmful effects of Cucs, 6) Luperini beetles expand into new ecological niches developing specific receptors for Cuc detection, 7) Luperini beetles develop high blood and tissue levels of cucurbitacin conjugates for defense against predators, and 8) domestication of species selects for fruits with non-detectable levels of Cucs. Presence of Cucs in the cotyledons[17], the inducibility of Cucs in leaves[56], and the attraction of beetles to cucurbit blossoms maintains the relationship of many Diabroticites with cultivated Cucurbitaceae.

Cucurbitacins as allomones for Cucurbitaceae

There can be no ambiguity about the role of the Cucs as protective semiochemicals for the Cucurbitaceae against herbivore attacks. Cucurbitacins are the bitterest substances known and can be detected by humans at dilutions as great as 1 ppb[37]. Trace amounts produce an almost paralytic response on the lips and mouth and a persistent aftertaste. Moreover, Cucs are extremely toxic to mammals (LD_{50} values for mice intraperitoneally: Cuc A 1.2, Cuc B 1.1 and Cuc C 6.8 mg per kg[12], and orally: Cuc I 5.0 and Cuc E glycoside 40.0 mg per kg[54]). Cattle and sheep that fed on bitter *Cucumis* and *Cucurbita* fruits during drought conditions have been poisoned[58] and outbreaks of human poisoning have resulted from ingestion of the fruits of *Cucurbita* cultivars that have reverted to the heterozygous *Bi* alleles for bitterness[16,48].

Cucurbitacins are feeding deterrents for a number of arthropods including leaf beetles *Phyllotreta* spp., *Phaedon* spp., and *Cerotoma trifurcata*, the stem borer *Margonia hyalinata* and red spider mites[11,37,40]. In an interesting twist of evolution, Cucs and conjugates may be used as defensive compounds by *Diabrotica* and *Acalymma* adults[18]. Chinese praying mantids rejected 72%, 46%, and 24% of the *D. balteata, D. u. howardi,* and *D. v. virgifera,* respectively, that were fed bitter *C. andreana* hybrid fruits as adults, whereas none of the beetles fed a pollen diet were ever rejected[18]. In a later study, four glucoside conjugates of Cucs were isolated from body extracts of *D. u. howardi*[4].

Cucurbitacins in Cucurbitaceae

Cucurbitacins, as the name suggests are peculiarly associated with the Cucurbitaceae where they have been characterized from at least 30 genera and more than 100 species[22,31,38,43,44]. Cucurbitacins are also found in a few genera of the related plant families Begoniaceae, Brassicaceae, and Datisceae[9,42], all of the superorder Violoflorae[57], and in a few species of Euphorbiaceae and Scrophulariaceae[14]. The genus *Momordica* lacks Cucs; however, the major bitter principles in the leaves are the cucurbitane triterpenoids, momordicines which inhibit feeding by *A. foveicollis*[62]. Cucurbitacins are typically found in the roots, stems cotyledons, leaves and fruits of Cucurbitaceae. The concentrations in the roots increase with age and in perennial plants can reach levels of 1% in *Citrullus* and *Acanthosicyos*[43]. In 18 species of *Cucurbita*, Cucs B–D were detected in the roots in 7 and Cucs E–I in 6, at levels up to 0.4% of fresh weight[38]. In the leaves of these species of *Cucurbita* examined, Cucs B–D were found in 7 species up to a maximum of 0.059% in *C. lundelliana* Bailey, and Cucs E–I in 6 species up to 0.1% of fresh weight in *C. okeechobeensis* Bailey[38]. Young rapidly growing leaves of *Citrullus vulgaris* and *C. ecirrhosa* contained only about 0.01% Cucs but the concentration reached 0.1–0.3% by the end of the vegetative season[43]. The fruits of a variety of Cucurbitaceae contain high concentrations of Cucs, > 1.0% in the fruits of *Citrullus colocynthis* and *C. ecirrhosa, Cucumis angolensis, C. longipes, C. myriocarpus,* and *C. sativus*[44]. From 18 species of *Cucurbita* examined, the fruits of 7 contained Cucs B–D up to 0.31% in *C. andreana* Naud., and the fruits of 5 contained Cucs E–I up to 0.23% in *C. foetidissima* HBK[38]. The concentration of Cucs in cotyledons was 0.0023% Cuc B in *C. pepo* cv. 'Black Zucchini', 0.0011% of Cuc B in *C. pepo* cv. 'Blackjack', and 0.0039% of Cuc E in *Citrullus lanatus* cv. 'Iopride'[17]. Of 19 species of wild *Cucurbita, Cucumis,* and Citrullus and 46 commercial cultivars, only 18 lacked detectable levels of Cucs in the cotyledons; *Cucurbita gracilior* was the only wild species that lacked Cucs in the cotyledons[17].

Chemical identity of cucurbitacins

At least 20 chemically different Cucs have been characterized from plants[22,31]. Cucurbitacin B is the predominant form found in about 91% of all species characterized followed by Cuc D (69%), Cucs G and H (47%), Cuc E (42%), Cuc I (22%), Cucs J and H (9%) and Cuc A (7%). Cucs C, F, and L were found only in a single species[43, 44]. Cucurbitacin B and Cuc E appear to be the primary Cucs and the other Cucs are formed by enzymatic processes occurring during plant development and maturation[31, 33, 34, 43]. Curcurbitacin B can be metabolized to Cucs A, C, D, F, G, and H and is characteristic of *Coccinia, Cucumis, Lagenaria,* and *Trichomeria*[44]. Similarly Cuc E can be metabolized to Cucs I, J, K, and L and is characteristic of *Citrullus*[43]. In *Cucurbita* there are two groups of species characterized by either Cuc B or Cuc E[38]. The Cucs of *Cucumis, Lagenaria,* and *Acanthosicyos* are present as free aglycones; however, in most species of *Citrullus, Echinocystis, Coccinea,* and *Peponium* the Cucs are present as glycosides[44]. In *Cucurbita* Cucs are present as aglycones in most species but glycosides are found in *C. cylindrata* Wats, *C. foetidissima, C. palmata* Wats, and *C. texana* Gray[38]. The presence of glycosides is related to the absence of B-glucosidase (elaterase) which may also be sequestered in intact plant tissues and released by crushing[15].

At least 5 independent genes are known to regulate the biosynthesis of Cucs[49]: a) a gene *Bi* that regulates synthesis in seedlings, b) a gene su^{Bi} that suppresses synthesis in fruits, c) a gene that controls quantity, d) a gene that determines the chemical nature of the Cuc formed, and e) a gene Mo^{Bi} that determines whether the Cuc exists as a free aglycone or as a glycoside. Bitterness in the Cucurbitaceae was originally thought to be regulated by a single dominant gene *Bi*[10, 45] and plants with non-bitter seedlings *Bi* did not synthesize Cucs. However, later evidence indicated that non-bitter fruits could develop from bitter seedlings[45] suggesting that a single recessive suppression gene su^{Bi} prevented bitterness in fruits, which could be expressed by the dominant allele Su^{Bi}[7].

Organospecific genes appear to control the qualitative and quantitative formation of Cucs in leaves, fruits, blossoms, and roots[43]. A single modifier gene Mo^{Bi} that acts only in the presence of *Bi* and Su^{Bi} alleles apparently controls the quantity of Cuc E-glycoside (elaterinide) formation in bitter fruit[15]. Mechanical damage to leaves of *C. moschata* and *C. pepo* induced within 40 min and 3 h, respectively, an increase in concentration of cucurbitacins that enhanced feeding by diabroticite beetles but inhibited feeding by two *Epilachna* spp.[56]. In *C. pepo* cv. 'Black', the levels of Cuc B doubled and Cuc D increased ca 8-fold in response to damage.

Diabroticite behavioral response to cucurbitacins

Cucurbitacins extracted from *Cucurbita* spp. by chloroform can be separated by thin-layer chromatography (TLC) on silica gel using mixtures of solvents such as chloroform:methanol 95:5[37, 38]. TLC plates so developed represent the spectrum of Cucs present in the plant and the Cucs attractive to the various species of rootworm beetles can be identified by allowing beetles to eat the silica gel where Cucs are present on the developed chromatograms (fig. 2)[17, 37, 38]. This 'beetle print' bioassay is sensitive to nanogram quantities of Cuc B using *D. u. howardi* (table 1).

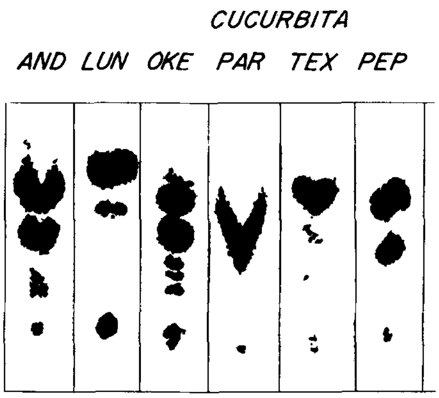

Figure 2. Beetle prints from feeding of *Diabrotica u. howardi* on thin-layer chromatograms of *Cucurbita* cotyledon extracts. Dark areas (eaten) indicate presence of various cucurbitacins. AND = *andreana*, LUN = *lundelliana*, OKE = okeechobeensis, PAR = *palmeri*, PEP = *pepo*, and TEX = *texana*[33].

Table 1. Limit of response (LR) of Diabroticites to pure cucurbitacins[33]

Diabrotica species	Cuc LR (μg)							
	B	D	E	F	G	I	L	E-gly
D. balteata	0.01			10	3	5		0.1
D. barberi	0.1		0.3					5
D. cristata	0.1	1.0	0.3	10	3	1.0	1.0	50
D. u. howardi	0.001	0.03	0.01	1.0	3	0.1	0.01	0.05
D. u. undecimpunctata	0.003		0.03					
D. v. virgifera	0.01	0.1	0.3	0.1	3	0.3	1.0	0.03
Acalymma vittatum	0.3		10					50

Beetle feeding has been used to characterize the spectrum of Cucs present in the roots, leaves, and fruits of 18 species of *Cucurbita*[37, 38]. The nature of the Cucs present and the beetle feeding responses agreed reasonably well with evolutionary groupings based on numerical taxonomy[46] and cross compatibilities[5]. The *Cucurbita* spp. were classified into Group (1) Cucs B–D forming species found in subgroup (a) *C. andreana* and *C. ecuadorensis* and subgroup (b) *C. gracilior, C. palmeri,* and *C. sororia.* Group (2) included Cucs E–I forming species found in subgroup (a) *C. martinezii* Bailey, *C. okeechobeensis,* and (b) *C. cylindrata* and *C. palmata.* The five domesticated *Cucurbita* cultivars *C. ficifolia* Bouche, *C. maxima* Duchesne, *C. mixta* Pangalo, *C. moschata,* and *C. pepo* showed no discernable beetle feeding. Although the Cucs were at undetectable levels in the roots, leaves, and fruits of the domesticated species of *Cucurbita,* damage by *D. u. howardi* and *Acalymma vittatum* to the cotyledons of *C. pepo* cultivars yielded a highly significant correlation ($p = 0.001$) between beetle feeding and Cuc content ($r = 0.78$, $n = 12$, $SD = 0.20$)[17, 38].

The beetle bioassay was used with 5 species of diabroticite beetles to demonstrate that there were no qualitative differences between their feeding patterns on a spectrum of Cucs including B, C, D, E, I, and E-glycoside. Almost identical 'beetle prints' were obtained with *A. vittatum* which is oligophagous on Cucurbitaceae and Fabaceae, *D. u. howardi* which is polyphagous on Cucurbitaceae, Fabaceae, Convolvulaceae, and Poaceae, and the corn rootworms *D. barberi* Smith and Lawrence and *D. v. virgifera* LeConte which are specialists on Poaceae (fig. 2). *D. cristata* Harris whose larvae feed only on the roots of native prairie grasses, especially big bluestem *Andropogon gerardi* Vitman[61] also fed avidly on the cucurbitacin-containing TLC plates, producing very similar 'beetle prints'[33, 34, 37, 38]. *Diabrotica speciosa* and *Cerotoma arcuata* Oliv. were observed feeding voraciously on the tubers of *Ceratosanthes hilariana,* a wild cucurbitaceous plant containing 0.08% fresh weight of Cucs B, E, and 23, 24-dihydrocucurbitacin B[41]. In contrast, *Cerotoma trifurcata,* the bean leaf beetle, is repelled by Cuc B[37]. *Aulacophora foveicollis* has also been shown to feed on crystalline Cuc E on filter paper[52]. Based upon the presence of a cucurbitacin response in diabroticite, aulacophorite and other Luperini species, it has been suggested that the entire group of rootworm beetles originally coevolved with the Cucurbitaceae and that preferences for other hosts, such as grasses, are relatively recent[33, 34].

Limits of response to pure cucurbitacins

The ultimate sensitivity of the various diabroticite species to pure crystalline cucurbitacins was determined by progressively decreasing the amounts of the various Cucs present on silica gel TLC plates to find the limit of response (LR in µg) or the least amount that produced a detectable feeding response in the beetle bioassay. LR values for 7 diabroticite species to 8 cucurbitacins are presented in table 1[33,34]. The LR values measure the relative degree of sensitivity of the receptors on the maxillary palpi of the beetles to the various cucurbitacins[34,37]. The information in table 1 shows the substantial differences in the LR values for the various diabroticite beetles to individual Cucs. These values have both evolutionary and behavioral significance. *D. u. undecimpunctata* and *D. u. howardi* consistently detected Cuc B at 0.001–0.003 µg and Cuc E at 0.01–0.03 µg on the silica gel plates while the LR values for the other species were somewhat higher[33, 34].

The conclusions that can be drawn from these data are: 1) Cuc B was consistently detected in the lowest amount, and is probably the parent Cuc to which Diabroticites' receptors are attuned, 2) Cuc B was consistently detected at levels of about 0.1 that Cuc E, 3) the acetoxy Cucs B and E were detected at levels about 0.1 those of the corresponding desacetoxy Cucs D and I respectively, 4) saturation of the desacetoxy Cucs at $C_{23}=C_{24}$ double bond (Cuc L) had little effect on level of detection, 5) sensitivity to the 2-OH, 3-C=O Cuc D was greater than to the 2-OH, 3-OH Cuc F, 6) the *fucata* subgroup (*D. balteata, D. u. undecimpunctata,* and *D. u. howardi*) is more sensitive to Cucs than the *virgifera* subgroup (*D. barberi, D. cristata,* and *D. v. virgifera*) and *A. vittatum* is substantially less sensitive to Cucs than *Diabrotica* spp.

Cucurbita blossom volatiles as diabroticite attractants

A recurrent theme in the host records of Diabroticites is the frequency of collection from blossoms of Cucurbitaceae species and their definite preference when given the choice between blossoms of various *Cucurbita* spp.[3, 20, 21]. Historically, *D. longicornis* and *D. v. virgifera* were first described from specimens collected from blossoms of the buffalo gourd, *Cucurbita foetidissima,* by the Long Expedition in 1824 and the Powell Expedition in 1868, respectively[53]. Various Diabroticites exhibit a distinct preference for the male blossoms of cultivated Cucurbitaceae. *D. barberi, D. u. howardi* and *A. vittatum* were found in greater abundance in the flowers of *C. maxima* Duchesne than in those of *C. pepo* L.[21]. In an interplanting of four *Cucurbita* spp., the order of preference by *D. barberi* and *D. v. virgifera* was *C. maxima* > *C. pepo* > *C. mixta* > *C. moschata*[20]. The relative abundance varied considerably between the two *Diabrotica* species; *D. barberi* were found almost exclusively in the blossoms of *C. maxima* whereas large numbers of *D. v. virgifera* were present in both *C. maxima* and *C. pepo* flowers. When cultivars of three *Cucurbita* species were randomly interplanted, *D. u. howardi* and *D. v. virgifera* also showed a preference for *C. maxima* over *C. moschata* and *C. pepo*[3].

The influence of various blossom characteristics (color, size and shape of the flower, volatile release, pollen content, and contact and gustatory stimulants) on beetle distribution is unclear from preference tests, although it was suggested that cucurbitacin content of blossoms partially explained beetle presence and feeding on floral tissue[3]. Headspace volatiles of *C. maxima* blossoms were fractionated by HPLC and a single component, indole, elicited a high level of electroantennographic response by *D. u. howardi*[2]. Field bioassays indicated low concentrations of indole attracted both *D. v. virgifera* and *A. vittatum,* but not *D. u. howardi.* In a systematic evaluation of the olfactory and gustatory cues inherent to cucurbit blossoms[3], preference for *C. maxima* cultivars generally corresponded to higher release rates of volatiles such as indole, cinnamaldehyde, cinnamyl alcohol, and β-ionone; and with the presence of Cucs. It was concluded that the high release rates of volatiles affected the rate of arrival of *Diabrotica* spp. and *A. vittatum* to blossoms and the presence of the non-volatile Cucs decreased the departure rate, thereby resulting in large aggregations of beetles in blossoms[3]. A subsequent study showed sticky traps baited with whole blossoms from *C. maxima,* cv. 'Blue Hubbard', caught a significant number of female *D. v. virgifera* and both sexes of *D. barberi*[39]. Beetle distribution in *C. maxima* and *C. moschata* blossoms was also mimicked by placing 30 g of shredded blossom material inside a paper carton coated externally with insect adhesive and the top covered with cheesecloth. After a 60-min period in a cucurbit plot, the average number of *D. v. virgifera* adults caught on four blossom-baited traps was 86.0 ± 30.6 for *C. maxima* cv. 'Blue Hubbard' blossoms, 11.3 ± 6.2 for *C. moschata* cv. 'Dickinson Field' blossoms, and 6.8 ± 7.5 for unbaited controls (unpublished data).

Isolation, identification, and bioassay of Cucurbita blossom volatiles

After the demonstration that olfaction played a dominant role in the location of *Cucurbita* blossoms by Diabroticites, a detailed analysis of *C. maxima* blossom constituents was initiated. Headspace volatiles were trapped by adsorption on a porous polymer, and the essential oil isolated by vacuum steam distillation[1,3]. More than 13 components were isolated from the headspace sample for Blue Hubbard and True Hubbard cultivars; benzyl alcohol, 1,4-dimethoxybenzene, 1,2,4-trimethoxybenzene, and a $C_{15}H_{24}$ hydrocarbon (probably cadinene or muurolene) comprised over 60% of the Blue Hubbard volatiles and over 90% of the True Hubbard. In a continuation of these studies, 30 volatile components were isolated from the steam distillate of these two *C. maxima* cultivars[1] and qualitatively, there was a preponderance of oxygenated cyclic compounds (table 2). The majority of the identified blossom components have been evaluated as attractants for several species of diabroticite beetles using sticky, cylindrical paper cartons[2,28-30] (and unpublished data). The phenylpropanoids indole, cinnamyl alcohol, and cinnamaldehyde and the cyclic terpenoid β-ionone have limits of response equal to or less than 1–3 mg for at least one of the three species of *Diabrotica* (table 2). Slopes from log dosage-response curves (the increase in trap catch with a logarithmic increase in dose) varied between compounds and Julian date (unpublished data).

Table 2. Volatiles from *Cucurbita maxima* blossoms as attractants for *Diabrotica* beetles

Volatile	Respose in field trapping[a] *D. barberi*	*D. u. howardi*	*D. v. virgifera*
1,4-dimethoxybenzene	0	0	0
1,2,4-trimethoxybenzene	0	+1	+1
benzyl alcohol	0	+1	0
benzaldehyde	0	0	0
phenylethanol	+1	+1	0
phenylacetaldehyde	0	+2	0
p-methoxybenzylalcohol	0	+1	0
p-methoxybenzaldehyde	0	+1	0
indole	0	+1	+4
cinnamyl alcohol	+4	+2	+1
cinnamaldehyde	+1	+4	+3
α-ionone	0	0	0
β-ionone	0	0	+4
nerolidol	0	0	0

[a] limit of response >200 mg (0), 30–100 mg (+1), 10–30 mg (+2), 3–10 mg (+3), 1–3 mg (+4)

All of the *Diabrotica* spp. examined to date are attracted to chemically related cyclic compounds; however, each species displays a distinctive pattern of response when exposed to a broad spectrum of candidate lures. *Diabrotica* species exhibit a cross response to some compounds (e.g. cinnamaldehyde attracts *D. u. howardi* and *D. v. virgifera*) and a species-specific response to others (e.g. β-ionone attracts only *D. v. virgifera*). *D. cristata* is attracted primarily to eugenol and cinnamyl alcohol which are attractants for *D. barberi*[29]. The influence of feeding behavior on the threshold of response by diabroticite beetles to attractants is suggested by the reduced response to volatile attractants when fresh silks and pollen are available in corn fields. Although indole was unequivocally shown as an attractant of *D. v. virgifera*, it significantly attracted beetles only in July, August and September[2,30]. The reduced trap catch in June corresponded to beetle feeding on tassels and silks of the corn. A similar corn phenology-beetle feeding influence on trap catch was noted for *D. u. howardi* and cinnamaldehyde; *D. v. virgifera* and estragole and 4-methoxycinnamaldehyde; and *D. barberi* and eugenol and cinnamyl alcohol[29], [30] (and unpublished data).

Combinations of components often exhibit synergistic effects on beetle response[28] (and unpublished data). The three component mixture of veratrole, indole, and phenylacetaldehyde caught 8 times as many *D. u. howardi* as the expected mean additive response with the individual components. A mixture of 1,2,4- trimethoxybenzene, indole and *trans*-cinnamaldehyde (TIC mixture), all blossom constituents (table 2), was particularly active for *D. u. howardi* and *D. v. virgifera* over a dosage range of 1 to 30 mg per trap. At 1 mg per trap, a mean of 15.2 ± 11.9 *D. u. howardi* and 15.5 ± 2.5 *D. v. virgifera* were caught and at 30 mg per trap 76.0 ± 21.4 and 77.2 ± 21.3 beetles per trap, respectively[28]. Subsequent tests with individual components, two component mixtures, and the three component TIC mixture indicated the two component mixtures were additive in their affect on beetle catch, but the complete mixture resulted in more than additive numbers of *D. v. virgifera* and *A. vittatum* (unpublished data).

The isolation of diabroticite attractants from *Cucurbita* spp. and structure-activity studies have produced an array of more than 20 single-component volatile lures. All of the species investigated, including *D. barberi, D. cristata, D. u. howardi, D. v. virgifera,* and *A. vittatum,* clearly respond to a simplified blossom mixture consisting of equal parts of trimethoxybenzene, indole, and cinnamaldehyde and exhibit a synergistic response to the complete mixture[28,29] (and unpublished). Response to indole is greatest in *D. v. virgifera* and *A. vittatum,* but is also occasionally evident in *D. u. howardi*[2,30]. Both *D. v. virgifera* and *D. u. howardi* are attracted to cinnamaldehyde, although the response by the latter species is greater[36]. *D. barberi* and *D. cristata,* two species often found together feeding on the pollen of prairie composites, respond strongly to cinnamyl alcohol[29]. Specificity of response is further demonstrated by the attraction of only one species (*D. v. virgifera*) to β-ionone[36]. Recent structure-activity studies have shown that volatiles not isolated from cucurbit blossoms are also attractive to various *Diabrotica* species; for example, the attraction of *D. barberi* to eugenol, isoeugenol and phenylpropanol; *D. v. virgifera* to *para*-methoxycinnamaldehyde and *para*-methoxycinnamonitrile; and *D. u. howardi* to cinnamonitrile[36] (unpublished).

Summary and conclusions

Phytochemicals unquestionably play a dominant role in the finding and acceptance of cucurbitaceous host plants by beetles in the tribe Luperini. The combined effects of the volatile attractants (phenylpropanoids and monoterpenes) and compulsive feeding stimulants (cucurbitacins) is to accelerate the arrival rate of the beetles to the plant and to delay their departure rate thus producing large aggregations of the beetles on blossoms and other plant parts. The antiquity of this ecological association is strongly supported by the following: 1) the affiliation of Old World Aulacophorites and New World Diabroticites with blossoms, foliage, fruit, or roots of wild and cultivated species of Cucurbitaceae, 2) the distinctive and widely distributed compulsive feeding response by *Diabrotica, Aulacophora, Acalymma,* and at least one species of *Cerotoma* to pure cucurbitacins; 3) the high sensitivity of *Diabrotica* and *Acalymma* species to the ancestral and most common cucurbitacin, Cuc B; and 4) the specific attraction of *Acalymma vittatum* and of *Diabrotica* spp. from two ecologically distinct subgroups (*virgifera* and *fucata*) to floral odors of *C. maxima* cultivars and synthetic cucurbit blossom mimics. Evidently, as the host range of this large taxon of herbivorous beetles expanded and diversified and life histories changed in response to environmental and ecological conditions the group retained a response to cucurbitacins and phenylpropanoid attractants. In the genus *Diabrotica,* the grass specialist species, such as *D. v. virgifera* and *D. barberi,* are less sensitive to Cucs than the generalist feeders like *D. u. howardi* and *D. balteata.*

Based upon admittedly a limited number of diabroticite species, the beetle responses to *Cucurbita* blossoms volatiles appear to have strong ecological and evolutionary significance. The sensitive and specific responses by Diabroticites from two distinct taxonomic groups (*fucata* and *virgifera*) to volatiles produced in *Cucurbita* blossoms is viewed as a complementary coevolutionary association to that resulting from the Cucs arrestants. The multiple species response of *Diabrotica* spp. to simplified blossom component mixtures (such as TIC) coupled with the species-specific pattern of response to individual components must be related to evolutionary shifts in host selection. *D. u. howardi* responds primarily to phenylpropanoids with unsubstituted aryl rings, e.g. cinnamaldehyde, while *D. v. virgifera* responds characteristically to those with *para*-methoxy substituents, e.g. 4-methoxycinnamaldehyde[36]. Both unsubstituted and *para*-methoxy substituted aromatics abound in squash blossom volatiles[1,23]. *D. barberi* responds primarily to unsaturated and saturated phenylalkanols, such as cinnamyl alcohol and 3-phenylpropanol (unpublished data).

The attraction of Diabroticites to cucurbit blossoms and individual volatile kairomones may have arisen as a means of finding ephemeral sources of pollen. This association may have been mutually profitable between the primitive Cucurbitaceae and Diabroticites for pollination. Olfactory cues were selected from the distinctly 'floral' component of the blossom odor, e.g. indole, cinnamaldehyde, cinnamyl alcohol, and β-ionone. As evolution continued, Diabroticites expanded into other host niches such as the Poaceae and Fabaceae, but they retained olfactory organs responding to a wide range of phenylpropanoids and terpenoids. Whereas species of the *virgifera* and *fucata* subgroups of *Diabrotica* differ in host preference and voltinism, the retention of a response to 'floral' volatiles may have been advantageous for finding a variety of food sources by these polyphagous pollen feeders.

Additional electrophysiological and behavioral studies with contact and olfactory kairomones of Diabroticites may help elucidate the evolution of chemoreception in this group. By examining the chemical ecology of Diabroticites, we may acquire a better understanding of insect-plant interactions and the use of semiochemicals in pest management.

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