Analysis of Southern Strain Plum Curculio (*Conotrachelus nenuphar*) Female Reproductive Development

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Abstract:

Reproductive development of lab-reared female plum curculio was assessed under different temperature regimes in same- and mixed-sex cohorts. Individuals were fed *ad libitum* and three individuals were dissected daily for each temperature and cohort type until full maturity was reached. Reproductive structures were dissected and assessed for number of visible oocytes, size of proximal and distal oocytes, and spermatheca development. Oocyte development appears to be temperature-dependent and unaffected by mating status. Developmental thresholds are estimated for the observed parameters.

Background

Plum curculio (figure 1) is a major native pest in commercial cherry and apple orchards east of the Rocky Mountains. There are two strains of plum curculio: northern and southern. Their habits are virtually identical except that the northern strain has an obligatory diapause requirement, while the southern strain has continuous overlapping generations.

Northern strain plum curculio overwinter as adults in the orchard floor or adjacent woodlands and emerge in the spring to mate and oviposit in developing fruit. While adults do cause some feeding damage on the fruit, it is minor when compared to the economic losses incurred from oviposition. The scar caused by oviposition is crescent-shaped, and in apple has a brown, fanlike appearance at harvest if the egg did not hatch. This scarring effectively removes the fruit from the fresh market. In addition to external damage, the developing larvae can completely consume the fruit's flesh (in cherries), or can cause the tree to abort the fruit (apples). Due to their continuous oviposition during the growing season, plum curculio larvae may still be present in fruit at harvest. This represents a serious issue for growers that send their produce to commercial processing facilities. There is a zero-tolerance for infested fruit in processed loads, and a single rejected load can cost a grower in excess of $30,000 in fees and lost revenue.

Figure 1. Adult Plum Curculio.

Need for Ovary Development Research

Timing of ovary development is important both for rearing purposes in the laboratory and for pest management decisions in the field. Plum curculio feed and oviposit in immature apples, and these apples can take up a considerable amount of rearing space. Knowledge of the non-fertile period allows for more efficient colony maintenance; caretakers can discard apples that have only been fed on rather than crowding larval emergence cages with apples that will not contribute to the next generation.

The use of effective insecticides that are registered for plum curculio control has become increasingly restricted under the 1996 Food Quality and Protection Act. New chemistries are less effective in killing plum curculio, and must be more effectively timed to maximize their control potential. Understanding reproductive progress and baseline developmental rates are key components for maximizing efficiency of spray applications and minimizing damage.

This study's objectives were to:

1) Determine the pre-ovipositional period for Southern strain plum curculio at rearing temperatures.
2) Determine the Degree-day base for reproductive development in southern strain plum curculio.

Materials and Methods

Source material:

Southern strain plum curculio were used from a continuous lab culture. This culture has been in constant production since 1997 with occasional additions from Florida cooperators. Main rearing conditions were continuous light at 25°C. Apples were provided *ad libitum* for food and ovipositional substrate for both rearing and experimental units.

Experimental design:

Beetles were grouped into 12-hour emergence cohorts from our main laboratory colony. There were three treatment groups: 1) 50 males and 50 females held together at 25°C (77°F), 2) 30 females at 25°C, and 3) 30 females at 18°C (64.4°F).

Three to five females were removed daily from each cohort and refrigerated in 70% ethanol for future dissection. In the mixed-sex cohort, an equal number of males was removed each day to maintain a 1:1 sex ratio. Dissections were performed in Ringer's solution using a Nikon SMZ1000 dissecting microscope with a 1.5x objective. Measurements were taken with an optical micrometer.

Measured parameters were mating status (Figure 3), number of oocytes in each of the four ovarioles, and size of the largest oocyte in each ovariole (Figure 4).

Figure 2a: This spermatheca appears transparent, indicating that there is no sperm contained inside.

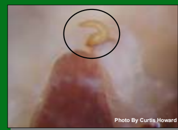

Figure 2b: The white coloration or cloudiness is due to the sperm contained within the organ.

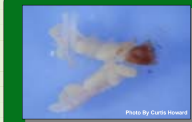

Figure 3a: Summer-caught plum curculio ovaries with mature eggs.

Figure 3b: Second-generation (fall) plum curculio ovaries. Note the absence of any oocyte development.

Results

Number of Oocytes per Female

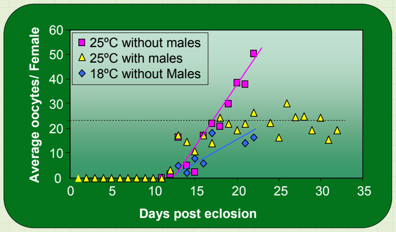

Figure 4. Female plum curculio develop oocytes in the absence of males and retain them until mating. Oocyte number in mature mated females reaches a plateau between 20 and 25. Development is faster at 25ºC than at 18ºC. Linear regression over the region of increasing development yields the following equations, which were used in subsequent degree-day base analysis.

25ºC: (Number of oocytes)= 4.307(days) - 50.737 R^2 = 0.71
18ºC: (Number of oocytes)= 1.426(days) - 13.513 R^2 = 0.21

Size of Largest Oocyte per Female

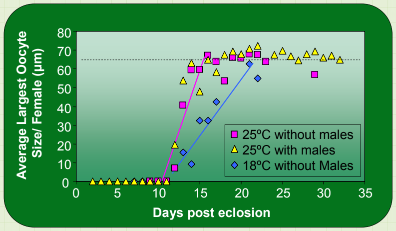

Figure 5. Female plum curculio oocyte size reaches a stable plateau of 62-70 µm after 15 days at 25ºC. Development is faster at 25ºC than at 18ºC. Linear regression over the region of increasing development yields the following equations, which were used in subsequent degree-day base analysis.

25ºC: (Largest Oocyte size)= 15.04 (days) - 153.95 R^2 = 0.43
18ºC: (Largest Oocyte size)= 5.29 (days) - 53.09 R^2 = 0.29

Pre-Ovipositional period at 25ºC

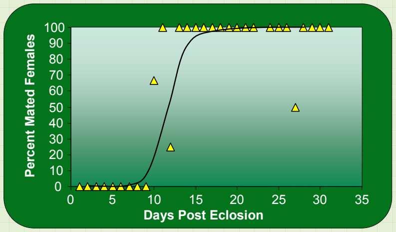

Figure 6. Females maintained in a mixed-sex cohort at 25°C were uniformly mated after 13 days (figure 5). The transition begins between days 9 and 10 and is complete on day 13.

Formula for Degree day analysis:

Basic Formula: *Degree Days = Days * (Temperature-DD base)*

With two constant temperature settings, one can set the degree days equal to one another and assess the amount of time it takes for insects held at each temperature to arrive at a specific developmental biofix. The rearranged equation is as follows:

$$\text{Degree Day Base} = \frac{(D1 \cdot T1 - D2 \cdot T2)}{(D1 - D2)} \quad \textit{Equation 1}$$

Where *T1* is Temperature1, *D1* is the days to developmental biofix at *T1*, and *D2* is the days to developmental biofix at *T2*.

Once the degree day base is calculated, degree days accumulated for any time period can be easily calculated.

Degree-Day Base Analysis

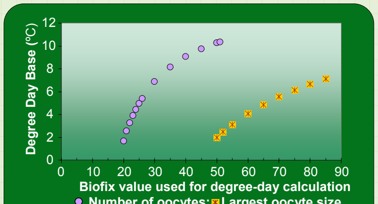

Figure 7

Method: Degree-day base determination was performed on both oocyte number and oocyte size data. The number of days required to get to a given biofix were calculated from regression equations and input into Equation 1 to determine the degree-day base.
Results: The calculated degree-day base was dependent on the choice of biofix for both reproductive parameters. Developmental plateau parameters of 22 oocytes and 65µm yield degree-day bases of 3.25ºC (38ºF) and 4.9ºC (41ºF), respectively.

Summary

- Mature oocyte size is estimated at 65µm and the stable total oocyte number in mated females is 22 (figures 4&5)
- Plum curculio females are mated after 13 days at 25ºC (77ºF) (Figure 6)
- Developmental rates of both oocyte size and number of oocytes appear to be temperature-dependent.
- Degree-day base calculated from mature parameters is approximately 4.4ºC (40ºF) (figure 7). The calculated degree - day base is dependent on the value that is used as the fixed comparison (biofix).

Discussion

These data are useful in addressing concerns of when cultured beetles are mature enough to oviposit. Normal culture practice involves a weekly transfer of apples from the adult colony to a larval emergence container. Guided by the results presented herein, we have begun discarding apples if the adults in that container are less than 10 days old, since there is no oviposition during that time at our rearing temperatures.

If northern strain plum curculio have the same degree-day base as the southern strain, then intensive early-season sampling and rapid reproductive measurement might be a useful tool for predicting the onset of maximum damage capacity (high proportion of fully-developed and mated females). Females are mated at 468 DD_{40} and have fully developed oocytes at 592 DD_{40}. Developmental status data from field collections could be input into the degree-day equations to determine where the field population is along the developmental continuum. This knowledge can then be coordinated with weather outlooks, resulting in better-optimized sprays and more informed management decisions. Rapid turnaround of such samples would be critical, however. The 40ºF degree-day base reported here is significantly lower than the accepted 55ºF activity threshold for this pest, plum curculio are likely to have accumulated heat units -- and reproductive capacity -- well before their first capture in activity traps.

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