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Short Communication

Rearing *Frankliniella zucchini* Nakahara & Monteiro (Thysanoptera: Thripidae) on zucchini (*Cucurbita pepo* L. 'Caserta') fruits

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ABSTRACT

Frankliniella zucchini transmits zucchini lethal chlorosis virus, causal agent of lethal chlorosis of zucchini squash. The characteristics of relationship between this virus with its vector have not been studied, one of the reasons being the lack of a method for rearing the thrips for laboratory studies. This work proposes a system for the rearing of *F. zucchini* on fresh virus free zucchini 'Caserta' fruits, offering a practical and efficient alternative for the supply of a large number of insects for later study of virus/vector relationship. In addition, to aid in the identification of this species of thrips, the immature and adult forms obtained from the colony were described.

The zucchini thrips, *Frankliniella zucchini* Nakahara & Monteiro (Thysanoptera: Thripidae) (Nakahara and Monteiro, 1999), is a species of thrips so far reported only in Brazil. It is the only species identified as a vector of the tospovirus zucchini lethal chlorosis virus (ZLCV), causal agent of lethal chlorosis of zucchini (*Cucurbita pepo* L. 'Caserta'); whereas transmission tests with *F. schultzei* Trybom and *F. occidentalis* Pergande were negative (Bezerra et al., 1999; ICTV, 2018; Monteiro et al., 2001). Moreira et al. (2014) monitored the thrips population in experimental plantations of zucchini and found the predominance of larvae and adults of *F. zucchini*, indicating that this species, besides transmitting ZLCV, also colonizes 'Caserta'.

In addition to *C. pepo* 'Caserta', ZLCV has been identified as naturally infecting other hosts, mainly cucurbitaceae: *Cayaponia tibiricae*, *Cucurbita maxima*, *C. moschata*, *C. moschata* × *C. maxima*, *Cucumis anguria*, *C. sativus*, *Citrullus lanatus*, *Luffa aegyptiaca* and *Sechium edule*; and solanaceae *Datura stramonium* (Camelo-García et al., 2015; Giampan et al., 2007; Yuki et al., 2000).

Characteristics of the relationship between zucchini thrips and ZLCV, such as acquisition and transmission access periods, incubation period, virus replication in the vector, have not been studied, although necessary for a better understanding of epidemiological aspects of the disease in the field. Studies of virus/vector relationships, however, require the availability of virus free colonies of thrips for larvae and adult trials. The absence of a continuous and standardized source of insects in the field, as well as the difficulty of collecting and identifying large numbers of individuals, makes the rearing of thrips indispensable for the development of these studies (Lopes and Alves, 2000). The objective of the present work was to develop a system of mass rearing of *F. zucchini* under laboratory conditions to support studies of its relationship with ZLCV. This colony was also used to further describe immature and adult forms to expand the taxonomic knowledge of this thrips species.

Rearing of *F. zucchini* was initiated from insects collected from zucchini plants 'Caserta' grown in the experimental field at the Department of Phytopathology and Nematology, ESALQ/USP, Piracicaba, SP, Brazil, where high incidence of thrips and ZLCV have been observed since 1996 (Camelo-García et al., 2015). Periodic identification of thrips specimens was performed by means of examination of slides mounted following the technique proposed by Mound and Marullo (1996) and using the key to *Frankliniella* species in Brazil proposed by Cavalleri and Mound (2012). Voucher specimens are deposited in the Entomological Collection of the Entomology and Acarology Department ESALQ/USP, Piracicaba, SP, Brazil. Leaves and flowers collected from these plants were examined under a stereomicroscope for collection of adults with the aid of a fine brush. The adults were then transferred to fresh virus free zucchini 'Caserta' fruits (~20 cm long) previously washed with 0.5% sodium hypochlorite and subsequently with running

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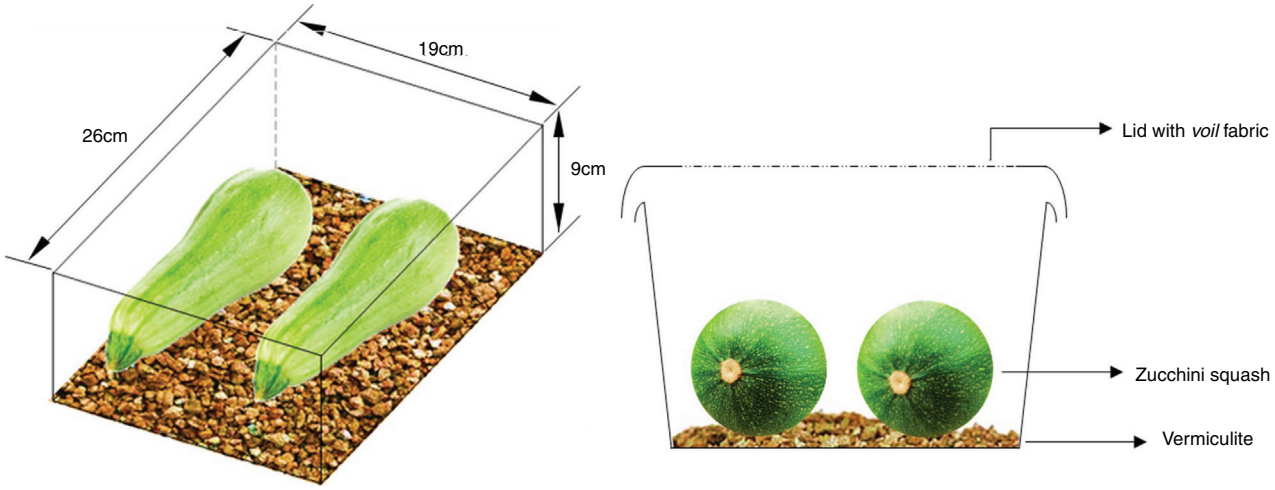

Fig. 1. Containers for rearing *Frankliniella zucchini* on fresh zucchini fruits.

water in abundance. The fruits were placed in plastic boxes (26 cm long × 19 cm wide × 9 cm high) with a lid protected with *voil* fabric to allow air exchange and a layer (2 cm) of expanded vermiculite sterilized in the bottom of the box. The plastic boxes with the fruits were kept in a BOD (Biothec) incubator, with a 12 h light and temperature of 24.5 °C ± 0.5 °C followed by 12 h of dark and 20 °C ± 0, 5 °C (Fig. 1). The fruits were renewed between 8 and 10 days, transferring larvae of the old fruits with the aid of a fine brush, keeping the old fruits in the box for a few more days to make sure that larvae could be collected after possibly hatching. Once a month, a complete cleaning of the plastic box with the vermiculite exchange was carried out.

The results obtained during 12 months of studies indicated the efficiency and feasibility of the use of fresh zucchini 'Caserta' fruits for rearing *F. zucchini*. In general, it was possible to estimate approximately 500 adults of thrips per plastic box. Attempts to rear *F. zucchini* on bean pods (*Phaseolus vulgaris*), and plants of zucchini 'Caserta', *Cucurbita maxima* 'Exposição', long neck squash (*C. moschata* 'Menina Brasileira'), cucumber (*Cucumis sativus* Safira') and common bean grown in a BOD (Biothec) incubator, with a 12 h light and temperature of 24.5 °C ± 0.5 °C followed by 12 h of dark and 20 °C ± 0, 5 °C were not successful, due mainly to the poor development of these species under such conditions.

The identification of immatures in Thysanoptera is restricted to a few species (Vierbergen et al., 2010) and is largely concentrated in the comparison of second instar larvae. In *Frankliniella*, De Borbon (2007) and Skarlinsky and Funderburk (2016) provided keys to some species of the Americas, however the characterization of this stage for *F. zucchini* is still unavailable. Second instar immatures of the species collected from the colony were used to characterize the morphology of larval stage in the zucchini thrips, which will allow certifying its possible host-association in different plant species. Slides of the immature forms were studied under Zeiss AxioLab A1 light microscope. In addition, adults were examined in light and scanning electron microscopy LEO 435 VP (Fig. 2) to provide new information on the morphology of *F. zucchini*.

Adult females of *Frankliniella zucchini* (Fig. 2a) are distinguished from other congeneric species by the simple pedicel on antennal segment III, ocellar setae pair III on position 3, absence of at least one postocular seta I (described below) (Fig. 2b), presence of a pair of campaniform sensilla on metanotum and posteromarginal comb on abdominal tergite VIII complete and regularly spaced with pointed teeth (Fig. 2c). The species is closely related to *F. distinguenda* Bagnall and *F. gemina*. The first is regarded usually as a smaller species, with ocellar setae III and pronotal anteromarginal setae smaller in comparison with *F. gemina* and *F. zucchini*. Although *F. zucchini* is regarded as host specific to *C. pepo* and *F. gemina* as more polyphagous, there is no certainty that discrete biological entities are involved (Cavalleri and Mound, 2012).

The only major difference found between *F. zucchini* and *F. gemina* is the absence of at least one postocular seta in the pair I in the first species (usually both setae are absent) (Cavalleri and Mound, 2012; Nakahara and Monteiro, 1999). The examination of the specimens obtained from rearing and surveys from other regions revealed that the absence of at least one seta of the postocular pair I is constant, as no specimen exhibited both setae, and in most individuals (about 90%) the pair was completely absent (Fig. 2b). In addition, a more recently used character to distinguish *Frankliniella* species (see Gunawardana et al., 2017) was examined among more than 300 specimens: *F. zucchini* has usually only one (sometimes zero) small microtrichia on the dorsal surface of the hind coxae (Fig. 2d), while *F. gemina* has three or four more developed microtrichia (Fig. 2e). Studies involving both molecular and morphologic biology should be carried out to reveal more consistent species delimitations.

Among the species close to *F. zucchini*, only *F. gemina* has second instar larvae described by De Borbon (2007). The second instar morphology of both species are similar, and the setal lengths exhibit overlaps (Table 1).

The morphological characterization of *Frankliniella zucchini* larvae II (Fig. 2f) is as follows: head with setae weakly pointed

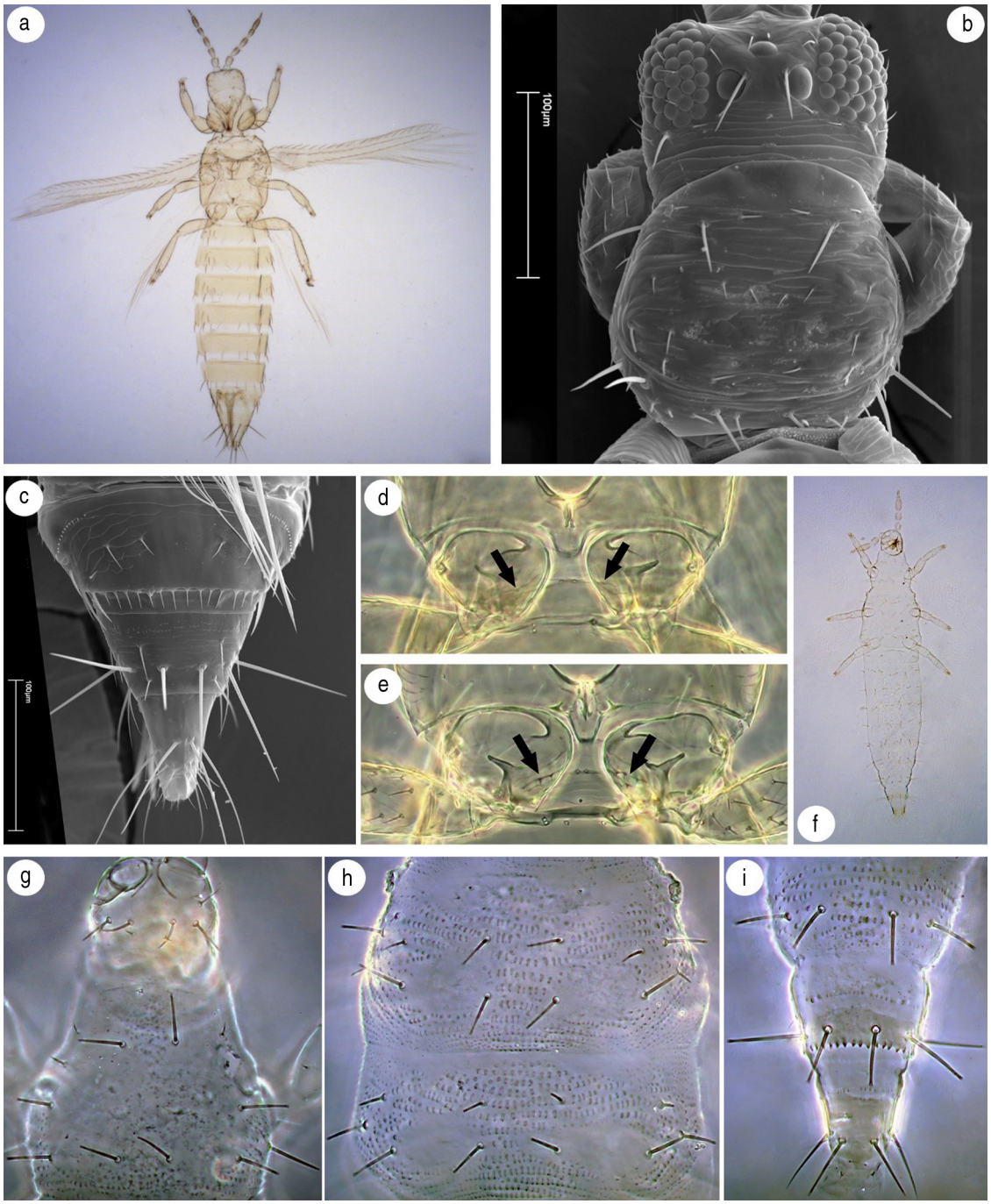

Fig. 2. Morphology of adult female and instar II immature. Adult female (*Frankliniella zucchini*): (a) habitus; (b) head and pronotum; (c) abdominal tergites VIII–X; (d) hind coxae upper surface. (e) Hind coxae upper surface of *Frankliniella gemina*. Instar II immature (*F. zucchini*): (f) habitus; (g) head and pronotum; (h) meso- and metanotum; (i) abdominal tergites VIII–X.

to capitate (Fig. 2g), D1 setae 5–10 μm long, D2 15–30 μm long, D3 12.5–17.5 μm long, D4 20–25 μm long; pro-, meso- and metanotal main setae capitate, pronotum medially without integument plaques, meso-and metanotum with rounded and elongated plaques medially, plaques on the posterior region smaller and sometimes weakly dentate (Fig. 2g and h); abdominal tergites with capitate to weakly expanded setae and rounded elongated integument plaques; tergite IX with sclerotized band extending from D1 setae 1.0–1.5 times the diameter of a setal insertion, not reaching the campaniform sensilla, posteromarginal comb usually with 16–18 teeth pointed and regularly spaced, of which 4–6 between D1 setae, teeth not longer than D1 seta insertion, transverse line of integument plaques present medially at the level of campaniform sensilla (Fig. 2i), D1 setae 30–40 μm long, D2 35–50; tergite X with sclerotized band in the posterior half (Fig. 2i); sternite IX posteromarginal comb with teeth small, about one third of dorsal teeth length.

Table 1
Setal lengths in *Frankliniella gemina*[a] and *F. zucchini* (in microns).

Sclerite	Setae	*F. gemina*	*F. zucchini*
Head	D1	7.5–10.0	5.0–10.0
	D2	15–22.5	15.0–30.0
	D3	15.0–18.8	12.5–17.5
	D4	22.5–32.5	20.0–25.0
Pronotum	D1	18.8–25.0	22.5–25.0
	D2	20.0–31.3	27.5–30.0
	D3	7.5–13.8	7.5–10.0
	D4	12.5–21.3	7.5–12.5
	D5	22.5–32.5	25.0–30.0
	D6	27.5–40.0	35.5–35.0
	D7	22.5–32.5	25.0–27.5
Tergite II	D1	16.3–23.8	15.0–20.0
	D2	17.5–27.5	17.5–22.5
	D3	16.3–25.0	12.5–17.5
Tergite VII	D1	20.0–30.0	25.0–27.5
	D2	25.0–35.0	25.0–30.0
	D3	27.5–37.5	30.0–35.0
Tergite VIII	D1	25.0–32.5	30.0–32.5
	D2	27.5–35.0	30.0–32.5
	D3	30.0–40.0	30.0–35.0
Tergite IX	D1	30.0–36.3	30.0–40.0
	D2	40.0–55.0	35.0–50.0

[a] Based on De Borbon (2007).

Conflicts of interest

The authors declare no conflicts of interest.

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