Preliminary study in artificial rearing of Chinese citrus fruit fly, *Bactrocera minax* (Enderlein)

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Background

Chinese citrus fruit fly (*Bactrocera minax*) is an univoltine frugivorous specialist. Recorded most from China, the host range is restricted to species of citrus. Adults emerge annually in early May. Females lay eggs in the fruits in July. Larvae develop inside until they drop to the soil, and pupate in early November. Pupal stage enters diapause for overwinter and last 6 months in the field. The current infestation areas covered 8 provinces in South-western and Central China, ranging from temperate to subtropical regions. There has been a outbreak of *B. minax* in 2008 in Sichuan province, which caused several billion dollars economic losses nationwide. The tremendous losses discouraged fruit farmers and caused the Chinese government seek effective measures to control *B. minax*. Laboratory rearing and knowledge of diapause of *B. minax* are key aspects to a potential development of SIT for this species.

Materials and methods

Pupae were collected from the field and transferred to plastic trays with sterilized soil. Trays were placed in various temperatures (5, 10, 15, 20 ℃) at different time intervals (30, 60, 90, 120, 150d) and then transferred to 25℃, respectively. The percentage of emergence was recorded every day.

Emerged adult flies were fed with white sugar and yeast at a ratio 4:1, aside from the availability of water. In the cages we provided citrus branches to mimic the natural conditions in the field and supplied green oranges for egg laying.

Oranges with laying-egg marks were removed from cages and transferred to the incubator at 26℃ to trigger egg hatch. Larvae were shifted to artificial diet.

Ovary development of female flies and embryonic development of eggs in different stages were observed and identified under a stereomicroscope.

Results

Cold treatment for pupal diapause termination

Among different treatments, the highest percentage of emergence was obtained at 5℃ and 60 days, while at constant 25℃ few pupae emerged as adults (3%). On the other hand, there is approximate 10% non-diapause individuals in the studied population.

Adult flies rearing

Reared in cages with simulated environment, adult flies show a strong vitality as field population. They become sexual maturation in two or three weeks and then copulate. Egg laying occurs about 12 days after copulation and lasts for several weeks. Flies have a lifetime of 3 months under the artificial rearing conditions (Fig. 1) . Eggs are always laid beneath the peel of the orange. Early hatched young larvae suck the juice for nutrition (Fig. 2).

Fig. 1. Reared in laboratory: a) flies in cage; b) orange branches for field simulation; c) bending ovipositor; d) copulation; e) post-copulation; f) green oranges for oviposition; g) laying eggs.

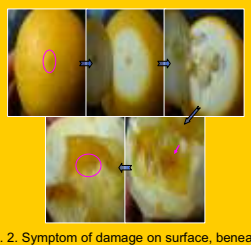

Fig. 2. Symptom of damage on surface, beneath peel and egg location inside

Ovary development of females

We determined ovarian development and identified five stages of development. 1. follicle in previtellogenic development; 2. initiation of vitellogenesis; 3. late vitellogenesis; 4. ovaries with mature oocytes; 5. onset of oviposition (Fig. 3).

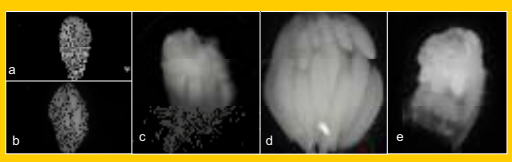

Fig. 3. Ovarian development: a) stage 1; b) stage 2; c) stage 3; d) stage 4; e) stage 5.

Larval rearing

Larval development usually take five months in the field. The early hatched young larvae are fragile so we kept them to grow inside fruits for about 15 days. Then larvae were transferred to artificial diets. Mature larvae drill out diet and pupate in moist sands (Fig. 4). We used several diet formulations recommended from other fruit flies rearing. Evaluation was carried out based on two parameters: larval developmental duration, and pupal recovery. Young larvae grow well inside different varieties of citrus fruits (Fig. 5).

Fig. 4. Larvae and pupae in the laboratory: a) larvae in navel orange; b) larvae; c) diet based on wheat bran; d) diet based on agrose; e) pupae; f) collecting pupae from sands

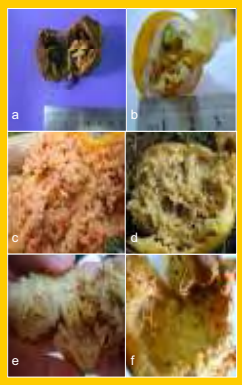

Fig. 5. Larvae in different varieties of citrus fruits: a) little peel-dried fragrant orange; b) little fragrant orange; c) blood orange; d) navel orange; e) fragrant orange; f) satsuma orange.

Embryonic development

The main visible embryonic developmental stages are as follows. Newly laid eggs are filled with white cytoplasm. Over a period of time the internal changes initiate with anterior and posterior of eggs becoming transparent. A series of mitosis occur before some obvious cells with blastomere nuclei appearing in the middle or tip of transparent eggs. These cells reproduce and separate from the posterior of eggs by forming furrow. A series of migrations and divisions commence among its cells. Meanwhile, various parts of embryo acquire characteristics as primordial organs. These complicated processes indicate the beginning of gastrulation. A clearly cephalic furrow occurs at this stage and divides the whole germ-band to protocephalo and protocorm. Subsequent events take place until blastoinesis. In the phase of blastoinesis, mouthpart segments and spiracular tracheas become visible in both poles of embryo. Fluoresing mid-gut of this phase shows tripartite structure. In the following stage of rapid histodifferentiation, fluoresing mid-gut get thin and integral coiled replacing the initial 3 yolk-filled regions. Pigment deposit in mouth hook and highly developed dorsal tracheas can easily be observed. After histodifferentiation, yolk resorption and onset of muscular activities of larvae arise frequently until hatching.

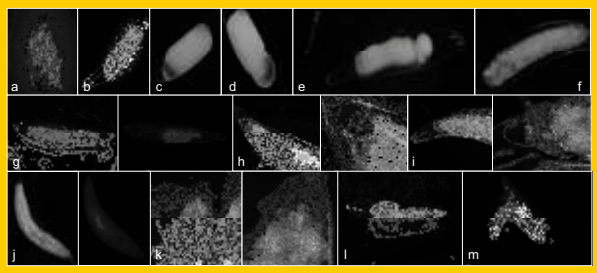

Fig. 6 embryoic development: a) newly laid egg; b) transparent egg; c) obvious blastomere nuclei in tip of egg; d) furrow formation; e) cephalic furrow occurrence and separation between protocephale and ventral wavelike protocorm; f) blastokinesis stage; g) 3 yolk-filled regions mid-gut under microscope and UV light (right); h) mouthpart segments; i) spiracular tracheas; j) thin and coiled mid-gut and highly developed dorsal tracheas; k) pigment deposit in mouth hook; l) yolk resorption and onset of muscular activities; m) hatching.

The major concern

- The termination of pupal diapause remains to be solved although there are limited amounts of non-diapause pupae in the population of *B. minax*.
- The selection of appropriate artificial oviposition devices for the female flies rather than laying eggs in natural citrus fruits.
- The comparison and evaluation of suitable larval diets for artificial rearing of *B. minax*.

Acknowledgements

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