COMPARISON OF BEETLE DIVERSITY AND INCIDENCE OF PARASITISM IN DIABROTICINA (COLEOPTERA: CHRYSOMELIDAE) SPECIES COLLECTED ON CUCURBITS

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Abstract

Diabroticina (Chrysomelidae: Galerucinae: Luperini) beetles were sampled under field conditions on two host plants of the family Cucurbitaceae, *Cucurbita okeechobeensis* ssp. *martinezii* L. Bailey (bitter, wild cucurbit) and *C. moschata* (Lam.) Poiret (non bitter, cultivated cucurbit). Seventeen species of Diabroticina were collected. *Acalymma blomorum* Munroe & Smith was the most abundant species on both host plants. The only parasitoid found was *Celatoria compressa* Wulp (Diptera: Tachinidae). This parasitoid attacked more beetle species on the cultivated cucurbit (65%) than on the bitter cucurbit (20%). However, the percentages of parasitism observed in all species were low (0.4% to 12.5%). These data suggest that host plant species might have an effect on parasitism.

Key Words: *Acalymma*, *Celatoria compressa*, *Cucurbita okeechobeensis* ssp. *martinezii*, *Cucurbita moschata*, host plant association, Diabroticina beetles

Resumen

Diecisiete especies de Diabroticina (Chrysomelidae: Galerucinae: Luperini) fueron colectadas en dos plantas hospederas de la familia Cucurbitaceae, *Cucurbita okeechobeensis* ssp. *martinezii* L. Bailey (amarga, silvestre) y *C. moschata* (Lam.) Poiret (no amarga, cultivada). *Acalymma blomorum* Munroe & Smith fue la especie más abundante en ambas hospederas. Se obtuvo únicamente el parasitoide *Celatoria compressa* Wulp (Diptera: Tachinidae) sobre las poblaciones de Diabroticina. Este parasitoide atacó mayor número de especies de escarabajos en la calabaza cultivada (65%) que en la calabaza amarga (20%). Los porcentajes de parasitismo observados se consideraron bajos (0.4% a 12.5%). Los datos sugieren que las plantas hospederas pudieron haber tenido un efecto sobre el parasitoide.

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Diabroticina (Chrysomelidae: Galerucinae: Luperini) are native to Mexico and Central America (Webster 1895). Few data, however, are published on host plant associations (Eben & Espinosa de los Monteros 2003) and natural enemies from this area (Eben & Barbercheck 1996).

In the Diabroticites (subtribe Diabroticina), the association of a number of species with plants in the family Cucurbitaceae is a well-known example for the effect of plant secondary chemistry on a plant-insect interaction (Chambliss & Jones 1966; Howe et al. 1976; Metcalf et al. 1982; Metcalf 1986). Host preferences of Diabroticites are strongly influenced by the presence of cucurbitacins (tetracyclic triterpenoids) in many wild cucurbit hosts. These non-volatile secondary compounds act as arrestants and feeding stimulants for these beetles (Chambliss & Jones 1966; Metcalf & Lampman 1989).

Furthermore, it has been proposed that Diabroticina species sequester cucurbitacins for their chemical defense. Studies of tritrophic effects demonstrated that cucurbitacins are deterrents for natural enemies such as mantids (Ferguson & Metcalf 1985), passerine birds (Nishida & Fukami 1990), the pathogenic fungus *Metarhizium anisopliae* (Moniliales: Moniliaceae) (Tallamy et al. 1998), and entomopathogenic nematodes (Barbercheck et al. 1995). On the other hand, no negative effects on general predators such as carabid larvae, mites, and centipedes (Brust & Barbercheck 1992) have been found. To date, no clear pattern has emerged from these studies. Field studies on larval host associations in the natural habitat are difficult due to the fact that Diabroticina larvae are root feeders.

Interestingly, although parasitoids are intimately associated with their hosts, and third trophic level effects of plant secondary compounds are described for a number of plant-insect associations (Gauld et al. 1992; Rowell-Rahier et al. 1995; Agrawal et al. 2002), no data exist for the cucurbit/Diabroticina/parasitoid system.

The objective of the present field study was to compare beetle abundance and diversity on two *Cucurbita* spp. that differed in presence, *Cucurbita okeechobeensis* ssp. *martinezii* L. Bailey, or absence, *C. moschata* (Lam.) Poiret, of secondary

compounds. Both cucurbits, the bitter *Cucurbita o. martinezii* and the cultivated *C. moschata*, are the most common cucurbits in the study area. Their morphology is similar, but the bitter species has smaller and paler flowers, and smaller leaves. Furthermore, the bitter species produces secondary compounds characteristic for Cucurbitaceae, the cucurbitacins (Metcalf et al. 1982; R. Ventura, unpubl. data). Moreover, parasitoid incidence in adult beetles was monitored and compared between individuals collected from the two host plants.

MATERIAL AND METHODS

Study Area and Host Plants

All adult insects were collected in the central zone of the state of Veracruz, Mexico. Mean annual temperature fluctuates between 18 and 25°C, with three distinct seasons: a dry-cool season (November-March), a dry-warm season (April-May) and a wet-warm season (June-October). Annual rainfall varies between 800 and 2500 mm, with a peak in the second warm season (Soto & García 1989). Common crops in the area are sugarcane, coffee, corn, squash, and beans. The original vegetation at lower altitudes is deciduous forest, whereas remnants of tropical cloud forest are found at higher altitudes (Gómez-Pompa 1977).

Within the study area, six locations for each *Cucurbita* spp. were identified. These locations were separated by at least nine km (i.e., 12 sample areas in total). They differed in altitude and climatic conditions (Table 1). To avoid collections of beetles which might recently have moved between hosts, sites with coexistence of both cucurbits were not accepted. Due to the fact that beetle abundance is affected by the presence of flowers (pers. observ.), insects were collected once or twice per week on flowering plants only. When plants began to dry out, they were replaced by others in flowering stage within the same area. At each collection date we recorded the diversity and abundance of beetles found on both plants.

Areas of approximately 100 m² covered by cucurbit vines were measured at each location to define the collection site.

Beetle Collection

Beetles were collected from August to December 2001 and from May to November 2002. Collection dates were based on previous studies (Rodriguez & Magallanes 1994; Eben & Barbercheck 1996; Cabrera & Cabrera 2004) which found a clear seasonality for Diabroticinas with peak abundance from early summer to fall. Plants were visually inspected for Diabroticina adults. The sampling unit was the number of beetles collected per person in one hour. Field collected adults were separated by species, location, and collection date. In order to allow for parasitoid emergence, the colonies of adult field collected beetles were maintained in the laboratory (25 ± 3°C), with a photoperiod of 13:11 (L:D), in transparent plastic containers (15 cm diameter × 25 cm length), with a gaze cover for ventilation. Beetles were fed fruits of *Cucurbita pepo* L. (zucchini) and artificial diet (Branson et al. 1975). Abundance of Diabroticina were analyzed by one-way ANOVA ($P < 0.05$) with SigmaStat™ statistical software version 2.0 (Jandel Scientific 1992-1997), after square root transformation.

Parasitism Rates

All cages with beetles were checked daily to collect and count parasitoid pupae (Eben & Barbercheck 1996). In addition, dead beetles with an entire abdomen were dissected to determine presence or absence of immature parasitoids. Percentage parasitism was calculated as the number of immature and adult parasitoids obtained for the total number of each beetle species, date, and location. Data were analyzed by a chi-square test (Zar 1999). Correlation between beetle abundance and percentage parasitism was analyzed by linear regression (Zar 1999).

RESULTS

In the study area, *C. moschata* is cultivated for human consumption. For this reason it was commonly found along road sides and in mixed corn-squash plots, mostly in direct sunlight. *Cucurbita o. martinezii* is grown in shadier places, with oth-

TABLE 1. CLIMATIC ZONES OF THE SIX COLLECTIONS AREAS IN THE STATE OF VERACRUZ.

Area	Altitude (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Geographical location
Coatepec	1200	19.2	1926.0	19°27'N/96°58'W
Jalcomulco	340	24.0	1125.0	19°20'N/96°46'W
Naolinco	1540	16.0	1639.7	19°39'N/96°52'W
Teocelo	1160	18.1	1797.0	19°39'N/96°58'W
Tlalnelhuayocan	1640	18.0	1009.0	19°39'N/96°58'W
Xalapa	1460	18.0	1509.1	19°32'N/96°55'W

ers plants as climbing structures. It was most abundant in and around coffee plantations.

We found 17 species of Diabroticinas from five genera (Table 2). All species were collected from *C. moschata*, 15 species were collected from *C. o. martinezii*. The abundance of three species, *A. blomorum*, *D. balteata*, and *I. tetraspilota*, differed between both cucurbit hosts ($P = 3.5 \times 10^9$, $P = 0.0011$, and $P = 0.048$, respectively). The other 12 beetle species were not more abundant in any of the two host plants. Proportions of all species were different in the two cucurbits. In *C. moschata*, *Acalymma blomorum* was the most abundant beetle species, followed by *Diabrotica balteata*, *D. scutellata*, and *D. viridula*, and in *C. o. martinezii*, *Isotes tetraspilota*, *A. fairmairei*, and *D. scutellata*.

The most diverse genus was *Diabrotica* with six species in the *fucata* group, *D. balteata*, *D. dissimilis*, *D. nummularis*, *D. sexmaculata*, *D. tibialis*, *D. undecimpunctata duodecimnotata*, and three species in the *virgifera* group: *D. porracea*, *D. scutellata*, and *D. viridula*. *Diabrotica balteata* and *D. scutellata* were the most abundant species within either group. *Cerotoma atrofasciata*, *Gynandrobrotica lepida* and *G. nigrofasciata* were most common on the foliage of *C. moschata* (Table 2).

Incidence of Parasitism

The only parasitoid found was a tachinid species, *Celatoria compressa* Wulp. Parasitoids were obtained in June and July 2002 from beetles collected on the bitter cucurbit. No parasitoids were found in beetles collected on this plant in 2001. In beetles collected on the cultivated cucurbit, parasitoids were present throughout the collecting period in both years. In general, parasitoid pupae were obtained during the first 48 h after collecting the host beetle. Adult parasitoids emerged from all parasitoid pupae ($n = 169$). The presence of other parasitoids was not observed.

The tachinid parasitoid was found in three of the 15 beetle species collected on the bitter cucurbit (20%), and in 11 of the 17 beetle species collected on the cultivated cucurbit (65%). On the bitter cucurbit, the parasitoid attacked *A. blomorum*, *A. fairmairei*, and *D. balteata* at percentages of 0.9%, 7.7%, and 5%, respectively (Table 2). In the species collected on the cultivated cucurbit, *A. blomorum*, *A. fairmairei*, *A. innubum*, *C. atrofasciata*, *D. balteata*, *D. porracea*, *D. scutellata*, *D. sexmaculata*, *D. tibialis*, *D. viridula*, and *G. nigrofasciata* were parasitized. The percentage of

TABLE 2. DIABROTICINA BEETLE ABUNDANCE COLLECTED ON *CUCURBITA OKEECHOBEENSIS* SSP. *MARTINEZII* (A) AND *C. MOSCHATA*; (B) (MEAN BEETLES COLLECTED PER PERSON PER HOUR), AND PERCENTAGE PARASITISM IN THE TOTAL NUMBER COLLECTED OF EACH BEETLE SPECIES IN THE YEARS 2001 AND 2002.

Species	2001				2002			
	Mean		%		Mean		%	
	A	B	A	B	A	B	A	B
Acalymma blomorum Munroe & Smith*	3.83	50.1	0	3.7	8.17	57.71	0.9	0.6
A. fairmairei (Fabricius)	3.61	4.29	0	0.4	2.31	1.99	7.7	0.7
A. innubum (Fabricius)	0.33	5.19	0	0.8	0.15	5.78	0	0
A. trivittatum Mannerheim	0.5	2.33	0	0	0.04	3.22	0	0
Diabrotica group *fucata*								
D. balteata LeConte*	1.11	0.61	0	0	0.44	35.1	5	3.7
D. dissimilis Jacoby	0	0.04	0	0	0	0.02	0	0
D. nummularis Harold	0.11	0.74	0	0	0.02	0.09	0	0
D. sexmaculata Baly	0	0.22	0	0	0.19	0.31	0	5.9
D. tibialis Baly	0.11	0.72	0	0	0.58	14.11	0	4.6
Diabrotica group *virgifera*								
D. porracea Harold	0.5	0.24	0	0	0.23	1.81	0	2.5
D. scutellata Baly	1.65	5.54	0	1.4	3.15	1.78	0	0.8
D. undecimpunctata duocecimnotata Harold	0.39	0.06	0	0	0.08	0.01	0	0
D. viridula (Fabricius)	0.11	0.37	0	0	0.15	5.4	0	0.2
Cerotoma atrofasciata Jacoby	0	0.28	0	12.5	0.83	3.33	0	2.5
Gynandrobrotica lepida (Say)	0	0.83	0	0	0.02	0.31	0	0
G. nigrofasciata (Say)	0	1.53	0	2.5	0	0.02	0	0
Isotes tetraspilota Baly*	0.44	0.03	0	0	8.56	0.01	0	0

*Significant differences between abundance per cucurbit ($P < 0.05$), and 0: No beetles nor parasitoids were found.

parasitism in these species varied between 0.4% and 12.5% (Table 2). Highest percentages of parasitism were found in *C. atrofasciata*. No significant differences in parasitism between beetle species were detected. Also, no correlation between beetle abundance and percentage parasitism was found. We found, however, significantly higher numbers of parasitoids in beetles collected on the cultivated cucurbit ($X^2_{(1, 0.05)}$ = 6.46). During the present study, parasitism was not observed in *A. trivittatum*, *D. dissimilis*, *D. nummularis*, *G. lepida*, and *I. tetraspilota*.

DISCUSSION

The diversity of Diabroticina species on *C. o. martinezii* and *C. moschata* was similar. Nevertheless, *Diabrotica scutellata* and *Isotes tetraspilota* were more abundant on bitter *C. o. martinezii*, whereas *Acalymma blomorum* and *D. balteata* were more abundant on the cultivated *C. moschata*. *Acalymma blomorum* was the most abundant species in both *Cucurbita* spp. These results are similar to data obtained by Cabrera & Cabrera (2004) with respect to the abundance of *Acalymma* spp. in Cucurbitaceae in Argentina. Within the *fucata* group, *D. balteata* was the most abundant species, and within the *virgifera* group *D. scutellata* was dominant. These results agreed with data reported by Rodriguez & Magallanes (1994) for *D. balteata* in Tamaulipas and Veracruz, and by Eben & Barbercheck (1996) for *D. scutellata* in Veracruz. In the present study, *Diabrotica dissimilis* and *G. nigrofasciata* were not collected on the bitter cucurbit. The other 12 species had a continuously low abundance on both cucurbits.

Acalymma blomorum and *D. balteata* were collected most frequently in *C. moschata*, perhaps as a result of the high quantities of pollen in this cucurbit. We observed that the beetles visited these plants to feed on petals and flowers. *Isotes tetraspilota* was the only species that was found feeding on the leaves of *C. o. martinezii*, and it was never seen on the cultivated cucurbit *C. moschata*. *Acalymma blomorum*, *A. fairmairei*, *C. atrofasciata*, and *D. balteata* were found on the cultivated cucurbit even when the plants began to dry. *Gynandrobrotica nigrofasciata* was collected only in 2001 on the cultivated cucurbit and furthermore, in much higher numbers, on a leguminous plant (*Pachyrhizus erosus* (L.) Urban) growing in the vicinity. Fabaceae are reported as host family for this genus (Jolivet & Hawkeswood 1995).

Celatoria compressa was the only parasitoid species obtained. It was reported for the first time by Eben & Barbercheck (1996) in Diabroticite beetles. Previously, *Celatoria bosqi* Blanch. (Heineck-Leonel & Salles 1997) *C. diabroticae* Shimer and *C. crawii* Coquillett had been collected from some *Diabrotica* spp. (Chittenden 1905; Sell 1915; Gordon et al. 1987) and *A. vittatum* (Walton 1914). The differences in parasitism in the species collected on both cucurbits, three species in *C. o. martinezii* vs. 11 species in *C. moschata*, were notable and might suggest that floral odor, or secondary compounds sequestered by beetles from the bitter host plant, had an effect on the adult parasitoid. To date, no study has tried to corroborate this for parasitoids of Diabroticina beetles. On the other hand, Diabroticina beetles were more abundant on the flowers and leaves of *C. moschata*, perhaps because this species offered greater amounts of pollen than *C. o. martinezii*. Consequently, *C. compressa* might have simply responded to the abundance of host insects in *C. moschata*. In general, the incidence of the tachinid in the guild of beetle species collected in both cucurbits was low, with parasitism ranging from 0.4% to 12.5%. In an earlier study similar parasitism rates of 1.0% to 11.1% were found (Eben & Barbercheck 1996). Parasitism by *C. bosqi* in *D. speciosa* ranged from 0.1 to 30.2% (Heineck-Leonel & Salles 1997) and *C. diabroticae* parasitized *D. u. howardi* with rates of 3 to 15% (Meinke & Gould 1987; Elsey 1988).

Given our observations, it would be interesting to investigate if cucurbitacins sequestered by the beetles collected on *C. o. martinezii* function as a repellent for *C. compressa*. Our data suggest that a possible effect of plant secondary compounds is stronger on adult parasitoid behavior (i.e., host acceptance) than on immature physiology, since all parasitoid larvae that eclosed from beetles pupated and developed successfully into adults.

During the course of our study a larger number of isolated plants of the cultivated than the bitter cucurbit species was found. This situation was contrary to our observations in previous years. It might be the consequence of the rapidly declining number of coffee plantations in the central area of Veracruz. In this area, the main habitat of *C. o. martinezii* are coffee plantations, where it is found growing in a vertical fashion upon the coffee bushes as climbing structures. By contrast, *C. moschata* grows horizontally, covering bare areas exposed to plain sunlight. These differences in habitat and microclimate might have influenced the abundance and species composition of Diabroticina beetles present in both plants as well as the searching behavior of *C. compressa*.

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