Journal of Chemical Ecology (©2006)
DOI: 10.1007/s10886-005-9007-6

FORAGING BEHAVIOR OF *Anastrepha ludens*, *A. obliqua*, AND *A. serpentina* IN RESPONSE TO FECES EXTRACTS CONTAINING HOST MARKING PHEROMONE

MARTIN ALUJA[1,*] and FRANCISCO DÍAZ-FLEISCHER[2,3]

[1]*Instituto de Ecología, A.C., Apartado Postal 63, 91000 Xalapa, Veracruz, Mexico*
[2]*Campaña Nacional Contra Moscas de la Fruta, Apartado Postal 368, 30700 Tapachula, Chiapas, Mexico*

(Received January 27, 2005; revised June 1, 2005; accepted October 13, 2005)

Abstract—Following oviposition, females of many Tephritid flies deposit host marking pheromones (HMPs) to indicate that the host fruit has been occupied. We describe the foraging behavior of these three economically important species (*Anastrepha ludens* and *A. obliqua* from the *fraterculus* species group and *A. serpentina* from the *serpentina* species group) when they encounter an artificial fruit (green agar spheres wrapped in Parafilm) marked with intra- and interspecific feces extracts that contain, among other substances, host marking pheromone. When flies encountered fruit treated with either 1 or 100 mg/ml feces extract, there were drastic and statistically significant reductions in tree residence time, mean time spent on fruit, and in the number of oviposition attempts or actual ovipositions when compared to the control treatment (clean fruit). These responses were almost identical irrespective of extract origin (i.e., fly species), indicating complete interspecific HMP cross-recognition by all three *Anastrepha* species tested. We discuss the ecological and practical implications of our findings.

Key Words—*Anastrepha*, Tephritidae, foraging behavior, host marking pheromones, infochemicals, evolution.

* To whom correspondence should be addressed. E-mail: alujam@ecologia.edu.mx
[3] Current address: LABIOTECA, Universidad Veracruzana, Apartado Postal 250, 91090 Xalapa, Veracruz, Mexico.

INTRODUCTION

When resources for foraging insects are fixed in space (e.g., leaf, fruit, larvae), the efficiency of females searching for an adequate oviposition substrate increases if they are able to quickly recognize and avoid already occupied hosts. A successful strategy to minimize overcrowding and to elicit movement away from areas with already occupied resources is the use of epideictic pheromones (among them host marking pheromones or HMPs) (Prokopy, 1981). According to Nufio and Papaj (2001), a marking pheromone is a "chemical signal associated with the host resource that signals occupation by conspecifics. Typically perceived by contact chemoreception, these pheromones are generally produced by females and placed onto or within larval resources following egg-laying.'' Interestingly, these infochemicals can either deter or enhance oviposition depending, among other factors, on concentration (Papaj and Aluja, 1993). The existence of HMPs has been reported in the insect orders Coleoptera, Diptera (particularly in the family Tephritidae), Hymenoptera, Lepidoptera, and Neuroptera (Prokopy, 1981; Nufio and Papaj, 2001).

In the case of true fruit flies (Diptera: Tephritidae), host marking behavior has been described in the frugivorous genera *Anastrepha*, *Bactrocera*, *Ceratitis*, *Paraceratitella*, and *Rhagoletis* (work reviewed by Aluja et al., 2000a; Díaz-Fleischer and Aluja, 2000; Díaz-Fleischer et al., 2000; Nufio and Papaj, 2001). In nonfrugivorous tephritids, the phenomenon is less well studied but has been reported for *Tephritis bardanae* (Schrank) (Straw, 1989), *Chaetorellia australis* Hering (Pittara and Katsoyannos, 1990), and *Terellia ruficauda* (Fabricius) (Lalonde and Roitberg, 1992). Flies in the genera *Anastrepha*, *Ceratitis*, and *Rhagoletis* mark hosts by dragging of the aculeus tip on the fruit surface (Roitberg and Prokopy, 1987; Aluja et al., 2000a), while in the case of the olive fruit fly, *Bactrocera oleae* Gmelin, marking is achieved through labellar spreading of fruit juices oozing from an oviposition puncture (Cirio, 1971; Girolami et al., 1981).

The most striking behavioral responses exhibited by foraging female fruit flies upon encountering fruit covered with HMP are as follows: an increase in overall movement (e.g., number of leaf landings per minute, distance of between-tree displacements) and fruit visitation rates, reduction in tree residency time (Roitberg and Prokopy, 1984; Aluja and Boller, 1992a), reduction or increase in clutch size depending on HMP concentration and fruit size (Papaj et al., 1990, 1996; Papaj and Aluja, 1993), and a reduction in the propensity of a female to initiate oviposition (Nufio and Papaj, 2001). HMP recognition is contingent upon previous experience (Roitberg and Prokopy, 1981) but sensitivity to the pheromone is reduced if exposure is continual, apparently due to central habituation or peripheral adaptation of certain sensilla in the tarsi (Aluja and Boller, 1992a). In the case of males of species exhibiting

a resource–defense mating system (e.g., *R. pomonella*, *R. cerasi*), an encounter with an HMP-marked fruit causes arrestment (Prokopy and Bush, 1972; Katsoyannos, 1975).

Host marking behavior has been described for *A. suspensa* (Prokopy et al., 1977), *A. fraterculus* (Prokopy et al., 1982a), and *A. ludens* (Papaj and Aluja, 1993). In *Anastrepha*, as with *Ceratitis capitata* and some *Rhagoletis* species (Prokopy, 1972; Papaj et al., 1989), the presence of the HMP does not automatically deter other conspecifics from ovipositing and re-marking a fruit. *A. ludens* females laid eggs in grapefruit marked up to three previous times by conspecifics (Papaj and Aluja, 1993). Low concentrations of the HMP seem to stimulate continued host marking and an upper concentration threshold must be reached to deter further oviposition. These findings lead Papaj and Aluja (1993) to postulate that host marking in *A. ludens* is regulated by sensory adaptation or habituation to HMP, in conjunction with dosage-dependent restoration of inhibition of locomotory behavior.

The HMPs of *R. cerasi* (*N*[15(β-glucopyranosil)oxy-8-hydroxypalmitoyl]-taurine) and *A. ludens* (2-[2,14-dimethyl-pentadecanoylamino]-pentane-dioic acid) were extracted from the feces of sexually mature females (Boller and Hurter, 1985; Hurter et al., 1987; Aluja et al., 2003; Díaz-Fleischer et al., 2004). They show some degree of similarity as both contain a long fatty acid and an amino acid. The *Anastrepha* HMPs are polar compounds (easily dissolved in water and methanol), which are remarkably stable under varying pH values and environmental light and temperature conditions (Díaz-Fleischer et al., 2004).

A topic of particular interest in the study of host marking behavior is the phenomenon of interspecific pheromone recognition. In some insects, this was attributed to phylogenetic relatedness among species (i.e., lineage effect). For example, in *Rhagoletis*, females within a species group (e.g., the *cingulata* group) recognize HMPs of heterospecifics, while females from unrelated groups (e.g., *cingulata* vs. *pomonella* groups) are usually not deterred by each other's HMP (Prokopy et al., 1976). In other insects, interspecific pheromone recognition may reflect an adaptive response to competition for common oviposition resources (see Nufio and Papaj, 2001 for details).

Here, we describe the foraging behavior of *A. ludens*, *A. obliqua*, and *A. serpentina* in response to their own and heterospecific fecal extracts, as these are known to contain the HMPs (Aluja et al., 2003; Díaz-Fleischer et al., 2004). Our hypothesis, based on the work on *Rhagoletis* and recent *Anastrepha* phylogenies (Norrbom et al., 2000; Norrbom, 2002; McPheron et al., 2000), was that interspecific recognition of the pheromone would exist for two species from the same intrageneric group (*A. ludens* and *A. obliqua*) but not for a species from a different group (*A. serpentina*) (Norrbom et al., 2000) given that in nature these three species rarely compete for resources.

METHODS AND MATERIALS

Study System. Fruit flies of the genus *Anastrepha* (Diptera: Tephritidae) are distributed from southern United States to northern Argentina (Hernández-Ortíz and Aluja, 1993). Of the ca. 200 species described so far (Norrbom et al., 2000), seven are considered important agricultural pests: *A. grandis* (Macquart), *A. fraterculus* (Wiedemann), *A. ludens* (Loew), *A. obliqua* (Macquart), *A. striata* Schiner, *A. suspensa* (Loew), and *A. serpentina* (Wiedemann) (Aluja, 1994). The three *Anastrepha* species we selected for this study are polyphagous species with different oviposition strategies. While *A. obliqua* deposits a single egg per oviposition bout, *A. ludens* and *A. serpentina* females can lay from 1 to over 20 eggs per oviposition (Aluja et al., 2000a). *A. ludens* attacks mainly fruit in the family Rutaceae (e.g., *Sargentia greggii* Coult., *Casimiroa edulis* Llave & Lex., *Citrus* spp.). It also attacks mangoes (*Mangifera indica* L.), but mainly at higher elevations (>800 m) (Aluja et al., 1987, 1996). *A. obliqua* preferentially attacks fruit in the family Anacardiaceae, especially in the genus *Spondias* (e.g., *S. mombin* L., *S. purpurea* L., *S. radlkoferi* J.D. Smith) (Díaz-Fleischer and Aluja, 2003b). It also attacks mangoes in lowland areas (therefore, simultaneous use of a mango fruit by *A. obliqua* and *A. ludens*, although possible, is not common) (Aluja et al., 1996). We note that mangoes were introduced by the Spaniards ca. 500 yr ago and therefore, from an evolutionary perspective, the interaction between the latter two *Anastrepha* species in such an exotic host is quite recent. Finally, *A. serpentina* attacks fruit in the family Sapotaceae (e.g., *Chrysophyllum caimito* L., *Manilkara zapota* (L.) P. Royen, *Mammea americana* L.) (Aluja et al., 1987, 2000b).

Study Site. All experiments were performed on the grounds of the Medfly Program at Metapa de Domínguez, Chiapas, México.

Research Arena. General bioassay protocols are described by Aluja and Boller (1992a). Eighteen potted mango trees (*M. indica* cultivar "criollo'') were placed in the center of a cylindrical 3 m (diam) × 2 m (height) field cage (Chambers et al., 1983), on top of wooden stools at different heights so as to mimic the canopy of a mango tree (ca. 2 m × 1.90 m). The cage was protected by a 25-m^2 metal frame with a corrugated fiberglass roof, and shading was provided by black greenhouse screen (2 mm fabric) placed on both the sides and the top. Approximately 35% of all leaves were removed to facilitate detection of a foraging fly within the canopy (details in Aluja et al., 1989 and Aluja and Prokopy, 1993), and trees were arranged so that a 50-cm walkway was left free to allow an observer to move around without encountering obstacles. Throughout this study (i.e., during fly maintenance phase and experiments), 3-cm-diam fruit mimics made of agar (Bacteriological Agar; Sigma, St. Louis, MO, USA) colored with green food dye (Colorante Alimenticio, Verde, McCormick, Herdez, Mexico) and wrapped in Parafilm (American National Can, Neenah,

WI, USA) (Boller 1968) were used instead of real fruit to control for intrinsic variations in both fruit quality and chemistry, as these influence fruit fly oviposition behavior (Papaj et al., 1992). There is ample evidence indicating that female flies accept such artificial oviposition substrates without perceptible behavioral differences with respect to natural hosts (Freeman and Carey, 1989; Jácome et al. 1999; Díaz-Fleischer and Aluja, 2003a).

Insects. All flies used were collected from infested fruit in the Soconusco Region, Chiapas and the vicinity of Xalapa, Veracruz, Mexico: *A. ludens* from *C. aurantium* L. and *M. indica*, *A. obliqua* from *Spondias* spp., and *M. indica*, whereas *A. serpentina* came from *M. americana* L. Details on handling of larvae and pupae are described elsewhere (Aluja et al., 1987).

Newly emerged females and males (25 of each sex per cage) were placed in 30 × 30 × 30 cm wooden screened cages with unlimited access to water and a sucrose/hydrolyzed protein mixture (3:1) (yeast hydrolysate enzymatic; ICN Biochemicals, Aurora, OH, USA) and held at 26°C, 70–85% RH, 12L:12D until 1 d prior to testing. Each cage had one *M. indica* branch with ca. 8 leaves to provide resting places for flies and maintain high RH levels. The latter was important because flies in bare cages break their wings by constantly hitting the walls, and relative air humidity influences the function of the tarsal chemoreceptors (Städler et al., 1987). In addition, 40 agar spheres were placed in each cage (ca. 10 hung from the roof and the rest on the floor) and replaced at least twice daily to avoid HMP accumulation on them, as flies maintained in an HMP-saturated environment become habituated/desensitized and lose their ability to discriminate between marked and clean fruit (Aluja and Boller, 1992a). The oviposition substrates were also important to familiarize females with bioassay conditions, and allow them to oviposit and mark fruit. Both aspects are critical in foraging behavior studies related to HMPs, because host acceptance in flies with a full complement of eggs increases after a period of host deprivation (Roitberg and Prokopy, 1983), which in turn "can make an individual prone to accept a host that would otherwise be rejected'' (Jaenike and Papaj, 1992). Furthermore, females require prior experience with the HMP before they discriminate between marked and unmarked fruit (Roitberg and Prokopy, 1981).

Only sexually mature, mated females were used and their ages varied between 16 and 30, 18 and 32, and 22 and 30 d for *A. obliqua*, *A. ludens*, and *A. serpentina*, respectively. On the day prior to testing, two cages with flies and agar spheres were manually transferred from the lab to a field cage to allow flies to acclimate to test conditions. During this period, we constantly provided them with fresh oviposition substrates to avoid high oviposition drive and habituation/desensitization to high pheromone concentrations.

Test Compounds. Crude pheromonal extracts were obtained from *A. ludens*, *A. obliqua*, and *A. serpentina* feces, which, as documented by Aluja et al. (2003)

and Díaz-Fleischer et al. (2004), contain HMPs. For each species, ca. 3500 adult flies (extremely high density) were placed in a 30 × 30 × 30 cm glass cage with two 13 × 25 cm sheets of glass to increase the exposed surface to flies. Food and water were offered continuously and after 30 d, the feces on the glass surfaces (without insect parts) were meticulously collected by scraping with a razor blade or a metal spatula. The samples were transferred to a ca. 500-ml glass bottle and frozen at −15°C until needed (further details in Aluja et al., 2003). The pheromone extracts (100 mg/ml) were prepared by mixing 1 g feces with 10 ml distilled water or methanol and were shaken manually for 15 min. The liquid was then centrifuged at 12,000 rpm for 20 min, and the resulting supernatant was used to treat test fruit for the bioassays. The same technique was used to prepare the 1 mg/ml concentration, but in this case only methanol was used as it proved to be a more efficient solvent than water (Díaz-Fleischer et al., 2004).

Bioassay Procedure. Following the protocol of Aluja and Boller (1992a), a single female was released into the cage and her behavior observed for a maximum of 1 hr or until it left the tree and flew to the cage walls. Every fly was released at exactly the same location (i.e., release leaf) and only used once (i.e., no repeated measures on same individual throughout study). In each assay, there were eight spheres, hung equidistant and at ca. the same height from branches in the external part of the canopy. The spheres were either pheromone treated (raw HMP extracts of the three fly species used during this study) or clean (treated with either water or methanol depending on which had been used as the solvent). On any given day, we tested all four treatments. An individual fly was either exposed to eight agar spheres treated with the HMP of one of the three *Anastrepha* species, or eight untreated control spheres (i.e., no choice conditions). Once observations with a particular fly were concluded (i.e., 1-hr time limit reached or fly flying to cage walls before time limit was over), another individual was released and exposed to spheres treated with the HMP of a different *Anastrepha* species or to untreated spheres. At the end of the daily ca. 4-hr observation period, the raw HMP extracts of all three *Anastrepha* species as well as the control treatment were tested (i.e., one replicate per treatment per day). Order of testing was randomized every day. Parameters recorded were total time on tree, time spent on fruit, time spent moving, cleaning and resting, and number of fruit visited. These variables are good descriptors of foraging activity (Roitberg et al., 1982) and, indirectly, of fly oviposition "drive'' as flies will visit more fruits and generally spend more time on trees with unmarked fruits, than in those with HMP-marked ones (Prokopy et al., 1987; Aluja and Boller, 1992a). Once a fly landed on a sphere, time spent exploring [actively moving while head butting (repeatedly touching surface of fruit with frons)] or resting, attempting to oviposit or actually ovipositing and time spent dragging (walking on fruit surface with extruded aculeus through which HMP is released) were recorded. We also computed a "fruit irritation

TABLE 1. ENVIRONMENTAL CONDITIONS AT METAPA DE DOMÍNGUEZ, CHIAPAS, MEXICO, WHEN STUDYING THE RESPONSES OF *A. ludens*, *A. obliqua*, AND *A. serpentina* FEMALES TO THEIR OWN AND HETEROSPECIFIC FECAL HOST MARKING PHEROMONE (HMP)

Month and year	Aug. 1993	Sep. 1993	Oct. 1993	Nov. 1993	Jul. 1994	Aug. 1994	Sep. 1994	Oct. 1994	Nov. 1994	Dec. 1994	Jan. 1995
Temperature (°C)	24.4	26.31	25.7	25.26	26.33	25.7	24.98	24.7	23.76	22.42	20.97
RH (%)	77.34	76.25	71.48	68.56	71.57	77.34	82	81.48	80.48	79.37	77.79
A. obliqua											
A. serpentina											
A. ludens											

Time at which tests were conducted indicated for every *Anastrepha* species. Values represent mean.

index'' (number of behavioral transitions while on fruit [walking–cleaning–resting–walking–cleaning] (Boller et al., 1987), which is a good indicator of host acceptance (Aluja and Boller, 1992a).

Assays were conducted approximately between 08:15 and 12:15 hr every day, but the actual time varied according to the different species-specific biorhythms (i.e., *A. obliqua* starts ovipositing earlier than *A. ludens* and *A. serpentina*; Aluja and Birke, 1993; Aluja et al., 2000a). Experiments were conducted between July and January, depending on host availability, with the 100 mg/ml HMP concentration assays being carried out in 1993 and the 1 mg/ml concentration during 1994–1995. In all cases, there was sufficient overlap in time of day and time of year to warrant formal comparisons between species (Table 1).

Data Analysis. As the two HMP concentrations were not simultaneously tested, we only compared one concentration at a time. Data were first analyzed by means of a two-way MANOVA (fly species × HMP origin), followed by a two-way ANOVA given the significance detected (O'Brien and Kaiser, 1985). Multiple comparisons were carried out using a least square means *t*-test (SAS, 2003).

RESULTS

All HMP extracts tested, at concentrations of 1 and 100 mg/ml, elicited strong behavioral responses in the three *Anastrepha* species studied. MANOVA revealed differences among species (Pillai trace = 0.295, $F_{12.568} = 8.2$; $P < 0.001$; Pillai trace = 0.129; $F_{12.562} = 3.2$; $P < 0.001$; for 1 and 100 mg/ml, respectively) and HMP origin (Pillai trace = 0.921, $F_{18.855} = 21.0$; $P < 0.001$; Pillai trace = 0.975; $F_{18.846} = 22.6$; $P < 0.001$; for 1 and 100 mg/ml, respectively). There was also a significant interaction between fly species and HMP origin (Pillai trace = 0.546, $F_{36.1728} = 4.8$; $P < 0.001$; Pillai trace = 0.432; $F_{36.1710} = 3.7$; $P < 0.001$; for 1 and 100 mg/ml, respectively). Two-way ANOVA (summarized in Tables 2 and 3) revealed that clean fruit mimics (untreated agar spheres), treated with both HMP concentrations, elicited a drastic and statistically significant reduction in female tree residence time. Importantly, differences between species were not significant, independent of pheromone concentration. With respect to the interaction, it was nonsignificant at a concentration of 1 mg/ml and significant at 100 mg/ml (Figure 1, Tables 2 and 3). In the case of mean time spent on fruit, we found a statistically significant reduction in HMP-treated hosts at both pheromone concentrations. As was the case with total time spent on the tree, we found no differences among species when comparing total time spent on fruit. Nevertheless, the interaction was nonsignificant at a concentration of 1 mg/ml and significant at 100 mg/ml

TABLE 2. TWO-WAY ANOVA OF THE BEHAVIORAL RESPONSES EXHIBITED BY *A. ludens*, *A. obliqua*, AND *A. serpentina* FEMALES WHEN FORAGING IN FIELD-CAGED MANGO TREES AND ENCOUNTERING ARTIFICIAL FRUIT (I.E., GREEN-COLORED AGAR SPHERES WRAPPED IN PARAFILM) COVERED WITH THEIR OWN AND HETEROSPECIFIC FECAL HOST MARKING PHEROMONE (HMP) EXTRACTS (VALUES ROUNDED TO SECOND DECIMAL)

Dependent variable	Source of variation	*df*	Sum of squares	Mean square	*F* value	*P* value
Tree residence time	HMP	3	263,456,037.13	87,818,679.04	220.3	<0.0001
	Species	2	1,441,810.33	720,905.16	1.8	0.16
	HMP * Species	6	1,853,392.74	5308,898.79	0.8	0.59
	Residual	288	114,814,184.64	398,660.36		
Fruit residence time	HMP	3	197,119,923.48	65,706,641.16	204.6	<0.0001
	Species	2	340,946.13	170,473.06	0.5	0.59
	HMP * Species	6	2,373,760.70	395,626.78	1.2	0.29
	Residual	288	92,467,245.04	321,066.82		
Fruit visited	HMP	3	73.29	24.43	8.7	<0.0001
	Species	2	5.46	2.73	1.0	0.38
	HMP * Species	6	56.01	9.33	3.3	0.003
	Residual	288	803.52	2.79		
Oviposition attempts	HMP	3	14,513.77	4837.92	61.4	<0.0001
	Species	2	895.02	447.51	5.7	0.004
	HMP * Species	6	2766.02	461.00	5.8	<0.0001
	Residual	288	22,683.92	78.76		
Ovipositions	HMP	3	1100.84	366.95	142.2	<0.0001
	Species	2	198.29	99.14	38.4	<0.0001
	HMP * Species	6	483.07	80.51	31.2	<0.0001
	Residual	288	743.04	2.58		
Irritation index	HMP	3	0.18	0.06	9.9	<0.0001
	Species	2	0.01	0.05	7.8	0.0005
	HMP * Species	6	0.04	0.01	1.0	0.42
	Residual	288	1.75	0.016		

HMP extracts tested at a concentration of 1 mg/ml.

(details on degrees of freedom, *F* and *P* values in Tables 2 and 3). *A. obliqua* females behaved differently after landing on unmarked control hosts when compared to the other two fly species (Figure 2).

Presence of HMP significantly altered the total number of fruit visited (Figure 3). Females visited more control fruit than treated ones at both HMP concentrations (details on statistical analyses in Tables 2 and 3). In the case of the number of oviposition attempts, we found differences between treatments and fly species at both HMP concentrations (also the case with respect to the interaction between factors). Consistent with the fact that flies tested here exhibit different oviposition strategies in nature (i.e., *A. obliqua* invariably lays

TABLE 3. TWO-WAY ANOVA OF THE BEHAVIORAL RESPONSES EXHIBITED BY *A. ludens*, *A. obliqua*, AND *A. serpentina* FEMALES WHEN FORAGING IN FIELD-CAGED MANGO TREES AND ENCOUNTERING ARTIFICIAL FRUIT (I.E., GREEN-COLORED AGAR SPHERES WRAPPED IN PARAFILM) COVERED WITH THEIR OWN AND HETEROSPECIFIC FECAL HOST MARKING PHEROMONE (HMP) EXTRACTS (VALUES ROUNDED TO SECOND DECIMAL)

Dependent variable	Source of variation	*df*	Sum of squares	Mean square	*F* value	*P* value
Tree residence time	HMP	3	253,603,018.41	84,534,339.47	174.8	<0.0001
	Species	2	363,233.01	181,616.50	0.4	0.69
	HMP * Species	6	24,686,197.26	4,114,366.21	8.5	<0.0001
	Residual	285	139,282,133.52	483,618.52		
Fruit residence time	HMP	3	300,068,550.08	100,022,850.02	64.5	<0.0001
	Species	2	7,318,537.71	3,659,268.85	2.4	0.10
	HMP * Species	6	23,081,114.72	3,846,852.45	2.5	0.02
	Residual	285	446,587,792.08	1,550,652.06		
Fruit visited	HMP	3	213.10	71.03	10.3	<0.0001
	Species	2	13.45	6.72	1.0	0.38
	HMP * Species	6	109.01	18.17	2.6	0.02
	Residual	285	1991.68	6.92		
Oviposition attempts	HMP	3	32,850.55	10,950.19	68.0	<0.0001
	Species	2	2105.08	1052.54	6.5	0.002
	HMP * Species	6	6143.73	1023.96	6.4	<0.0001
	Residual	285	45,912.23	161.1		
Ovipositions	HMP	3	1765.64	588.546	48.0	<0.0001
	Species	2	296.60	148.302	12.1	<0.0001
	HMP * Species	6	712.67	118.779	9.7	<0.0001
	Residual	285	3495.11	12.264		
Irritation index	HMP	3	0.35	0.115	31.3	<0.0001
	Species	2	0.01	0.002	0.6	0.52
	HMP * Species	6	0.01	0.002	0.6	0.69
	Residual	285	1.06	0.004		

HMP extracts tested at a concentration of 100 mg/ml.

eggs singly, whereas *A. ludens* and *A. serpentina* tend to do so in clutches), responses to the control treatment (unmarked fruit) varied among species (Figure 4). With respect to actual ovipositions (aculeus insertion followed by dragging), we found significant differences when comparing all four treatments at both HMP concentrations. We also detected differences between species, as

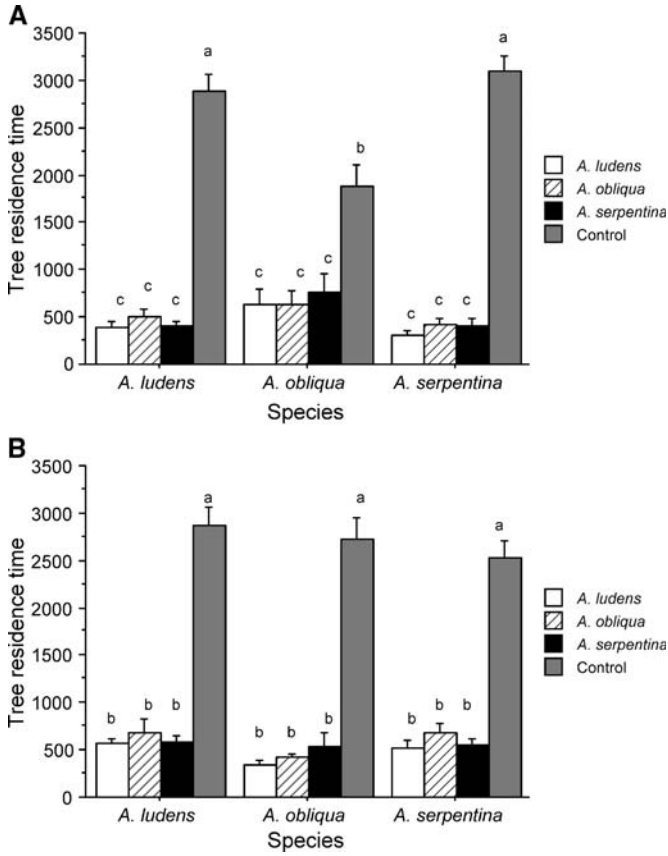

FIG. 1. Tree residence time (sec) (mean ± SE) exhibited by three *Anastrepha* species exposed to untreated and HMP-treated oviposition substrates. (A) HMP crude extract dissolved in H_2O at 100 mg/ml; (B) HMP crude extract dissolved in MeOH at 1 mg/ml. $N = 25$ in every bar. Means followed by the same letter are not significantly different (least square means *t*-test).

A. obliqua females oviposited more often than *A. ludens* and *A. serpentina* females (Figure 5). The interaction was significant at both concentrations (Tables 2 and 3). Remarkably, these responses were almost identical irrespective of HMP origin (least square means *t*-test; SAS, 2003). That is, there was complete interspecific HMP cross-recognition by all three *Anastrepha* species tested.

Finally, the irritability index was significantly higher in HMP-treated than in control (clean) fruit at both concentrations (Figure 6). *A. serpentina* females were significantly less irritated than females of the other two species when

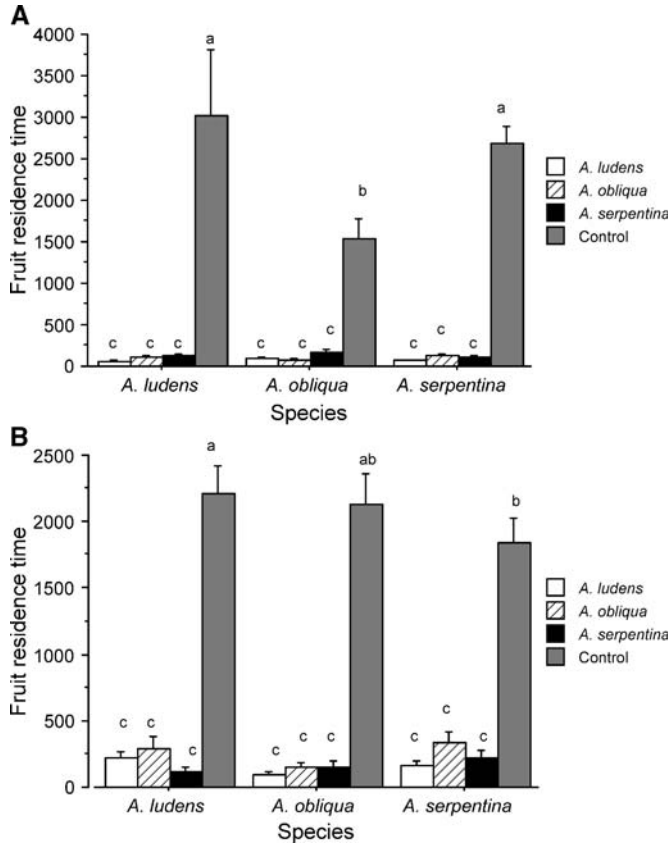

FIG. 2. Fruit residence time (sec) (mean ± SE) exhibited by three *Anastrepha* species exposed to untreated and HMP treated oviposition substrates. (A) HMP crude extract at 100 mg/ml; (B) HMP crude extract at 1 mg/ml. $N = 25$ in every bar. Means followed by the same letter are not significantly different (least square means *t*-test).

exposed to fruit covered with HMP at a concentration of 1 mg/ml, but not at a higher concentration. The interaction was not significant at either concentration (Tables 2 and 3).

DISCUSSION

We were able to document a clear pattern of HMP cross-species recognition between *A. ludens*, *A. obliqua*, and *A. serpentina*. Interestingly, although

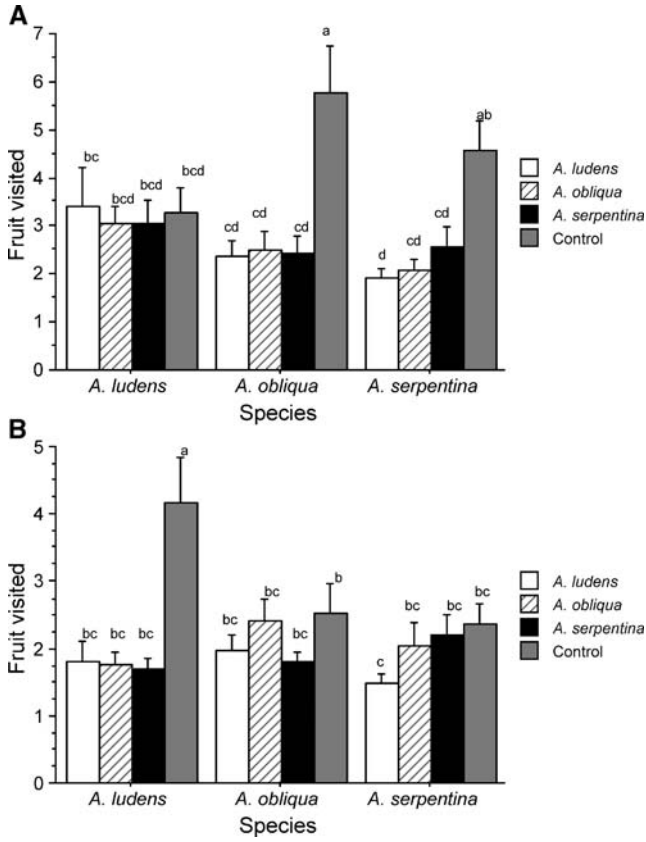

FIG. 3. Mean number (± SE) of host visited by three *Anastrepha* species exposed to untreated and HMP treated oviposition substrates. (A) HMP crude extract at 100 mg/ml; (B) HMP crude extract at 1 mg/ml. $N = 25$ in every bar. Means followed by the same letter are not significantly different (least square means *t*-test).

responses to fruit treated with the two HMP concentrations were practically identical among the three *Anastrepha* species (complete cross-recognition), when comparing the data on untreated, control fruit, clear behavioral differences were detected between the single egg/clutch layer *A. obliqua* and the multiple egg/clutch layers *A. ludens* and *A. serpentina* (discussion at the end of this section). Overall, the response pattern of females to HMP-marked and clean fruit closely resembled the one previously reported for *R. cerasi* (Aluja and Boller, 1992a and references therein). Results from the present study suggest that *Anastrepha* females increase patch persistence time when encountering unmarked hosts, but decrease search allocation following encounters with

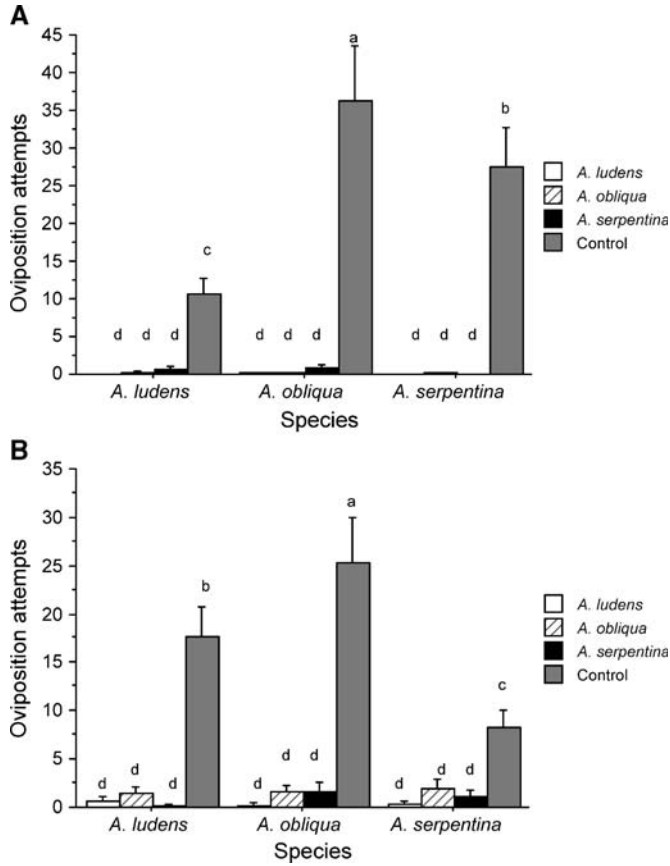

FIG. 4. Mean number (±SE) of oviposition attempts by three *Anastrepha* species exposed to untreated and HMP treated oviposition substrates. (A) HMP crude extract at 100 mg/ml; (B) HMP crude extract at 1 mg/ml. $N = 25$ in every bar. Means followed by the same letter are not significantly different (least square means *t*-test).

marked hosts. Furthermore, females not only increase host-searching time, but they also visited more hosts when they were unmarked. These responses are parallel to those reported by Prokopy et al. (1987) for *C. capitata* flies. Importantly, both HMP concentrations tested, although different by a hundred-fold factor, deterred oviposition in treated fruit. Flies respond strongly to the infochemical, even when present at low concentrations (1 mg/ml HMP in our case). Such a pattern of response to HMPs is similar to the one reported in other insects such as parasitoids (Bernstein and Driessen, 1996).

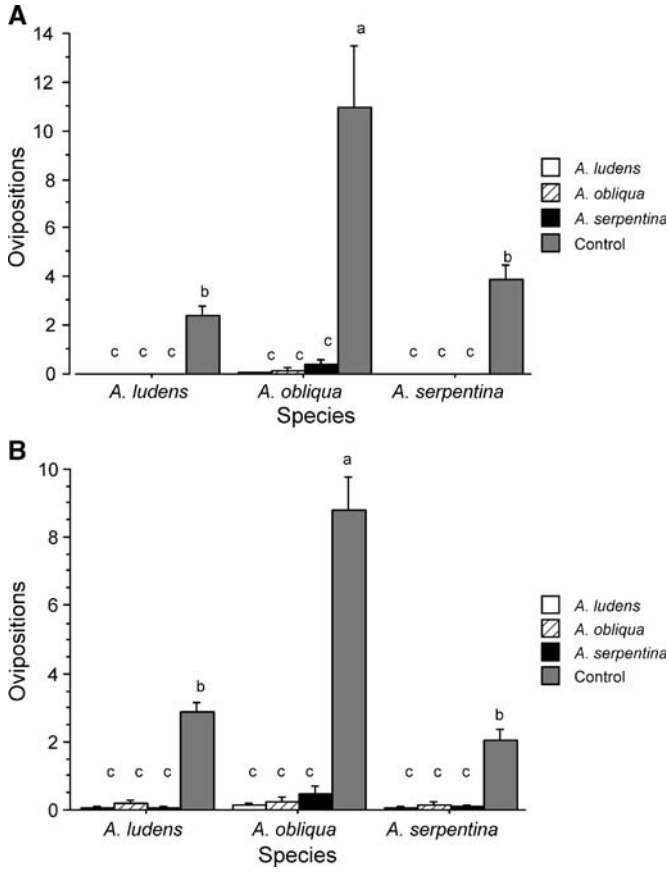

FIG. 5. Mean number (±SE) of ovipositions by females of three *Anastrepha* species exposed to untreated and HMP treated oviposition substrates. (A) HMP crude extract at 100 mg/ml; (B) HMP crude extract at 1 mg/ml. $N = 25$ in every bar. Means followed by the same letter are not significantly different (least square means *t*-test).

The fact that interspecific HMP cross-recognition exists in *Anastrepha* is particularly interesting given that individuals of the species studied rarely exploit the same resources in nature (Norrbom, 2003) and, at least in two cases, are not closely related according to the most recent phylogenies for the group (McPheron et al., 2000; Norrbom et al., 2000). Our prediction that due to a lineage effect there would be HMP cross-recognition between *A. ludens* and *A. obliqua* (*fraterculus* species group), but not *A. serpentina* (*serpentina* species group), was not supported. This finding, together with additional data (M. Aluja and F. Díaz-Fleischer, unpublished data) suggests the possibility of shared

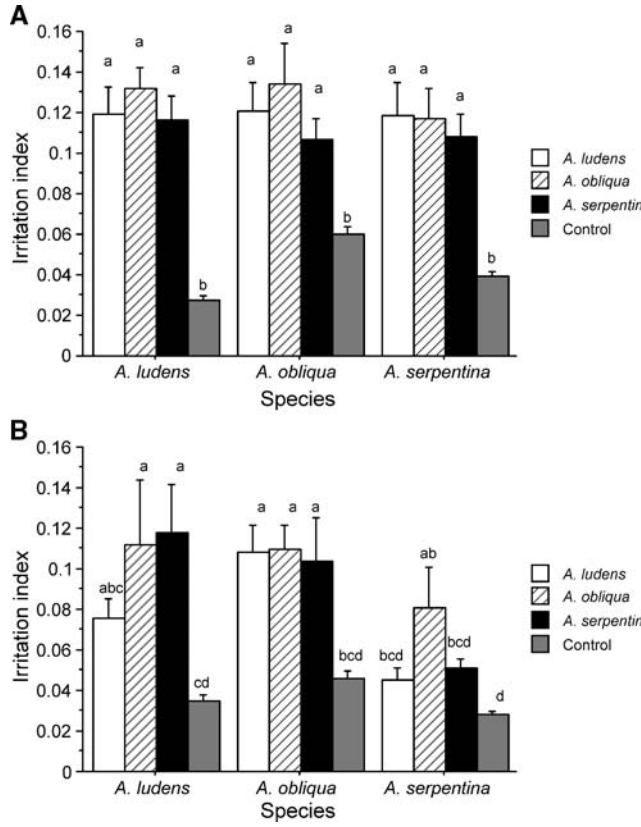

FIG. 6. Mean irritation index (±SE) exhibit by females of three *Anastrepha* species exposed to untreated and HMP treated oviposition substrates. (A) HMP crude extract at 100 mg/ml; (B) HMP crude extract at 1 mg/ml. $N = 25$ in every bar. Means followed by the same letter are not significantly different (least square means *t*-test).

active molecules in the HMP of all *Anastrepha* species). As only the HMP of *A. ludens* has been described (Aluja et al., 2003), it is too early to postulate meaningfully whether this pattern can be explained by convergent evolution or represents a plesiomorphy or synapomorphy. Our results differ from those for other tephritid flies (various species of the genus *Rhagoletis*), as Prokopy et al. (1976) found that "individuals from different species belonging to different species groups did not recognize each other's marking pheromones'' and that "different species within the same species group varied in reaction from complete to no cross-recognition.'' However, it is important to note that these differences could be possibly related to bioassay conditions. Prokopy et al.

(1976) worked under artificial laboratory conditions using real fruit marked by live females (resulting in a variable HMP concentration and a possible effect of fruit volatiles on fly response), while we exposed live females to known concentrations of HMP extracts applied on artificial hosts in a field cage containing abundant foliage and foraging space. Thus, these different approaches could affect results, as deterrent activity of HMPs is dose-dependent (Bowdan, 1984; Schoonhoven, 1990; Papaj and Aluja, 1993), and varying numbers of natural marks are needed to obtain similar discrimination coefficients, at least when comparing different *Anastrepha* species (M. Aluja and F. Díaz-Fleischer, unpublished data).

The HMP cross-recognition pattern we report for *A. ludens*, *A. obliqua*, and *A. serpentina* is similar to several cases seen in Lepidoptera. In one case, two species within the same genus were involved (*Pieris rapae* L. and *P. brassicae* L.), with females of *P. rapae* recognizing the crude pheromone extracts of *P. brassicae* (Schoonhoven, 1990; Schoonhoven et al., 1990). In another case, a number of tortricid species from different genera, *Lobesia botrana* (Denis & Schiffermüller), *Eupoecilia ambiguella* (Hübner), *Cydia molesta* (Busck), and *C. pomonella* L., also showed cross-recognition (Thiery and Gabel, 1993; Gabel and Thiery, 1994). In the latter case, Thiery et al. (1992, 1995) and Gabel and Thiery (1996) suggested that a mixture of straight chain fatty acids and esters of fatty acids were most likely responsible for the observed deterrent effect, and the same mechanism may be working in *R. cerasi* and *A. ludens,* as the HMP of these two fruit fly species also contain long chain fatty acids (Hurter et al., 1987; Aluja et al., 2003).

Thiery and Gabel (1993) proposed the term "oviposition regulating synomone'' for molecules or blends that influence the interspecific spacing of eggs when females of different species compete for the same oviposition resources in nature. However, in the genus *Anastrepha*, this condition only occurs occasionally. For example, *A. striata* and *A. fraterculus* may compete for various species of fruit in the genus *Psidium* (*P. guajava*, *P. sartorianum*, *P. guineense*) (Aluja et al., 2000b; Sivinski et al., 2004). Consequently, one must ask why is there such strong interspecific HMP recognition between *A. ludens*, *A. obliqua*, and *A. serpentina* when they rarely share the same oviposition resources in nature. As mentioned earlier, one explanation is the presence of shared active molecules in the HMP of all *Anastrepha* species. All HMP may contain substances intimately related to oogenesis (as they are only released by sexually mature females and not by males; Boller and Hurter, 1985; Städler et al., 1992), and could explain, in part, the presence of HMP in feces of species that do not exhibit marking behavior (Aluja et al., 2003; M. Aluja and F. Díaz-Fleischer, unpublished data). HMP is secreted into and accumulated in the gut lumen, to be released with other gut contents in both the marking trail and feces (Prokopy et al., 1982b). The fact that host marking is present in several unrelated groups of insects (Diptera, Lepidoptera, Coleoptera) and that

HMPs may have similar components (fatty acids in Diptera and Lepidoptera) suggest that this could reflect an ancestral physiological process that has remained unchanged over evolutionary time (a plesiomorphy). Such speculation is supported by the fact that, at least in the case of *R. cerasi*, HMP is related to cerebrosides and gangliosides, compounds found in nerve cell membranes (Hurter et al., 1987). Differences in the life histories of species in the genera *Rhagoletis* and *Anastrepha* may also help explain why the chemical structures of their HMPs differ, as most *Rhagoletis* species are monophagous and univoltine (Zwölfer, 1983; Prokopy and Papaj, 2000), whereas the *Anastrepha* species under study here are polyphagous and multivoltine (Aluja et al., 2000a). Thus, digestive processes used to deal with fruit defenses may be quite different as specialist insects develop specific adaptations to confront plant defenses (Lambdon, 2001). If this is the case then, the specialization in *Rhagoletis* flies could have resulted in the HMP molecule, purportedly a by-product of the digestive process, becoming more complex than that observed in *Anastrepha*.

Anastrepha obliqua, an egg-limited species (Aluja et al., 2001; Díaz-Fleischer and Aluja, 2003b), exhibited more oviposition attempts and had more oviposition bouts than either *A. ludens* or *A. serpentina*, two time-limited species (Figures 4 and 5). These behaviors are probably adaptations to cope with highly ephemeral resources [fruit that appear and disappear (fall to the ground) within 4 wks] and could partly explain why *A. obliqua* exhibited a high and uniform irritation index when exposed to low concentrations of all three pheromone sources (it is highly sensitive to fruit quality) (Figure 6).

HMPs have potential as a means of control of insect pests, as shown for *R. cerasi* (Aluja and Boller, 1992b; Aluja et al., 2003). Our present results show that, there is a possibility that one synthetic HMP could offer a viable alternative to the widespread use of insecticides for the management of all three *Anastrepha* species examined in this study. However, when designing deployment strategies, one must remember that HMPs evolved as messages not deterrents (Roitberg and Prokopy, 1987; Schoonhoven, 1990). Consequently, females deprived of suitable oviposition sites may end up exploiting available fruit even in the presence of HMPs (i.e., there is no absolute oviposition deterrence). Furthermore, and as documented by Quiring and McNeil (1984) and Averill and Prokopy (1987), heavy rains can potentially reduce efficacy due to wash off given the water-soluble nature of the substance (but see Katsoyannos and Boller, 1976, 1980), and plants along crop edges should remain unsprayed to maintain high response thresholds to the HMP. Additional research is required to exploit the full potential of this management tool.

Acknowledgments—We are indebted to Jesús Reyes-Flores, former director of the Mexican Campaña Nacional Contra Moscas de la Fruta for encouraging us to conduct this research and for facilitating our research. We thank S. Aceituno, J. Arredondo, J.L. Márquez, and F. Avendaño (all

Programa MoscaMed, Tapachula, Chiapas, Mexico) for expert assistance during field collections, fly feces collections and maintenance of study insects. We thank Walther Enkerlin, Dina Orozco, Juan Rull, Carlos Fredersdorff, and Pablo Montoya (all Programa MoscaMed, Tapachula, Chiapas, Mexico) for providing materials, laboratory equipment, and providing critical administrative support. Javier Valle Mora (El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico) offered statistical advice and Nicoletta Righini (Instituto de Ecología, A.C.) helped during the writing and formatting stages. Finally, we acknowledge the insightful reviews by the two referees and an associate editor. This work was principally financed by the Mexican Campaña Nacional Contra Moscas de la Fruta (Secretaría de Agricultura, Ganadería, Desarrollo Rural y Pesca–Instituto Interamericano de Cooperación para la Agricultura [SAGARPA–IICA]). We also received a series of anonymous donations.

REFERENCES

ALUJA, M. 1994. Bionomics and management of *Anastrepha*.. *Annu. Rev. Entomol.* 39:155–178.

ALUJA, M. and BIRKE, A. 1993. Habitat use by *Anastrepha obliqua* flies (Diptera: Tephritidae) in a mixed mango and tropical plum orchard. *Ann. Entomol. Soc. Am.* 86:799–812.

ALUJA, M. and BOLLER, E. F. 1992a. Host marking pheromone of *Rhagoletis cerasi*: foraging behavior in response to synthetic pheromonal isomers. *J. Chem. Ecol.* 18:1299–1311.

ALUJA, M. and BOLLER, E. F. 1992b. Host marking pheromone of *Rhagoletis cerasi*: field deployment of synthetic pheromone as a novel cherry fruit fly management strategy. *Entomol. Exp. Appl.* 65:141–147.

ALUJA, M. and PROKOPY, R. J. 1993. Host odor and visual stimulus interaction during intratree host finding behavior of *Rhagoletis pomonella* flies. *J. Chem. Ecol.* 19:2671–2696.

ALUJA, M., GUILLÉN, J., DE LA ROSA, G., CABRERA, M., CELEDONIO, H., LIEDO, P., and HENDRICHS, J. 1987. Natural host plant survey of the economically important fruit flies (Diptera: Tephritidae) of Chiapas, Mexico. *Fla. Entomol.* 70:329–338.

ALUJA, M., PROKOPY, R. J., ELKINTON, J. S., and LAURENCE, F. 1989. Novel approach for tracking and quantifying the movement patterns of insects in three dimensions under seminatural conditions. *Environ. Entomol.* 18:1–7.

ALUJA, M., CELEDONIO-HURTADO, H., LIEDO, P., CABRERA, M., CASTILLO, F., GUILLÉN, J., and RIOS, E. 1996. Seasonal population fluctuations and ecological implications for management of *Anastrepha* fruit flies (Diptera: Tephritidae) in commercial mango orchards in Southern Mexico. *J. Econ. Entomol.* 89:654–667.

ALUJA, M., PIÑERO, J., JÁCOME, I., DÍAZ-FLEISCHER, F., and SIVINSKI, J. 2000a. Behavior of flies in the genus *Anastrepha* (Trypetinae: Toxotrypanini), pp. 375–406, *in* M. Aluja and A. Norrbom (eds.). Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL, USA.

ALUJA, M., PIÑERO, J., LÓPEZ, M., RUÍZ, C., ZÚÑIGA, A., PIEDRA, E., DÍAZ-FLEISCHER, F., and SIVINSKI, J. 2000b. New host plant and distribution records in Mexico for *Anastrepha* spp., *Toxotrypana curvicauda* Gerstacker, *Rhagoletis zoqui* Bush, *Rhagoletis* sp., and *Hexachaeta* sp. (Diptera: Tephritidae). *Proc. Entomol. Soc. Wash.* 102:802–825.

ALUJA, M., DÍAZ-FLEISCHER, F., PAPAJ, D. R., LAGUNES, G., and SIVINSKI, J. 2001. Effects of age, diet, female density and the host resource on egg load in *Anastrepha ludens* and *Anastrepha obliqua* (Diptera: Tephritidae). *J. Insect Physiol.* 47:975–988.

ALUJA, M., DÍAZ-FLEISCHER, F., EDMUNDS, A. J. F., and HAGMANN, L. 2003. Isolation, structural determination, synthesis,biological activity and application as control agent of the host marking pheromone (and derivatives thereof) of the fruit flies of the type *Anastrepha* (Diptera: Tephritidae). United States Patent No. US 6,555,120 B1. 30 p .

AVERILL, A. L. and PROKOPY, R. J. 1987. Residual activity of oviposition-deterring pheromone in *Rhagoletis pomonella* (Diptera: Tephritidae) and female response to infested fruit. *J. Chem. Ecol.* 13:167–177.

BERNSTEIN, C. and DRIESSEN, G. 1996. Patch-marking and optimal patterns in the parasitoid *Venturia canescens*. *J. Anim. Ecol.* 65:211–219.

BOLLER, E. F. 1968. An artificial egging device for the European cherry fruit fly *Rhagoletis cerasi*. *J. Econ. Entomol.* 61:850–852.

BOLLER, E. F. and HURTER, J. 1985. Oviposition deterring pheromone in *Rhagoletis cerasi*: Behavioral laboratory test to measure pheromone activity. *Entomol. Exp. Appl.* 39:163–169.

BOLLER, E. F., SCHONI, R., and BUSH, G. L. 1987. Oviposition deterring pheromone in *Rhagoletis cerasi*: Biological activity of a pure single compound verified in semi-field test. *Entomol. Exp. Appl.* 45:17–22.

BOWDAN, E. 1984. Electrophysiological responses of tarsal contact chemoreceptors of the apple maggot fly *Rhagoletis pomonella* to salt, sucrose and oviposition-deterrence pheromone. *J. Comp. Physiol A.* 154:143–152.

CHAMBERS, D. L., CALKINS, C. O., BOLLER, E. F., ITÔ, Y., and CUNNINGHAM, R. T. 1983. Measuring, monitoring and improving the quality of mass-reared Mediterranean fruit flies, *Ceratitis capitata* (Wied.). *Z. Angew. Entomol.* 95:285–303.

CIRIO, U. 1971. Reperti sul meccanismo stimolo-risposta nell'ovideposizione del *Dacus oleae* (Gmelin) (Diptera: Trypetidae). *Redia* 52:577.

DÍAZ-FLEISCHER, F. and ALUJA, M. 2000. Behavior of tephritid flies: A historical perspective, pp. 39–69, *in* M. Aluja and A. Norrbom (eds.). Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL, USA.

DÍAZ-FLEISCHER, F. and ALUJA, M. 2003a. Clutch size in frugivorous insects as a function of host firmness: The case of the tephritid fly *Anastrepha ludens*. *Ecol. Entomol.* 28:268–277.

DÍAZ-FLEISCHER, F. and ALUJA, M. 2003b. Behavioral plasticity in relation to egg and time limitation: The case of two fly species in the genus *Anastrepha* (Diptera: Tephritidae). *Oikos* 100:125–133.

DÍAZ-FLEISCHER, F., PAPAJ, D. R., PROKOPY, R. J., NORRBOM, A. L., and ALUJA, M. 2000. Evolution of fruit fly oviposition behavior, pp. 811–841, *in* M. Aluja and A. Norrbom (eds.). Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL, USA.

DÍAZ-FLEISCHER, F., ALUJA, M., HURTER, J., ENKERLIN, W., and BOLLER, E. F. 2004. Propiedades físico-químicas de la feromona marcadora de hospedero (FMH) de tres especies de moscas de la fruta del género *Anastrepha* (Diptera: Tephritidae). *Folia Entomol. Mex.* 43:43–53.

FREEMAN, R. and CAREY, J. R. 1989. Interaction of host stimuli in the oviposition response of the Mediterranean fruit fly (Diptera: Tephritidae). *Environ. Entomol.* 19:1075–1080.

GABEL, B. and THIERY, D. 1994. Semiochemicals from *Lobesia botrana* (Lepidoptera: Tortricidae) eggs deter oviposition of the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *Eur. J. Entomol.* 91:353–359.

GABEL, B. and THIERY, D. 1996. Oviposition response of *Lobesia botrana* females to long chain free fatty acids and esters from its eggs. *J. Chem. Ecol.* 22:161–171.

GIROLAMI, V., VIANELLO, A., STRAPAZZON, A., RAGAZZI, E., and VERONESE, G. 1981. Ovipositional deterrents in *Dacus oleae*. *Entomol. Exp. Appl.* 29:177–188.

HERNÁNDEZ-ORTÍZ, V. and ALUJA, M. 1993. Listado de especies del género neotropical *Anastrepha* (Diptera: Tephritidae) con notas sobre su distribución y plantas hospederas. *Folia Entomol. Mex.* 88:89–105.

HURTER, J., BOLLER, E. F., STÄDLER, E., BLATTMAN, H., BUSER, R., BOSSHARD, N. U., DAMM, L., KOZLOWSKI, M. W., SCHONI, R., RASCHDORF, F., DAHINDEN, R., SCHLUMPF, E., FRITZ, H., RICHTER, W. J., and SCHREIBER, J. 1987. Oviposition-deterring pheromone in *Rhagoletis cerasi* L.: Purification and determination of the chemical constitution. *Experientia* 43:157–164.

JÁCOME, I., ALUJA, M., and LIEDO, P. 1999. Impact of adult diet on demographic and population parameters in the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). *Bull. Entomol. Res.* 89:165–175.

JAENIKE, J. and PAPAJ, D. R. 1992. Behavioral plasticity and patterns of host use by insects, pp. 245–264, *in* B. D. Roitberg and M. B. Isman (eds.). Insect Chemical Ecology: An Evolutionary Approach. Chapman and Hall, New York, USA.

KATSOYANNOS, B. I. 1975. Oviposition-deterring, male arresting, fruit marking pheromone in *Rhagoletis cerasi* L. *Environ. Entomol.* 4:801–807.

KATSOYANNOS, B. I. and BOLLER, E. F. 1976. First field application of oviposition-deterring pheromone of European cherry fruit fly, *Rhagoletis cerasi*. *Environ. Entomol.* 5:151–152.

KATSOYANNOS, B. I. and BOLLER, E. F. 1980. Second field application of oviposition-deterring pheromone of European cherry fruit fly, *Rhagoletis cerasi*. *Z. Angew. Entomol.* 89:278–281.

LALONDE, R. G. and ROITBERG, B. D. 1992. Host selection behavior of the thistle-feeding fly: Choices and consequences. *Oecologia* 90:534–539.

LAMBDON, P. W. 2001. Do plant toxins impose constraints on herbivores? An investigation using compartmental analysis. *Oikos* 93:168–176.

MCPHERON, B. A., HAN, H.-Y., SILVA, J. G., and NORRBOM, A. L. 2000. Phylogeny of the genera *Anastrepha* and *Toxotrypana* (Trypetinae: Toxotrypanini) based upon 16S rRNA mitochondrial DNA sequences, pp. 343–361, *in* M. Aluja and A. Norrbom (eds.). Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL, USA.

NORRBOM, A. L. 2002. A revision of the *Anastrepha serpentina* species group (Diptera: Tephritidae). *Proc. Entomol. Soc. Wash.* 104:390–436.

NORRBOM, A. L. 2003. Host plant database for *Anastrepha* and *Toxotrypana* (Diptera: Tephritidae: Toxotrypanini), Diptera Data Dissemination Disk (CD-ROM). North American Dipterist's Society, Washington DC.

NORRBOM, A. L., ZUCCHI, R. A., and HERNÁNDEZ-ORTÍZ, V. 2000. Phylogeny of the genera *Anastrepha* and *Toxotrypana* (Trypetinae: Toxotrypanini) based on morphology, pp. 299–342, *in* M. Aluja and A. Norrbom (eds.). Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL, USA.

NUFIO, C. R. and PAPAJ, D. R. 2001. Host marking behavior in phytophagous insects and parasitoids. *Entomol. Exp. Appl.* 99:273–293.

O'BRIEN, R. G. and KAISER, M. K. 1985. MANOVA method for analyzing repeated measures designs and extensive primer. *Psychol. Bull.* 97:316–333.

PAPAJ, D. R. and ALUJA, M. 1993. Temporal dynamics of host-marking in the tropical tephritid fly, *Anastrepha ludens*. *Physiol. Entomol.* 18:279–284.

PAPAJ, D. R., ROITBERG, B. D., and OPP, S. B. 1989. Serial effects of host infestation on egg allocation by the Mediterranean fruit fly: A rule of thumb and its functional significance. *J. Anim. Ecol.* 58:955–970.

PAPAJ, D. R., ROITBERG, B. D., OPP, S. B., ALUJA, M., PROKOPY, R. J., and WONG, T. T. Y. 1990. Effect of marking pheromone on clutch size in the Mediterranean fruit fly. *Physiol. Entomol.* 15:463–468.

PAPAJ, D. R., AVERILL, A. L., PROKOPY, R. J., and WONG, T. T. Y. 1992. Host-marking pheromone and use of previously established oviposition sites by the Mediterranean fruit fly (Diptera: Tephritidae). *J. Ins. Behav.* 5:583–598.

PAPAJ, D. R., GARCÍA, J. M., and ALONSO-PIMENTEL, H. 1996. Marking of host fruit by male *Rhagoletis boycei* Cresson flies (Diptera: Tephritidae) and its effect on egg-laying. *J. Insect Behav.* 9:585–598.

PITTARA, K. and KATSOYANNOS, B. I. 1990. Evidence for a host-marking pheromone in *Chaetorellia australis*. *Entomol. Exp. Appl.* 54:287–295.

PROKOPY, R. J. 1972. Evidence for a pheromone deterring repeated oviposition in apple maggot flies. *Environ. Entomol.* 1:326–332.

PROKOPY, R. J. 1981. Epideictic pheromones that influence spacing patterns of phytophagous insects, pp. 181–213, *in* D. A. Nordlund, R. L. Jones, and W. J. Lewis (eds.). Semiochemicals: Their Role in Pest Control. Wiley, New York.

PROKOPY, R. J. and BUSH, G. L. 1972. Mating behavior in *Rhagoletis pomonella*. III. Male aggregation in response to an arrestant. *Can. Entomol.* 104:275–283.

PROKOPY, R. J. and PAPAJ, D. R. 2000. Behavior of flies of the genera *Rhagoletis, Zonosemata*, and *Carpomya* (Trypetinae: Carpomyina), pp. 219–252, *in* M. Aluja and A. Norrbom (eds.). Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL, USA.

PROKOPY, R. J., REISSIG, W. H., and MOERICKE, V. 1976. Marking pheromone deterring repeated oviposition in *Rhagoletis* flies. *Entomol. Exp. Appl.* 20:170–178.

PROKOPY, R. J., GREANY, P. D., and CHAMBERS, D. L. 1977. Oviposition deterring pheromone in *Anastrepha suspensa*. *Environ. Entomol.* 6:463–465.

PROKOPY, R. J., MALAVASI, A., and MORGANTE, J. S. 1982a. Oviposition deterring pheromone in *Anastrepha fraterculus* flies. *J. Chem. Ecol.* 8:763–771.

PROKOPY, R. J., AVERILL, A. L., BARDINELLI, C. M., BOWDAN, E. S., COOLEY, S. S., CRNJAR, R. M., DUNDULIS, E. A., ROITBERG, C. A., SPATCHER, P. J., TUMLINSON, J. H., and WEEKS, B. L. 1982b. Site of production of an oviposition deterring pheromone component in *Rhagoletis pomonella* flies. *J. Insect Physiol.* 28:1–10.

PROKOPY, R. J., PAPAJ, D. R., OPP, S. B., and WONG, T. T. Y. 1987. Intra-tree foraging behavior of *Ceratitis capitata* flies in relation to host fruit density and quality. *Ent. Exp. Appl.* 45:251–258.

QUIRING, D. T. and MCNEIL, J. N. 1984. Intraspecific competition between different aged larvae of *Agromyza frontella* (Rondani) (Diptera: Agromyzidae): Advantages of an oviposition-deterring pheromone. *Can. J. Zool.* 62:2192–2196.

ROITBERG, B. D. and PROKOPY, R. J. 1981. Experience required for pheromone recognition in the apple maggot fly. *Nature* 292:540–541.

ROITBERG, B. D. and PROKOPY, R. J. 1983. Host deprivation influence on response of *Rhagoletis pomonella* to its oviposition deterring pheromone. *Physiol. Entomol.* 8:69–72.

ROITBERG, B. D. and PROKOPY, R. J. 1984. Host visitation sequence as a determinant of search persistence in fruit parasitic tephritid flies. *Oecologia* 62:7–12.

ROITBERG, B. D. and PROKOPY, R. J. 1987. Insects that mark host plants. *BioScience* 37:400–406.

ROITBERG, B. D., VAN LENTEREN, J. C., VAN ALPHEN, J. J. M., GALIS, F., and PROKOPY, R. J. 1982. Foraging behavior of *Rhagoletis pomonella*, a parasite of hawthorn. *J. Anim. Ecol.* 51:307–325.

SAS INSTITUTE INC., 2003. JMP version 5.0.1.2 Cary, NC, USA.

SCHOONHOVEN, L. M. 1990. Host marking pheromones in Lepidoptera, with special references to two *Pieris* spp. *J. Chem. Ecol.* 16:3043–3052.

SCHOONHOVEN, L. M., BEERLING, E. A. M., KLIJNNSTRA, J. W., and VAN VUGGT, Y. 1990. Two related butterfly species avoid oviposition near each other's eggs. *Experientia* 46:526–528.

SIVINSKI, J., ALUJA, M., PIÑERO, J., and OJEDA, M. 2004. Novel analysis of spatial and temporal patterns of resource use in a group of tephritid flies of the genus *Anastrepha*. *Ann. Entomol. Soc. Am.* 97:504–512.

STÄDLER, E., SCHONI, R., and KOZLOWSKI, M. W. 1987. Relative air humidity influences the function of the tarsal chemoreceptor cells of the cherry fruit fly (*Rhagoletis cerasi*). *Physiol. Entomol.* 12:339–346.

STÄDLER, E., ERNST, B., HURTER, J., BOLLER, E., and KOZLOWSKI, M. W. 1992. Tarsal contact chemoreceptors of the cherry fruit fly, *Rhagoletis cerasi*: Specificity, correlation with oviposition behavior, and response to the synthetic pheromone, pp. 143–146, *in* S. B. J. Menken, and P. Harrewijin (eds.). Proc. 8th Int. Symp. Insect–Plant Relationships. Kluwer Academic Publishers, Dordrecht.

STRAW, N. A. 1989. Evidence for an oviposition-deterring pheromone in *Tephritis bardanae* (Schrank) (Diptera: Tephritidae). *Oecologia* 78:121–130.

THIERY, D. and GABEL, B. 1993. Inter-specific avoidance of egg-associated semiochemicals in four torticids. *Experientia* 49:998–1001.

THIERY, D., GABEL, B., FALRKAS, P., and PRONIER, V. 1992. Identification of an ovipostion-regulating pheromone in the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae). *Experientia* 48:697–699.

THIERY, D., GABEL, B., FARKAS, P., and JARRY, M. 1995. Egg dispersion in codling moth: Influence of egg extract and of its fatty acid constituents. *J. Chem. Ecol.* 21:2015–2026.

ZWÖLFER, H. 1983. Life systems and strategies of resource exploitation in tephritids, pp. 16–30, *in* R. Cavalloro (ed.). Fruit Flies of Economic Importance. Balkema, Rotterdam.