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Scientific Note

# Infestation indices and characterization of injuries of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) in Barbados cherry

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**Abstract.** Females of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) have a serrated ovipositor that causes severe physical damage to soft fruit. This behaviour results in the early ripening and accelerated rotting of infested fruit. In addition, feeding by larvae softens of the fruit peel, causing in a depreciation in commercial value. In this study, results of infestation indices, pupal viability, egg-adult period, degradation time of infested fruit, and description of physical injuries caused by oviposition and larval feeding of *D. suzukii* in Barbados cherries (*Malpighia emarginata* DC., Malpighiaceae) are described. Overall, our results and discussion contribute to the understanding of the status of *D. suzukii* as an insect pest of the Barbados cherry.

**Keywords:** Acerola; Depreciated fruit; Fruit damage; Insect pest; Spotted-wing drosophila

*Drosophila suzukii* Matsumura (Diptera: Drosophilidae) is an important insect pest in fruit orchards around the world. An invasive species, it probably originating in Asia and has spread to North America, South America and Europe (Deprá *et al.* 2014; Asplen *et al.* 2015). It is a polyphagous fly that prefers to oviposit on ripe, red, soft fruit (Lee *et al.* 2011, 2015; Diepenbrock *et al.* 2016).

*Drosophila suzukii* is also known as spotted-wing drosophila due to the males characteristic dark spot on the edge of each wing. Females do not have wing spots. However, they have a serrated ovipositor that allows them to infest and physically damage fruit. These male and female characteristics distinguish *D. suzukii* from other drosophilids (Walsh *et al.* 2011; Miller *et al.* 2017; Nikolouli *et al.* 2018).

The importance of *D. suzukii* is due to the damage caused by females in fruit production. Using their serrated ovipositor, females penetrate the peel of soft fruit, resulting in a minor injury to the peel. A few days after oviposition, the larvae hatch and begin to feed on the fruit pulp. The injury caused by oviposition opens a door for attack by other insects, fungal infections and bacterial diseases. In addition, feeding by the larvae results in soft, brown, sunken spots on the fruit (Walsh *et al.* 2011). This causes premature depreciation of fruit, resulting in yield losses and economic damage to the fresh fruit chain (Goodhue *et al.* 2011; Walsh *et al.* 2011).

The first reports of *D. suzukii* in Brazil were in 2013 in the states of Santa Catarina and Rio Grande do Sul, Brazil (Deprá *et al.* 2014). After its detection, *D. suzukii* managed to disperse through several Brazilian regions (Andreazza *et al.* 2017; Zanuncio-Junior *et al.* 2018), probably through the transportation of infested fruit intended for commercial sale (Vilela & Mori 2014). In Brazil, *D. suzukii* has been characterized as an insect pest mainly targeting red fruit such as strawberries [*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier] (Santos 2014; Andreazza *et al.* 2016), with potential damage to several other fruit crops (Benito *et al.* 2016; Wollmann *et al.* 2019).

The Barbados cherry (*Malpighia emarginata* DC., Malpighiaceae) is a fruit of economic importance in Brazil. In 2017 its production reached 142,992 metric tons in 7,724 ha (IBGE 2017). The fruit is soft and reddish when ripe. The Barbados cherry is a natural host of *D. suzukii* in the field. Potentially *D. suzukii* is a primary pest for this fruit crop (Louzeiro *et al.* 2019). Considering the impact of *D. suzukii* as a fruit pest, and its potential be a pest for a variety crops, our objectives were to evaluate the infestation of the Barbados cherry with respect to the duration of exposure to mature *D. suzukii* females and to characterise the injuries caused by infestation in laboratory.

Barbados cherry fruit were collected in the municipality of Mogi Mirim, São Paulo state, Brazil (22°31'13.48" S

47°00'36.83" W) in June 2019. Fruit that were reddish in colour were collected directly from the trees and were immediately brought to the Economic Entomology Laboratory, Instituto Biológico, where they were washed in sodium hypochlorite solution (0.5% v/v) and allowed to dry. The females used in this study came from the *Drosophila* Stock Center at the Departamento de Genética, Evolução, Microbiologia e Imunologia, Instituto de Biologia, UNICAMP, and were raised on an artificial diet based on molasses and cornmeal (Freire-Maia & Pavan 1949). Fruit were deposited individually into 50 mL glass tubes containing a moistened cotton pad in the base. Ten 16-day-old *D. suzukii* females were inserted into each tube and the tubes were sealed with cotton balls. After infestation, individual fruit were placed in 50 mL plastic pots with a vermiculite layer at the inferior base. The plastic pots were sealed with voile fabric and fastened with an elastic. The fruit were kept in a room at 23 ± 0.5 ºC and 65 ± 5% relative humidity during storage and the infestation experiments. The infestation experiments were performed to evaluate infestation indices (puparia/fruit and puparia/gram of fruit) during the infestation period and characterise the injuries caused by oviposition and larval feeding by *D. suzukii*. The infestation indices were evaluated after 12, 24 and 48 h of exposure to *D. suzukii*. Eight fruit were used at each time of exposure, totalling 24 fruit; each one was considered a replica. Twenty days after infestation, puparia and adults of *D. suzukii* were counted. To characterise infestation injuries, we used six infested fruit that were subjected to 24 h of exposure. The injuries caused by oviposition were recorded using a camera (Canon - PowerShot A650 *IS*) coupled with a stereoscope microscope (Zeiss-Stemi 2000-C) 24 h after infestation. Injuries caused by larval feeding were recorded every two days. Percentage of pupal viability was calculated by the formula: (number of adults/number of puparia) x 100 in each replica. The relationships between fruit exposure time versus the values of infestation indices (puparia/fruit and puparia/gram of fruit) and between puparia per fruit versus percentage of pupal viability were analysed by Pearson's correlation at 95% significance ($p$-value ≤ 0.05) using SAS University Edition software (Version 3.8) (SAS Enterprise Miner 13.1. SAS Institute Inc., Cary, NC.).

Infestation indices and pupal viability of *D. suzukii* in Barbados cherries varied with fruit exposure time (Table 1). The highest infestation indices were 70.0 puparia per fruit and 14.6 puparia per gram observed in the shortest exposure time (12 h). A weak, negative and non-significant correlation was detected between exposure time and puparia per fruit ($r$ = -0.2399, d.f. = 23, $p$-value = 0.2588), and between exposure time and puparia per gram ($r$ = -0.1982, d.f. = 23, $p$-value = 0.3532). The highest pupal viability occurred in the shortest exposure time (12h), when the infestation indices were higher than other times. A strong, positive and significant correlation was observed between puparia per fruit and pupal viability (r = 0.7129, d.f. = 23, $p$-value < 0.0001). The egg-adult period ranged from 13 to 16 days. The shortest egg-adult period occurred in the highest infestation index (Table 1). The characterisation of the infestation of *D. suzukii* in Barbados cherries showed that a fruit is susceptible to multiple punctures (Figure 1A). The oviposition of *D. suzukii* causes a circular and/or semicircular wound approximately 0.2-0.3 mm in diameter where the female inserts eggs into the fruit (Figure 1B). The ovipositions may be near to each other (Figure 1C), or far from each other (Figure 1D). Peeling of the skin at the oviposition site was observed and fruit degradation progressed as the larvae developed. During the larval feeding period, the pulp of the fruit softened, accompanied by a change in the colour of the peel at the attack site. Five days after the initial infestation, the fruit were completely depreciated (Figure 1E).

In this study, in contrast to our initial hypothesis, the infestation indices of *D. suzukii* were not directly proportional to the fruit exposure time. It is probable that the maximum infestation level a unique Barbados cherry can withstand was reached in the first few hours of infestation. Females of *D. suzukii* may have avoided oviposition once they perceived that the fruit could collapse and would not provide enough substrate for larval development. *Drosophila suzukii* likely leaves host chemical markers, as do many other insects (Roitberg & Prokopy 1987). Some studies have reported that the number of ovipositions of *D. suzukii* vary, mainly due to host preference, maturation stage and °Brix of the fruit (Lee *et al.* 2011; Walsh *et al.* 2011; Diepenbrock *et al.* 2016).

Based on the infestation indices, pupal viability and short egg-adult period, the Barbados cherry is a substrate favourable for the development of *D. suzukii* immatures. This evidence is based on positive and significant correlation between puparia per fruit and pupal viability. Probably the physicochemical characteristics of the Barbados cherry, such as pH (ranging from 3.0 to 3.5), maturity index (total soluble solids/titratable acidity) (from 5.0 to 5.8) and vitamins (Santos *et al.* 2012; Sagar *et al.* 2013) contributed to the high developmental rates of immature *D. suzukii*.

Even with a few hours of fruit exposure, the fly can perform multiple ovipositions and achieve good egg-adult development. Infestation conditions in the laboratory, with 10 females per fruit, do not correspond to field infestations, which generally range from one to five puparia per fruit (Louzeiro *et al.* 2019), but demonstrate the host potential of Barbados cherry and its influence on the demography. This preliminary study demonstrates that under high populational pressure (10 female *D. suzukii*) the Barbados cherry can endure high levels of infestation. In backyards, isolated crops or mixed orchards, Barbados cherries may sustain the *D. suzukii* population, especially in tropical regions where some crops may be harvested all year long. As a polyphagous fly *D. suzukii* can use successive host (Diepenbrock *et al*. 2016; Wollmann *et al*. 2019) to maintain its population in the field throughout the year. This increases the risk of damage to commercial fruit orchards.

The puncture, oviposition and larval feeding of *D. suzukii* inflict significant injuries on Barbados cherries. This can jeopardise their production and commercial sale due to further infection by pathogens that contribute to fruit rot (Walsh *et al.* 2011), as well as the total depreciation of the fruit in a few days due to larval feeding. Our results help expand the understanding of the potential pest status of *D. suzukii* for the Barbados cherry

**Table 1.** Mean values (± standard error) of Barbados cherries (length, diameter, weight) tested under laboratory infestation (N = 8 per treatment), infestation indices, pupal viability, and life cycle of *Drosophila suzukii* (Drosophilidae) after different durations of fruit exposure.

| Time (h) | Length (cm) | Diameter (cm) | Weight (g) | Puparia per fruit | Puparia per g | Pupal viability (%) | Egg-adult period (day) |
|---|---|---|---|---|---|---|---|
| | Mean (± SE) | Mean (± SE) | Mean (± SE) | Mean (± SE) | Mean (± SE) | Mean (± SE) | Mean (± SE) |
| 12 | 1.8 (± 0.03) | 2.1 (± 0.05) | 4.4 (± 0.28) | 39.5 (± 5.9) | 9.1 (± 1.3) | 90.6 (± 2.6) | 14.2 (± 0.3) |
| 24 | 1.6 (± 0.06) | 1.8 (± 0.06) | 3.3 (± 0.30) | 18.7 (± 7.3) | 4.9 (± 1.7) | 56.0 (± 14.2) | 14.6 (± 0.4) |
| 48 | 1.7 (± 0.07) | 1.8 (± 0.07) | 3.4 (± 0.50) | 24.0 (± 8.2) | 6.1 (± 1.8) | 75.4 (± 12.3) | 15.5 (± 0.1) |


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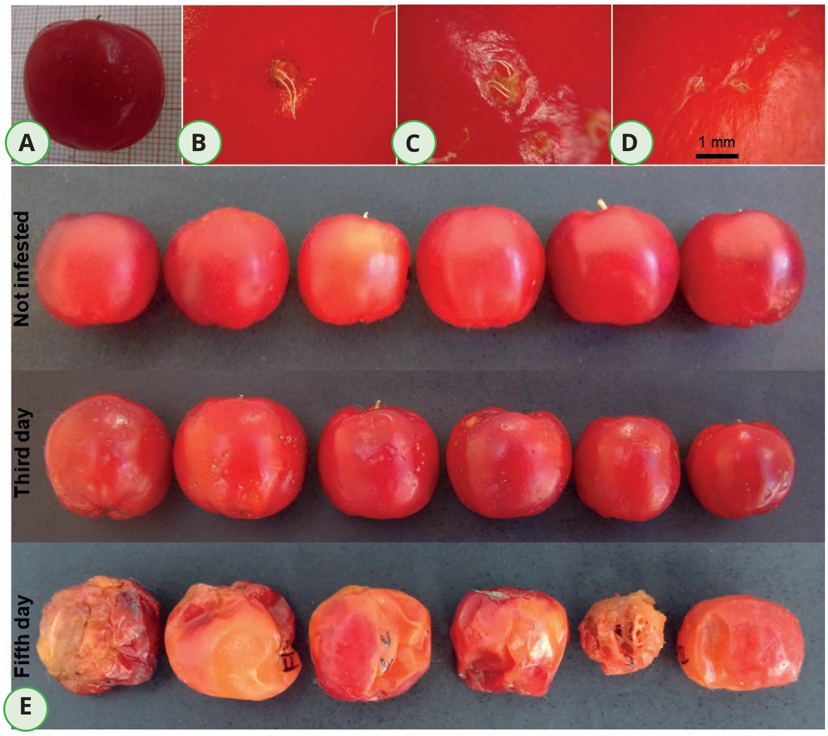


**Figure 1.** Multiple ovipositions of *Drosophila suzukii* (Drosophilidae) (A), injury of puncture and oviposition (B), eggs close to each other (C), eggs far from each other (D), and ovipositions and evolution of Barbados cherry degradation due to larval development (E).

crop. Infestation indices, pupal viability and egg-adult period can help in studies of biology, ecology and the relationship between fly/host fruit. Moreover, characterisation of the injuries caused by *D. suzukii* infestation differs from that of other frugivorous flies that infest Barbados cherries. Further, basic and applied studies on the biology, ecology and behaviour of *D. suzukii* in fruit orchards are necessary for a better understanding of its status a fruit pest.

In conclusion, we show for the first time that *D. suzukii* infestation indices are not correlated with the fruit´s exposure time to females. To detect any correlation, we suggest the study of short time intervals (hourly intervals up to 9-10 h). This result was obtained in Barbados cherries and must be further investigated to assess its generalisation to other fruit. The Barbados cherry proved to be an important host for *D. suzukii* because it allows rapid multiplication of the fly and supports high viability rates for immatures. However, the physicochemical characteristics of the host can influence the life cycle of frugivorous flies. We recommend that applied studies on this topic be presented. *Drosophila suzukii* has potential to be a pest in the production and commercial sale of *M. emarginata*. This potential is associated with physical injuries and rapid depreciation of the fruit caused by oviposition and larval feeding.

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