TEMPORAL AND SEASONAL DIFFERENCES IN MOVEMENT OF THE CARIBBEAN FRUIT FLY LARVAE IN GRAPEFRUIT AND THE RELATIONSHIP TO DETECTION BY ACOUSTICS

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ABSTRACT

Larvae of the Caribbean fruit fly Anastrepha suspensa (Loew) can be detected by the sounds they make while feeding using an acoustical detection system. Efforts to improve and determine the efficiency of the system led to detailed studies of the feeding behavior of larvae in grapefruit. Movement, feeding and growth are related to the maturity of the fruit. As the fruit matured, it became more sweet and larvae fed more consistently and voraciously, moved into the pulp portion earlier, and developed more rapidly. Larvae were detected within hours after they hatched from eggs, when they are often too small to be seen in the fruit with the unaided eye. The efficiency of the system in detecting infested fruit has been demonstrated to be more efficient than the accepted method of cutting and visual examination.

RESUMEN

Larvas de la mosca de frutas del Caribe, Anastrepha suspensa (Loew), se pueden detectar por el sonido que hacen cuando comen usando un sistema de detección acústica.
Esfuerzos para mejorar y determinar la eficiencia del sistema nos dirigió hacia estudios detallados del comportamiento de las larvas cuando comen toronjas. El movimiento, alimentación y crecimiento están relacionados con la madurez de la fruta. A medida que la fruta madura, se pone más dulce, y las larvas se alimentaron más consistente y vorazmente, se movieron hacia las porciones de pulpa más temprano, y se desarrollaron más rápidamente. Las larvas se detectaron a pocas horas de salir de los huevos cuando a menudo todavía eran muy pequeñas para verse a simple vista en la fruta. Se ha demostrado la eficiencia del sistema en detectar frutas infestadas y es más eficiente que el aceptado método de cortar y examinar visualmente.

The Caribbean fruit fly, Anastrepha suspensa (Loew) (caribbeanby), has inhabited Florida since 1966. Although most of its hosts are wild and dooryard fruits, it does occasionally infest citrus, particularly grapefruit and oranges (Swanson & Baranowski 1972). Although it has never been found to cause extensive damage in citrus groves, the threat of the presence of its larvae in citrus fruit being shipped to other tropical and subtropical areas has caused it to become subject to quarantine regulations. Florida citrus is now quarantined by Arizona, California, Hawaii, Texas and Japan.

Prior to 1983, fumigation by ethylene dibromide (EDB) successfully controlled eggs and larvae in fruit. However, after the withdrawal of this compound, citrus exporters were only able to use methyl bromide, cold treatment and shipment of fruit from fly-free areas as means of overcoming the quarantine restrictions. In all cases, because these methods are more complicated and less effective than EDB, a large sample of fruit must be examined by cutting to determine the presence or absence of caribbean larvae.

When it was discovered that sounds of feeding by larvae of the caribbean could be detected by use of an accelerometer (Webb & Landolt 1984), a new technology for the detection of fruit fly infested fruit was developed. Subsequent improvements in the system eliminated the accelerometer and the frequency of the feeding sounds produced were altered at the detector due to the physics of the system (Webb et al. 1988). To improve on the efficiency of the system and to help explain the variability in the sounds detected, it was necessary to determine the feeding behavior of the larvae in the target fruit. It also became necessary to determine patterns of movement in relation to age of larvae producing the audible signal and the maturity of the fruit so that standard comparisons could be made between different equipment modifications.

**MATERIALS AND METHODS**

Grapefruit in Central Florida blooms in March. The fruit picking starts in late October and extends to May of the following year. Grapefruit (var. Marsh White) used in this study were picked every four weeks from November, 1984 to April, 1985 from a grove maintained on Merritt Island, Florida. The fruits were transported to the Insect Attractants, Behavior and Basic Biology Research Laboratory in Gainesville where they were washed and infested with eggs from gravid female cariibflies. Fruits were placed into cages containing large numbers of sexually mature flies and the females were observed for probing and oviposition activity. The fruits were exposed to the flies for 6 hours, then were removed from the cage and placed on trays and incubated for 3 days at 25 °C. From each collection, 45 to 60 fruits were infested, 15 on each of 3 days.

After 3 days, the fruits were placed individually on the sound detector (Webb et al. 1988) to see if eggs had hatched and the larvae had begun feeding. Under ideal conditions in the laboratory at 25 °C., eggs hatch in 3 days. If feeding sounds were detected, the date of detection was written on the fruit and the fruit was returned to the incubation tray. If no sound was heard, the fruits were examined acoustically each day thereafter until larval sounds were apparent.
Fig. 1. The three major regions of a citrus fruit. Flavedo is the colored outside layer containing the oil glands. The albedo is the white fibrous inner layer. The pulp is the edible portion from which the juice is extracted.

The three main layers of fruit are illustrated in Figure 1. The flavedo is the yellow portion of the grapefruit peel. It contains most of the oils in the peel. The albedo is the white layer between the flavedo and the pulp and is made up mostly of pectin, but several chemicals including naringin and limonin also occur there (Greany et al. 1983, Kefford & Chandler 1970). The pulp is the yellow edible center portion of the fruit. Each of these portions of the fruit were dissected to locate larvae.

Three fruits were dissected to locate larvae at 2-day intervals after the first larval sounds were heard during the months of November through February. During March, dissections were made daily for 5 days because larval development and movement were so rapid. During dissections, the fruits were first cut in half and then into quarters. Then a cut was made to separate the albedo from the pulp. After each cut, the fruit portion was examined carefully with a 2X magnifier/lamp or with a 10X dissecting microscope for feeding trails or larvae. The flavedo and the albedo were carefully teased apart with forceps and probes. Neonate larvae are very small and identical in color to the albedo layer in grapefruit which makes them difficult to see. As they become larger, they and their feeding trails were more easily found. An example of a feeding trail is shown in Figure 2.

Data were recorded on a diagram of the fruit indicating where each larva was located. The distance that the larva moved from the outer perimeter of the fruit was measured with a direct line from the outer edge to the center. No attempt was made to determine lateral distances the larvae may have moved while feeding because of the great variations found and the difficulty in following the complete feeding route.

**RESULTS**

The average location of larvae and the number of days after acoustic detection for each month are shown in Figures 3 and 4 for each month from November through March. The lines on either side of the dot is the range of depths that larvae had pene-
Fig. 2. An example of a feeding trail in the albedo of a grapefruit made by a Caribbean fruit fly larva.

trated by that day. Because growth and movement of larvae in November and December were slow, the figure only indicated locations at 4-day intervals.

Fruits picked in November were just beginning to turn yellow. The peel was still firm to the touch. In all cases when eggs were discovered, always in the flavedo, they were found to be laid singly rather than in aggregates. During the first 4 days after larval chewing was detected, the larvae were only found in the flavedo. By day 8, larvae were found in the albedo and in the outer regions of the pulp. On day 12, most of the larvae found were in the pulp with a few still in the albedo. On day 14, one mature larva left the fruit to pupate. By day 16, all of the larvae found were in the pulp. This was the last observation made because all of the infested fruit from the November picking date had been cut for examination. The number of larvae found on the days of examination ranged from 11 to 33, more larvae were found in the later examination periods as might be expected because they were larger in size and easier to see.

In December, larvae were recorded only in the flavedo on day zero. On day 4, they were found in both the flavedo and the albedo. On day 8, they were found in all 3 regions and by day 12 and 16 were found exclusively in the pulp (Figure 3). The first larva emerged from the fruit on day 13.

From January through March, fruits were collected every 2 weeks so that 60 fruits could be infested each week. They were examined every two days after larval sounds were detected. Five fruits per day were cut and examined. During the first 2 days after egg hatch, larvae were found exclusively in the flavedo. On day 4, most of the larvae were found in the albedo with a few still in the flavedo and 2 larvae were found at the border between the pulp and the albedo. By day 6, although most of the larvae were still in the albedo a few larvae had penetrated the pulp, one as deep as 26 mm. On day 8, most of the larvae were found in the pulp with a few still found at the border between the pulp and the albedo. The number of larvae found per fruit ranged from 14 to 44.
Fig. 3. The mean location of Caribbean fruit fly larvae in grapefruit picked during November and December at specified days after larval eclosion.

From day 10 on, almost all of the larvae were found in the pulp (Figure 4). The first larvae to emerge during the first week's infestation was on day 13. Later in the month, the first emergence of mature larvae occurred on days 12, 10 & 10 for the next 3 weeks, respectively. The number of larvae found ranged from 13 to 55/fruit.

In February, larvae were already found in the albedo on day 2 and one larva was found in the pulp at the interface between the pulp and the albedo. By day 4, a few
larvae were moving into the pulp, one as deep as 5 mm while most were at the interface. By day 6, most of the larvae were in the pulp as deep as 26 mm (Figure 4). Most of the larvae had completed development and had exited the fruit before the 14th day. Unfortunately, the date of first emergence was not recorded.

During March, fruits were examined every day for 5 days. On day zero, the first day larval sounds were detected, all of the newly hatched larvae were still in the flavedo. On day 1, several had already migrated into the albedo. By day 2, most were found in the albedo. By day 3, although most were in the albedo, a few had already entered the pulp as deep as 2 mm. On days 4 and 5, the larvae were found both in the albedo and the pulp. The first larva began exiting the fruit on day 6 (Figure 4). Feeding
and growth were very rapid in fruit picked in March. The number of larvae found ranged from 16 to 81/fruit. The feeding passages were difficult to follow because there was such a large number of larvae in each fruit and the feeding was so extensive. The fruit also became infested with fungi and mold and broke down very quickly, partly as a consequence of the extensive larval feeding.

**DISCUSSION**

Results of this study indicate that the maturity of the fruit has a great influence on the development rate and movement of larvae within the fruit. The rate of egg hatch in November took from 6 to 12 days to hatch. In February, the egg hatch occurred within 4-6 days. An interesting side observation of this study was that about 50% of the eggs were found in the oil glands and about 50% between the oil glands in fruit from all picking dates. Greany et al. (1983) observed a high mortality of eggs and newly-hatched larvae that were inside of oil glands. The increase in development rate and movement of larvae into the pulp occurred earlier in consecutive months from November through March. The greatest increase occurred in March when complete larval development occurred as early as 5 days after egg hatch. During this month the fruit has reached a stage of maturity when grapefruit is truly susceptible to attack from the Caribfly. Feeding sounds of larvae are most easily detected by acoustical techniques when fruit is most mature because larvae feed almost continually at this stage (Webb, unpublished data). There are also several changes in the chemistry of the peel with maturity and, these appear to affect feeding and development rates early in the season. (Shaw & Calkins, unpublished).

![Diagram](image)

**Fig. 4.** The mean location of Caribbean fruit fly larvae in grapefruit picked during January, February and March at specified days after larval eclosion.
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REFERENCES CITED


USING INSECT SOUNDS TO ESTIMATE AND MONITOR THEIR POPULATIONS

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ABSTRACT

Accurate estimates of population size are needed to understand the population dynamics of any species. They are also needed to determine when to implement a specific control tactic, and to measure whether that control tactic has been effective. This paper discusses the use of acoustic signals produced by insects and the feasibility of using these signals to census populations.

Insect sounds are either incidental (produced as a by-product of some activity) or non-incidental (produced to cause a response in some other animal). Incidental sounds differ from non-incidental sounds with respect to several features that are important to using sound to census populations. These features include species specificity, frequency content, ease of localization, distance traveled, and the duration and timing of sound production.

Studies of crickets show that information about which individuals in a population are producing sound, when the individuals produce sound (seasonally and daily), and the probability that individuals produce sound during census periods must be known to accurately estimate the size of a population.

RESUMEN

Se necesitan estimados precisos del tamaño de la población para entender el dinamismo de la población de cualquier especie. También se necesitan para determinar