

**RATE OF FOOD PASSAGE THROUGH
THE BOLL WEEVIL GUT**
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Abstract

There is considerable variation in the contents of the digestive tracts of trap-captured weevils. If the rate at which the digestive tract empties after feeding were known, observation of gut condition might be useful in estimating the time elapsed since a weevil last fed. Because this information is currently unavailable, we examined the rate of food (cotton pollen) passage through digestive tracts of boll weevils held at 12.8, 18.3, 23.9, and 29.4°C (55, 65, 75, and 85°F, respectively). At 29.4°C, intact pollen grains were no longer visible in the midgut 12 h after last feeding. At 23.9 and 18.3°C, pollen grains were no longer distinguishable in the midgut after 48 h. At 12.8°C, intact pollen grains were no longer present in the sacular portion of the midgut after 48 h, but remained visible in the tubular section of some weevils for up to 12 d. The majority of weevils, regardless of temperature, retained remnants of food in the hindgut. Our study indicates that examination of food content in the tubular portion of the weevil midgut is the best indicator for assessing the recent feeding history of trap-captured weevils. Weevils containing solid food in this portion of the gut can be presumed to have fed within the past 24 to 48 h. This technique should prove useful in studies to infer the previous food source of weevils captured in traps, and provide insight into their potential origin.

Introduction

Boll weevil eradication programs rely almost exclusively on pheromone traps for population assessments and treatment decisions. Despite this reliance, there is little assurance that trap-captured weevils originated from adjacent cotton fields. While several investigators have examined seasonal patterns in sex ratio or the physiological status of trap-captured weevils (Merkl and McCoy 1978, Mitchell et al. 1972, Mitchell and Hardee 1974, Segers et al. 1987, Wolfenbarger et al. 1976), evidence or methods linking captured weevils to their recent origin is generally lacking.

In the spring of 2000, we initiated a field study intended to provide insight into potential source(s) of trapped weevils, and ultimately improve our ability to interpret trap catches. While performing dissections in this field study, we noted distinctive differences in the midgut contents of weevils collected from cotton fields compared with those captured in traps bordering cotton fields. Because weevils captured in traps no longer have access to a food source (e.g., cotton squares), we hypothesized that digestion and elimination of remaining food in trapped weevils was primarily responsible for these differences. No information currently exists regarding the rate of food passage through the boll weevil gut. Such information may provide a means of estimating the time elapsed since last feeding for field-collected or trap-captured weevils. This estimate, in combination with other morphological characters, could provide valuable insight regarding the source of trap-captured weevils. Our objective was to examine the rate of passage of cotton pollen through the boll weevil gut following a period of starvation, and to develop a rating system for determining when weevils recently collected or captured from fields last fed on cotton.

Materials and Methods

Weevil Source

Adult boll weevils were reared from infested cotton squares collected from plants in commercial fields near College Station, TX. Infested squares were held in screened cages (20 x 20 x 20 cm) within an environmental chamber set at 29.4°C and a photoperiod of 13:11 (L:D) h. Squares were checked for pupae 5 d after collection and daily thereafter. Pupae were removed from squares and placed on a thin layer of moistened vermiculite in 100 x 15 mm plastic Petri plates (approx. 40 to 50 pupae/plate), and returned to the same environmental chamber. Petri plates were examined at least twice daily for newly emerged adults, which were removed and sexed by the tergal notch method (Sappington and Spurgeon 2000).

Experimental Design

Newly eclosed adults were held in same-sex groups of five weevils within 100 X 15 mm Petri dishes. Each group was provided 3 cotton squares (5 to 9 mm diam.; bracts intact) for 3 d. Squares were replaced with freshly picked squares on the 2nd d. Weevils were held at 29.4°C and a photoperiod of 13:11 (L:D) h throughout the feeding period. After the 3rd d, squares were removed and weevils were starved for 20 to 24 h. Weevils then were transferred individually into capped 25-ml diet cups and provided a single square (5 to 9 mm diam.; debracted) for 4 to 6 h. These squares were removed, inspected for feeding punctures, and discarded. Cups, lids, and squares were examined for fresh frass. Only weevils that punctured squares and deposited fresh frass were used in the experiment. Immediately after the inspection process, 10 randomly selected weevils of each sex were dissected (time interval 0) to ensure that weevils were full of cotton pollen, and to establish a baseline gut rating. The remaining weevils (75 to 90 adults of each sex) were placed in an environmental chamber without food or water, and held at a constant temperature of either 12.8, 18.3, 23.9, or 29.4°C and a photoperiod of 13:11 (L:D) h. At selected intervals (determined in preliminary studies) following the time of last feeding, 10 weevils of each sex were randomly selected for dissection. Weevils held at 12.8°C were dissected on d 1, 2, 4, 6, 8, 10, 12; and 14; those at 18.3°C were dissected on d 1, 2, 4, 6, 8, 10, and 12; those at 23.9°C were dissected on d 1, 2, 3, 4, 5, and 6; and those at 29.4°C were dissected at 12, 24, 36, 48, 60, 72, and 96 h following the last feeding period. The experiment was repeated twice at each temperature. The two replications at each temperature were pooled for analysis.

Midgut and Hindgut Classifications

The two major regions of the midgut (sacular and tubular) were rated separately. The condition of cotton pollen in each section was rated as either **intact** (pollen grains intact and readily identifiable) or **disintegrated** (no distinguishable intact pollen grains present, food liquefied and light to dark tan in color). The hindgut was also divided into two sections (anterior and posterior) with the bend in the hindgut dividing the two sections. Each hindgut section was classified as having "food" **present** or **absent**.

Statistical Analysis

The proportion of weevils with the sacular portion of the midgut devoid of intact pollen was calculated at each temperature and time interval using the SAS procedure PROC FREQ (SAS Institute 1998). The time at which 100% of weevils had no visible pollen in the sacular midgut section was determined for each temperature. The same was done for the tubular portion of the midgut. A logistic function was fitted to determine the time at which no intact pollen was visible in the tubular portion of the midgut using the SAS procedure PROC NLIN (SAS Institute 1998). The form of the model was $Y = e^{(BO + B1*TIME)} / [1 + e^{(BO + B1*TIME)}]$ where Y was the proportion of weevils with no intact pollen, TIME was the number of days since last feeding, and BO and B1 were estimated. The logistic function was only fitted to the data for weevils held at 12.8°C because degradation of cotton pollen in the tubular portion of the midgut occurred too rapidly to permit similar analysis in weevils held at the other temperatures. The

proportion of weevils with the anterior half of the hindgut classified as empty was calculated at each temperature and time interval. The same calculations were made for the posterior half of the hindgut.

Results and Discussion

Sub-samples of dissected weevils (time interval 0) indicated that the digestive tract of all weevils used in the study were initially full of cotton pollen. At 29.4 and 23.9°C, intact pollen grains were no longer visible in the midgut 24 h after weevils were removed from food. At 18.3°C, intact pollen grains were no longer visible in the sacular portion of the midgut after 24 h, or in the tubular portion after 48 h. At 12.8°C, intact pollen grains were no longer visible in the sacular portion of the midgut after 48 h, but remained visible in the tubular portion of some weevils for up to 12 d (Fig. 1). There was a strong relationship between time since last feeding and absence of intact pollen grains in the tubular midgut section of weevils held at 12.8°C ($F = 582.46$; $df = 2, 8$; $P < 0.001$; $R^2 = 0.99$). The predicted time for complete absence of intact pollen in the tubular section was between 6 and 8 d.

After intact pollen grains were largely eliminated from the midgut, midgut contents assumed a light to dark tan color and appeared liquefied. Other observations (C. Suh and D. Spurgeon, unpublished data) suggest that the color and texture of contents in the weevil midgut, and to a lesser extent hindgut, vary with food type. Midgut contents of weevils fed leaves, petioles, or terminals initially appeared green, but rapidly (<12 h) turned dark maroon and appeared fluid-like. Midgut contents of weevils fed bolls also had a distinctive initial appearance, but with increasing time became less readily distinguishable from those of weevils fed squares. These data indicate that presence of intact pollen grains in the weevil midgut may be used to accurately identify weevils that have recently fed on squares.

Assessments of the hindgut appeared less useful than those of the midgut for identifying recently fed weevils. The majority of weevils at each temperature and time interval retained some material in the hindgut at the conclusion of the experiment. Contents in the hindgut initially appeared solid and yellowish in color, then gradually took on a whitish chalky appearance. This transformation generally occurred first in the posterior half of the hindgut. Although the proportion of weevils classified as having an empty anterior hindgut generally increased with time, no definitive relationship could be established because the condition and amount of material in this section was highly variable. At 29.4 and 23.9°C, >97% of weevils at each time interval retained some material in the posterior end of the hindgut. Surprisingly, weevils held at the two cooler temperatures (12.8 and 18.3°C) had a greater proportion (up to 20%) of posterior hindguts classified as empty. We suspect that exposure to cooler temperatures hastened emptying of the posterior hindgut because many freeze-intolerant insects empty the digestive tract in preparation for winter (Leather et al. 1993).

Conclusions

Our results indicate that examination of the midgut, and to a lesser extent the hindgut, can be used to identify the recent feeding history of field- or trap-collected weevils. Absence of intact pollen grains in the midgut, at temperatures permitting capture of weevils in traps, reliably indicates that a weevil has not fed on cotton squares for >1 to 2 d. This information, in combination with other morphological characters such as testicular or ovarian development, should provide insight into the recent origin of trap-captured weevils.

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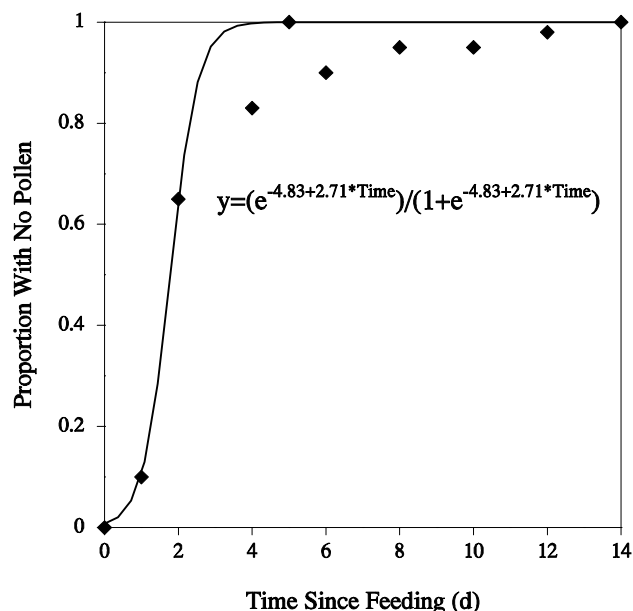


Figure 1. Observed and predicted (logistic equation) relationships between time since last feeding and absence of intact cotton pollen grains in the tubular midgut section of boll weevils held at a constant temperature of 12.8°C.