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Research article

Plant Resistance

Effect of Resin Ducts and Sap Content on Infestation and Development of Immature Stages of *Anastrepha obliqua* and *Anastrepha ludens* (Diptera: Tephritidae) in Four Mango (Sapindales: Anacardiaceae) Cultivars

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Abstract

We determined the influence of resin ducts, sap content, and fruit physicochemical features of four mango cultivars (Criollo, Manila, Ataulfo, and Tommy Atkins) on their susceptibility to the attack of the two most pestiferous fruit fly species infesting mangoes in Mexico: *Anastrepha ludens* (Loew) and *Anastrepha obliqua* (Macquart). We performed three studies: 1) analysis of resin ducts in mango fruit exocarp to determine the density and area occupied by resin ducts in each mango cultivar, 2) assessment of mango physicochemical features including fruit sap content, and 3) a forced infestation trial under field conditions using enclosed fruit-bearing branches to expose mangoes to gravid *A. ludens* or *A. obliqua* females. Infestation rates, development time from egg to prepupae and pupae, pupal weight, and percent of adult emergence, were assessed. 'Ataulfo' and 'Tommy Atkins' cultivars exhibited the highest resin duct density and sap content, the lowest infestation rate, and had a negative effect on immature development and pupal weight. In sharp contrast, 'Manila' and 'Criollo' cultivars, with the lowest resin duct density and sap content, were highly susceptible to *A. ludens* and *A. obliqua* attack. We conclude that sap content and the number, size, and distribution of resin ducts as well as firmness in mango fruit exocarp are all involved in the resistance of mango to *A. ludens* and *A. obliqua* attack.

Key words: plant resistance, fruit fly, Tephritidae, host status, constitutive defense

Host plant defense against phytophagous insects include both constitutive and induced defensive mechanisms. The former consists of physical and chemical barriers like waxes, trichomes, resin, or latex, whereas the latter includes the synthesis of toxic or antifeedant secondary metabolites in response to insect attack (Agrawal 2007, Howe and Jander 2008). Many wild and cultivated plant species exhibit both defensive mechanisms. For example, resins stored under pressure in plant secretory ducts can mechanically defend plants (e.g., imped insect movement or cause death by asphyxiation) and can act as toxic or antifeedant chemicals (Fahn 1979, Becerra et al. 2001).

Tephritid fruit flies (Diptera: Tephritidae) are major pests of fruit plants of economic importance worldwide (White and Elson-Harris 1992). It has been suggested that physical and chemical traits that vary widely among fruit cultivars are closely associated with resistance to tephritid fly infestation (Aluja and Mangan 2008). For example, the role of secondary metabolites on host resistance to fruit fly attack has been reported among cultivars for tomatoes (*Solanum lycopersicum* L.) infested by *Bactrocera tryoni* (Froggatt) (Balagawi et al. 2005), apples (*Malus domestica* Borkh.) infested by *Anastrepha ludens* (Loew) (Aluja et al. 2014a), olives (*Olea europaea* L.) attacked by *Bactrocera oleae* (Gmelin) (Burrack and Zalom 2008), and mangoes (*Mangifera indica* L.) infested by *Bactrocera dorsalis* Hendel (Verghese et al. 2012). Physical (e.g., fruit thickness, hardness) and chemical (e.g., sugar concentration) characteristics among cultivars have been evaluated and correlated with fruit fly infestation in the cases of walnut (*Juglans regia* L.) with *Rhagoletis completa* Cresson (Guillén et al. 2011), and mangoes with *B. dorsalis* Hendel (Rattanapun et al. 2009), *Ceratitis capitata* Wiedemann (Joel 1980), *A. ludens* (Loew), and *Anastrepha obliqua* (Macquart) (Aluja et al. 2014b).

Mangoes (Sapindales: Anacardiacea) are one of the most important tropical commercial fruit, extensively grown in Latin American, African, and Asian countries (Litz 2009). Several fruit fly species, including *A. obliqua*, *A. ludens*, *Bactrocera frauenfeldi* Schiner, *B. dorsalis*, *Ceratitis cosyra* (Walker), and *C. capitata*, attack mangoes causing serious economic losses (White and Elson-Harris 1992, Aluja and Mangan 2008, Badii et al. 2015). *Anastrepha obliqua*, the West Indian fruit fly, and *A. ludens*, the Mexican fruit fly, are important mango pests in Mexico and Central America (Jirón and Hëdstrom 1991, Aluja et al. 1996, Niklaus–Ruiz Borge and Basedow 1997), and in the case of *A. obliqua*, also in South America (Carrejo and González 1994, Korytkowski 2001, Martínez and Serna 2005). Both *A. ludens* and *A. obliqua* are polyphagous, attacking fruit of economic and noneconomic significance, but have different host fruit preferences (Norrbom and Kim 1988, Hernández-Ortíz and Aluja 1993, Aluja 1994). They also differ in that *A. ludens* lay eggs in clutches of up to 40 eggs, whereas *A. obliqua* invariably lay a single egg per oviposition bout (Aluja et al. 2000a,b; Díaz-Fleischer and Aluja 2003a).

Integrated pest management (IPM) approaches for fruit fly control combines different compatible techniques such as chemical, biological, microbial, and cultural control; quarantine treatments; the Sterile Insect Technique; use of bait station in massive trapping (Aluja et al. 2009, Ndiaye et al. 2008, Badii et al. 2015); and to a lesser extent, the use of resistant cultivars (Aluja and Rull 2009, Peña et al. 2009).

Mangoes seem to be constitutively defended against fruit fly attack through a complex resin secretory system in the exocarp (peel) that mechanically (Joel 1980), and probably, chemically defend the fruit. The mango resin duct system has an unusual structure, with a network of ducts branching and anastomosing in all directions (Joel 1978). According to Joel (1980), each mango cultivar has a typical resin duct system rendering some cultivars more resistant than others. With respect to the resin concentration, Joel (1980) found it to drop drastically when the fruit reaches maturity and this may reduce its effectiveness against fruit fly attack. Correlation between resistance to fruit fly attack and degree of fruit ripeness has been reported for several fruit such as mangoes, citrus, olives, and guavas (Burrack and Zalom 2008, Greany et al. 1985, Greany 1989, Díaz-Fleischer and Aluja 2003b, Birke and Aluja 2011).

Despite the economic importance of mangoes, few studies have examined the influence of exocarp features in the resistance to insect attack. To address this issue, we used mango cultivars 'Criollo', 'Manila', 'Ataulfo', and 'Tommy Atkins' to analyze the influence of the number and area occupied by resin ducts in the fruit exocarp, sap volume, fruit weight, and firmness on infestation by *A. ludens* and *A. obliqua*, and their effects on flies offspring performance. We chose these cultivars, as we had previously shown that two exhibit resistance to these *Anastrepha* species ('Ataulfo' and 'Tommy Atkins') and two were susceptible to their infestation ('Criollo' and 'Manila').

Materials and Methods

Study Insects

Anastrepha ludens were obtained from naturally infested grapefruit collected in Rancho la Palmilla (20° 0′32.03″ N, 97° 10′49.97″ W; 256 masl), Tlapacoyan, Veracruz, Mexico, and *A. obliqua* from naturally infested hog plums, *Spondias* sp. (Anacardiaceae), collected in Salto de Eyipantla (18° 23′16.01″ N, 95° 12′8.38″ W; 195 masl) and Sihuapan (18° 26′13.35″ N, 95° 10′42.00″ W; 258 masl), San Andrés Tuxtla, Veracruz, Mexico. Infested fruit were taken to the Red de Manejo Biorracional de Plagas y Vectores (RMBPV) laboratory at the Instituto de Ecología, A. C. (INECOL) in Xalapa, Veracruz, Mexico, where they were placed in perforated plastic trays on top of plastic washbowls containing vermiculite as a pupation medium. Vermiculite was inspected every second day to search for pupae; recovered pupae were placed inside a 250-ml labeled plastic container with vermiculite which was covered with organza fabric. Pupae were held at 27 ± 1°C, 75 ± 5% relative humidity (RH), and photoperiod of 12:12 (L:D) h. Pupae were moistened as needed to prevent desiccation with a 0.2% (wt/vol) sodium benzoate solution until adult emergence. Once emerged, sets of 30 females and 15 males of *A. obliqua* or *A. ludens* were placed in 30- by 30- by 30-cm plexiglass cages and fed ad libitum with a 3:1 mixture of sugar and yeast hydrolysate and water offered in a plastic container with a piece of soaked cotton.

Study Site

The study was performed during the 2012 mango fruiting season in Actopan, Veracruz, Mexico. Mango trees of 'Ataulfo', 'Manila', and 'Criollo' cultivars were located along the margins of commercial stands of sugarcane (19° 27′48.2″ N, 96° 30′12.9″ W; 118 masl), and 'Tommy Atkins' trees were in an orchard (19° 26′28.1″ N, 96° 30′9.46″ W; 105 masl). Mean annual temperature in the area was 24.8°C and mean rainfall was 863 mm (Servicio Metereológico Nacional).

Study 1. Analysis of Resin Ducts in Mango Fruit Exocarp

Three unripe, green-ripe, and ripe mango fruit were harvested from three different trees of each cultivar to obtain nine fruits per cultivar. Immediately, two thick and lengthwise "slices" were cut off from each fruit (from opposite sides of the fruit) and placed into 500-ml plastic containers with 250 ml of FAA fixative (100 ml formaldehyde, 500 ml ethanol, 50 ml acetic acid, and 350 ml distilled water; López et al. 1998). Samples were taken to the Laboratorio de Anatomía Vegetal at the INECOL for processing.

Following López et al. (1998), both slices of each fruit, previously fixed in FAA, were randomly labeled "A" and "B". A sample (1.5 cm^3) from the middle portion of each mango slice was then cut. An incision was made on the back of each cube to mark their orientation. Immediately, cubes were rinsed with water in individual 500-ml plastic containers every 2 h for four times to eliminate the FAA solution. Then, cubes were dehydrated and embedded in paraffin according to Sandoval-Zapotitla et al. (2005). Paraffin blocks were prepared to set the samples as described in López et al. (1998) and slices of 20–25 µm thickness were obtained with a rotary microtome (LEICA RM2125RT). The paraffin slices were flattened in a hot-water flotation bath, as described in López et al. (1998). To remove the paraffin, the slices were placed in an oven at 60 °C for 15–20 min and subsequently immersed in a series of products for complete removal of paraffin and staining, as described in Sandoval-Zapotitla et al. (2005). The tissues were mounted on slides of 10 by 10 mm using acrolein (Luzitron, made by Politec, Rodin, S.A de C.V. Venado 163, Col. Los Olivos, Tlahuac, C.P. 13210, Mexico, D.F.) following the protocol of López et al. (1998).

Twelve histological sections of each ripeness stage and cultivar (6 from side "A" and 6 from side "B") were microphotographed with a Nikon SZM1500 stereomicroscope and Digital Sight DS-2Mv camera, at 12× magnification to perform measures with the Nis-elements (AR) image analyzing software (Nikon Corporation Copyright 1991–2006). Considering the ovipositor size of the fly species tested, 3.2–5 mm for *A. ludens* and 1.4–1.7 mm for *A. obliqua* (Hernández-Ortíz 1998), measurements were done at 2 mm depth (2 by 5 mm = 10-mm^2 section) and at 5 mm depth (5 by 5 mm = 25-mm^2 section; Fig. 1). To corroborate the depth of penetration in our analysis, we measured the aculeus of 20 *A. ludens* and *A. obliqua* females randomly selected from Study 3 (see forced

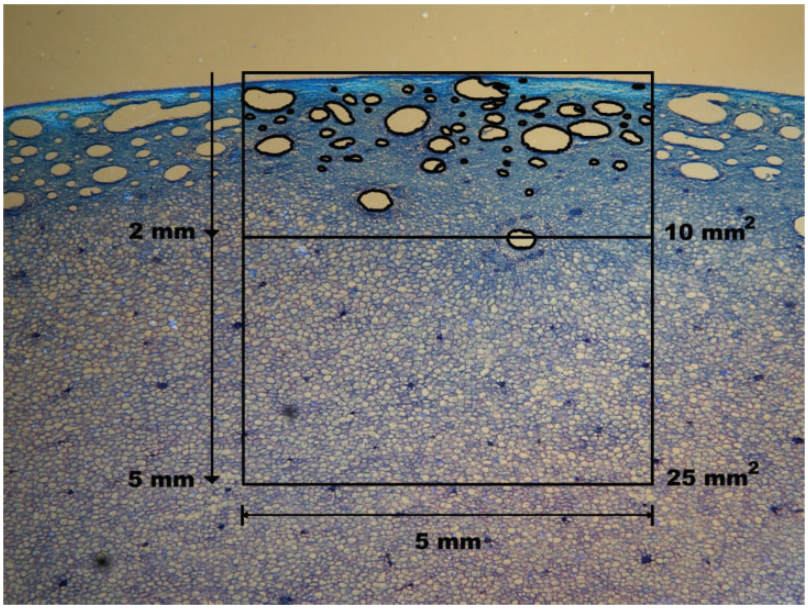

Fig. 1. Cross section of mango fruit exocarp ('Ataulfo' cultivar, green-ripe fruit), depicting the resin ducts, which were marked to easy recognition. The measurements were taken in the 10-mm^2 area (2 by 5 mm) and 25-mm^2 area (5 by 5 mm).

infestation below), recording an aculeus length of 3.69 ± 0.08 mm (mean ± SE) for *A. ludens* and 1.54 ± 0.01 mm for *A. obliqua*. Sampled sections were used to measure: 1) number of resin ducts (NRD; i.e., duct density) and 2) percentage of area occupied by resin ducts (PAOD) at 2 and 5 mm depth.

Study 2: Sap Volume, Physical Features, and Fruit Sugar Content

Sap Volume

Twenty unripe fruits from different trees of each cultivar were individually processed to estimate their sap content in a unit of measurement. Unripe fruit were used because they contain larger amounts of sap than ripe fruit (Joel and Fahn 1980), are easier to measure, and also because at this fruit physiological stage, mango fruit becomes attractive to *A. ludens* and *A. obliqua* flies (Díaz-Fleischer and Aluja 2003b). Each fruit was picked from the tree without the peduncle and immediately tipped onto a 100-ml plastic beaker, allowing the sap to flow freely for 1 min. Collected sap was measured with a 1-ml glass pipette. The length, width, and weight of each fruit were recorded. Fruit from each cultivar were sampled at the same period (12:00–14:00 h) to avoid changes in sap volume due to sampling hour (Amin et al. 2008).

Physical Features and Fruit Sugar Content

Five fruits from eight trees of each cultivar were harvested, and their weight, length, and firmness measured in the laboratory within 3 h after collection. Fruit firmness was measured with a penetrometer (AMETEK Mansfield & Green division Accuforce III), and expressed as the average of two measurements taken in the apical part of each fruit. Length was measured with a manual Vernier. Sugar concentration (°Brix) of 10 unripe, 10 green-ripe, and 10 ripe fruits from each cultivar were measured with a hand-held refractometer (Pocket Refractometer Pal-1, Atago Tokio tech. Award).

Study 3. Fruit Forced Infestation by *A. ludens* and *A. obliqua* Under Field Conditions

The field study was performed from March to May 2012 following the methodology of Aluja and Mangan (2008). Branches (bearing unripe fruit) from six trees of each cultivar were bagged with chiffon bags to prevent natural fruit fly infestation. Four bagged branches containing three fruits were chosen on two sides of each tree. When fruit reached the green-ripe stage, six mated females, with no previous oviposition experience, and three males of *A. ludens* or *A. obliqua* were released into one bag in each tree side (Aluja et al. 2004). Six replicates were completed for each cultivar, each one consisting of a tree with two bagged branches for each species of *Anastrepha*. When the assay was set up, the age of the females ranged from 8–22 d for *A. obliqua*, and 13–17 d for *A. ludens*.

The bags were labeled with satin ribbons according to cultivar, replicate, and fly species. Paper napkins soaked in a mixture of water, protein, and sugar, and water in a plastic vial with a piece of soaked cotton were hung inside the bags to feed flies. After 3 d of confinement, flies were recaptured and placed on a plastic container with 70% ethanol to measure aculeus length (see Study 1 section for details). Seven days after insect removal, fruit were harvested and immediately taken in labeled bags to the RMBPV laboratory.

Each fruit was weighed on an analytical scale (OHAUS Precision Plus) and placed in a 1-liter plastic container with a thin layer of vermiculite. The containers were sealed with elastic fabric and arranged on counter tops, at room temperature.

Infestation Rates

Four days after fruit collection, fruit and vermiculite were inspected every second day during a 30-d period to recover pupae. Total number of pupae recovered was recorded and pupae from the same fruit and day were placed together in a 250-ml plastic cup (5.2 cm diameter at the base, 7.2 cm diameter at the top, and 6 cm tall) with vermiculite. Cups were maintained at 27 ± 1°C, 75 ± 5% RH, and photoperiod of 12:12 (L:D) h.

Immature Development Time, Pupal Weight, and Adult Emergence

Time elapsed between oviposition and the prepupal stage was recorded daily for each individual (pupa). Pupae were individually weighed when they were 4 d old using an analytical balance (Sartorius CP64), and then returned to its container. Every third

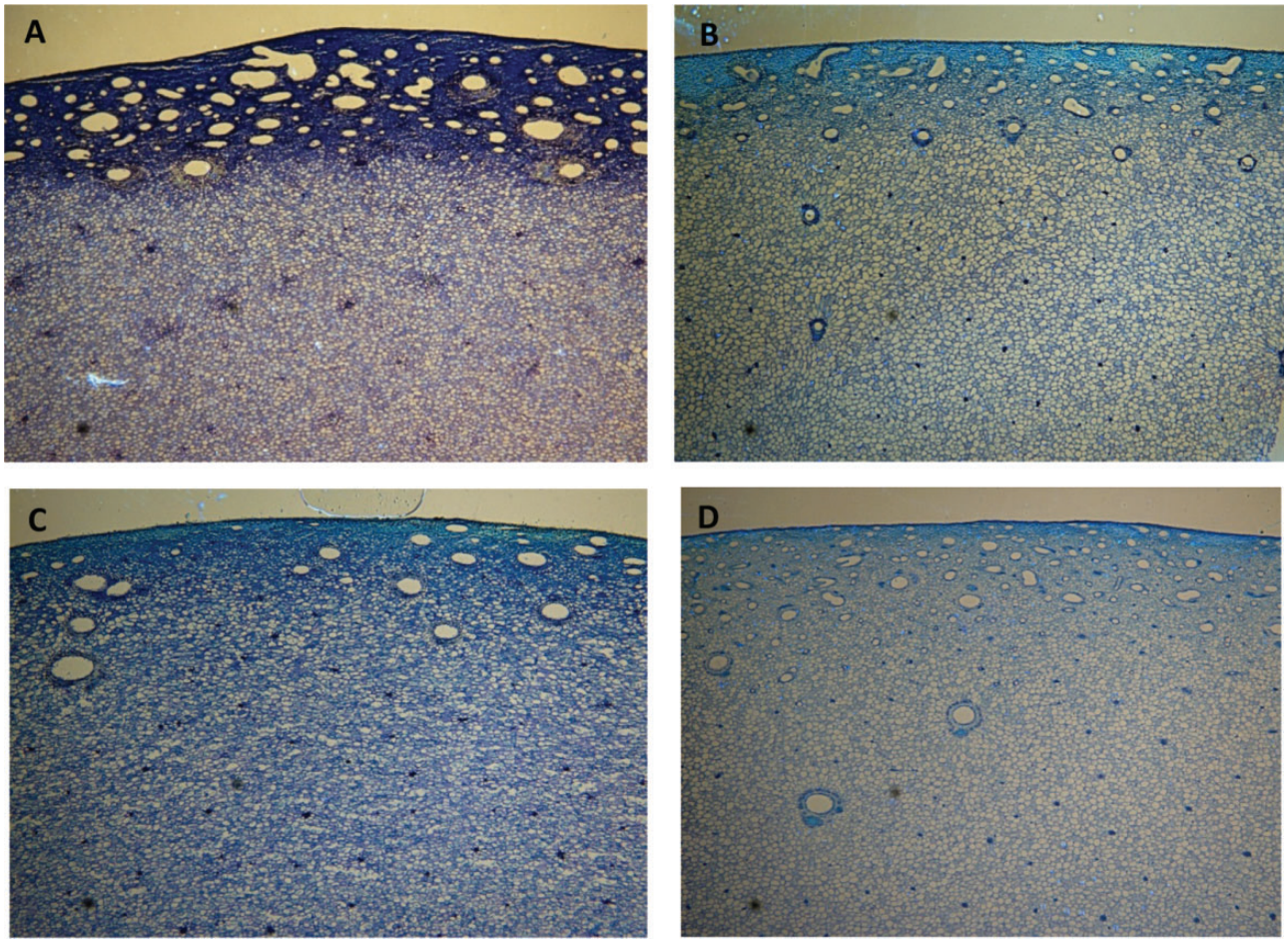

Fig. 2. Examples of histological sections of green-ripe mango fruit exocarp showing the pattern (number, size, and distribution) of resin ducts in four mango cultivars: (**A**) 'Ataulfo', (**B**) 'Criollo', (**C**) 'Manila', and (**D**) 'Tommy Atkins'.

day, vermiculite was moistened with a sodium benzoate solution to prevent pupal desiccation. Containers with pupae were daily inspected to record number and date of adult emergence.

Statistical Analyses

All data were checked for normality and homogeneity of variances with Kolmogorov–Smirnov and Lilliefors tests. The statistical tests were run using STATISTICA (StatSoft, Inc. 2004).

Study 1

We used a factorial ANOVA to compare the number and area occupied by resin ducts (10-mm^2 and 25-mm^2 sections) in cultivars 'Ataulfo', 'Criollo', 'Manila', and 'Tommy Atkins' at different physiological stages of the fruit (unripe, green-ripe, and ripe) and two fruit sides ("A" and "B"). The variable "fruit side" was considered as a factor to elucidate if this feature is homogenous in the fruit. Fisher LSD tests were used for means comparison.

Study 2

To avoid any effect of mango fruit size in sap volume, a sap index was generated (ml of sap shed per kg per fruit). Means were compared by Fisher LSD tests. To ascertain if sap content had any influence on infestation level, a correlation analysis was performed. Fruit parameters were analyzed with one-way ANOVAs and their means were compared with a Fisher LSD test. The nonparametric Kruskal–Wallis test was used to analyze sugar concentration (°Brix) for degree of fruit ripeness and cultivar. Means were compared via Student–Newman–Keuls tests.

Study 3

To compare infestation rates (pupae per fruit), data were square root transformed, as model assumptions were not meet, and then analyzed with a Generalized Linear Model (GLZ (Generalized Linear Model in Statistica program) procedure in Statistica) with a Poisson Error Distribution. Data were back-transformed for graphical presentation. General Linear Models (GLM procedure in Statistica) with a factorial ANOVA were used to analyze immature development time (from egg to prepupae), pupal development time, pupal weight, and percent of fly emergence as a function of fly species and cultivar. Means were compared using Fisher LSD test. Correlations were used to establish the relationship between infestation rates (pupae per fruit) and fruit features (sugar concentration, area occupied by ducts in 10 mm^2, sap volume, firmness, and weight).

Results

Study 1. Analysis of Resin Ducts in Mango Fruit Exocarp

The NRD in the 10-mm^2 section differed significantly among cultivars (Figs. 2 and 3; Table 1) and fruit physiological stage, and it was

Table 1. ANOVA results when comparing resin ducts in mango fruit exocarp according to cultivar, fruit side, and physiological state

Parameters	df	Peel section							
		10 mm^2				25 mm^2			
		Number of ducts		Percentage of occupied area by ducts		Number of ducts		Percentage of occupied area by ducts	
Effect		*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Cultivar	3/408	249.85	0.0001	356.23	0.0001	256.74	0.0001	282.84	0.0001
Physiological state (PS)	2/408	14.99	0.0001	15.10	0.0001	10.62	0.0001	5.12	0.01
Fruit side (FS)	1/408	1.53	0.216	5.26	0.05	3.14	0.077	3.09	0.079
Cultivar*PS	6/408	12.34	0.0001	15.53	0.0001	15.37	0.0001	10.52	0.0001
Cultivar*FS	3/408	0.27	0.850	2.85	0.05	0.77	0.510	1.01	0.388
PS*FS	2/408	9.27	0.0001	2.91	0.0557	11.90	0.0001	10.36	0.0001
Cultivar*PS*FS	6/408	1.70	0.119	5.64	0.0001	1.70	0.120	3.65	0.005

also influenced by the interactions, cultivar*physiological state and physiological state*fruit side (Table 1, Fig. 3A). Fruit side and the interactions cultivar*fruit side and cultivar*physiological state*fruit side were not significant in the ANOVA model (Table 1, Fig. 3A). 'Ataulfo' followed by 'Tommy Atkins' and 'Criollo' cultivars had the largest number of resin ducts (Fig. 3A).

A similar pattern was observed in the PAOD (Fig. 3B). PAOD was highly significant among cultivars and physiological stages, and it was influenced by the interactions cultivar*physiological state and cultivar*physiological state and fruit side. The interaction physiological state*fruit side was not significant (Table 1, Fig. 3B). 'Ataulfo' had the highest percentage of PAOD with 12.41 ± 0.25% (mean ± SE) and 'Criollo' cultivar the lowest (6.24 ± 0.25%), followed by 'Manila' and 'Tommy Atkins' (Fig. 3B).

The NRD in the 25-mm^2 section was highly different among cultivars, physiological state, and the interactions cultivar*physiological state and physiological state*cultivar (Table 1, Fig. 4A). As in the 10-mm^2 section, NRD did not differ significantly in fruit side or in the interactions between fruit side and cultivar, and in the interaction cultivar*physiological state*fruit side (Table 1, Fig. 4A).

The PAOD at 25 mm^2 was highly different among cultivars and was also highly influenced by the interactions cultivar*physiological state and physiological state*fruit side (Table 1). Physiological state and the interaction cultivar*physiological state*fruit side were also significant (Table 1). PAOD was not affected by fruit side or by the interaction between cultivar and fruit side (Table 1, Fig. 4B). Both parameters NRD and PAOD at 25-mm^2 showed the same pattern than NRD and PAOD at 10-mm^2 section.

Study 2. Physical and Chemical Fruit Features

Sap volume yield in unripe fruit was significantly different among cultivars ($F = 35.766$; df = 3, 76; $P < 0.001$). 'Ataulfo' oozed significantly more sap than the other cultivars (Fig. 5). Independently of the cultivar and fruit size, sap volume and the area occupied by ducts at 10 mm^2 were also highly correlated ($r = 0.9940$, $P < 0.0001$). Fruit parameters (weight, firmness, length, and °Brix) were different among cultivars (Tables 2 and 3). Unripe and green-ripe fruit of all cultivars had similar sugar concentrations (Table 3). In general, sugar concentration of unripe and green-ripe fruit was significantly lower than in ripe fruit.

Study 3. Forced Infestation of Mango by *A. obliqua* and *A. ludens* Under Field Conditions

Infestation rates differed significantly between fly species (Wald $\chi^2 = 27.69$; df = 1; $P < 0.0001$) and among cultivars (Wald $\chi^2 = 128.02$; df = 3; $P = < 0.0001$; Fig. 6). The cultivar 'Criollo' was the most infested by both fly species and the cultivar 'Tommy Atkins', the least (Fig. 6). The interaction fly species*mango cultivar affected significantly infestation rates ($F = 17.81$; df = 3, 256; $P < 0.0001$).

Pupal weight differed significantly between fly species ($F = 295.04$; df = 1, 183; $P < 0.001$) and among cultivars ($F = 25.97$; df = 3, 183; $P < 0.0001$). Similarly, the interaction between cultivar and fly species was significant ($F = 4.57$; df = 3, 183; $P < 0.005$). For both fly species, pupae from 'Criollo' cultivar exhibited the lowest weight (Table 4).

Development time from egg to prepupae was significantly different among cultivars ($F = 14.79$; df = 3, 183; $P < 0.0001$) and fly species ($F = 117.78$; df =1, 183; $P < 0.0001$). The interaction between cultivars and fly species was also significant ($F = 2.03$; df = 3, 183; $P = 0.11$). In general, *A. ludens* needed more time to reach the prepupal phase than *A. obliqua* (Table 4). Egg to prepupa development time of *A. ludens* (27.11 ± 0.96 d) and *A. obliqua* (22.88 ± 1.21 d) was slower in 'Manila' cultivar (Table 4).

Pupal development time differed significantly among cultivars ($F = 7.21$; df = 3, 150; $P < 0.001$), and fly species ($F = 40.44$; df = 1, 150; $P < 0.0001$). The interaction between cultivar and fly species was also significant ($F = 4.94$; df = 3, 150; $P < 0.005$). Pupation time was longer for *A. ludens* than for *A. obliqua*. *Anastrepha obliqua* pupae from 'Tommy Atkins' exhibited a shorter pupation time (Table 4).

Percentage of adult emergence differed significantly between fly species ($F = 108.1$; df = 1, 174; $P < 0.0001$) and among cultivars ($F = 5.42$; df = 3, 174; $P < 0.005$). The interaction between cultivars and fruit fly species was significant ($F = 10.91$; df = 3, 174; $P < 0.0001$). With the exception of 'Tommy Atkins', the emergence of adult *A. ludens* in all other cultivars was higher than *A. obliqua* (Table 4).

The relationship between the different fruit features and the infestation rates per fly species was negative in all cases (Table 5). The infestation by both fly species was higher in cultivars with the lowest amount of sap, number of ducts, and the area occupied by ducts at 10 mm^2 and 25 mm^2 (Figs. 3–6).

Discussion

Both the NRD and the PAOD were significantly different among mango cultivars and were negatively correlated with *A. ludens* and *A. obliqua* infestation rates. 'Tommy Atkins' and 'Ataulfo' cultivars with the most NRD were the least infested, and 'Criollo' and 'Manila' cultivars with the fewest NRD, the most infested. Mango

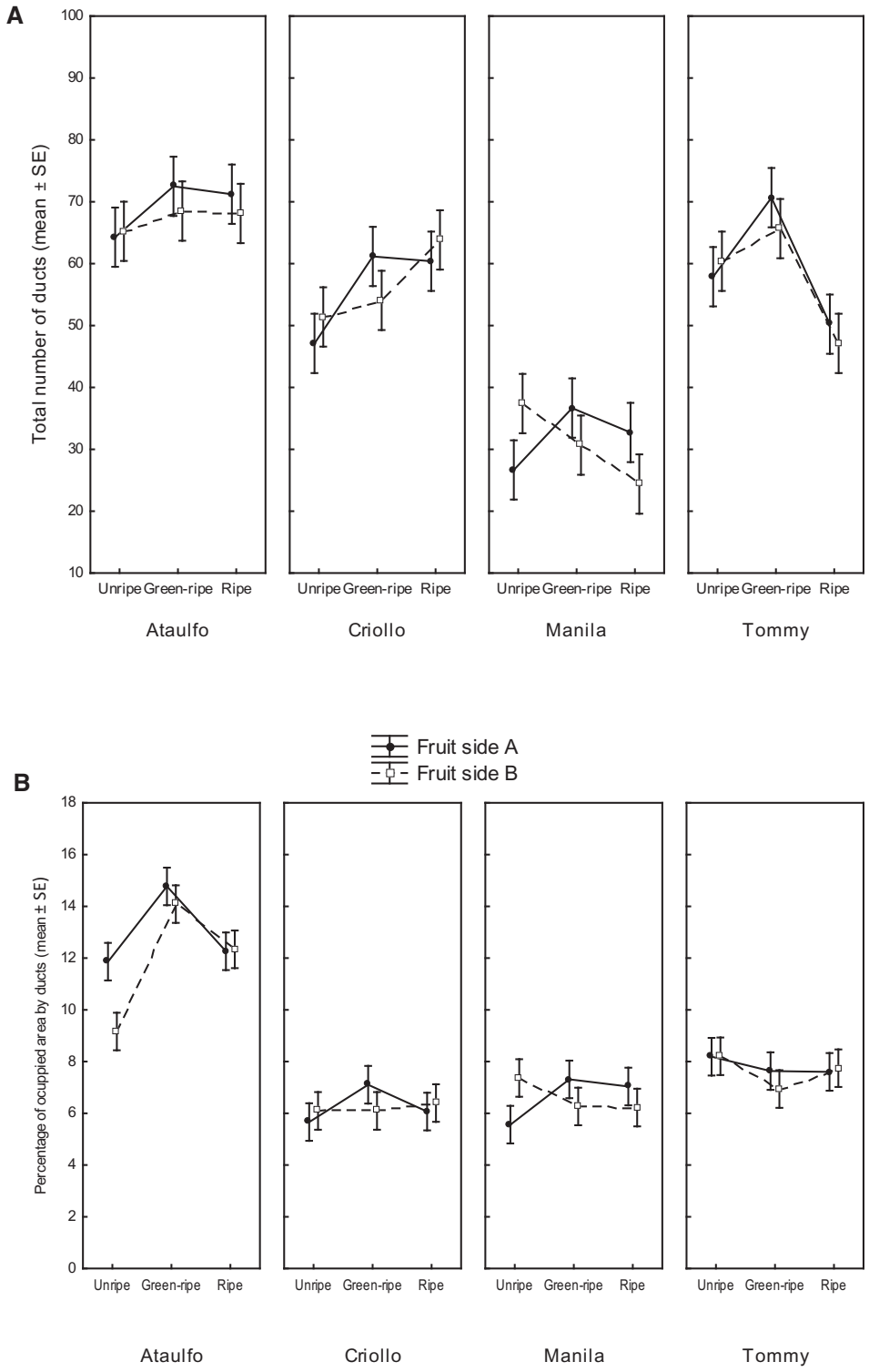

Fig. 3. (**A**) Number of resin ducts (NRD) in mango fruit exocarp in a 10-mm^2 section from different fruit ripening stages. (**B**) Percent of area occupied by resin ducts by fruit physiological state in four mango cultivars in a 10-mm^2 section.

cultivars exhibit a distinct resin duct system which renders some of them more susceptible than others to fruit fly attack (Joel 1980). In this study, although 'Ataulfo' and 'Tommy Atkins' cultivars had similar NRD, the PAOD of 'Ataulfo' was higher owing to the larger diameter size of its ducts (Fig. 2). The higher number and larger size of ducts in 'Ataulfo' cultivar could explain its resistance to fly attack. In the case of 'Tommy Atkins', which had the lowest fly infestation level, but similar PAOD than the susceptible cultivars 'Criollo' and 'Manila', its resistance could be owing to the pattern of its resin duct system. The latter is characterized by many small ducts homogenously distributed in the exocarp. In cultivars with ducts more spatially distributed, eggs might be oviposited in free-resin duct areas. However, while moving inside the fruit, the larvae might come into contact with them. Therefore, *A. ludens*

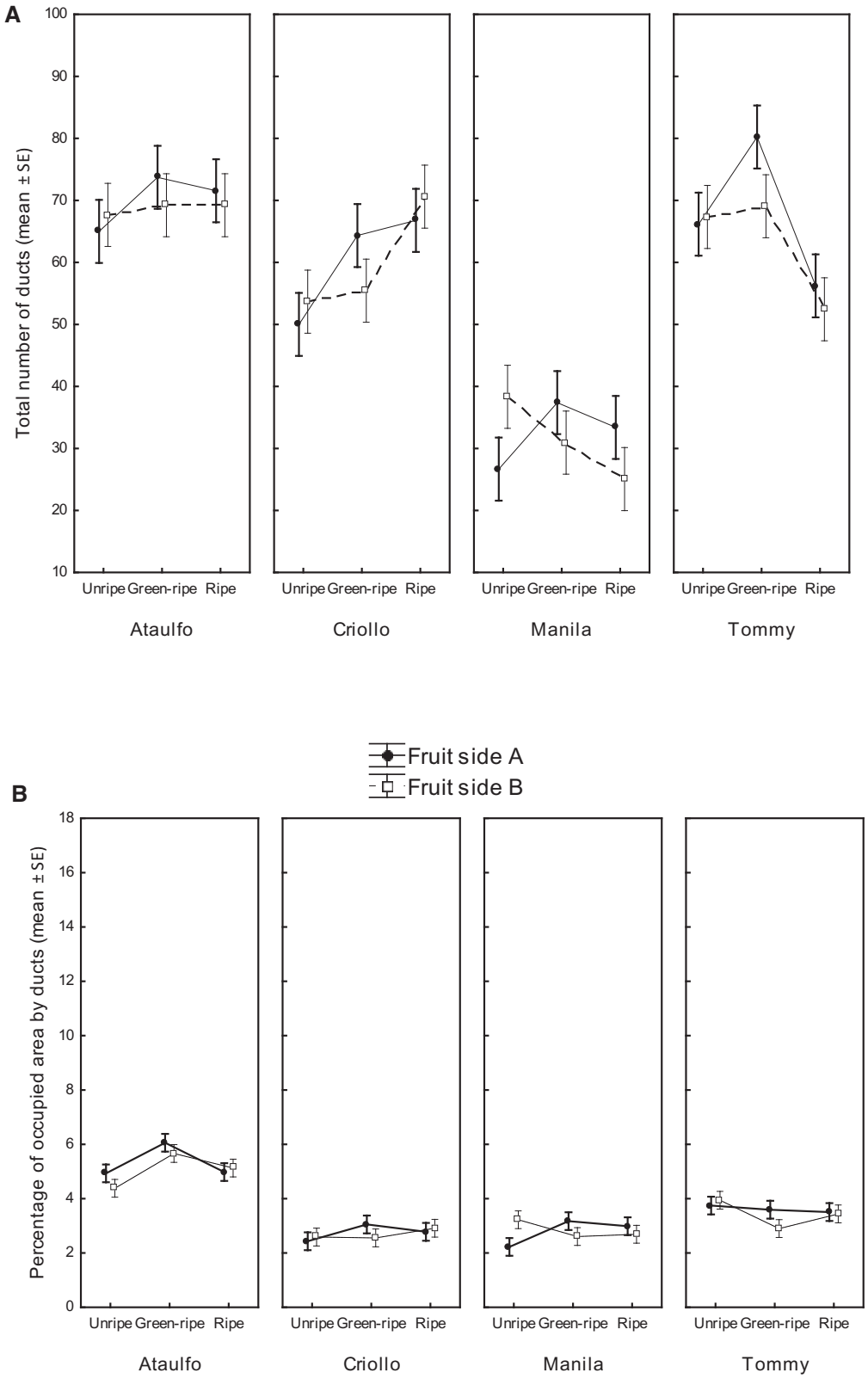

Fig. 4. (**A**) Number of resin ducts as influenced by fruit ripening stage in four mango cultivars in a 25-mm^2 section. (B) Percent of area occupied by resin ducts by fruit physiological state in four mango cultivars in a 25-mm^2 section.

and *A. obliqua* females are more likely to reach a resin duct with their aculei while trying to oviposit in 'Ataulfo' than in other cultivars.

NRD and PAOD in unripe and mature mango exocarp were very similar in both fruit sides (Figs. 3 and 4). Although these results are not surprising, they are important because it dispels the doubt

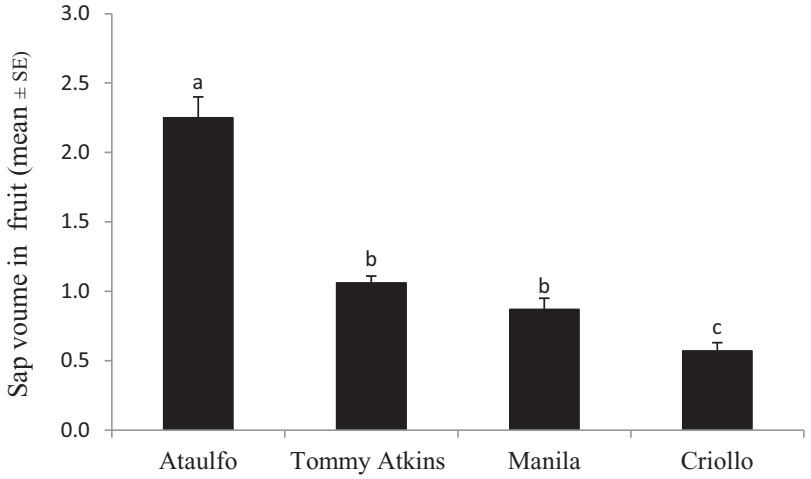

Fig. 5. Sap volume shed by unripe mango fruit of four cultivars. Means followed by the same letter do not differ by Fischer LSD test $\alpha = 0.05$.

Table 2. Physical features of four mango cultivars at two fruit ripeness stages

Stage of ripeness	Cultivar	Weight (g) Mean ± SE[a]	Length (cm) Mean ± SE[a]	Width (cm) Mean ± SE[a]	Firmness (N) Mean ± SE[b]
Unripe $F_{3,156}/P^c$	Ataulfo	205.62 ± 7.28a	10.29 ± 0.14a	6.54 ± 0.08a	11.85 ± 0.52a
	Criollo	136.20 ± 6.52b	8.34 ± 0.14b	6.02 ± 0.10b	10.34 ± 0.47b
	Manila	177.51 ± 9.07c	11.28 ± 0.26c	5.88 ± 0.12bc	8.86 ± 0.52c
	Tommy Atkins	320.73 ± 10.14d	10.18 ± 0.12a	8.03 ± 0.08d	11.25 ± 0.42ab
		$F = 89.28/P < 0.0001$	$F = 49.55/P < 0.0001$	$F = 98.62/P < 0.0001$	$F = 7.26/P < 0.0001$
Green-ripe $F_{3,156}/P^c$	Ataulfo	234.56 ± 7.98a	10.19 ± 0.15a	6.83 ± 0.08a	12.18 ± 0.67a
	Criollo	159.85 ± 5.29b	8.45 ± 0.16b	6.32 ± 0.09b	8.86 ± 0.55b
	Manila	246.22 ± 9.42a	11.99 ± 0.18c	6.55 ± 0.08bc	10.36 ± 0.64bc
	Tommy Atkins	447.83 ± 10.75c	10.97 ± 0.13d	8.96 ± 0.09d	10.21 ± 0.42bd
		$F = 205.23/P < 0.0001$	$F = 90.55/P < 0.0001$	$F = 203.7/P < 0.0001$	$F = 5.57/P < 0.001$

[a] Mean of five fruits/stage of ripeness/plant/cultivar (eight replicates).
[b] Mean of two measurements taken in the apical part of each fruit (five fruits/stage of ripeness/plant/cultivar, eight replicates).
[c] One-way ANOVA tests, df = 3,156, *F*-value/*P*-value.
Means followed by the same lowercase in the line do not differ by Fischer LSD test $\alpha = 0.05$.

Table 3. Mean (± SE) sugar content (°Brix) in four mango cultivars at three ripeness stages

Cultivar	Unripe	Green-ripe	Ripe
Ataulfo	7.95 ± 0.49aA	9.30 ± 1.08aAB	16.52 ± 0.97bA
Tommy Atkins'	5.57 ± 0.41aB	6.12 ± 0.71aC	15.36 ± 0.40bA
Manila	6.65 ± 0.74aAB	10.00 ± 0.90aA	15.20 ± 0.77bA
Criollo	6.49 ± 0.53aAB	6.92 ± 0.34aBC	13.71 ± 0.97bA

Means followed by the same lowercase in the line and uppercase in the column do not differ by Student–Newman-Keuls test $\alpha = 0.05$.

about the uniformity of these characteristics in the fruit, which could influence the oviposition behavior of fruit flies and explain the differences in infestation among fruits. In *B. dorsalis*, it was observed that females preferred to oviposit in the top area of the fruit which was the softer part and also exhibited lower sugar content (Rattanapun et al. 2010). In our study, we did not compare the avoidance of resin ducts in top, middle, and bottom areas, but they could be different. Regardless of cultivar, NRD and PAOD were very similar in both 10-mm^2 and 25-mm^2 sections (Figs. 3 and 4), because most of the ducts were concentrated in the 10-mm^2 section at 2 mm depth.

Here, we estimated the fruit sap volume of each cultivar, and found a positive correlation with NRD and PAOD, and a negative correlation with the infestation of both fly species. Our study clearly showed that *A. ludens* and *A. obliqua* infestation was lower in 'Ataulfo' and 'Tommy Atkins' cultivars which had the largest amount of sap volume. The role of sap on mango resistance to fruit fly infestation is not well understood. Sap pressure could be expelling the eggs when they are laid into resin ducts. Rattanapun et al. (2009) observed that when *B. dorsalis* flies oviposited in the pericarp of unripe mango, the resin flowed out immediately and pushed the eggs out of the fruit. This was also recorded by Joel (1980) in *C. capitata* ovipositing in mango. Alternatively, sap and resin compounds could be toxic to eggs and larvae. In fact, mango fruit sap is highly acidic and contains terpenes, protein, polyphenols, and enzymes such as polyphenol oxidase peroxidase (Loveys et al. 1992; Saby et al. 1999, 2003; Musharraf et al. 2016). Some of these compounds are associated with plant defense against insect attack (Maffei 2010, Verghese et al. 2012, Aluja et al. 2014b).

Our results are in close agreement with Aluja et al. (2014b), who found that 'Ataulfo' mango was among the least susceptible cultivars to fruit fly attack and that 'Criollo' mango was one of the most susceptible. These authors proposed a relationship between mango susceptibility and the cultivar volatile profile. In our case, we document instead a clear relationship between mango infestation and the number of resin ducts and PAOD.

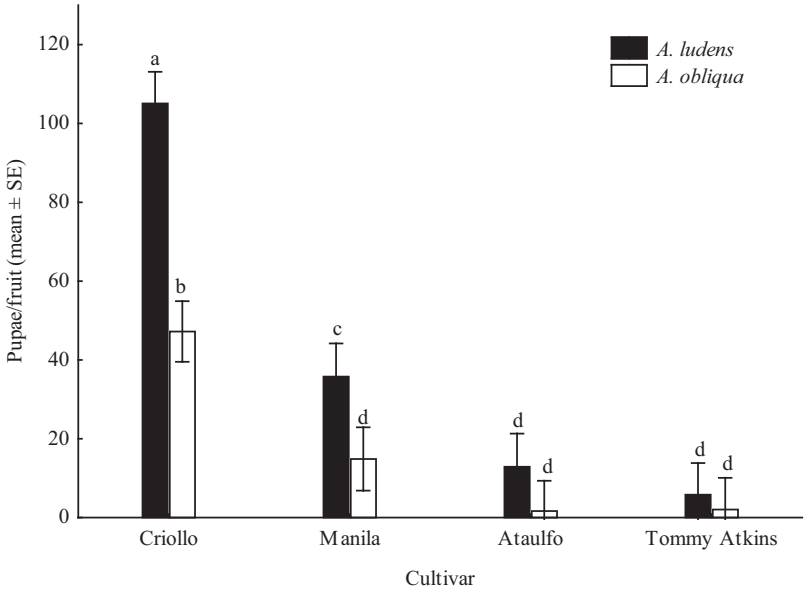

Fig. 6. Infestation level of *A. ludens* and *A. obliqua* in four mango cultivars.

Table 4. Parameters related with the fitness of *A. ludens* and *A. obliqua* reared on four mango cultivars (mean ± SE)

Fly species cultivar	Immature development time (d)	Pupa development time (d)	Pupa weight (mg)	Adult emergence (%)
***A. ludens*:**				
Criollo	24.88 ± 0.62A	14.63 ± 0.07ACD	15.38 ± 0.59A	61.31 ± 2.28A
Manila	27.11 ± 0.96B	14.37 ± 0.06AC	20.18 ± 0.21B	82.49 ± 2.71B
Ataulfo	24.38 ± 0.65A	14.79 ± 0.09AD	19.74 ± 0.49B	72.99 ± 6.16B
Tommy	24.18 ± 0.59A	14.52 ± 0.34ACD	16.28 ± 1.07A	40.20 ± 6.38C
***A. obliqua*:**				
Criollo	17.94 ± 0.35C	14.33 ± 0.13AC	9.16 ± 0.37C	16.28 ± 2.56D
Manila	22.88 ± 1.22A	13.83 ± 0.24B	12.01 ± 0.74D	29.15 ± 10.06D
Ataulfo	16.67 ± 0.53C	13.95 ± 0.56BC	11.34 ± 1.07D	12.91 ± 6.03D
Tommy	17.51 ± 0.81C	12.75 ± 0.36B	12.46 ± 1.84D	46.37 ± 16.54AC

Means with the same letter(s) are not significantly different at the 0.05 level using Fischer LSD.

All the fruit features we measured varied among cultivars and most of them were negatively correlated with *A. ludens* and *A. obliqua* infestation rates. For example, peel firmness of green-ripe fruit was correlated negatively with infestation. 'Ataulfo' and 'Tommy Atkins' were the cultivars with the firmest peel, suggesting that mango resistance to fly attack could also be partly related with this fruit feature. Fruit firmness has been associated with oviposition preference by fruit flies (Lalonde and Mangel 1994, Messina et al. 1991, Rattanapun et al. 2009). Díaz-Fleischer and Aluja (2003b) found that fruit firmness influences the oviposition strategies of *A. ludens* females, with them laying larger egg clutches into unripe fruit than into ripe fruit. Unripe fruit often have tougher skin than fully-ripe fruit (Rattanapun et al. 2010), and this represents a limitation to fruit fly oviposition (Balagawi et al. 2005). Furthermore, Jones and Kim (1994) suggested a structural degradation (i.e., wear) of the aculeus tip in the tephritid flies *Rhagoletis pomonella* (Walsh), *Rhagoletis mendax* Curran, *C. capitata*, and *B. oleae* caused by constant oviposition in fruit with hard skin.

'Criollo' and 'Manila' cultivars were most severely infested by *A. ludens* than by *A. obliqua*. This could be due to two factors. First, because *A. ludens* females lay eggs in clutches, whereas *A. obliqua* invariably oviposit one egg per oviposition bout (Díaz-Fleischer and Aluja 2003b, Birke et al. 2013); and second, because *A. ludens* with an aculeus twice as large (3.69 ± 0.08 mm) than that of *A. obliqua* (1.54 ± 0.01 mm) could lay its eggs deeper, avoiding with this the main resin duct area. In fact, it has been shown that the length of the aculeus is an important factor that allows females to successfully exploit host fruit (Birke et al. 2006, Papachristos and Papadopoulos 2009). We observed that 95% of the resin ducts are found within the first 2 mm of the exocarp (Figs. 3 and 4) and, therefore, *A. ludens* females could easily lay eggs in parts of the fruit falling

Table 5. Correlations between "infestation rates" of *A. ludens* and *A. obliqua* reared on four mango cultivars and certain fruit features

Fruit features	*A. ludens*		*A. obliqua*	
	r	*P*	*r*	*P*
Sugar concentration	−0.156	0.08	−0.184	0.05
Firmness	−0.161	0.01	−0.564	0.0001
Sap volume	−0.529	0.0001	−0.479	0.0001
Area occupied by ducts at 10 mm^2	−0.474	0.0001	−0.434	0.0001
Weight	−0.440	0.0001	−0.421	0.0001

below the maximum resin duct concentration. In contrast, *A. obliqua* had no option but to lay eggs in the middle of highest resin duct concentration. This situation favors *A. ludens* over *A. obliqua* when exploiting mangoes. However, the latter did not to confer a definitive advantage to overcome mango resistance mechanisms, as there was no difference in infestation between these two *Anastrepha* species in the case of the most resistant cultivars ('Ataulfo' and 'Tommy Atkins'). The latter suggests that maybe *A. obliqua* larvae have an internal mechanism (e.g., gut microbiota) to cope with toxic components of resin and sap, or to better assimilate nutrients in mango flesh (Ben-Yosef et al. 2008, 2014; Ridley et al. 2012; Douglas 2015). Proteomic studies in xylem and phloem sap have unraveled the occurrence of key proteins associated with self-maintenance and defense, including proteins related to cell wall metabolism, pathogen defense, proteolysis, and redox response (Kim et al. 2013, Rodríguez-Celma et al. 2016). Future studies should examine the chemical composition of mango resin, as this might be a factor contributing to mango resistance to fruit fly attack. Proteomic and metabolomic studies with a powerful mass spectrometer will likely shed light on the molecular diversity of mango fruit sap and resin.

Mangoes are native to Asia (Litz 2009), so the relationships among *A. obliqua*, *A. ludens*, and mango fruit are recent from an evolutionary perspective. Both fly species are considered polyphagous but in the case of *A. obliqua*, the most preferred native host fruit belongs to the family Anacardeacea, to which mangoes also belong. Thus, although *A. ludens* has the advantage of having a longer aculeus, probably *A. obliqua* has the advantage to be more adapted to the chemistry of mango fruit. The latter is related to the chemical coevolution hypothesis, one of the most accepted theories to explain host use pattern in herbivorous insects (Ehrlich and Raven 1964).

Pupal weight was different between fly species, which is not surprising, as *A. ludens* adult and pupae are naturally bigger than *A. obliqua* (Table 4). Both fly species performed better (i.e., had heavier pupae and high emergence rates) in the 'Manila' cultivar. Females of several species of fruit flies prefer to oviposit in a host where their offspring perform well (Rattanapun et al. 2009, 2010; Balagawi et al. 2013). In this study, larvae of both fly species spent more time feeding in 'Manila' fruit, which could have contributed to their better performance as they had more time to accrue nutrients.

We conclude that resistance of 'Tommy Atkins' and 'Ataulfo' cultivars to *A. ludens* and *A. obliqua* can be related to fruit constitutive defenses, particularly by the area occupied by ducts, the distribution of the ducts in the exocarp, as well as peel firmness and unknown chemical components of resin and sap. Our results reinforce the idea that resin ducts and sap in the exocarp play an important role in mango fruit resistance to fruit fly attack and provide valuable information for IPM or area-wide management schemes, which should consider the use of resistant cultivars to minimize the use of insecticides (Aluja and Rull 2009). However, more studies are needed to fully understand the mechanisms involved in resistance of mango cultivars to fruit fly attack.

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