

## COMPARATIVE STUDIES OF COURTSHIP BEHAVIOR OF *CERATITIS* SPP. (DIPTERA: TEPHRITIDAE) IN REUNION ISLAND

S. QUILICI, A. FRANCK, A. PEPPUY, E. DOS REIS CORREIA, C. MOUNIAMA AND F. BLARD  
 CIRAD-FLHOR Réunion, Station de Bassin Martin, Laboratoire d'Entomologie  
 BP180 F-97455, Saint-Pierre Cedex, France

### ABSTRACT

Three species of *Ceratitis* (Diptera: Tephritidae) are damaging fruit crops in the French island of La Réunion, in the Indian Ocean. A comparison is given on the status of knowledge of mating behavior in these three species. Aspects examined include male pheromone calling (behavioral sequences, circadian rhythm, factors influencing calling), lek formation and pre-copulatory behavior. While *Ceratitis capitata* courtship behavior has been heavily studied in many countries, research data are still scarce for *Ceratitis rosa* and are only preliminary for *Ceratitis catoirii*.

Key Words: *Ceratitis*, fruit flies, mating behavior, pheromone calling, lek

### RESUMEN

Tres especies de *Ceratitis* (Diptera: Tephritidae) se encuentran dañando cultivos frutícolas en la isla francesa de La Reunión, en el Océano Indico. Se presenta una comparación con respecto al grado de conocimiento que se tiene del comportamiento de apareamiento en estas tres especies. Los aspectos examinados incluyen llamado a través de feromonas del macho (secuencias de comportamiento, ritmo circadiano, factores que influyen en el llamado), formación de áreas específicas para el cortejo y comportamiento pre-copulatorio. Mientras el comportamiento de cortejo en *Ceratitis capitata* se ha estudiado considerablemente en muchos países, la información obtenida a través de investigaciones es todavía escasa para el caso de *Ceratitis rosa* y apenas se está iniciando para *Ceratitis catoirii*.

The Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann), belongs to a large genus currently comprising some 70 species, with main subgenera *Ceratitis* (9 spp.), *Ceratalaspis* (29 spp.), *Pardalaspis* (10 spp.) and *Pterandrus* (24 spp.) (Hancock 1984, Freidberg 1991, Meyer 1996, Hancock & White 1997).

In La Réunion, a French island situated in the Indian Ocean, 200 km north-east of Mauritius, 3 *Ceratitis* species are damaging a large variety of fruit crops. The Natal Fruit Fly, *Ceratitis* (*Pterandrus*) *rosa* Karsch is by far the main one in terms of economic importance. This is due to its large geographical distribution throughout the island, from sea-level up to an altitude of 1500 m, and its wide polyphagy (Etienne 1982, Quilici 1989). It largely dominates the other *Ceratitis* spp. in interspecific competition in most areas of the island. *C. capitata* is found in the lowland and mid-altitude areas, and is particularly abundant in the lee-ward, drier side of the island. The third species, *Ceratitis* (*Ceratitis*) *catoirii* Guérin-Mèneville, may be found at low population densities in eastern and southern lowlands areas.

While *C. capitata* has a nearly worldwide distribution, *C. rosa* is present in many African countries, particularly in the eastern and southern parts of the continent. *C. catoirii* is an endemic species from the Mascarenes, found in Mauritius and La Réunion.

In the last twenty years, a large amount of research has been conducted on the sexual behavior of *C. capitata*. This has recently been reviewed by Eberhard (2000). One of the main objectives of this research was to improve the quality of mass-reared males used in SIT (Sterile Insect Technique) programs against this pest around the world. Comparatively very little knowledge is available on the sexual behavior of *C. rosa*, despite the economic importance of this species in many african countries and recent projects for implementing SIT against it in South Africa. The sexual behavior of *C. catoirii*, as is most of its biology, is too date, totally unknown.

Most of the research done in CIRAD Réunion on sexual behavior of *C. rosa* or *C. catoirii* is unpublished, and in this paper we summarize the main results obtained. This will provide a preliminary comparison of sexual behavior characteristics in these 3 *Ceratitis* species.

### MATERIAL AND METHODS

#### Pheromone Calling

For the study of circadian rhythm and factors influencing male calling in *C. rosa*, we used wild flies collected as larvae in infested fruits of guava, *Psidium guayava* L. or Chinese guava, *Psidium cattleianum* Sabine. Freshly emerged males are

placed in cages (30 × 20 × 34 cm) by groups of 10 males per cage. The cages are set in a room exposed to natural light, so that the adults may perceive the decrease of light at dusk. Climatic conditions within the rearing room are measured with a luxmeter (Bioblock Scientific Lx-101 K 32 523) and a thermo-hygrograph (Jules Richard, France). For the study of rhythm, before and during the experiments, males receive a complete food regime consisting of sugar, protein hydrolysate and water. The experiment is conducted during two days (from 0600 h to 1815 h) when males are 13 and 15 days old, with 5 replicates (5 cages). The number of calling males, as well as light intensity, are recorded every half an hour.

For the study on the influence of food regime on *C. rosa* male calling, males are subjected to 4 different food regimes: (1): complete food regime (water, sugar, and yeast), (2): water and sugar, (3): water and yeast, (4): water only. The methodology is similar to that of the previous experiment, except that calling males are recorded every half an hour during the period of maximum calling (from 1600 h to 1800 h). For the study of the influence of food shortage on *C. rosa* male calling, males receive a complete food regime until they are 20 days old. They are then subjected to periods of food shortage of 0, 4, 8, 12 and 28 h, with 4 replicates for each treatment.

The circadian rhythm of male calling in *C. catovirii* was studied in a small cubic cage (a = 30 cm) where a recent lab colony is maintained in our laboratory. The cage, containing adults of both sexes, is placed in a laboratory room exposed to natural light, and calling males are recorded every half an hour during the whole photophase, with 3 replicates (3 days).

To study the behavioral sequences of pheromone calling and pre-copulatory behavior of the 3 *Ceratitis* spp. we used the standard methodology for video recordings, that was agreed upon within the FAO-IAEA Co-ordinated Research Program. Adults used for the experiments were of wild origin (collected as larvae in infested fruits), except for the rather rare *C. catovirii*, for which adults from a recent lab-rearing were used. Adults had reached sexual maturity when used for the video recordings.

#### Lek Formation

For the study of lek behavior we used wild *C. rosa*, collected as larvae in infested fruits from various hosts-plants. For *C. capitata*, that was more difficult to obtain from the wild in the study period, we used flies from a recent lab-rearing. Males are separated from females the day following emergence and maintained in small cubic cages (a = 30 cm) until the experiment. Experiments are conducted in a greenhouse insectarium (4 × 4 × 3 m), receiving only the natural light

and submitted to climatic conditions resembling those of outdoors: temperature varying from 17 to 23°C during the night and 20-30°C during the day, R.H: 45-90% and photoperiod close to L11: D13. Thirty potted Citrus plants are evenly distributed on the ground in order to provide a more natural environment.

To study lek formation, 300 males of a given species are released in the insectarium. The number of males calling on each Citrus plant is then recorded every 15 min. from 1000 h to 1600 h for *C. capitata*, and from 1600 h to 1815 h for *C. rosa*. Two replicates are done for each species.

## RESULTS AND DISCUSSION

### Pheromone Calling

*Behavioral sequences.* The release of a pheromone by males of *C. capitata*, attracting females, was shown in the early sixties by Feron (1962). Later, various authors studied the courtship behavior in this species (Prokopy & Hendrichs 1979, Sivinski et al. 1989, Shelly et al. 1993). The basic sequences of pheromone calling have been described by Feron (1962):

- in stage I, the male exerts an anal ampulla, with the abdomen bent dorsally, and emits a pheromone. The lateral pleura of the abdomen are also strongly inflated.
- in stage II, the male initiates a continuous wing vibration (fanning), while bending its abdomen tip ventrally. Abdominal pleura are still strongly inflated. This stage is initiated by the visual perception of another fly in the immediate surroundings.

The first data on the courtship behavior of *C. rosa* were given by Myburgh (1962), who mentioned the rectal ampulla (thought to be the aedeagus), wing fanning, and erection of tibial hair. More recently, field-cage studies and video recordings allowed us to precisely describe the courtship sequences of *C. rosa* males (S. Q., unpublished data). As the two first stages broadly correspond to what is observed in *C. capitata*, the nomenclature of Feron (1962) may be used for their description:

- in stage I, the *C. rosa* male exhibits an anal ampulla bent dorsally. Video recordings clearly show that this ampulla is tri-lobed, while it appears more or less spherical in medfly. The abdominal pleural distension is as conspicuous as in *C. capitata*. Another characteristic of the calling male is the erection of black hair present on male mid-tibias in this species (Myburgh 1962). Wings are maintained perpendicular to the body axis.
- the initiation of stage II is very similar to what is observed for the medfly male: at the approach of another adult, the male faces it

and initiates wing fanning while the abdomen is bent downwards. If the intruder is another male, the calling male then returns to stage I.

Experiments in an insectarium allowed us to confirm that calling males of *C. rosa* attract conspecific females but not those of *C. capitata*, while calling males of *C. capitata* attract conspecific females but not those of *C. rosa* (S. Q., unpublished data). However it is not yet known if this is due to pheromone specificity or to circadian rhythms of female receptivity.

Lab cage observations, macrophotographs and video recordings have recently given us a preliminary description of pheromone calling in *C. catovirii* (S. Q., unpublished data):

- stage I appears very similar to what is observed in medfly male. The exerted anal ampulla is more or less spherical, and appears at the tip of the abdomen, bent dorsally. Abdominal pleura are also strongly inflated.
- stage II also appears similar to the corresponding stage in medfly: male initiates wing fanning while bending its ampulla downwards. During both stages, hair of the male fore-femur are typically erected.

**Circadian rhythms.** In *C. capitata*, various authors have shown that males congregate in leks in the morning and early afternoon, depending on climatic and experimental conditions (Prokopy & Hendrichs 1979, Arita & Kaneshiro 1989, Whittier et al. 1992).

Myburgh (1962) showed that the sexual activity of *C. rosa* occurred at the end of the day, as in

many tephritid species. Most of the matings he observed were in periods when the light intensity was between 0 and 20 foot candles. The results of our study in laboratory cages (Fig. 1), but also studies in field cages and observation in orchards confirmed this pattern in *C. rosa* (S. Q., unpublished data).

Recent lab-cage observations showed that the pheromone calling of *C. catovirii* occurred during the morning, with a maximum at 10.00 h (Fig. 2) (S. Q., unpublished data), confirming that sexual behavior in this species is very similar to that of the medfly.

**Factors influencing male calling.** The age at which adults of *C. capitata* become sexually mature varies with environmental conditions. Lab-reared males may reach their sexual maturity as early as 3 days old (Feron 1962). The availability, quantity, and quality of food resources also influence pheromone production in tephritids (Nation 1989).

In lab studies of *C. rosa*, calling was shown to be initiated some 10 days after adult emergence. The percentage of calling males then increases quickly in subsequent days and remains more or less stable until males are at least 35 days old (S. Q., unpublished data).

In lab studies, food quality also had a marked influence on the calling propensity of male *C. rosa*. When food consisted of sugar or yeast only, a decrease was observed in the maximum calling rate (20%) compared with adults fed with sugar + yeast (80%). For males previously fed for 20 days with a complete regime (sugar + yeast), food deprivation periods of 4, 8, 12 or 28 h had no effect on the calling rate of males (S. Q., unpublished data).

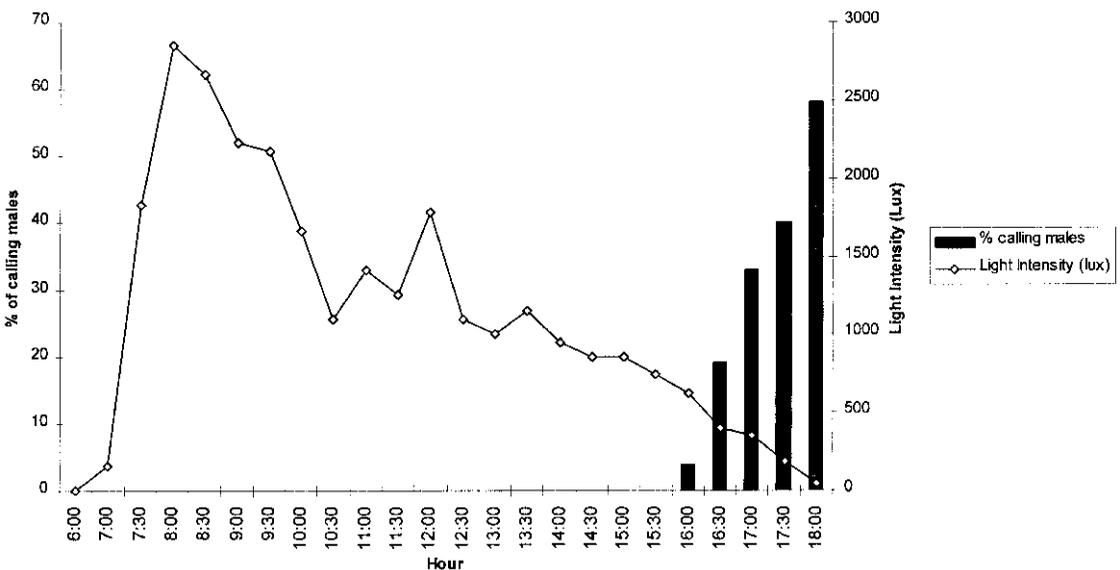


Fig. 1. Circadian rhythm of pheromone calling of male *Ceratitis rosa* in a lab cage, in relation with light intensity (males 15 days-old; mean of 5 replicates).

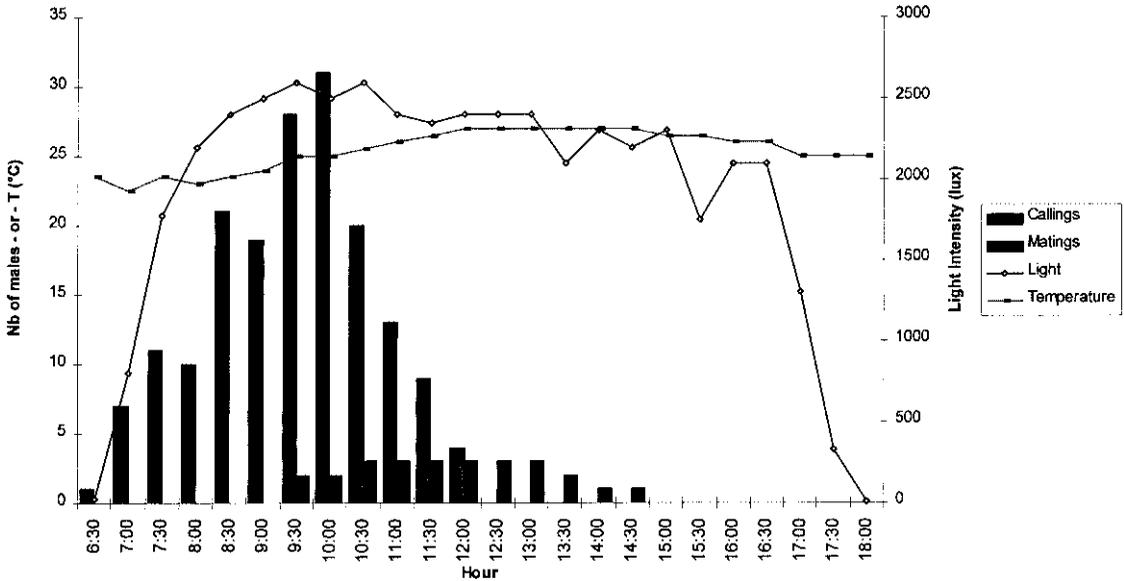


Fig. 2. Circadian rhythm of pheromone calling and mating of *Ceratitis catovirii* in a lab cage containing both sexes, in relation with abiotic factors (2nd replicate; males older than 12 days; N = 53 males).

Lek Formation

Since the first study by Prokopy & Hendrichs (1979), many authors have described the lekking behavior of *C. capitata* (Arita & Kaneshiro 1985, 1989, Whittier et al. 1992, Shelly et al. 1993).

Preliminary field-cage observations showed that males of *C. rosa* also aggregate in leks (S. Q., unpublished data). Our insectarium experiments with wild flies in the presence of potted citrus plants showed that males began aggregating and calling around 1715 h. A sharp increase in calling was observed, with 20% of released males calling at 1800 h, then this percentage dropped to 10% at 1815 h (S. Q., unpublished data). The mean number of calling males per plant reached a maximum of only 1,5, with the biggest aggregation observed including 10 males. Within a lek, males were situated on the underside of leaves, occupied and defended territories, as described previously in *C. capitata*.

More recently, observations were conducted in a citrus orchard during the harvest period. On two successive days, *C. rosa* leks were observed on different trees. Nearly all calling males occupied the underside of a fruit. Interestingly, a study of the circadian rhythm of daily activities of both sexes in this orchard showed that peak female oviposition activity takes place late in the afternoon (S. Q., unpublished data). In certain periods of the year, the situation of calling sites on fruits, and the temporal coincidence of sexual activities with the preferred oviposition period of the females, may constitute an advantage for maximizing the probability of males encountering females. Interestingly, citrus volatiles are known to be strongly

attractive in the selection of oviposition sites by females of *C. rosa* (S. Q., unpublished data). Moreover, in artificial rearing conditions, the number of eggs laid by *C. rosa* females is strongly increased when adding pieces of citrus fruit, or citrus juice, into oviposition devices.

Precopulatory Behavior

In *C. capitata*, the final sequences of mating behavior preceding the mating attempt (close range interactions between male and female) have been studied by various authors (Briceno et al. 1996, Eberhard 2000), as well as the factors influencing male mating success (Whittier et al. 1994).

Feron (1962) defined as stage III, the large movements of wings, moved rhythmically forwards and backwards while still vibrating. This stage, also called "approach song" (Sivinski et al. 1989), "buzzing", or "intermittent wing buzzing" (Eberhard 2000), frequently precedes a mating attempt. It is associated with movements of the head ("head-rocking") performed in bursts (Eberhard 2000).

A limited number of video recordings have provided us preliminary data on close-range interactions between males and females of *C. rosa*. They failed to show any type of "buzzing" or "head-rocking" during this stage. Though this is not visible on all sequences, it appears that the male throws its mid-legs forward just before mounting.

In *C. capitata*, the sexually dimorphic capitata bristles on the anterior surface of the male's head may be displayed visually to the female during

“head-rocking” (Eberhard 2000). As such bristles are not present in *C. rosa*, it may be hypothesized that the conspicuous black hair of male mid-tibia could play a similar role in this species. Close-range interactions between sexes of *C. rosa* appear to follow a simpler scheme, and to have a shorter duration than in medfly.

Similar preliminary video recordings of interactions between males and females of *C. catoirii* showed that males of this species display some “buzzing” but very little, if any, “head-rocking”. The capitate bristles of *C. catoirii* are white colored, which may limit their possible role in a visual display.

In future studies, it would be worth enlarging such comparisons to various african species of *Ceratitidis*. In species within the subgenus *Pterandrus*, the males present more or less developed black hair on the mid-tibias and/or mid-femurs. The males of species within the subgenus *Ceratitidis*, such as *Ceratitidis malgassa* Munro, also possess capitate bristles as in *C. capitata* and *C. catoirii*.

More video recordings studies will be necessary in order to better understand and quantify the sequences of courtship behavior of *C. rosa* and *C. catoirii*. Additional observations of leks in orchards and experiments in field-cages will also be necessary to understand the factors that are involved in male mating success in these species.

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