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HOT PEPPERS AS A HOST FOR THE MEXICAN FRUIT FLY *ANASTREPHA LUDENS* (DIPTERA: TEPHRITIDAE)

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On the 28th of April, 2003, a shipment of manzano chile peppers (*Capsicum pubescens* Ruis & Pavon cv Rocoto) entering the United States at Pharr, Texas, was found to be infested with insect larvae. USDA inspectors first noted maggots crawling in the bed of the truck underneath the 16 cardboard boxes (240 Kg) containing the chile peppers. Further inspection confirmed that the larvae were in, and emerging from, the fleshy pods. Two of the larvae were immediately preserved in alcohol while 50 more larvae were kept alive. All specimens were hand carried to the nearby USDA-ARS laboratory in Weslaco, Texas for identification. Microscopic examination established that the larvae had the morphological characteristics of the Mexican fruit fly, *Anastrepha ludens* (Loew), as described by Steck et al. (1990). However, this identification was tentative because there are approximately 200 described species in this genus (Norrbom et al. 1999) and the larval stages are known for only thirteen. Several kinds of maggots will breed in rotting vegetable matter including chile peppers, but these are non-pest species, and this incident involved sound fruit (Fig. 1). No dipterans are listed as economic pests of chile peppers by English & Lewis (2004). Baker et al. (1944) cited incidents of *A. ludens* in "bell peppers and chili peppers" and there are equally ambiguous reports of another tephritid, *Zonosemata vittigera* (Coquillet), taken in "peppers" (Cole 1969). *Zonosemata electa* (Say) is known as the "pepper maggot" (Peterson 1960) and has been reared from "*Capsicum annuum* L." (Smith & Bush 1999). The latter solanaceous plant species includes both hot and sweet peppers. The usual host plants for *Zonosemata* spp. are members of the genus *Solanum* (Norrbom 2002). To confirm the specific identity of the larvae infesting the manzano peppers, the available live larvae were placed in culture and maintained in the laboratory to obtain adults. Larval specimens that died before pupariation were preserved in alcohol and sent to Bruce

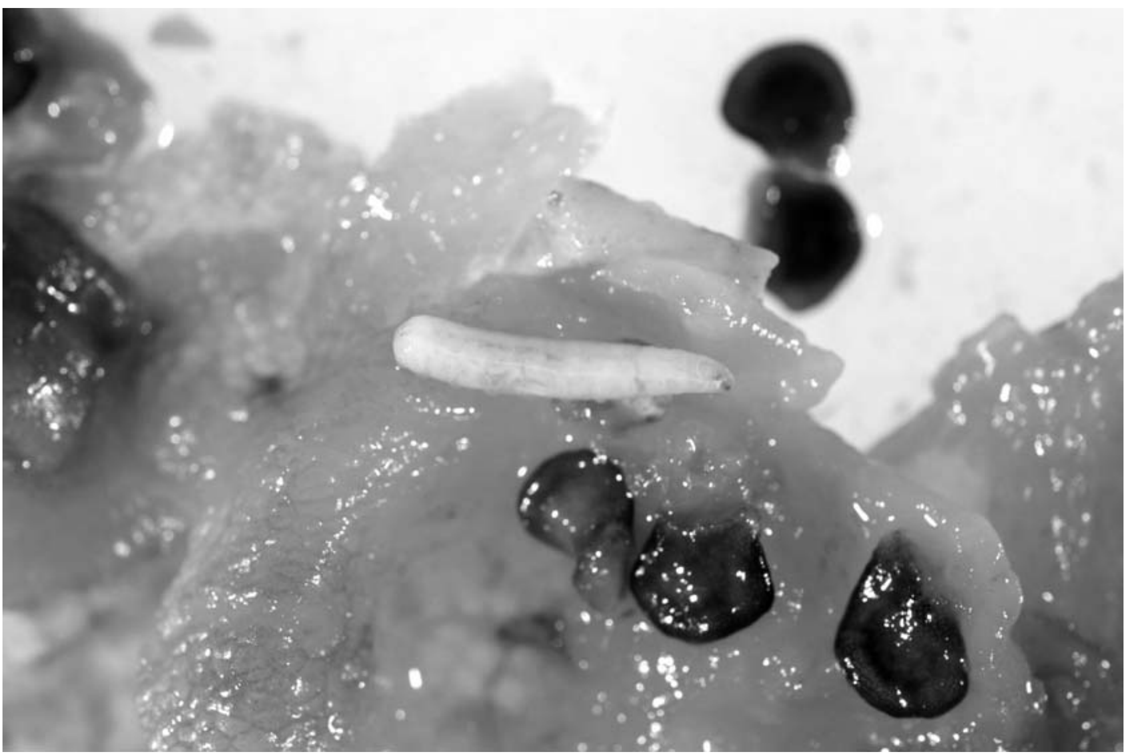

Fig. 1. Larvae of *Anastrepha ludens* infesting a manzano pepper intercepted at the U.S.-Mexico border. Note the black seeds characteristic of *Capsicum pubescens*.

A. McPheron of Pennsylvania State University for genetic fingerprinting. Based on sequencing of a fragment of the mitochondrial 16S ribosomal RNA gene, the specimens were indistinguishable from sampled populations of *A. ludens* (Silva et al. 2001). This gene has been studied and is diagnostic for 40 of the most important species of *Anastrepha* (McPheron et al. 1999).

A total of 42 larvae pupariated and of these eleven eclosed as adults. All were *A. ludens*, a determination confirmed by Allen L. Norrbom of the USDA-ARS Systematic Entomology Laboratory in Washington D.C. At Weslaco, all non-eclosed puparia were examined and the number of tubules on the anterior spiracles did not differ from those in puparia of *A. ludens*. On the 2nd of May, the 16 boxes of embargoed manzano peppers were taken to a disposal site for burial. At that time additional larvae were seen egressing the fruit and some of these were collected as voucher specimens by USDA-APHIS personnel.

Because records indicated that shipments of manzano peppers had cleared customs in the days immediately previous to the discovered infestation, an effort was made to track these shipments to their destinations. Manzano peppers infested with larvae were recovered from Chicago, IL; Detroit, MI; Atlanta, GA; Richmond, VA; and at two retail outlets in Pinellas County, FL. Two weeks later, on 16 May 2003, an adult *A. ludens* was found in a fruit fly trap in Orlando, FL. Because the previous detection of this species in Florida was in Sarasota in 1972 (Steck 1998) the new detection was presumed to have originated with the infested manzano pepper shipments.

Anastrepha ludens is a major pest of citrus and mangoes with a wide host range known to include at least 60 varieties of fruit (Norrbom & Kim 1988). Sweet peppers, cultivars of *Capsicum annuum* that lack the alkaloid capcaicin, are occasionally infested by *A. ludens*, but confirmed records of hot peppers (cultivars containing capcaicin) as larval hosts have not been reported. According to the inspectors who first discovered the infested shipment, just standing next to the open truck with the manzano peppers caused their eyes to water. On the Scoville scale manzano peppers (also marketed as “rocoto” or “perón” peppers) are rated at 12-30K (by comparison, jalapeños are rated 2.5-8K Scoville Units) (DeWitt & Gerlach 1990). Although manzano peppers are a low volume specialty item, accounting for much less than 1% of all peppers exported by Mexico (McClure 2003), chile pepper species in aggregate are a major commodity imported to the United States. Because of its non-host status, chile pepper importations had not required a disinfestation treatment or more than cursory inspection. In response to this incident, higher than normal inspection rates were implemented on all peppers, and a stricter protocol established for shipments destined to citrus producing states. Nonetheless, the more intense inspections failed to result in further interceptions of infested chile peppers of any species.

In order to further our understanding of hot peppers as potential hosts of *A. ludens*, a series of experiments were conducted. To provide material for these tests, arrangements were made with the International Services branch of USDA-APHIS to provide fresh manzano peppers from Mexico, inasmuch as these peppers are not commercially cultivated in the United States. One box (15 kilos) of manzano peppers was acquired at a market in Mexico City and shipped by air to our satellite laboratory in General Teran, Nuevo Leon, Mexico. On arrival, technicians discovered that these peppers also were heavily infested with *A. ludens* larvae.

Questions raised by these incidents include whether other species of hot peppers are susceptible hosts for oviposition by *A. ludens*; whether the host status of chile peppers is determined primarily by physiological or ecological factors; whether the flies infesting the manzano peppers were adaptively different from other populations of *A. ludens* (host-races); and are flies reared on chile peppers reproductively competent.

The flies used in these experiments were from two sources. One line was established from adults reared from the initial interception of manzano peppers at Pharr, Texas in April 2003. The second source was the research colony of *A. ludens* maintained at the USDA-ARS laboratory in Weslaco, Texas. This laboratory colony originated with specimens collected from yellow chapote, *Casimiroa greggii* (S. Wats.) Chiang, in Nuevo Leon, Mexico in 1994. Yellow chapote is a wild Rutaceae and the primary native host of *A. ludens* in Mexico (Plummer et al. 1941).

Two sets of experiments were conducted. In the first set of tests the flies were offered fresh fruits in both choice and non-choice configurations under laboratory conditions. In the second set of experiments the flies were released into a green house within the Weslaco quarantine facility with potted pepper plants to determine the acceptability of the living, undehisced pods as oviposition sites.

The Weslaco colony flies were reared on an artificial larval media described by Spishakoff & Hernandez-Davila (1968). Because wild flies are reticent to lay eggs in the artificial substrate used in mass-rearing colonization, the flies bred from the manzano peppers were offered fresh fruit for oviposition. Placed in the cage with these adults were manzano peppers, bell peppers, grapefruit and mangoes. Although the flies were observed “stinging” all of these fruits with the aculeus, only the mangoes became infested.

Flies and fruit were distributed among separate fine mesh screen cages, $30 \times 30 \times 30$ cm in dimension. The cages were maintained in an environmental chamber at 24°C, 12:12 DL. Each cage contained a glass vial filled with distilled water plugged by a cotton wick and an open petri dish with granulated sugar and torula yeast. All flies were females of 15 d age that had been caged with males up until the time of the experiment. Females are capable of laying eggs at age 11 d when maintained at 24°C (Liedo et al. 1993). The test fruits were set in the cage on short wooden pegs so that flies could access the bottom side of the fruit. Aluja et al. (1999) cite field observations that *A. ludens* always "sting" oranges on the bottom side.

Test 1: This test used the Nuevo Leon strain from the USDA colony. Ten female flies were released into each cage. In order to approximately equalize surface area of fruit, one sour orange (*Citrus aurantium* L.) was considered equivalent to four peppers or four chapotes. The sour oranges were picked fresh from trees on the day previous to testing. Habanero peppers (*Capsicum chinense* Jacq.) were purchased from a local grocer. Because yellow chapote does not grow in the U.S., fruits were collected in Nuevo Leon, Mexico and transported in coolers to our laboratory, under permit, the week prior to the test.

The fruits were distributed in six cages as follows: habanero peppers only, sour orange only, yellow chapote only, one sour orange combined with habanero peppers, four yellow chapote combined with four habanero peppers, and four yellow chapote combined with one sour orange.

At the end of 24 h the fruit was removed from the cages and placed individually in cups containing moistened vermiculite for pupariation and held in the chambers at 24°C. In the three cages where only one choice was provided, new fruits replaced those exposed, but a sour orange was placed in the cages that held the habanero peppers and habanero peppers placed in the cages provided orange or chapotes. The rationale of this design was to demonstrate that any failure to oviposit was due to choice and not due to reproductive incapacity. After 24 h these fruits were removed and maintained in separate cups as those previously exposed. After ten days (normal larval development time in the laboratory) the fruits were cut open to determine degree of infestation, if any.

Test 2: Because the only fruits infested in the first test were those offered on the second day, it was reasoned that oviposition response required more than a 24 h entrainment. For the second test, no rotation of fruit was included, but instead, exposure time was increased to 48 h. Also, manzano peppers acquired from Mexico City replaced the yellow chapote in this test. This test was conducted with the USDA colony flies with the same numbers and conditions as the previous test.

Test 3: For this test, mango (*Mangifera indica* L. cv Tommy Atkins) was substituted for the sour orange and only manzano peppers were offered, alone or in combination with the mango. This test was also conducted with the Nuevo Leon strain with the same numbers and conditions as the previous test.

This test was conducted with progeny of the larvae found in the intercepted manzano chile peppers at Pharr in April 2003. These were reared in mangoes maintained under a constant temperature and light regime. Both sexes of adult flies were maintained together until the flies were 11 d old. The test was conducted in the ARS quarantine security green house with naturally cycling temperatures and light regime. Ten females were released into a large screened cage ($78 \times 48 \times 32$ cm) containing three potted chile pepper plants with mature fruit. A bell pepper plant (*Capsicum annuum*) with two mature (red) pods; a habanero pepper plant with seven mature (orange) pods; and an Anaheim pepper plant (*Capsicum annuum*) with three mature (red) pods. All potted pepper plants had been grown together in the greenhouse from seedlings. These particular plants were selected on the basis that the surface area of the pods on each plant was approximately the same, although the number of pods differed. In the cage, the flies were provided with wicked water and open petri dishes with sugar and yeast as before. The flies were exposed to the potted plants for 48 h. After that time, the potted plants were removed to an environmental chamber and held for three weeks. At that time, the pods were cut open to determine infestation. The test was then repeated by installing new plants and replacing the females that had died during the first test with flies from the same cohort.

The majority of the live larvae that were recovered from the intercepted manzano peppers (42 of 50) successfully pupariated. From these 42 puparia only 11 adults successfully developed and eclosed, about 25%. However, survival of larvae collected from citrus and yellow chapote fruit in nature and brought into the laboratory is typically less than 50%. Because wild flies do not readily accept the artificial oviposition medium used in mass rearing, these adults were offered a variety of fruits including sweet and hot peppers, oranges and mangoes. The F_1 generation (4 females and 3 males surviving to maturity) successfully infested and produced progeny only in the mangoes, with over 50 larvae developing. The larger F_2 generation oviposited in both peppers and mangoes. We recovered 27 pupariating larvae from the manzano peppers, 21 larvae from red Tommy Atkins mango and 36 from yellow Manila mango.

In the first test with the Nuevo Leon strain, none of the offered fruit became infested during the 24 h exposure although "stinging" was observed. Evidently, oviposition had not become entrained because after the subsequent 24 h exposure, third instars were found in both the sour orange (2 larvae) and the habanero peppers

(4 larvae). A typical clutch oviposited by a female *A. ludens* is 5-6 eggs according to Berrigan et al. (1988). Habanero peppers are the most pungent of the hot chile pepper species with a rating of 100-500K Scoville Units. Evidently, capcaicinoids do not inhibit oviposition or larval development. It is interesting that the yellow chapotes did not become infested even though it is the native, and therefore presumably, the preferred host fruit for this fruit fly species.

In the second test, manzano peppers were substituted for the yellow chapote and the test extended to 48 h to allow entrainment of oviposition. In subsequent dissection, one larva developed in the manzano pepper and pupariated. However, no adult emerged. In the habanero peppers, one fruit was infested with nine larvae. All pupariated and five adults emerged. The sour orange was not infested.

In the third test, mango was substituted for the sour orange and tested against the manzano peppers. None of the manzano peppers became infested. The mango combined with the peppers produced two larvae which pupariated. The mango by itself produced 72 pupariating larvae. The results of these tests suggest that under laboratory conditions mango, though a non-native host, is preferred by *A. ludens* as an oviposition site compared both to the citrus and the chile peppers. But the results also suggest that chile peppers are as acceptable as citrus, which is the normal host.

A factor which can influence the acceptability of a fruit for oviposition is its ripeness and its status pre- and post-dehiscence. Perhaps this is why the yellow chapotes were not infested. The laboratory tests established that hot peppers are physiologically acceptable as breeding hosts for *A. ludens*. Greenhouse tests were conducted to test if fruits on the bush were similarly acceptable. Bell peppers ($n = 2$) were not infested. Anaheim peppers (0.5-2.5 Scoville Units) were infested, with five larvae found in one pod and six in another. The larvae were placed in vermiculite for pupariation with five of the larvae pupariating and four eclosing as adults. Of the seven habanero peppers, one was found infested with 11 larvae. Three pupariated and two eclosed as adults. In the second replicate only the Anaheim peppers became infested. Of the six larvae recovered, four pupariated and three eclosed as adults.

The results of these tests demonstrate that even the hottest chile peppers are adequate hosts for development of *A. ludens* larvae and that *A. ludens* females are not deterred from oviposition by the capcaicin alkaloids. Such being the case, the important question is why then is the incidence of infestation in commercially grown chile peppers so infrequent? Manzano pepper is unlike other commercial cultivars of peppers in both its growth habit and habitat. The three most commonly cultivated species, *Capsicum frutescens* L. *C. annuum* and *C. chinense* are low (up to 2 m), herbaceous perennials, grown in commercial plantings as a row crop. *Capsicum pubescens* is native to the high elevations of the Andes Mountains of Peru and Bolivia (Eshbaugh 1979) where its growth is reportedly bushy and reaches considerable size, up to 2 m according to Rick (1950). However, the commercial cultivars grown in Mexico are viney in habit, much like tomato plants. In April 2004, the author visited the manzano pepper growing areas in Mexico. In the region around Patzcuaro, Michoacan, cited by Andrews (1984) as the primary commercial production area, the crop is known locally as "chile perón." This region is better known for its commercial production of avocados, *Persea americana* (Mill.). The manzano pepper plants are rooted in the shade of the avocado trees and grow as a vine using the branches of the avocado tree for support. *A. ludens* is trapped in this area but is not considered to be a pest of avocados (Aluja et al. 2004), and local growers were unaware of infestations in the manzano peppers. At the time of the visit in April the peak harvest season was well past, with only late season fruit remaining. We examined mature pods in the avocado groves at Tacambaro and in the local markets of Patzcuaro and none were infested with fruit fly larvae. The habitat at Tacambaro is cool and dry with an elevation of ca. 2200 m. The native vegetation in the area is pine and oak forest.

The infested manzano peppers intercepted at Pharr, TX, were in boxes labeled "Ixhuatlan de Cafe, Veracruz." At Ixhuatlan the product is known locally as "Chile de Arbol." In this region the peppers are grown as an understory plant intercropped with coffee plants. The pepper plants are staked to provide support. The elevation at Ixhuatlan is ca. 1,400 m and the climate is cool but humid. The overstory trees are those indigenous to the tropical montane forest on the eastern slope of the Sierra Madre Oriental (*cf.* Rzedowski 1983). The phenology is apparently variable because this April the plants were in flower with only a few having green immature pods (Fig. 2). Moreover, we found no manzano peppers for sale in the local markets. According to the growers, the manzano pepper is a relatively new crop to this area, introduced only in recent years. They were aware of the fruit fly problem but were also experiencing serious problems with plant diseases, especially fungal pathogens. The high humidity in this region may be less favorable for manzano pepper production compared to the traditional growing area in Michoacan.

Aluja et al. (1999) cite observations in citrus that *A. ludens* females shun exposed fruit in favor of those in the well-shaded parts of the tree. It may be that *A. ludens* avoids most peppers because it avoids open exposed habitats in favor of groves, forests, and shaded urban settings. If the primary factors influencing oviposition are behavioral and

Fig. 2. Manzano pepper plant in a coffee finca at Ixhuatlan de Cafe, Veracruz where the infested peppers originated.

ecological, then manzano peppers may be more susceptible to infestations than other commercial pepper varieties. If so, this information is relevant to quarantine and import inspection protocols.

Summary

Hot chile peppers were not previously considered to be hosts for the Mexican fruit fly. Laboratory tests demonstrate that cultivars with high levels of capcaicinoids are acceptable to ovipositing females, even when given a choice between peppers and citrus, and are adequate for larval development. Recent intercepts of manzano peppers infested with larvae are the first indication that such infestations occur in nature and their import is a potential risk for entry by this invasive tephritid species. Reasons for the low incidence of natural infestations in hot peppers are discussed.

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