Behavior and Predation of Fruit Fly Larvae (*Anastrepha* spp.) (Diptera: Tephritidae) After Exiting Fruit in Four Types of Habitats in Tropical Veracruz, Mexico

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ABSTRACT   *Anastrepha* spp. (Diptera: Tephritidae) larval behavior on the ground was quantified, and biotic and abiotic mortality factors were identified from the moment larvae exit fruit until they secure pupation sites in the soil in four environments that differed in climate, soil structure, and fruit tree composition in tropical Mexico (State of Veracruz). Distribution of pupae was influenced by fruit position on the ground but not by shade, litter depth, average soil temperature, soil pH, vegetation cover, and number of predacious insects per surface unit. At all sites, most larvae (90%) entered the soil after exiting the fruit within 10 min, but only at one site did larvae die because of sun exposure during the hottest part of the day. The most important biotic mortality factor at all sites was predation by ants, which in turn was influenced by temperature, humidity, and soil dampness, and varied significantly among host trees within a site. Ants commonly attacked larvae within 5 min of exit from fruit at all but one site. In laboratory experiments, larvae exposed to ant attack pupated at greater depths than larvae exposed to ants but that were not attacked (which in turn pupated at greater depths than larvae not exposed to ants).

KEY WORDS   predation, mortality factors, antipredator behavior, *Anastrepha*, Tephritidae

PREDATION IS A POTENTIALLY IMPORTANT, but relatively underexplored, force in the evolution of host specialization in phytophagous insects and the understanding of their population dynamics (Price et al. 1980, 1986, Zalucki et al. 2002). In many life tables, "predators and unknown losses" is the most substantial category, yet assumed predation is not often quantified (Price 1987, Bernays and Graham 1988). Two exceptions to the latter are represented by the relatively recent work of Thomas (1993, 1995) and Hendrichs et al. (1994). Thomas (1993), working with the Mexican fruit fly [*Anastrepha ludens* (Loew)], performed detailed studies on larval and pupal predation in northeast Mexico (General Terán and Linares, Nuevo León) and Texas (Weslaco) and discovered, among others, that in the Mexican sites (not in Texas) deer mice [*Peromyscus leucopus* (Rafinesque) and *P. boylii* (Baird)] killed (by eating them) 38% of 4,800 experimentally exposed *A. ludens* pupae. Furthermore, the same author (Thomas 1995) found that fire ants [*Solenopsis geminata* (Fabricius)] inflicted high mortality (up to 94%) in warm months (June–November), but that the onset of cold weather (December and January) reduced mortality to only 2.5% because fire ant colonies became inactive. Importantly, high mor-

tality caused by fire ants was only observed in Weslaco, TX, but not in the Mexican sites (General Terán and Linares, Nuevo León). Hendrichs et al. (1994) elegantly described how yellow-jacket wasps (*Vespula germanica* L.) used the pheromone plumes generated by calling Mediterranean fruit fly males [*Ceratitis capitata* (Wiedemann)], aggregated in leks, to locate and prey on them.

Just as predation is often unmeasured, the antipredator behaviors of large classes of phytophagous prey, such as the tephritid fruit flies, are seldom described. Of the descriptions of antipredator adaptations of Tephritidae, the majority concerns the adult insect and not the larval stage (Greene et al. 1987, Mather and Roitberg 1987, Prokopy and Papaj 2000, White 2000). Thus, while predation of larval fruit flies and their responses to predators are essential aspects of their evolution and their potential biological control, these subjects have been left largely unexamined.

While inside ripening fruit, the fate and behavior of immature Tephritidae is difficult to observe, it seems that, with the exception of fruit consumption by vertebrates (Grewal and Kapoor 1986, Drew 1987), the larvae may confront relatively few predators (Díaz-Fleischer et al. 2000). However, larvae of fruit infesting *Anastrepha* species typically leave fallen fruit to pupate in the soil (Aluja 1994), and while still on the soil surface, they are exposed to a variety of predators and often unfavorable environmental conditions. Among the predators commonly encountered are ants

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(Formicidae) and rove beetles (Staphylinidae), which have been observed to cause substantial mortality (Bateman 1968, Newell and Haramoto 1968, Wong et al. 1984, Eskafi and Kolbe 1990, Thomas 1995, Hodgson et al. 1998).

Skwarra (1934) suggested that larvae move haphazardly during their potentially dangerous searches for suitable pupation sites, whereas Thomas (1995) refers to a more methodical approach, albeit in areas where larval predation was minimal. Darby and Kapp (1934) indicated that pupation sites were chosen on the basis of soil pH. Temperature and soil moisture may also be involved in pupation site selection because they influence mortality, rate of development, and size of emerging adults (McPhail and Bliss 1933, Baker et al. 1944, Aluja et al. 2000). The depth at which larvae eventually pupate affects predation risks (Hodgson et al. 1998), as well as the dangers of desiccation, disease, and parasitism (Bateman 1976, Baeza-Larios et al. 2002, Guillén et al. 2002).

In the following series of observations and experiments, larval behavior after exiting the fruit and before pupation is described, as are larval responses to various biotic and abiotic mortality risks. Larvae from sites that differed in terms of host fruit, local fauna and flora, and soil type were compared. This study follows a previous study on depth of pupation and survival of *Anastrepha* spp. pupae in the same study areas (Hodgson et al. 1998).

Materials and Methods

Study Sites. The study sites were all located in the central part of tropical Veracruz, Mexico. The behaviors of mature larvae (i.e., third stage) and the dangers they faced were studied in the vicinity of four host tree species (study sites): *Spondias purpurea* L., *Spondias mombin* L., *Psidium guajava* L., and *Citrus sinensis* Osbek/*C. aurantium* L. The *Anastrepha* species infesting each type of fruit, and therefore used in the various experiments described below, were *A. obliqua* (Macquart) (*S. purpurea* and *S. mombin*), *A. fraterculus* (Wiedemann) (*P. guajava*) and *A. ludens* (Loew) (*C. sinensis*) (Hodgson et al. 1998). Figure 1 shows the mean daily fluctuations in temperature and humidity in the four study sites while experiments were being conducted. Further details on general climatic conditions and original native vegetation can be found in Hodgson et al. (1998).

Spondias purpurea (tropical plum locally known as ciruela). Investigations were performed under the canopies of two ≈6-m (ht) trees (trees 1 and 2) that were located adjacent to a managed mango orchard situated on the outskirts of Apazapan (19°19′ N; 96°42′ W; altitude, 347 m). We worked during the hottest and driest months of the year (May and June), which coincided with the highly ephemeral and synchronous fruiting season of *S. purpurea* trees, which carry no foliage while bearing fruit. Trees of *S. purpurea* produce hundreds (depending on tree size, thousands) of fruit, which on reaching maturity, fall to the ground within 2–4 wk (details in Aluja and Birke
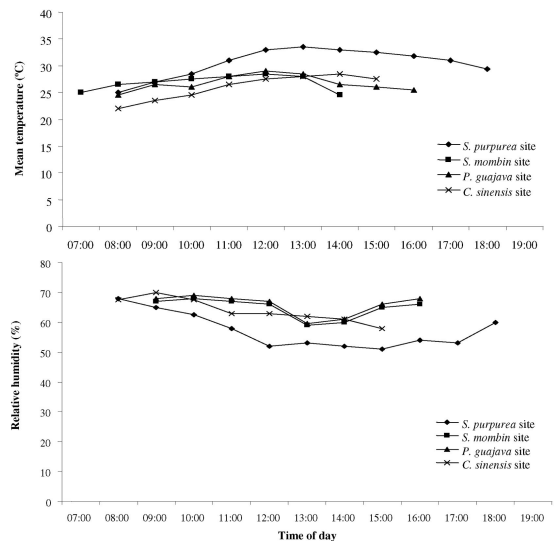

Fig. 1. Climatic conditions (mean diurnal temperature and humidity) at the four study sites during the study periods.

1993). As described by Hodgson et al. (1998), during the first week of the study period, the site's ground cover was <40% (both grasses and broad-leaved species), but subsequent rainfall resulted in an increase to >60% ground cover.

Spondias mombin (tropical plum locally known as jobo). One ≈15- to 17-m (ht) tree was selected at the base of the Ixtla Vieja canyon located ≈3 km from the village of Llano Grande, at latitude 19°22′ N, longitude 96°53′ W and an altitude of 680 m. Single trees of *S. mombin* can produce thousands of fruit which ripen synchronously in a short period of time (Díaz-Fleischer and Aluja 2003). The site, divided laterally by a stream, was located in a semimanaged coffee plantation, shaded by tree canopies and with ground cover consisting of broad-leaved species, varying between 0 and 70%. The southern portion of the site was characterized by 12-cm-deep litter, whereas in the portion north of the stream, the litter depth was <3 cm. The study period encompassed the months of August and September.

Psidium guajava cultivar criollo (guava locally known as guayaba). The four study trees (≈5–6 m in height) were scattered in a backyard orchard (locally known as huerto de traspatio) in the village of Tejería, latitude 19°22′ N, longitude 96°54′ W (altitude of 1,050 m). Guava trees bear ripe fruit over a period of 1–2 mo in this locality. Ground cover varied between the study trees; beneath some, this exceeded 90% (predominantly grasses), whereas beneath others, it was <15%. As was the case with *S. mombin*, the study period encompassed the months of August and September.

Citrus sinensis cultivar Valencia (orange locally known as naranja dulce) and *C. aurantium* (naranja cucha). This site was designated as *C. sinensis*, even though larvae used in experiments stemmed from

the *C. aurantium* trees in patch ('Valencia' oranges seldom get infested in study region). This field site was located ≈200 m from the village of Tejería (details on location above). The four study trees (≈5 m) were located in a mixed coffee plantation with oranges and bananas (ground cover was <40%). In stark contrast with *S. purpurea* and *S. mombin,* orange trees bear ripe fruit over extended periods of time (>4 mo). Observations were made during October.

Experiment 1: Natural Pupal Distribution Beneath the Host Tree Canopy. In the laboratory, *Anastrepha* larvae have been observed to move distances of a meter or more after exiting from the host fruit (Darby and Kapp 1934; J. Sivinski, unpublished data). Therefore, the aim of this experiment was to study if the latter is the case in nature and if so, whether this is a response which has evolved to take advantage of desirable microhabitat conditions in the soil to reduce mortality risks.

The experiment was performed under the canopies of *S. purpurea* and *P. guajava* using knowledge acquired during a previous study on depth of pupation and *Anastrepha* spp. pupal survival (Hodgson et al. 1998). The sampling area extended from a point level with the tree trunk to beyond the canopy with ≈50% of sampling quadrants (described below) under canopy cover and 50% outside canopy cover. The sampling areas therefore extended through a variety of microhabitat conditions that differed in quantity of fallen fruit, shade, vegetation, and soil conditions (i.e., humidity, pH, temperature). Within the study area, nine transect lines were marked out in three groups of three (A, B, and C). Each line consisted of 50 by 50-cm quadrants, 26 per line in *S. purpurea* and 15 in *P. guajava.* Sampling was carried out on three dates, extending from early to late within the fruiting season of each respective fruit.

On each sampling date, one line of quadrants was sampled from each of the groups A, B, and C. For each quadrant, the following characteristics were recorded: shade, as a percentage cover when looking vertically upward; vegetation cover, as percentage cover; litter depth; number of fruit; and ground predator activity.

At two hourly intervals throughout the sampling period, soil temperature and pH were recorded (Portable Meters; Hannapro S.A. de C.V., Mexico City). In each quadrant, pupae were searched for at three levels: surface, 0–2 cm, and 2–5 cm (as described in Hodgson et al. 1998, pupae were almost never [$n = 1$] found at depths >5 cm). Soil was sieved using colanders (≈1.5-mm pore size). In the guava site where soil was wet and like clay, it was necessary to mix it with water in a bucket before passing it through the colander. The number of pupae and pupal cases found at each level was recorded.

Experiment 2: Observations of Larvae After Exiting the Fruit. The experiment was designed to describe the behaviors and fates of larvae between exiting the fruit and entering the soil and was run in the four experimental sites. The main difference in procedure between sites involved the number of trees used. This was dependent on availability. Four, four, two, and

one *P. guajava, C. sinensis, S. purpurea,* and *S. mombin* trees were used, respectively. In the last case, although only one tree was used, its large canopy area was sufficiently large to offer a variety of conditions.

The first method was to mimic natural larval exit conditions as much as possible, by obtaining larvae by shaking a branch of the tree gently so that only the fruit that were ready to drop fell. Five infested fruit were placed together on the ground. Larval exit and subsequent larval behaviors and their fate were noted as follows: distances moved across the soil surface and time taken to do so; interactions with predators; attempts at burrowing; successful entry into the soil and location of entry in relation to start point; killed by ants; and death from climatic exposure.

Fruit were observed for 1 h and dissected to determine remaining larval content. Temperature and humidity were measured throughout the experiment at 30-min intervals. The soil surface conditions were also noted (i.e., whether wet or dry).

We were only able to use method 1 in the case of the two *S. purpurea* trees, where larval exit from the fruit occurred shortly after the fruit hit the ground. In the other three field sites, 30 freshly fallen fruit (*C. aurantium* in the case of the *C. sinensis* site) were observed for 1 h without a single larval exit being recorded. Method 2 therefore consisted of removing mature third-instar larvae (ready to pupate) from field-collected fruit. Larvae were placed on the top of the fruit so they could detect its presence and perhaps direct their movement toward a specific pupation site relative to the fruit position as opposed to placing larvae next to a fruit without prior contact with it.

Experiment 3: Pupal Recovery. The aim of this experiment (a follow-up of the previous one) was to study the relationship between distances moved from the fruit and pupation depth and to give an indication of mortality caused by ants when the larvae enter the soil. We hypothesized that predators might orient to fallen fruit and that larvae burrowing nearer fruit might pupate at greater depths to avoid high concentrations of predators.

Given that larvae exiting *S. purpurea* fruit almost always crawled underneath it (i.e., did not move any significant distance away from fruit), this experiment was only run in the *S. mombin, P. guajava,* and *C. sinensis* sites using the larval release method described as method 2 under experiment 2. The entry points of larvae were marked with sticks placed vertically in the ground at all sites. After 2 h, the soil in that area was searched to detect the presence/absence of prepupae and to measure their burial depth. In comparison to fully developed third-instar larvae, prepupae can be recognized by their shorter fattened body, which prevents further burrowing. A soil sample from each site was removed (the top 4 cm depth were used) to determine particle size and classify soils on the basis of texture. Soil analyses were performed by specialized personnel at the Departamento de Ecología de Suelos, Instituto de Ecología, A.C., Xalapa, Veracruz.

Experiment 4: Investigation of Larval Exit Times from the Fruit. The aim of this experiment was to study the period that larvae spend in fruit on the ground and to detect any diurnal pattern of exit. We only used *P. guajava* and *C. aurantium* because the other fruit were unavailable at the time the study was run. Collections were made in the middle of the fruiting season by shaking trees so that ripe fruit fell to the ground. Ten kilograms of fruit from each tree was placed in plastic mesh baskets with holes in the base sufficiently large to allow the passage of larvae from fruit to soil. The basket was balanced on a large plastic bowl. The bowl contained soil (4 cm deep) so that falling larvae would be encouraged to pupate. Initially, the soil was sifted daily to search for pupae. However, once larvae started to exit, the number of pupae (and larvae) in the soil was noted at 4-h intervals until a decline in exiting larvae was evident. This intensive sampling period lasted 3 d for each fruit type.

Experiment 5: Observations of Fallen Fruit to Determine Position of Exit Holes. Larvae were observed to exit through holes present in the soft part of the fruit that they occupied. Typically, this only constituted a small portion of the fruit (20%). These perforations are probably used initially as breathing holes or to allow fermentation gases to escape (Aluja et al. 2000). Observations were designed to study the position of these holes as an indication of the position of the larvae within the fruit and also the location of the point of exit in relation to the lie of the fruit on the ground. Both of these factors might influence mortality.

With the exception of *P. guajava*, in each of the study sites, at least 80 freshly fallen, randomly chosen fruit, were studied (*C. aurantium* in the case of the *C. sinensis* site). The positions of the holes were scored as either top, bottom (i.e., under fruit body), or side. In the case of *P. guajava*, it was not possible to identify fruit fly holes from beetle holes, so it was not possible to conduct observations.

Experiment 6: Effect of Ant Interactions on Pupal Depth. The depth at which a larva pupates can have a significant effect on its survival (Hodgson et al. 1998). Ground predators are an important mortality factor whose activity is negatively correlated with depth (Bateman 1976). If ants, as one of the most frequently reported predators, are encountered by the larva before pupation, we hypothesized that an appropriate response might be to pupate at a greater depth. A total of 90 third-instar *A. ludens* larvae removed from oranges (*C. aurantium*) were used, 30 for each of the following three treatments:

• Treatment 1. Larvae were individually placed in a plastic container enclosing 50 ants (*S. geminata*). All larvae were attacked within a time period of 6 min. As soon as this happened, the ant in the act of biting was removed. The pupation sites were in the form of two 1-liter plastic bowls containing a known quantity of soil, compacted to an equal depth (15 cm) in each bowl. After ant attack, larvae were placed on the soil surface of one of the bowls

(15 larvae/bowl) and allowed to burrow into the soil and pupate. After 24 h, pupae were located and their burial depth noted.
• Treatment 2. The same procedure was followed as in treatment 1, except this time the ants were prevented from attacking the larvae. The larvae did, however, spend the same period of time in the ant arena and had physical contact with ants. Pupation sites were exactly the same as in treatment 1.
• Treatment 3. Larvae were handled in the same way as in treatments 1 and 2 but were placed in a clean arena that had never contained ants for 6 min.

Statistical Analyses. The relationships between the distribution of pupae (number per quadrant) and distribution of fallen fruit (number per quadrant), shade, litter depth, average soil temperature, soil pH, vegetation cover, and number of predacious insects per quadrant were studied using generalized linear interactive modeling package (GLIM; release 4; NAG, Oxford, United Kingdom; as were other data sets; details follow). To study mortality, data were analyzed using a binomial error structured model that initially incorporated all measured explanatory variables. These were removed in turn to study their individual importance. In the case of *S. purpurea*, the only site where there were two clearly distinguishable mortality factors (ant predation and exposure), data were analyzed first to study total mortality and then to study the two mortality factors individually. The relationship between distance from the fruit and pupal depth among sites as well as larvae–ant interactions on pupal depth were analyzed by analysis of variance (ANOVA) using GLIM.

Results

Experiment 1: Natural Pupal Distribution Beneath the Canopy of the Host Tree. The only variable that could explain a significant proportion of the variance in pupal distribution was fruit distribution ($F = 45.94$; df = 1,232; $P < 0.001$). Shade, litter depth, average soil temperature, soil pH, vegetation cover, and number of predacious insects (i.e., ants) per quadrant did not contribute significantly in explaining variance in pupal distribution.

Experiment 2: Observations of Larval Fate After Exiting the Fruit. Table 1 compares the proportion of larval entry into the soil, attack by ants, and death caused by exposure among sites.

Figure 2 shows the frequency distribution of time taken to enter the soil by the larvae in the four study sites. In the *S. mombin*, *P. guajava*, and *C. sinensis* sites, >90% of the larvae entered within the first 10 min of the experiment (most of these within the first 2 min). In the *S. purpurea* site, however, <50% of the larvae achieved entry within the first 10 min of the experiment, and the range of "times to entry" was broader (1–120 min). Mean time taken to enter the soil was significantly different among sites ($F = 80.73$; df = 3,605; $P < 0.01$). In the *S. purpurea* site, the time taken was greatest (20.09 ± 2.64 min; $n = 51$), whereas in

Table 1.   Fate of larvae after exiting the fruit. Total mortality, mortality caused by ant attack, and mortality caused by exposure are given as a percentage of the total sample size for each study site

	S. purpurea site	*S. mombin* site	*P. guajava* site	*C. sinensis* site
Percent total mortality	54.5	6.6	14.7	6.8
Percent killed by ants	32.2	6.6	14.7	6.8
Percent killed by exposure	22.3	0.0	0.0	0.0
Percent entered soil	45.5	93.4	85.3	93.2
Sample size	170	183	129	183

S. mombin site, the mean entry time was least $(2.21 \pm 0.26$ min; $n = 170)$. In the two remaining sites (*P. guajava* and *C. sinensis*), mean entry times were similar (*P. guajava:* 4.18 ± 0.42 min; $n = 166$; *C. sinensis:* 5.2 ± 0.43 min; $n = 219$).

In the *S. purpurea* site, ants attacking larvae on the surface were *S. geminata*, whereas in all other sites, both *S. geminata* and *Pheidole* sp. actively predated larvae. Figure 3 shows that the mean time taken for ants to attack larvae was significantly different among the four field sites (ANOVA: $F = 5.99$; df $= 3,138$; $P < 0.01$). In the *S. purpurea, S. mombin,* and *P. guajava* sites (10.64 ± 1.34 min, $n = 78$; 14.33 ± 3.78 min, $n = 9$; and 6.94 ± 1.09 min, $n = 32$; respectively), ≈50% of the larvae were attacked within the first 5 min. However, in the *C. sinensis* site, where mean time to attack was 19.2 ± 2.13 min ($n = 24$), only 8% of the larvae attacked experienced the aggressive act within this time period.

Only in the *S. purpurea* site did larvae die because of environmental exposure alone (mainly direct sunshine). Such larvae typically managed to move only <10 cm from the fruit before they began to wriggle violently. The mean time taken to die was 4 ± 1.63 min, with some larvae dying as quickly as 2 min after the beginning of the experiment. The larvae only suffered this mortality between 1000 and 1400 hours (Fig. 4). The most "hazardous" time appeared to be between 1100 and 1200 hours, when 91% of the larvae died. One of the aims of experiment with method 1 (shaking the tree to reproduce natural events) was to study whether larvae leave the fruit at the time of day when mortality risk is high. Our results showed that they do.

Table 2 compares the relative importance of explanatory variables on larval mortality for all sites. In the *S. purpurea* site (two study trees), when total mortality (mortality caused by ants and exposure) was considered, significantly less mortality occurred at higher humidity ($\chi^2 = 9.04$; df $= 1,59$; $P < 0.01$). Both experimental method and tree were also shown to affect mortality significantly. When mortality caused by ant attack was considered individually, the variables that explained a significant amount of the variance were temperature ($\chi^2 = 9.72$; df $= 1,59$; $P < 0.01$), humidity ($\chi^2 = 4.83$; df $= 1,59$; $P < 0.05$), and soil dampness ($\chi^2 = 13.58$; df $= 1,59$; $P < 0.05$). Ant predation decreased with increasing temperature and increased with increasing humidity. Mortality caused by ants was significantly higher at tree 1 than tree 2 and on dry soil as opposed to wet soil. Mortality caused by exposure was significantly affected by date ($\chi^2 = 5.58$; df $= 1,59$; $P < 0.05$), soil dampness ($\chi^2 = 13.58$; df $= 1,59$; $P < 0.01$) and tree ($\chi^2 = 5.90$; df $= 1,59$; $P < 0.05$). Mortality decreased throughout the sampling period (mainly because of rainfall that increased with soil dampness) and was greater at tree 1.

In the *S. mombin* site (only one tree), the only recorded variable that could explain a significant amount of the variance in mortality (entirely caused by ants) was tree (i.e., there was greater mortality under the canopy north of the stream; $\chi^2 = 9.19$; df $= 1,59$; $P < 0.01$).

In the *P. guajava* site, difference among the four study trees was the only significant ($\chi^2 = 4.73$; df $= 1,42$; $P < 0.05$) explanatory variable describing larval mortality caused by ant attack, whereas at the
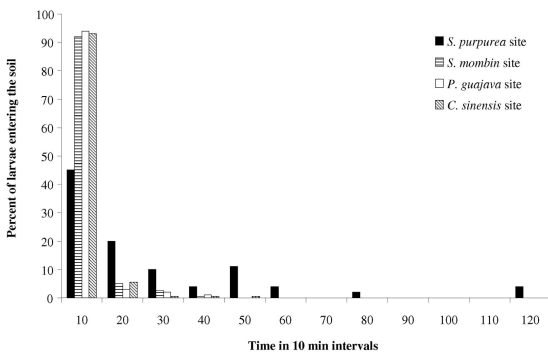

Fig. 2. Time taken for *Anastrepha* spp. larvae to enter soil. Measured in minutes between natural exit from fruit (*S. purpurea* site) or artificial release on top of fruit (all other sites).
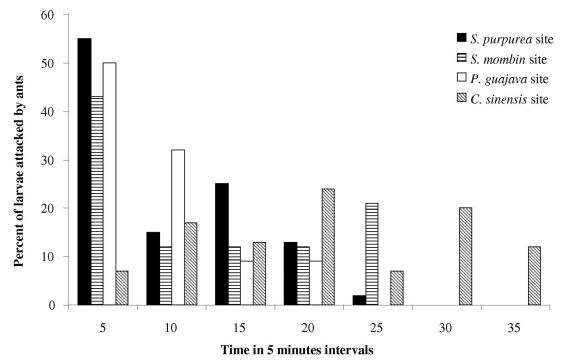

Fig. 3. Time taken for ants to initiate attack on *Anastrepha* spp. larvae. Measured in minutes between natural exit from fruit (*S. purpurea* site) or artificial release on top of fruit (all other sites).
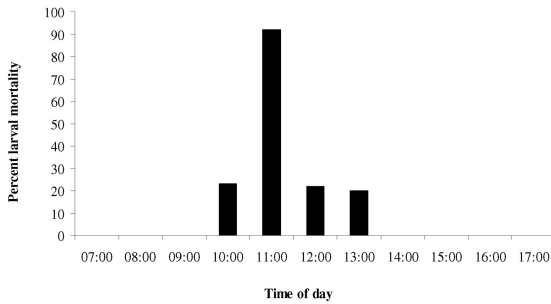
Fig. 4. Diurnal *A. obliqua* larval mortality caused by exposure to the elements (particularly sun) in Apazapan, Veracruz, Mexico. Observations made under the canopy of a tropical plum tree (*Spondias purpurea*).

C. sinensis site (also four study trees), mortality showed significant increases with decrease in both temperature ($\chi^2 = 12.2$; df = 1,86; $P < 0.01$) and humidity ($\chi^2 = 8.63$; df = 1,86; $P < 0.01$).

Experiment 3: Pupal Recovery. Analyses of soil samples revealed the following: clay-loam in the *S. mombin* and *P. guajava* sites (Llano Grande and Tejería) and clay in the *C. sinensis* site (also Tejería). Soil in the *S. purpurea* site (Apazapan, not considered in this experiment), was sand-loam. Percentages of pupae recovered from the original sample (larvae released) were similar for all sites (*S. mombin* site: 60% [$n = 134$]; *P. guajava* site: 54.7% [$n = 137$]; *C. sinensis* site: 63.5% [$n = 241$]). The relationship between distance from the fruit and pupal depth differed among the sites. In the *S. mombin* site, pupae retrieved at a greater distance from the fruit were deeper ($F = 4.65$; df = 1,151; $P < 0.05$); in the *P. guajava* site, there was no relationship ($F = 0.28$; df = 1,100; $P > 0.05$); and in the *C. sinensis* site, pupae closer to the fruit were at a greater depth ($F = 9.7$; df = 1,71; $P < 0.01$).

Experiment 4: Larval Exit Times from the Fruit. Larvae started to exit from *C. aurantium* on the fourth day after fruit collection and continued for the next 4 d. In the case of *P. guajava*, they began to exit on the tenth day after collection and continued to do so for 3 d. Figure 5 shows diel exit patterns. Exit of larvae from *C. aurantium* occurred throughout the 24-h pe-

riod, with most exits occurring between 0400 and 1200 hours (>60%). Overall, larvae exiting from *P. guajava* showed a marked preference to exit between 0400 and 0800 hours (79% of the larvae exited on the second day of emergence). No larvae exited between 2000 and 0000 hours.

Experiment 5: Observations of Fallen Fruit to Determine Position of Exit Holes. In the *S. purpurea* site, the majority of the exit holes (69.1%) were under the fruit, 9.5% were found on top of the fruit, and 21.4% were found on the sides of fruit ($n = 84$). In the *S. mombin* and *C. sinensis* sites, the positions of the holes were more evenly distributed (39.3, 21.5, and 39.2%, $n = 112$; 28.1, 33.7, and 38.2%, $n = 89$).

Experiment 6: Effect of Ant Interactions on Pupal Depth. Mean pupal depths for treatments 1, 2, and 3 ($n = 30$ per treatment) were shown to be significantly different ($F = 6.482$; df = 2,87; $P < 0.05$). The larvae that were bitten by ants pupated at an average depth of 2.92 ± 0.01 cm. The larvae that were presented to ants pupated at a depth of 2.48 ± 0.02 cm. The larvae that had not encountered ants pupated at the shallowest mean depth of 2.33 ± 0.01 cm.

Discussion

Natural Pupal Distribution Beneath the Canopy of the Host Tree. In contradiction to the belief of Darby and Kapp (1934) that larvae often searched considerable distances for pupation sites, here we found that all pupae detected were within 50 cm of a fruit. Movement away from the fruit could be desirable if larvae search for pupation sites with optimal abiotic conditions (e.g., moist, softer soil; see for example, Thomas 1995) or if displacement away from kairomone-emitting fruit prevented aggregated patterns of pupae that might attract and hold predators and parasitoids (Guillén et al. 2002). However, there is likely to be a trade-off between the advantages of securing an ideal pupation site and the perils (i.e., predators, parasitoids, heat or exposure to burning from sun light), which are associated with being exposed on the soil surface. In our study, >75% of larvae entered the soil within a 5-cm radius of their host fruit. When possible, larvae entered the soil directly beneath the fruit. Gen-

Table 2. Fate of *Anastrepha* spp. larvae after exiting fruit

| | *S. purpurea* site | | | *S. mombin* site | *P. guajava* site | *C. sinensis* site |
	Total	Ants	Exposure			
Date	NS	NS	5.58[a]	NS	NS	NS
Hour	NS	NS	NS	NS	NS	NS
Temperature	NS	9.27[b]	NS	NS	NS	12.20[b]
Humidity	9.04[b]	4.83[a]	NS	NS	NS	8.63[b]
Tree	7.21[b]	NS	5.9[a]	9.19[b]	4.73[a]	
Soil dampness	NS	4.42[a]	13.58[a]			
Sample size	60	60	60	60	43	87

Effect of explanatory variables (i.e., date, hour, temp, humidity, and soil dampness) on larval mortality (caused by ant attack and exposure to the elements).

Numbers before superior letters are χ^2 values.

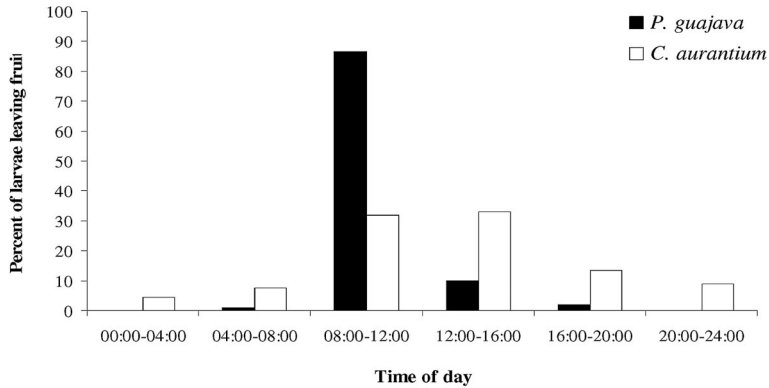[a] $P < 0.05$; [b] $P < 0.01$.

NS, not significant.

Fig. 5. Pattern of diurnal exit of *Anastrepha* spp. larvae from infested *P. guajava* and *C. aurantium* fruit over a 3-d period.

eralist predators, the main mortality factor during this stage, did not seem to use individual fruit odor as a cue to increase search activity in areas where larvae may have concentrated (i.e., they were not more abundant in areas with greater fruit density). However, it has been shown that many ant species, when encountering a persistent food source (such as continuing seed fall), deposit trails of glandular secretions (Hölldobler and Wilson 1990). Such a foraging strategy could concentrate ant activity under the canopy of fruiting trees, although not necessarily close to individual fruit. Because the most abundant and widespread predator in most of our study sites (*S. geminata* ants) does not seem to be effective underground (details in Hodgson et al. 1998), immediate larval burrowing may be in response to environmental exposure and predation risk, both of which are greatest when larvae or pupae are on the soil surface (Hodgson et al. 1998). A similar result was obtained by Thomas (1995) working in Weslaco, TX, a place with large fire ant populations. We note, however, that the same author (Thomas 1995) working in a different site (General Terán and Linares, Nuevo León, northeast Mexico), found that, if predation pressure is low, burrowing behavior is driven by "an adaptive emphasis on location of a suitable pupation site rather than an effort to reduce the vulnerability to predation." Under such conditions, larvae may cover significant distances and take as long as an hour before finally burying into the ground to pupate (Thomas 1995).

Observations of Larval Fate After Exiting the Fruit. While larvae minimize risk of exposure and predation by entering soil as soon as possible after exiting the fruit, good pupation sites may not be readily available. In the *S. purpurea* site, where larvae took a significantly greater time to burrow, the soil was very dry, sun-baked, and appeared difficult to penetrate (as noted before, soil type in this site was sandy loam). As larvae moved across the surface, they would frequently stop and attempt to enter. The highest percentage of larvae killed was at this site, and death was not only caused by ants but also to exposure. At the *P. guajava* site, in contrast, the mean time to initiation

of ant attack was shortest, perhaps because the mean time to enter the soil was also small. Similarly, the site where larvae entered the soil most rapidly was the *S. mombin* one, where the top 15 cm of the soil had a high organic content that served to make the soil less compact (therefore easing larval entry). Larvae frequently disappeared beneath leaf litter within the first minute. At this site, we observed the lowest number of ant attacks of our study and witnessed no death caused by exposure.

In the *S. purpurea* site, ants were mostly *S. geminata*, which as noted previously, are highly effective aboveground foragers (Hölldobler and Wilson 1990). Many of the larvae were located and aggressively attacked by ants in the first 2 min of the experiment. When a single ant encountered a larva, it immediately inserted its mandibles and soon was joined by as many as 50 conspecifics. In the other sites, the dominant ant species was *Pheidole* spp. These were not so abundant on the soil surface, and on average, the time for larval discovery was longer.

Death caused by exposure only occurred in the *S. purpurea* site at midday probably because of a combination of overheating (i.e., thermal stress), dehydration, and possibly burning of the cuticle by the hot soil surface. Larvae did exit fruit during the period when the risk of mortality was greatest, most likely because fruit pulp also became extremely hot at this time of day, and the only chance a larva has for survival is to exit the fruit and attempt to enter the soil (Aluja et al. 2000). Under conditions of extremely hot temperatures, *A. obliqua* larvae sometimes egress and fall from unabscised fruit on the tree to avoid death from overheating (Aluja and Birke 1993). In our study, death caused by exposure was influenced by soil dampness, which can reduce mortality in two ways: ease of entry into the soil and reduction of soil temperature. Furthermore, increases in temperature and decreases in humidity in the *S. purpurea* site reduced mortality caused by ants. This was probably a reflection of decreased ant activity during the hottest part of the day typical of diurnal foraging patterns of ant

species inhabiting warm climates (Hölldobler and Wilson 1990).

The only variable that influenced mortality (entirely caused by ants) in the *S. mombin* site was within-site location. This highlights the variability in predation within a small area, perhaps because foraging ants are swiftly affected by ambient temperature and humidity of the microenvironment (Hölldobler and Wilson 1990). The area under the tree canopy was divided by a stream and the area north of the stream was sloping and subject to rapid removal of leaf debris by run-off. Larval entry into the soil was quicker south of the stream, where leaves provided rapid escape from potential predators.

At the *P. guajava* site, location was again an important influence on ant-induced mortality. Here, ease of entry into the soil was not uniform and ants showed an aggregated distribution. Entry into the soil seemed to be influenced by vegetation. Under the trees where grass dominated (>85% cover), entry was facilitated by stems breaking the soil surface and providing a route for penetration. Under trees that were close to houses, the soil tended to be compacted and vegetation was scant. Burrowing under these conditions was more difficult, giving rise to longer exposure to ants before entry was achieved.

At the *S. purpurea* and *C. sinensis* sites, ant activity was reduced at high temperatures. However, in contrast with the *S. purpurea* site, increased humidity resulted in reduced larval mortality in the *S. mombin* site, because high levels of humidity coincided with rainfall that deterred ant activity (according to Skinner 1980a, b, rain halts ant foraging in places where drops pelt the ground and form small puddles and rivulets). In contrast, in the *S. purpurea* site, rainfall generally started after the daily study period, and high humidities coincided with lower temperatures and a suitable environment for ant foraging.

Depth of pupation may also be related to predation risk. While 47% of Mediterranean fruit fly, *Ceratitis capitata* (Wied.), pupated as deeply as 3 cm in coffee plantations in Guatemala, they still suffered substantial predation by ants and other insects (Eskafi and Kolbe 1990). However, under other circumstances, burial as shallow as 2 cm can significantly lower predation rates (Hodgson et al. 1998). Sivinski (1982) showed for scarab dung beetles that there is a negative relationship between distance from the dung patch and dung-ball burial depth. This is thought to be a response to aggregation of predation around the source of the ball. The same relationship was shown in the *S. mombin* site where pupae closest to the fruit buried deeper in the soil. This may be attributed to an increased threat of predation in the vicinity of fruit that could act as a lure to predators and an indication of buried pupae. In the *S. mombin* site, rove beetles (Staphilinidae) were commonly associated with the underside of the fruit and observed to attack larvae. These and other predators lurking under host fruit may select for deeper burrowing by larvae near fruit. Finally, distance and depth were positively correlated in the orange site with pupae closest to the fruit being

shallowest, perhaps because soil was harder to enter near fruit.

Larval Exit Times from the Fruit. Larvae exited *C. aurantium* and *P. guajava* fruit most frequently in the mornings, but what triggers this is unknown. The most important factor might be temperature and high relative air humidity, because 87% of the larvae exited from the guavas between 0400 and 0800 hours, the coldest and dampest time of the day. Such exit patterns contrast with those observed in the *S. purpurea* site, where the main stimulus seemed to be the impact of the fruit when it hit the ground (80% of mature larvae exited in the first 5 min after impact). Relatively small fruit in areas with high temperatures likely represent rapidly deteriorating environments that are best left quickly (John Sivinski, Tim Holler, and Ruy Pereira, unpublished data). This might be particularly true if within-fruit predators and parasitoids (e.g., figitid wasps; Ovruski and Aluja 2002) forage more efficiently in smaller fruit.

Differences in larval exit times between sites could be caused by different predator diel patterns (Aluja et al. 2000). It is possible that in the *P. guajava* and *C. sinensis* sites, low temperatures resulted in reduced predator activity, but in the *S. purpurea* site, observations during the cooler night revealed no lull in ant activity. The only time a decrease in ant activity was observed was during the hottest time of the day. However, death caused by exposure was greatest when ant activity was least. Therefore, there may be a trade-off in optimal exit time, and this could explain why there seems to be no preference. Rapid exit when fruit hits the ground may well be an appropriate strategy, because ants and rove beetles were often witnessed crawling on and into fruit, offering a potential risk to larvae that remain in the fallen fruit.

Observations of Fallen Fruit to Determine Position of Exit Holes. The location of exit holes from *C. aurantium* and *S. mombin* did not show a tendency to be positioned in a particular way in relation to the lie of the fruit. In *S. purpurea*, however, the majority of the perforations were under the fruit. Perhaps high temperatures led larvae to seek more immediate shelter. Beneath the fruit, entry into the soil may have been easier and therefore quicker as fruit juices could soften the soil. Larvae exiting from the fruit often crawled directly beneath it, effectively creating a semirefuge from ant attack.

Effect of Ant Interactions on Pupation Depth. Larvae that interacted with ants pupated at a greater depth. For this predatory avoidance mechanism to evolve, larvae and/or pupae must be subjected to ant attack in the soil, and the risk of this occurring must be depth dependant. Although Eskafi and Kolbe (1990) reported large proportions of Medfly pupae removed by predators at a depth of 3 cm, Hodgson et al. (1998) found that *Anastrepha* spp. pupal removal by ants was by far greater on the soil surface and recorded identical and much lower rates of removal for pupae buried at 2 and 5 cm of depth. The more extensive burrowing of attacked larvae may be to escape a present danger by outdistancing a predator or

an attempt to minimize future encounters predicted by a previous interaction with ants.

In summary, the objectives of conducting this series of experiments were to clarify the role of ground predators in the mortality of the late larval stages of the fruit fly life cycle and to ascertain larval behaviors that may have evolved in response to predation. It is apparent that the particulars of fruit fly–predator interactions are highly variable both between and within sites, resulting in different mortalities and different larval behaviors. The vulnerable time period in terms of ground predators commences when the fruit hits the ground. Once exposed on the soil surface, rapid entry into the soil may be the optimal strategy, and this is more important to survival than seeking an ideal pupation site (but see Thomas 1995). This strategy may be largely the result of selection caused by ant predation on the soil surface. Observations of larval burrowing more deeply in the soil after exposure to ants suggest that depth of pupation may be a result of predatory selective pressure. However, it is not clear that the relatively small differences (albeit significant) in pupation depths among larvae contacting or not contacting ants artificially are biologically meaningful. Previous experiments with buried pupae suggested little decreased mortality (i.e., pupal disappearance between pupae at depths of 2.5 and 5.0 cm; Hodgson et al. 1998). However, larvae in nature may leave kairomones as they burrow and so expose themselves to predators not involved in the burial experiments of Hodgson et al. (see Baeza-Larios et al. 2002).

In regard to recommendations for control through predation, the variability encountered in our study and the one by Thomas (1995) suggests techniques to be explored. We must consider the factors that seem important to mortality and which of these can be manipulated. The two most important factors governing mortality caused by predation seem to be ant species present and time taken for the larvae to enter the soil. Both these factors are strongly influenced by climate, soil physical properties, and ecological background (i.e., type of vegetation cover). Exploring the use of cultural practices to enhance performance of ground predators such as ants in tropical environments could result in reduction of tephritid fruit fly damage to crops, as it has been found for other insect pests (Hagen et al. 1999). However, as shown here and elsewhere by Thomas (1995) and Hodgson et al. (1998), the effectiveness of ants as biological control agents will vary greatly between species and sites. For example, here and in the sites described by Thomas (1995), *S. geminata* was more effective than *Pheidole* sp. Furthermore, evidence suggests that, at least in the case of *S. geminata*, effectiveness is contingent on environmental conditions (e.g., soil humidity) and degree of environmental perturbation. For example, predation levels were high in our *S. purpurea* site (Apazapan, Veracruz) and in Weslaco, TX (Thomas 1995), but low in more natural settings such as the premontane, riparian stands of yellow chapote (*Sargentia greggii* S. Wats.) commonly found in Nuevo León, Mexico (Thomas 1995). Interestingly, in such environments, deer mice exert a much higher predation pressure than ants (Thomas 1993).

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