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USE OF HOST FRUIT CHEMICAL CUES FOR LABORATORY REARING OF *DORYCTOBRACON AREOLATUS* (HYMENOPTERA: BRACONIDAE), A PARASITOID OF *ANASTREPHA* SPP. (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Doryctobracon areolatus (Szepligeti) (Hymenoptera: Braconidae) is a common parasitoid of *Anastrepha* spp. (Diptera: Tephritidae). An efficient method of laboratory rearing incorporates chemicals from pear fruits into oviposition units. Production for the F_1 and F_2 generations was 12.1 and 9.3 progeny per female, respectively. Mean daily progeny production by F_2 females was between 1-2 progeny per female for almost all ages from 9 to 22 days. A bioassay was designed to determine the source of chemical cues used for host location. Parasitoids were given a choice between two oviposition units: a positive control containing all possible cues, and a treatment unit with cues derived from either the host fly, host fruit, both, or none. The number of females active on each oviposition unit was recorded. This experiment demonstrated that chemical cues derived from the host fruit, probably the peel, are involved in host location.

Key Words: biological control, fruit fly, host location, oviposition

RESUMEN

Doryctobracon areolatus (Szepligeti) (Hymenoptera: Braconidae) es un parasitoide común de *Anastrepha* spp. (Diptera: Tephritidae). Un método eficiente de criarlos en el laboratorio incorpora unos químicos de la fruta de la pera en las unidades de oviposición. La producción en las generaciones F_1 y F_2 fueron 12.1 y 9.3 descendientes por hembra, respectivamente. El promedio de la producción diaria de los descendientes para las hembras de F_2 fué entre 1-2 descendientes por hembra para casi todas las edades de 9 a 22 dias. Un bioensayo fué diseñado para determinar la fuente de las señales químicas usadas para la ubicación del hospedero. Los parasitoides podian escoger entre dos unidades de oviposición: un control positivo que tenia todas las señales posibles, y una unidad de tratamiento con las señales derivadas ya sea de la mosca hospedera, de la fruta hospedera, ó ambas, ó ninguna de las dos. Se registró el número de hembras activas sobre cada unidad de oviposición. Este experimento demostró que las señales químicas derivadas de la fruta hospedera, probablemente la cascara, estan envueltas en la localización del hospedero.

Doryctobracon areolatus (Szepligeti) (Hymenoptera: Braconidae) is a widespread Neotropical parasitoid of *Anastrepha* Schiner spp. (Diptera: Tephritidae), ranging from Mexico to Argentina (Wharton & Marsh 1978). In Brazil, it is the dominant species, constituting between 62% and 89% of all *Anastrepha* parasitoids in various surveys (Canal et al. 1994, 1995; Leonel et al. 1995; Araujo et al. 1996; Aguiar-Menezes & Menezes 1997; Aguiar-Menezes et al. 2001). Furthermore, *D. areolatus* represented 43-59% of all parasitoids collected in the State of Veracruz, Mexico (Hernandez-Ortiz et al. 1994; López et al. 1999), and accounted for 33% of the parasitism in Venezuela (Katiyar et al. 1995).

Doryctobracon areolatus was introduced into Florida in 1969 for the control of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Baranowski & Swanson 1970). It is currently the dominant parasitoid in the interior region of south-central Florida. In a recent study we found that it parasitized up to 36% of the host larvae and constituted 61-100% of all parasitoids at various sites (unpublished data).

Due to the importance of *D. areolatus* as a parasitoid of *Anastrepha* spp., there is much interest in establishing laboratory cultures of this species. Rearing of several fruit fly parasitoids has been facilitated by the use of 'oviposition units' in which host larvae are presented to the females

within an artificial apparatus (Wong & Ramadan 1992). This is based on the finding that female *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) exhibit an ovipositional response to vibrations of the host larvae (Lawrence 1981). However, *D. areolatus* females show no response to hosts in such an apparatus, and rearing has been successful only through the presentation of fruit fly larvae within host fruit.

Several studies have demonstrated the importance of fruit-associated chemicals in host location by parasitoids of fruit flies. Greany et al. (1977) found that chemicals released by fungi associated with rotten fruits are attractive to *D. longicaudata* females. Messing & Jang (1992), using chopped ripe fruits placed in traps, demonstrated attraction of *D. longicaudata* females to various host fruits. Messing et al. (1996) demonstrated similar responses by *Psyttalia fletcheri* (Silvestri) (Hymenoptera: Braconidae) to odors of fresh cucumber and decaying pumpkin.

Parasitoids may also respond to cues associated with the host fly. Prokopy & Webster (1978) found that *Utetes canaliculatus* (=*Opius lectus*) (Gahan) (Hymenoptera: Braconidae) responds primarily to the host-marking pheromone of *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). Similarly, *Halticoptera rosae* Burks (Hymenoptera: Pteromalidae) was found to respond to the pheromone deposited by *Rhagoletis basiola* (Osten Sacken) (Diptera: Tephritidae) (Roitberg & Lalonde 1991).

In this paper we describe an efficient method of *D. areolatus* rearing by incorporating host chemicals into oviposition units. We demonstrate that the ovipositional response is to chemicals derived from the host fruit, and not the fly.

Materials and Methods

Laboratory Rearing

Insects. A parent generation of *D. areolatus*, a total of 128 females and 41 males, was reared from cattley guava, *Psidium cattleianum* Sabine, fruit collected mostly at LaBelle, Florida. Larvae of *A. suspensa* were obtained from a laboratory colony maintained for approximately 150 generations at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida.

Cage Setup. Adult parasitoids were maintained in 20 cm^3 metal-framed cages, the top and two side panels with 16-mesh (per inch) screens, and other panels Plexiglas. One of the side Plexiglas panels included a cloth sleeve. A brown paper towel was taped to the outside of the opposing Plexiglas panel, in order to reduce light intensity. Each cage was stocked over a period of several days (depending on the emergence rate) with up to 100 females and 100 males. Food was supplied daily in the form of a fresh block of honey agar set on an inverted 30 ml plastic cup, and a strip of honey on the Plexiglas side panel. Water was supplied in a 100 ml plastic cup with a cloth wick inserted through a hole in the lid; the external part of the wick was split in half and laid upon the lid. Cages were maintained at 25 ± 0.5°C, 45% R.H., and a light-dark cycle of 14:10.

Oviposition Unit. Oviposition units were composed of *A. suspensa* larvae in diet (Burns 1995) between two layers of cloth, topped with a layer of parafilm, all maintained within a 7.6 cm diameter plastic embroidery hoop. Before exposure to the parasitoids, the parafilm had been wrapped overnight on a fresh 'Anjou' pear (chosen because pears were commercially available throughout the year), previously placed for several hours in a cage with adult *A. suspensa*. The parafilm was placed in the unit with the side previously in contact with the host fruit facing out. This procedure, allowing transfer of fruit chemicals to the oviposition unit, was previously used by Papaj & Prokopy (1986) for fruit fly bioassays. Each sheet of parafilm was used on two consecutive days and, when not in use, was kept in a sealed and refrigerated plastic cup.

Approximately 40 cm^3 diet containing several hundred host larvae were placed in each oviposition unit. The larvae-diet mixture was selected from areas of the larval trays containing high densities of larvae, so that at least 50% of the volume was larvae. This was done to increase the chance of successful probing by the parasitoids. A greater amount of diet would have allowed larvae to migrate away from the oviposition surface and avoid parasitism. Less diet would have left parts of the unit devoid of hosts, thus decreasing the chance of a successful probing. Host larvae were usually 4 or 5 days old, corresponding to late second and/or early third instar; occasionally 3 or 6 day old larvae were used.

The oviposition unit was elevated onto an inverted 100 ml plastic cup to set it closer to the center of the cage, thus improving access of the parasitoids to it. Hosts were exposed to parasitoids for approximately 8 h daily. However, when high activity (15 or more parasitoids simultaneously on the oviposition unit) was observed, two successive exposures were performed, with units being replaced after 4 h. This was done to reduce the chance of superparasitism.

Parasitoids were first provided with hosts within several days of emergence. Exposure continued daily, depending on availability of suitable hosts, until the last female in the cage died. Because cages were stocked over several days, the exact age of ovipositing females could not be determined. Age was estimated as the difference between the exposure date and the median emergence date of all females in a particular cage. This age estimate for F_2 females was subsequently related with the number and sex ratio of their progeny.

Immature Stages and Adult Emergence. Upon completion of exposure, host larvae were transferred to 30 ml plastic cups, which were filled to the top with fresh diet. These cups were then placed upon moist fine vermiculite (15-20 ml water per 100 cm^3 vermiculite) in 500 ml plastic cups. Fully developed larvae emerged from the diet, dropping to the vermiculite in which they pupated. After allowing larvae to pupate for several days, the vermiculite was sieved, and host puparia transferred into fresh moist vermiculite within 100 ml plastic cups. These cups were covered with a solid lid, which was replaced after one week with a cloth lid. This procedure allowed the vermiculite to remain moist while minimizing development of fungi. Immature stages were maintained at the same environmental conditions as adults.

Number and sex of adult parasitoids were determined upon emergence, and adults were transferred to screened cages. Cups were discarded when no emergence was observed for several days.

Bioassay of Chemical Cues

Doryctobracon areolatus were reared successfully from host larvae in oviposition units with parafilm that had contained possible chemical cues from both adult fruit flies and host fruit (described above). A subsequent study was conducted to confirm that chemicals from the host fruit and/or adult fly were used as cues for host location and to further determine the source of these cues.

Insects. Adult *D. areolatus* used in the bioassay were F_3 individuals from the laboratory culture described above. Larvae of *A. suspensa* were obtained from the laboratory culture at the Division of Plant Industry described above.

Experimental Design. Cages were 30 cm long × 20 cm wide × 20 cm high. The bottom and two longer sides were Plexiglas, with a cloth sleeve in the middle of one of the side panels. The top panel was 52-mesh (per inch) screen, and the two smaller sides 16-mesh screen. Each of 6 cages was stocked with 100 female and 70 male *D. areolatus*. Dead females were replaced daily. Before experimentation, females were provided at least once with an oviposition unit containing both host fruit and fly chemicals. Oviposition units were as described above for the laboratory culture, except that the embroidery hoops were made of wood and not plastic.

Parasitoids in each cage were allowed to choose between two oviposition units, both placed upon inverted plastic containers. One unit ('Positive control') contained parafilm wrapped overnight on unwaxed 'Anjou' pears exposed to ovipositing *A. suspensa* females for several hours. This unit contained all possible chemical cues deriving from the host fruit and adult host fly, similar to units used in rearing the laboratory culture.

The second oviposition unit ('Treatment') contained parafilm with chemical cues from either the host fruit or fly, a combination of both, or without added cues. This treatment unit presumably represented a subset of the positive control unit, and response was expected to be either equal to or less than response to the positive control. Treatments were: (1) Untreated parafilm; (2) 'Intact fruit'—wrapped on fresh undamaged pear; (3) 'Punctured fruit'—wrapped on pear punctured approximately 200 times with a no. 0 insect pin (to simulate puncturing by ovipositing flies); (4) 'Damaged fruit'—wrapped on pear from which sections of pulp had been cut out (to simulate vertebrate damage); (5) 'Fly cues'—placed for several hours within a cage containing ovipositing *A. suspensa* females (flies oviposited through the parafilm from several to several hundred times); (6) 'Fly cues + punctured fruit'—as treatment (5) but subsequently wrapped on punctured pear.

The experiment was replicated on 12 of 13 consecutive days. On each day, each of the 6 cages contained a different treatment. Each treatment was replicated twice in each cage, placed alternately on the left and right side of the cage; the placement on any given day was random.

Response Variable and Statistical Analysis. The number of females active on each oviposition unit was recorded at 1, 4 and 8 h following placement of the units in the cage. An active female was defined as an individual either probing into the unit with its ovipositor, or one standing on the unit with ovipositor at a horizontal or below horizontal position; when the female is not reproductively active the ovipositor is curved slightly upward.

The difference between the 'Positive control' and 'Treatment' units ('diff') was calculated for each cage at each hour. This variable was submitted to the MIXED procedure of the SAS statistical software package (Verbeke & Molenberghs 1997), with the hourly observations treated as repeated measurements. This procedure produced t-statistics for each treatment, testing whether the variable 'diff' was different than zero, i.e., whether there was a significant difference between 'Positive control' and 'Treatment'. It further produced t-values comparing 'diff' among the various treatments.

RESULTS

Laboratory Rearing

Lifetime progeny production averaged 2.4, 12.1 and 9.3 for P_1, F_1 and F_2 females, respectively. Mean daily production by surviving F_2 females was between 1-2 progeny per female for almost all ages from 9 to 22 days (Fig. 1).

The sex ratio was 44.7, 62.5 and 48% males for the progeny of P_1, F_1 and F_2 females, respectively. The sex ratio of the progeny of F_2 females was relatively stable over time, averaging close to 50%

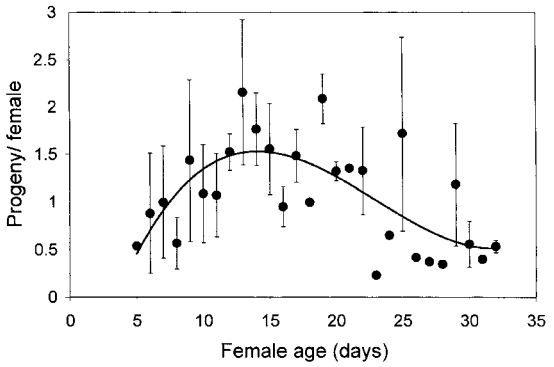

Fig. 1. Daily progeny production by F_2 *Doryctobracon areolatus* females.

(Fig. 2). However, at the oldest female ages the progeny sex ratio tended to be male-biased. This may be the result of sperm depletion, or perhaps lower mortality of unmated females.

Development time of immature stages at 25°C was 22.1 ± 1.1 days (range 19-35 days) for females and 20.6 ± 1.1 days (range 18-26 days) for males.

Bioassay of Chemical Cues

Figure 3 compares the number of active *D. areolatus* females on the oviposition unit among the various treatments. Ovipositional response to the intact and punctured fruit treatments was highest, and did not differ from the response to the positive control ($t = 1.77$, $p = 0.08$; $t = 0.26$, $p = 0.80$; respectively). Response to the fly cues + punctured fruit, damaged fruit, fly cues, and untreated parafilm treatments was significantly lower than to the positive control ($t = 2.24$, $p = 0.03$; $t = 3.83$, $p = 0.0003$; $t = 7.28$, $p < 0.0001$; $t = 6.80$, $p < 0.0001$; respectively).

Adult parasitoid response was greater to all fruit treatments, i.e., intact fruit, punctured fruit, damaged fruit and fly cues + punctured fruit, than to either fly cues only ($t = 3.90$, $p = 0.0003$; $t = 4.96$, $p < 0.0001$; $t = 2.44$, $p = 0.02$; $t = 3.56$, $p = 0.0008$; respectively) or to untreated parafilm ($t = 3.56$, $p = 0.0008$; $t = 4.63$, $p < 0.0001$; $t = 2.10$, $p = 0.04$; $t = 3.23$, $p = 0.002$; respectively). Additionally, response to punctured fruit odor was greater than that to odor of damaged fruit ($t = 2.53$, $p = 0.01$). All other comparisons among treatments were statistically insignificant.

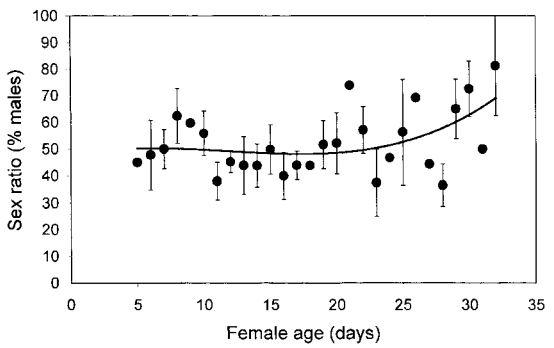

Fig. 2. Relationship between age of F_2 *Doryctobracon areolatus* females and sex ratio of progeny produced.

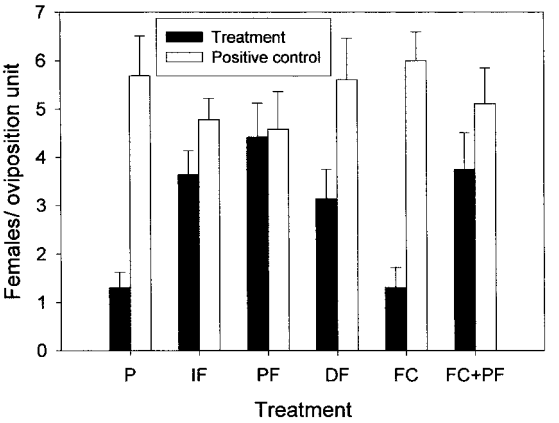

Fig. 3. Number of *Doryctobracon areolatus* females on oviposition units treated with chemical cues from various sources ('Treatment') and on units containing cues from fruit exposed to flies ('Positive control'). The mean value for three observations (1, 4 and 8 h following placement of the units in the cage) is presented. P = Untreated parafilm; IF = Intact fruit; PF = Punctured fruit; DF = Damaged fruit; FC = Fly cues.

Discussion

Parasitoids utilize a wide range of host-related stimuli for host location, often chemical (Godfray 1994). We found that chemical cues emanating from ripe host fruit elicit a significant ovipositional response in *D. areolatus*. Response to "damaged" fruit, in which pieces of peel are removed and the pulp exposed, is somewhat less than to the whole fruit, suggesting that the active chemical(s) may be located in the peel.

Chemical cues derived from the host fly have no apparent effect on *D. areolatus* females. *Utetes canaliculatus* and *H. rosae*, which were shown to respond to host fly pheromones (Prokopy & Webster 1978; Roitberg & Lalonde 1991), parasitize eggs or early-instar larvae, whereas *D. areolatus* prefers later instars. As the host pheromone is water-soluble, it would be degraded by precipitation. Species like *D. areolatus*, which attack the host larvae long after they were deposited as eggs, have less of an association with the pheromone, and thus are less likely evolve a response to it.

Vibration cues are insufficient to elicit a significant ovipositional response in *D. areolatus*. Vibrotaxis has been reported for *Diachasmimorpha*

mellea (Gahan) (Lathrop & Newton 1933), *Diachasma alloeum* (Muesebeck) (Glas & Vet 1983), and *Aganaspis pelleranoi* (Brethes) (Hymenoptera: Eucoilidae) (Ovruski 1994), and *D. longicaudata* can locate larvae within fruit solely by vibration sensing (Lawrence 1981). The lack of response to larvae alone does not imply that host vibrations have no role in host location in *D. areolatus*. Chemical cues may be used in the early stages of host location, as attractants or arrestants, with vibration stimulating probing behavior once the parasitoid is on the fruit.

Total progeny production for *D. areolatus* in the current study was less than half the 29.6 progeny per female reported for *D. longicaudata* (Greany et al. 1976). Similarly, daily progeny production for *D. longicaudata* peaked at nearly 4 progeny per female (Greany et al. 1976), which is approximately double the peak daily progeny production for *D. areolatus*. For both species, the number of mature eggs in the ovaries (*D. areolatus*, 64.3 ± 4.3, n = 6; *D. longicaudata*, 73.0 ± 6.3, n = 6; t = 1.18, p = 0.26; 7-day-old females not exposed to hosts, specimens reared in Mexico by M.A.) is much greater than the maximum number oviposited per day. Thus, the differences in progeny production between the two species are not due to differential egg supply. These may be the result of different experimental procedures or differential adaptability to laboratory conditions. However, they may also represent different reproductive strategies, whereby *D. longicaudata* produces large numbers of progeny in a short period of time, and *D. areolatus* smaller numbers over longer periods.

The rearing method reported here is an improvement over the procedure of rearing *D. areolatus* on host larvae within fruits, and could serve as a basis for the establishment of laboratory cultures for research. Such research could supply further information on life history traits, temperature tolerances, host location, competitive abilities, etc., of *D. areolatus*, which in turn could help explain field observations, e.g., differences in temporal and spatial distribution patterns between *D. areolatus* and *D. longicaudata* in Florida (Sivinski et al. 1998; Eitam unpublished data).

Further improvements in rearing techniques could make possible mass-production for purposes such as augmentative releases (see Sivinski et al. 1996, for an example of augmentative releases of *D. longicaudata*). For instance, chemical identification of fruit cues used for host location may totally eliminate the need for fruits in laboratory rearing.

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