Effects of Time of Day, Adult Food, and Host Fruit on Incidence of Calling by Male Caribbean Fruit Flies (Diptera: Tephritidae)

PETER J. LANDOLT AND JOHN SIVINSKI

Insect Attractants, Behavior, and Basic Biology Research Laboratory, USDA–ARS, Gainesville, Florida 32604

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ABSTRACT Male Caribbean fruit flies, *Anastrepha suspensa* (Loew), exhibited two distinct calling periods under greenhouse conditions, one within the first hour of daylight and another during mid to late afternoon. In the laboratory, males called only during the afternoon. In the greenhouse, this afternoon calling period was not observed when flies were deprived of food for that day. High rates of calling were observed in the afternoon if flies were provided either a mixture of hydrolyzed torula yeast and cane sugar, or pure sucrose, but not if provided yeast alone, or deprived of food. Deprivation of sugar for 8 h resulted in a 30% reduction in calling rates in the afternoon, whereas such deprivation for 23 h (from late afternoon to midafternoon) nearly eliminated calling during that period. Male Caribbean fruit flies kept in cages with green, ripe, or overripe guava fruit also exhibited calling (35–45%) near dawn, but only those flies kept in cages with overripe fruit called in the afternoon. Such a difference is likely due to males feeding on the surface of overripe fruit.

KEY WORDS Insecta, *Anastrepha suspensa*, signaling, pheromones

THE CARIBBEAN FRUIT FLY, *Anastrepha suspensa* (Loew), is a major quarantine pest of citrus fruit in Florida and infests many tropical fruits, including guava (Swanson & Baranowski 1972). As with other tephritid fruit flies of economic importance, methods are needed for monitoring and detecting populations and for control and eradication technology. Currently, only protein-based baits are available to attract and trap this species. These baits are considered weak and ineffective in detecting low populations and in attracting flies from some distance (Calkins et al. 1984).

Male Caribbean fruit flies produce a pheromone attractive to both males and females. Nation (1972) first reported the characteristic distension of pleural abdominal pouches (puffing) and the extrusion of an anal sac by males, a behavior often associated with sex pheromone release in tephritids. Nation (1972, 1975) also reported evidence of female attraction to live males and solvent extracts of males, using a tube olfactometer. Sexual attraction was first documented in the field by Perdomo et al. (1976), who reported the recapture of released females in traps baited with live males. Webb et al. (1983) provided evidence from field cage studies that the observed sexual attraction of Caribbean fruit flies is attributable at least partly to chemicals produced by males as well as to calling sounds. Together, these studies indicate that *A. suspensa* males produce a pheromone that may be used as an attractant for monitoring and detection.

Several chemicals have been identified as potential pheromone components of male Caribbean fruit flies (Nation 1972, 1975; Battiste et al. 1983, 1988; Chuman et al. 1988). However, no progress on the development of an attractant or lure based on pheromone chemistry has been reported. Information is needed on female and male behavioral responses to male sexual signals, including pheromones. A detailed account of behavioral interactions between male and female flies would provide a better means to assay critically components and blends isolated from males for pheromonal responses. It would also provide a better assessment of what attraction responses to expect in the field to a synthetic lure. As a prelude to the study of female pheromonal responses, experiments were conducted to determine the conditions necessary for male pheromonal calling.

It is likely that calling by male *A. suspensa* is influenced by environmental factors such as time of day, temperature, and light intensity (Burk 1983, Hendrichs 1986). Male calling, including pheromone release, also may be influenced by the location or occurrence of resources, such as food or oviposition sites. For example, species of *Drosophila* aggregate at food and oviposition sites (Spieth 1974), and *Drosophila* male-produced aggregation pheromones normally released at such sites may be synergistic with food odors for female attraction (Bartelt et al. 1988). The papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker, puffs its pleural abdominal pouches, releasing pheromone, while perched on papaya fruit, *Car-*

ica papaya L., in the field (Landolt & Hendrichs 1983).

In this study, temporal patterns of male *A. suspensa* calling behavior during the day and effects of the presence of host fruit and adult food on calling behavior are determined.

Materials and Methods

Flies used in these experiments were reared at the USDA–ARS Insect Attractants, Behavior, and Basic Biology Laboratory, Gainesville, Fla., as part of a colony maintained for ≈15 yr. Voucher specimens from this colony have been deposited in the Florida State Arthropod Collection, Gainesville. Rearing methods were as reported by Greany et al. (1976). Males were separated from females when immature (2–4 d old) and were held at 24 ± 1°C and 50 ± 10% RH under a 12:12 (L:D) photoperiod. Test flies were sexually mature (9–20 d old) and were held in screen cages (30 by 30 by 30 cm). Wet cotton wicks and a 3:1 mixture of refined cane sugar and hydrolyzed torula yeast were placed in cages as sources of water and food. Lighting from fluorescent lamps was 880 lux at the cage tops. The laboratory photophase was from 0700 to 1900 hours (EST); the natural outdoor photophase during experiments conducted in a greenhouse was from 0700–0730 to 1800–1830 hours. Maximum greenhouse light intensity under the shade cloth was about 8,000 lux. The greenhouse temperature was regulated with hot water pipes and evaporative coolers with ventilation fans. During experiments, the greenhouse was kept at 24 ± 2°C and 60 ± 15% RH.

Experiments were conducted to evaluate rates of male calling at different times of the day in the presence of adult food and host fruit. The effects of the duration of the absence of food on calling was also determined (calling here refers to the simultaneous puffing of the pleural abdominal pouches and extrusion of the anal sac). At each observation time, the number of calling males in each cage were counted and recorded. The behavior did not appear to be intermittent, and each cage check was brief (15–30 s). Although pheromonal calling appeared to be accompanied by wing buzzing, evidence of sound production (Webb et al. 1976) was not recorded.

Two experiments in the greenhouse tested for effects of the presence of food (a 3:1 mixture of unrefined cane sugar and hydrolyzed torula yeast in cake form) on calling throughout the day by males. In the first of these experiments, the 10 treated cages included three small pieces of adult food placed on the top of cages, with an accessible surface area of about 16 cm^2 in contact with the screened cage top. The 10 control cages did not include such food. Each cage contained a water source and 10 males. Cages of flies were placed on tables under a screen shade cloth at 1700 hours with hourly observations conducted on the following day from 0730 to 1730 hours. At each observation time, temperature, relative humidity, and light levels were recorded. In the second test, comparisons were made of calling by males throughout the day in cages with no food, with the sugar–yeast mixture, with yeast alone, or with sucrose alone. An aqueous yeast suspension or saturated sucrose solution was applied to the surface of a paper card (7 by 12.5 cm) placed face down on the cage top. This comparison was made with eight cages per treatment and four treatments. Ten flies were placed in each cage. Again, flies were set up the previous day at 1700 hours with calling rates documented the following day from 0730 to 1730 hours.

Because these tests were set up the evening preceding day-long observations, a comparison also was made of the effects of sucrose deprivation for different periods of time on male calling rates in midafternoon. This test followed indications in the preceding experiment that sugar deprivation had a profound effect on male calling. Rates of male calling were determined at 1430 hours for males in cages held without food for 0.5, 3.5, 7.5, 16, or 22 h. Food used was a 3:1 mixture of refined cane sugar and hydrolyzed torula yeast. Flies to be starved were removed from cages with food and placed in clean cages, with water only, at 1600 and 2330 hours on the previous day, and at 0700, 1100, and 1400 hours on the day of observations (made at 1430 hours). Ten flies were placed in each cage and four cages were used for each of the five treatments. The significance of a relationship between length of time of starvation and calling rates was determined by linear regression analysis after a log transformation of the data (Steel & Torrie 1960).

The pattern of calling by males with food over the course of a day was also assessed under laboratory conditions, which lacked dusk and dawn light intensities. Thirteen groups, each of 10 males, were held in glass chambers (270 mm long, 50 mm inside diameter) through which purified humidified air was passed. Counts were made of males calling at hourly intervals through the 12-h photophase. Lighting from overhead fluorescent lamps was ≈400 lux at the chambers.

An additional series of experiments in the greenhouse compared calling rates of males in cages with or without one guava fruit, *Psidium guajava* L., in each cage. Guava is the most important host of the Caribbean fruit fly in Florida (Swanson & Baranowski 1972). A separate experiment evaluated male calling with each of three fruit stages, either mature-sized (6 cm diameter, 8 cm long) but green fruit, yellow ripe fruit, or overripe fruit with the beginnings of brown rot. All fruit were obtained from a commercial guava grove in Dade County, Florida. Green and ripe fruit were cut from guava trees ≈20 h before the beginning of the test. The ripe guavas were sub-

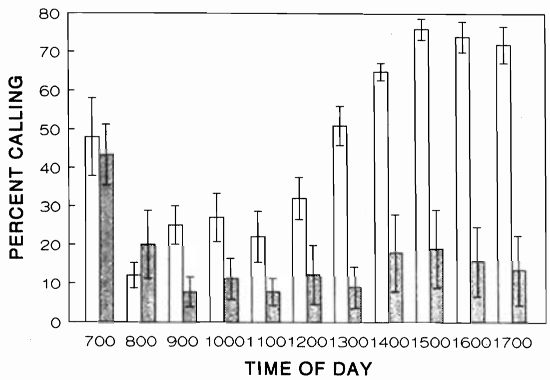

Fig. 1. Percentages ($\bar{x} \pm$ SE) of male Caribbean fruit flies calling in a greenhouse at hourly intervals throughout the day in cages supplied with food (open bars) and in cages without food (slashed bars).

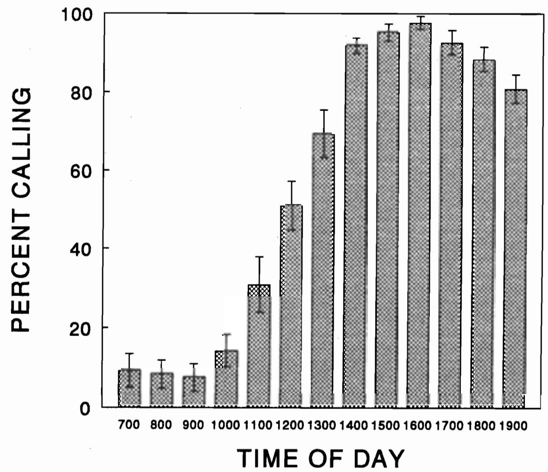

Fig. 2. Percentages ($\bar{x} \pm$ SE) of male Caribbean fruit flies in glass chambers in the laboratory with food calling at each hour of the 12-h photophase.

sequently tested as overripe fruit when they showed signs of rot (expanding brown areas on the skin). All three of these tests were carried out as paired replicates (six with a fruit, six without a fruit) with 10 flies per cage. All cages contained water on cotton wicks. Flies for these tests were set up in cages with fruit at 1700 hours; observations were made throughout the following day. A fruit was placed on top of a 5-cm-tall glass jar placed on the floor of each cage. Hourly counts were made of the numbers of flies on the fruit, in addition to the number of males calling in the cage.

Results

In the experiment conducted, comparing rates of male calling in cages in the greenhouse with and without the food mixture (sugar and yeast hydrolysate), two activity periods were evident. Both groups of flies exhibited calling (everted anal sacs and pleural abdominal pouches) during the first hour of daylight (0700–0800 hours), with calling rates of 40–50% (Fig. 1). Light intensities at this time were 55–200 lux. Most flies ceased calling by 0800 hours. Males in cages with food began calling again in early afternoon, with high rates of calling throughout the afternoon (1300–1700 hours). Light intensities at these times ranged from 500 to 4,000 lux at the cage tops. Only 10–20% of males in cages without food called in the afternoon, compared with >70% of males supplied with food (Fig. 1). Males deprived of food did not otherwise appear different, and mortality rates were similar for both treatments (<5%). Males in the laboratory that were supplied food exhibited high rates of calling throughout the afternoon only (Fig. 2). The morning activity period was not evident with these flies.

The further testing of the effects of sucrose versus enzymatic hydrolyzed torula yeast demonstrated a pronounced afternoon calling period for males supplied sucrose or a sugar–yeast mix. However, males given yeast or no food called little in the afternoon (Fig. 3). Sets of males given either the food mix or sucrose also exhibited two calling periods with a brief dawn period and a broad afternoon period. Most males denied food from 1700 hours on one day to 1400 hours on the following day did not call that afternoon. There was a significant relationship between the length of time that flies were deprived of sugar and the incidence of calling in the afternoon (Fig. 4) ($r^2 = 0.96$, $t = 7.99$, $P = 0.008$).

Dawn activity periods were observed in the day-long documentation of male *A. suspensa* calling in the presence of either no fruit, green guava fruit, ripe guava fruit, or overripe guava fruit (Fig. 5). Calling rates were near 40% in all cases, and light intensity was 55–200 lux during

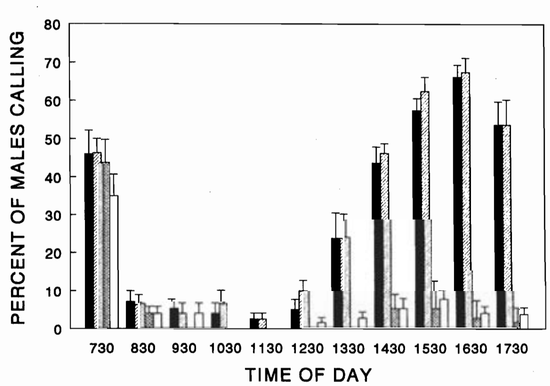

Fig. 3. Percentages ($\bar{x} \pm$ SE) of male Caribbean fruit flies in a greenhouse calling at hourly intervals throughout the day in cages supplied with either a sugar-hydrolyzed torula yeast mixture (closed bars), with sucrose (slashed bars), with hydrolyzed torula yeast (cross-hatched bars), or with no food (open bars).

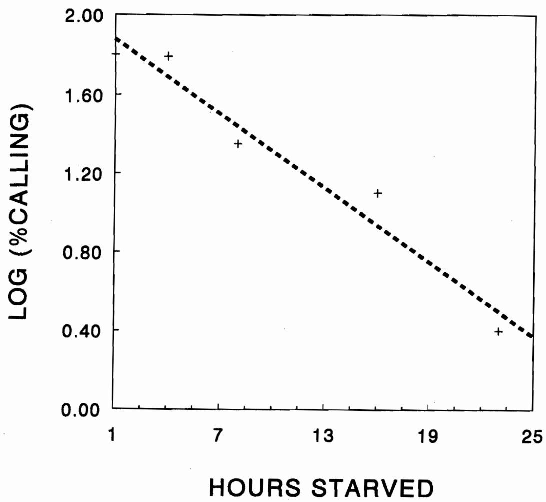

Fig. 4. Percentages of male Caribbean fruit flies in a greenhouse calling at 1500 hours (EST) following starvation for 1, 4, 8, 16, or 21 h. Best fit line equation was log $Y = 1.94 - 0.063X$.

this calling period near dawn. Afternoon calling by males in cages with water only, or in cages with green guava or ripe guava fruit, was very low (between 0 and 5%). Males in cages with overripe guava fruit, however, resumed calling in the afternoon, with calling rates of ≈20% (Fig. 5). Males visited fruit during these tests; more flies were consistently present on overripe fruit than were on ripe or green fruit (Fig. 6). Numbers of flies on green fruit were sharply higher in late morning compared with the rest of the day. Numbers of flies on overripe fruit were higher at all times of observation, except at 0730 hours. Flies on overripe fruit in this test appeared to be feeding at brown areas on the surface of the fruit. Flies were not observed calling on any fruit at any time during these studies.

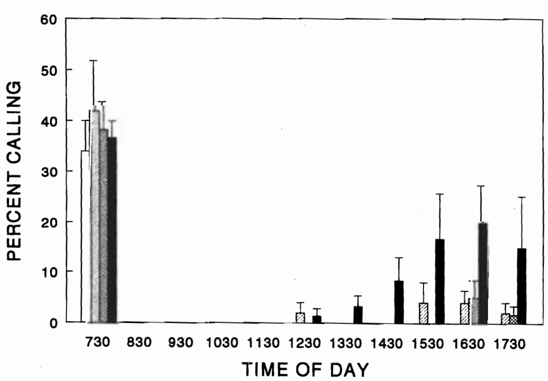

Fig. 5. Percentages ($\bar{x} \pm$ SE) of male Caribbean fruit flies in greenhouse calling at hourly intervals throughout the day in cages supplied with green guavas (slashed bars), ripe guavas (cross-hatched bars), rotten guavas (closed bars), or no fruit (open bars).

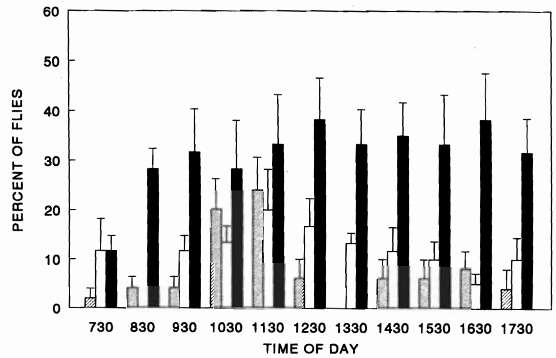

Fig. 6. Percentages of male Caribbean fruit flies in a greenhouse in cages with green (slashed bars), ripe (open bars), or rotten guavas (closed bars) that were on the fruit at hourly intervals throughout the day.

Discussion

Male Caribbean fruit flies in the greenhouse exhibited calling, including puffing of the pleural abdominal pouches and extrusion of the anal sac, in the early morning and again during much of the afternoon. This daily pattern of male calling was found by Hendrichs (1986), who reported a minor period of male sexual activity early in the morning and a long period of sexual activity in the afternoon among flies confined in a field cage encompassing a guava tree. Burk (1983) reported a pronounced increase in displaying by male Caribbean fruit flies during late afternoon in a guava grove in south Florida, with a peak at 1700–1800 hours (EST). He did not report male sexual display activity in early morning. However, such flies, if near the tops of host trees as indicated by Hendrichs (1986), may have not been visible in the field to Burk (1983). The absence of early morning calling in our laboratory study (Fig. 2) may be due to a lack of dawn lighting conditions.

The results reported here agree with the reported pattern of sexual activity observed by Hendrichs (1986), with a calling period at dawn of short duration and more extensive activity late in the afternoon. However, the finding that male calling during the afternoon was dependent on recent access to sugar has not been reported. Because both Burk (1983) and Hendrichs (1986) observed fly calling on host trees, natural sugar sources on those trees may have been sufficient for the observed calling and sexual displays in the afternoon. Honeydew from plant-sucking homopterous insects has been thought to provide fruit flies with food (Hagen 1958). The afternoon calling observed with flies held in cages with overripe guava fruit indicates that they probably obtain some sugar from feeding on the surface of such fruit rather than from feeding on the surface of green or ripe fruit. It seems plausible, then, that the distribution of natural sources of sugars in nature may profoundly affect the survival and distribution of *A. suspensa* males, influence the selection of calling sites by males, and affect male reproductive success. Additional studies are needed of the types of foods required by males and of the spatial relationships between suitable food sources and male calling sites. It is important to know if males remain close to such sources when they call or are merely limited to how far they can disperse from such a source before they need to feed again. Perhaps this is a condition of a favorable microhabitat leading to the formation of aggregations, as suggested by Sivinski (1989).

The two periods of calling may indicate different strategies by males to attract females. Previous studies of Caribbean fruit fly behavior have indicated that the principal male mating strategy is to participate in aggregations or leks on host plant foliage (Burk 1983). Males in such aggregations release pheromone and produce calling songs to lure females. Such aggregations may allow females to compare and select the males that are most fit. Lek formation and calling in leks is coincident with the afternoon calling period observed here. All studies to date of *A. suspensa* mating and sex pheromones appear to involve this afternoon activity period. Webb et al. (1983) reported that most captures of female *A. suspensa* in traps in field cages baited with live males occurred in late afternoon, but they did not record numbers of flies trapped before 0900 hours. Published records of daily patterns of mating by *A. suspensa* all indicate late afternoon as the usual mating period (Perdomo 1974, Burk 1983, Hendrichs 1986), with no indications of mating in early morning. Bihourly collections of possible pheromone chemicals produced by males showed increasing amounts of compounds released in the afternoon but did not indicate release in the early morning hours (Nation 1989).

At present, it is not clear what functions pheromonal calling play in male mating strategies. The observations of Hendrichs (1986) suggest that males call at dawn from the tops of trees, but there is yet no indication of courtship interactions or mating in such places. Caribbean fruit flies may exhibit a morning activity pattern similar to that of *Anastrepha fraterculus* (Wiedemann), which remain in treetops overnight and call, court, and mate in the morning in the upper canopy of trees (Malavasi et al. 1983).

The visitation rates of males on guava fruit differed somewhat with fruit maturity. This may indicate differential arrival rates, arrestment, or both. Throughout most of the day, few flies were observed on green guava fruit. The peak in such numbers, although comprising a small percentage of flies in the cages, did occur at the same time of day that such activity occurs in the field. Burk (1983) reported limited numbers of males on green guava fruit in the field but concentrated during the late morning hours. The much higher numbers of flies on the overripe guava fruit may be a result of a greater attractiveness of such fruit to hungry flies. Also, feeding by the flies on the fruit surface probably results in their remaining on the fruit for some time. No males on such fruit were observed to puff their pleural pouches or evert their anal sacs.

The results of these tests should be of use in the development of sex pheromone bioassays, in the collection and isolation of pheromone components from males, and characterization of a released blend of volatile compounds. Similarly, the likely release of pheromone from the abdominal pleural pouches and from the anal sac during the early morning and late afternoon indicates that these times would be most suitable to investigate behavioral responses by female and male flies to male-released pheromone. Two factors documented here as affecting male calling may have to be considered in the isolation and characterization of the pheromone. Given the two separate calling periods, multiple pheromone sources in this insect (Nation 1983), and the possibility for dual mating strategies by males, it may be desirable to characterize released volatile chemicals (as possible pheromones) from both time periods and under more natural lighting. The dependence on sugar feeding observed here also indicates that chemical collections made without regard to such feeding may give misleading results.

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