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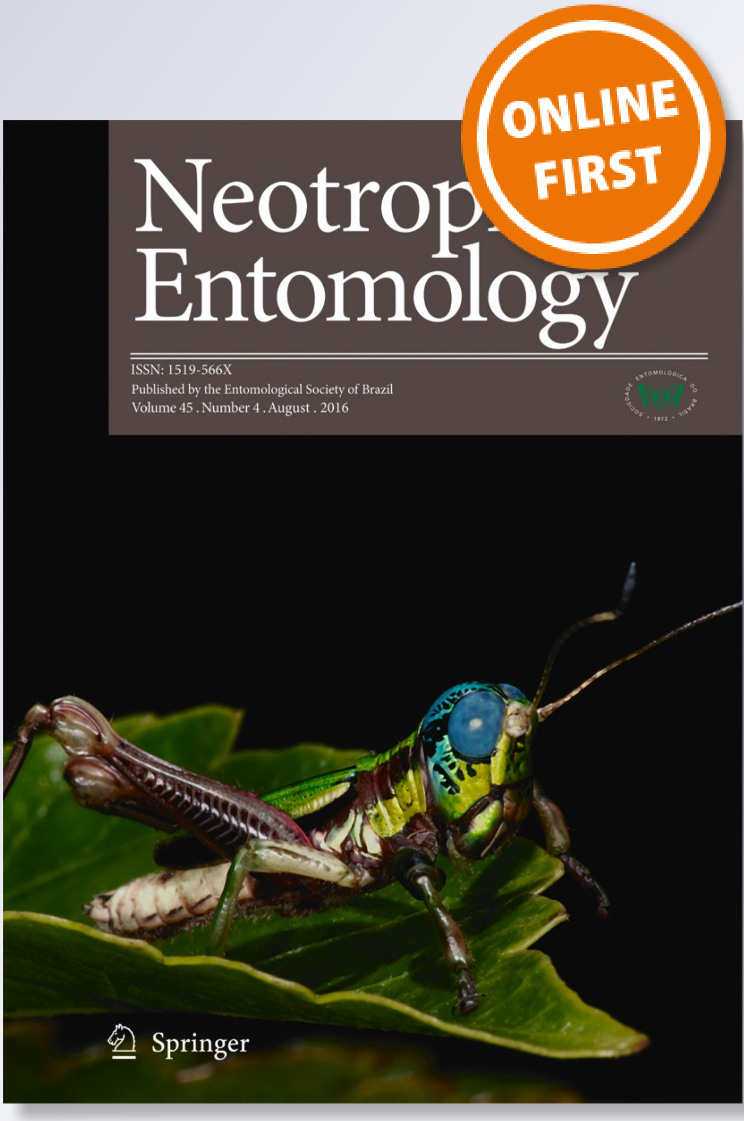

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ECOLOGY, BEHAVIOR AND BIONOMICS

Susceptibility and Interactions of *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Damaging Strawberry

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Keywords

Spotted wing drosophila, African fig fly, fruit preference, oviposition, ripening stage

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Abstract

Drosophila suzukii (Matsumura) has been recently detected causing damage to strawberries in Brazil. Infestation in strawberry culture has often been observed jointly with the presence of *Zaprionus indianus* Gupta. This study investigated the susceptibility of strawberries at three ripening stages to infestation of *D. suzukii* and *Z. indianus* and their interaction. In the laboratory, strawberries cv. Albion at different ripening stages (green, semi-ripe and ripe) were exposed to *D. suzukii* and *Z. indianus* for 24 h in choice and no-choice bioassays. Additionally, we evaluated the effects of mechanical damage incurred artificially or by *D. suzukii* oviposition on *Z. indianus* infestation. In no-choice bioassay, there were no significant differences in fruit susceptibility to *D. suzukii* infestation at different ripening stages. However, in choice bioassay, *D. suzukii* adults preferred to oviposit on R fruit. The presence of mechanical damage did not increase susceptibility of fruit to *D. suzukii* oviposition. For *Z. indianus*, there was greater susceptibility of R fruit in relation to SR and G fruit in both the choice and no-choice bioassays. There was a significant and positive interaction of mechanical damage and damage caused by *D. suzukii* to R fruit and infestation by *Z. indianus*, which was not observed in SR and G fruit. Although infestation of *Z. indianus* is related to attack damaged or decaying fruit, this work shows that this species has the ability to oviposit and develop in healthy strawberry fruit with and increased infestation level when the fruit has damage to its epidermis.

Introduction

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is one of the major pests associated with the cultivation of small fruits worldwide (Walsh *et al* 2011, Cini *et al* 2012, Santos 2014, Asplen *et al* 2015). This is attributed to its high polyphagia (Dreves *et al* 2009), rapid population growth (Tochen *et al* 2014) and dispersion capacity (Walsh *et al* 2011, Cini *et al* 2012). This species has spread rapidly across North America, Europe (Walsh *et al* 2011, Asplen *et al* 2015, Lee *et al* 2015) and South America (Deprá *et al* 2014, Santos 2014), where it has caused significant economic losses in fruit orchards, mainly in small fruits such as blackberry, cherry, raspberry, blueberry and strawberry (Goodhue *et al* 2011, Bellamy *et al* 2013, Santos 2014, De Ros *et al* 2015, Ioriatti *et al* 2015, Lee *et al* 2015). It has also recently been reported that *D. suzukii* has caused economic damage and significant losses in strawberry crops in southern Brazil (Santos 2014).

Fruit damage and economic losses are caused by *D. suzukii* females, which have a serrated ovipositor with the ability to lay eggs inside healthy ripe fruit (Walsh *et al* 2011, Cini *et al* 2012, Lee *et al* 2015). Injuries caused by external piercing and/or oviposition allow pathogens to penetrate, increasing the losses (Dreves *et al* 2009, Bolda *et al* 2010) in addition to causing the release of volatiles (Abraham *et al*

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2015) that attract other drosophilid species such as *Zaprionus indianus* Gupta (Diptera: Drosophilidae) (Van Timmeren & Isaacs 2013, Joshi *et al* 2014, Lasa & Tadeo 2015).

The African fig fly, *Z. indianus*, is considered the primary pest associated with fig culture worldwide (Raga *et al* 2003, Commar *et al* 2012), with occurrences in Brazil (Vilela *et al* 1999), Uruguay (Goñí *et al* 2001), Central America (Van Der Linde *et al* 2006), North America (Joshi *et al* 2014, Lasa & Tadeo 2015), Europe (Commar *et al* 2012) and Asia (Fartyal *et al* 2014). In figs, females oviposit on the fruit ostiole base and damage is caused by larvae when they penetrate the fruit tissue (Vilela *et al* 1999). However, *Z. indianus* is considered a secondary pest to more than 70 other fruit species due to its tendency to attack and feed only on decaying fruit (Joshi *et al* 2014). This is related to the inability of females to oviposit on ripe fruit without prior injuries or the presence of mechanical damage caused by other insect pests (Fartyal *et al* 2014).

Due to their high capacity for adaptation to different hosts in temperate regions (Ramniwas *et al* 2012), several recent studies have reported the occurrence of both *Z. indianus* and *D. suzukii* in grape cultivation in the United States (Van Timmeren & Isaacs 2013, Joshi *et al* 2014), in monitoring traps containing the hydrolysed protein CeraTrap™ in guava culture in Mexico (Lasa & Tadeo 2015) and, directly, in ripe strawberry fruit in southern Brazil (Nava *et al* 2015). Due to the simultaneous occurrence of *D. suzukii* and *Z. indianus* in commercial strawberry crops in southern Brazil (Andreazza *et al* 2015, Nava *et al* 2015), there is a need to i) verify the susceptibility of strawberry fruit at different ripening stages to infestation by *D. suzukii* and *Z. indianus* to support the management of these pests in the field and ii) determine the interactions between damage caused by oviposition of *D. suzukii* and by mechanical means on infestation by *Z. indianus* in strawberry fruit at different ripening stages.

Material and Methods

Insect populations and obtaining fruits

Populations of *D. suzukii* and *Z. indianus* were reared in the laboratory on an artificial diet based on corn flour, yeast and sugar (Emiljanowicz *et al* 2014) and banana and brewer's yeast (Nava *et al* 2007). Strawberry fruits of the cultivar Albion at different ripening stages (G- Green, SR – Semi-ripe and R – Ripe) with stems still to attached to the fruits were obtained from a commercial orchard. The strawberry plants were grown under plastic sheeting constructed as a 'shallow tunnel' without the application of insecticides during cultivation. The color determination was performed by subjective method based on the intensity of color variations perceptible to the human eye, according to the methodology proposed by Oliveira *et al* (2005). In the laboratory, the fruit were analysed for pH (AOAC 2005) and total soluble solids (TSS) using the methodology of Pregnolatto (1985). G fruit showed 100% green coloration, pH 3.1 and TSS 6.75 (average weight per fruit was 12.8 g); SR fruit had up to 30% red coloration, pH 3.6 and TSS 7.54 (average weight per fruit was 14.5 g); and R fruit showed 100% red coloration, pH 3.5 and TSS 7.96 (average weight per fruit was 14.3 g). The three fruit types were used to carry out choice and no-choice bioassays at the Laboratory of Entomology of Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, Brazil, in controlled room conditions of 25 ± 1°C, 60 ± 10% of RH and a 14:10 h (light:dark) photoperiod.

No-choice and choice bioassays

In the first step, we performed in no-choice bioassay. In the laboratory, G, SR and R strawberry fruit were examined under a stereo microscope (40x) to evaluate skin integrity and absence of eggs. Later, the fruit were individually placed in cages composed of transparent plastic cups (300 mL) flipped upside-down on a Petri dish (8 cm diameter) with a 4 m diameter hole cut in the top and sealed with fabric mesh to allow gas exchange and avoiding excess of moisture. Four mated females of *D. suzukii* or *Z. indianus* that were 4-5 days old were released into each cage, and distilled water was supplied to the adults via capillarity in cotton wool in 10 mL glass vials. Twenty-four hours after infestation (HAI), the flies were removed and eggs on the fruit (external surface + internal epidermis) were counted with the aid of a stereo microscope (40x). The fruits were then individually placed on a vermiculite layer (1 cm) inside plastic containers (100 mL) that were sealed at the top with Parafilm™ (Bemis Company, Inc.). The plastic containers were assessed daily to quantify fly emergence. The biological variables evaluated per fruit were number of eggs, number of adults and mean developmental time.

In the second step, we performed in choice bioassay. Strawberry fruits at the different ripening stages described above were examined for the presence of lesions and drosophilid eggs. Later, one G, one SR and one R fruit (ratio 1:1:1) were placed in each cage made of a plastic cup (700 mL, 15 cm in diameter) flipped upside-down on a Petri dish (18 cm diameter) with a 4 cm diameter hole at the top and sealed with 'mesh' fabric to allow gas exchange and avoid excess moisture. Four mated females of *D. suzukii* or *Z. indianus* that were 4-5 days old were placed in each cage and given distilled water. At 24 h after infestation, the flies were removed; from that moment forward, the same methodology as in the no-choice bioassay was followed.

Damage interaction of infestation by D. suzukii and Z. indianus on strawberry fruit

Two bioassays were conducted to determine whether mechanical damage (injuries) to the fruit epidermis facilitated

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infestation by *D. suzukii* or *Z. indianus* and whether damage caused by *D. suzukii* during oviposition facilitated infestation by *Z. indianus*. In the first bioassay, G, SR and R fruits were collected in the field and were damaged mechanically in the laboratory (20 perforations in the epidermis at a depth of 2 mm) with the aid of an entomological pin (0.25 mm). Afterwards, the fruits were submitted to infestation by *Z. indianus* following the same methodologies as previously described.

In the second bioassay, to determine whether injuries caused by *D. suzukii* facilitated infestation by *Z. indianus*, intact G, SR and R fruit were collected in the field over the same period as the first bioassay. In the laboratory, the fruits were offered to mated females of *D. suzukii* (four mated females/cage) that were 4-5 days old and were given distilled water for 24 h following the same procedures as previously described. Afterwards, the fruits were again placed inside the cages, and four mated females of *Z. indianus* that were 4-5 days old were released inside the cages for 24 h. Fruits were then removed, and eggs of *Z. indianus* were counted with the aid of a stereo microscope (40x) following the same methodologies as those described above.

Statistical Analysis

In all experiments (no-choice and choice bioassays and damage interaction) the experimental design was completely randomized with 50 fruits (G, SR or R) with each one considered replication per treatment. For the statistical analysis, all data were submitted to a studentized residual analysis to confirm the assumption of normality (Shapiro-Wilk test) using the PROC UNIVARIATE procedure in SAS® 9.1 (SAS Institute 2002). When data did not exhibit a normal distribution they were arcsine square root (x+0.5) transformed prior to the analysis. Afterwards, data were submitted to the variance analysis and the means were compared by the Tukey test ($P \leq 0.05$) (PROC ANOVA, SAS Institute 2002).

Results

Strawberry fruit susceptibility to infestation by D. suzukii and Z. Indianus

The no-choice bioassays showed no significant differences ($F_{2,\ 147} = 3.69$, $P = 0.07$) in susceptibility of strawberry fruit at different ripening stages to infestation by *D. suzukii* in terms of average number of eggs and adults insects per fruit/female (Fig 1). The choice bioassays showed a greater susceptibility to infestation in strawberry R fruit ($F_{2,\ 147} = 112.69$, $P < 0.0001$) compared with SR and G fruit (Fig 1).

For *Z. indianus*, there was an oviposition preference for R fruit in both the no-choice ($F_{2,\ 147} = 499.25$, $P < 0.0001$) and choice bioassays in relation to SR and G fruit (Fig 2). No eggs or emerged insects were observed for the SR and G fruits (Fig 2). The mean developmental times (egg to adult) were 11.2 ± 0.07 for *D. suzukii* (Table 1) and 14.1 ± 0.11 days for *Z. indianus* (Table 2), with viabilities (egg to adults) greater than 80% for *D. suzukii* and 70% for *Z. indianus*.

Damage interaction of infestation by D. suzukii and Z. indianus on strawberry fruit

There were no significant interactions for mechanical damage to the epidermis of G ($F_{1,\ 98} = 1.18$, $P = 0.30$), SR ($F_{1,\ 98} = 0.27$, $P = 0.07$) or R fruit ($F_{1,\ 98} = 3.42$, $P = 0.07$) and the susceptibility to infestation by *D. suzukii* compared to healthy fruits (Table 1). However, there was a significant ($F_{2,\ 144} = 4.83$, $P = 0.02$) interaction of damage caused by oviposition by *D. suzukii* or mechanical damage to the epidermis of R fruit and infestation by *Z. indianus* compared to healthy R fruit (Table 2). In the G fruit, this interaction was not significant ($F_{2,\ 147} = 1.69$, $P = 0.12$) in regarding to damage of either type compared to healthy G fruit without damage (Table 2). However, in the SR fruit, eggs were observed in the epidermis of fruits damaged mechanically. This result was significantly different ($F_{2,\ 144} = 36.92$, $P < 0.0001$) from those obtained for undamaged SR fruit or fruit damaged by *D. suzukii*, for which no eggs or subsequent adults insects were observed (Table 2). Regarding the period of larval development of *Z. indianus*, there were no significant differences ($F_{2,\ 147} = 4.11$, $P = 0.17$) between larvae that developed on R fruit without damage compared to fruits damaged by *D. suzukii* or by mechanically damaged fruit (Table 2). For all bioassays, we did not observe eggs or adults insects of *D. suzukii* or *Z. indianus* adults in G, SR and R fruit obtained from the field, showing that the fruit were free from the presence of these drosophilids.

Discussion

Oviposition of *D. suzukii* females occurred during the 24 h period in strawberry G, SR and R fruit in the no-choice bioassay, with no significant differences between the different stages of ripeness. In contrast, in the choice bioassay, females of *D. suzukii* showed a greater preference for oviposition on R fruits, corroborating previous studies reporting a higher oviposition rate of *D. suzukii* on ripe fruits of blackberry, blueberry, cherry, raspberry and strawberry (Lee *et al* 2011) and blueberry with lower skin firmness (Kinjo *et al* 2013).

Damage caused by *D. suzukii* is related to the cutting of the fruit skin by the ovipositor of the female and consumption of pulp by the developing larvae (Dreves *et al* 2009, Bolda *et al* 2010, Renkema *et al* 2013). These results and

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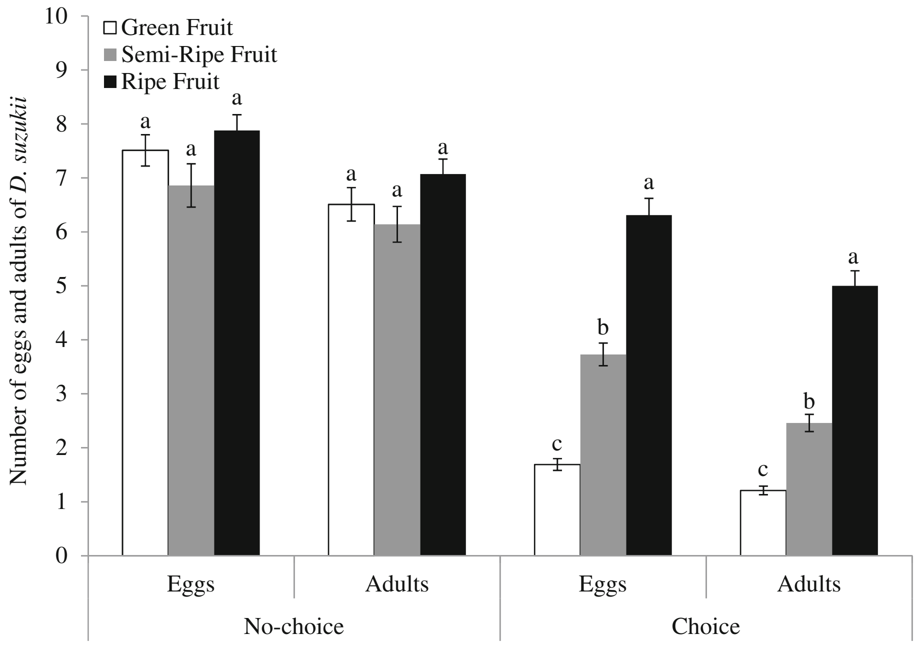

Fig 1 Number of *Drosophila suzukii* eggs and adults emerged per fruit/female (mean ± SE) in strawberry fruit cv. Albion at different ripening stages in choice and no-choice bioassays. Note: Different letters indicate significant differences within each group of bars representing the different combinations of insect developmental stage and bioassay type, according to the Tukey test ($P < 0.05$).

our data confirm that they do not require prior injuries in strawberry to deposit their eggs. However, the piercing of the fruit skin epidermis by mechanical damage or other insects may promote contamination and development of pathogens (Dreves *et al* 2009, Bolda *et al* 2010) and can promote infestation by other opportunistic drosophilid (Faucher *et al* 2013).

In this study, R fruits with previous damage caused by *D. suzukii* adults or artificial mechanical damage facilitated infestation by *Z. indianus*. We observed the presence of *Z. indianus* eggs inside the areas of damage caused by both *D. suzukii* females and mechanical means, showing the opportunistic ability of *Z. indianus* adults to infest damaged fruit. However, healthy R strawberry fruit also serve as a substrate for oviposition of *Z. indianus* because eggs were laid in the strawberry achene region or on the fruit epidermis, and nearly 1.5 days after oviposition, the larvae hatched and penetrated the fruit. This shows the ability of females to oviposit on healthy R strawberry fruit and generate viable off spring, which did not occur in SR and G fruit.

The ability of females to oviposit on healthy R strawberry fruit could be associated with the attraction of *D. suzukii* and

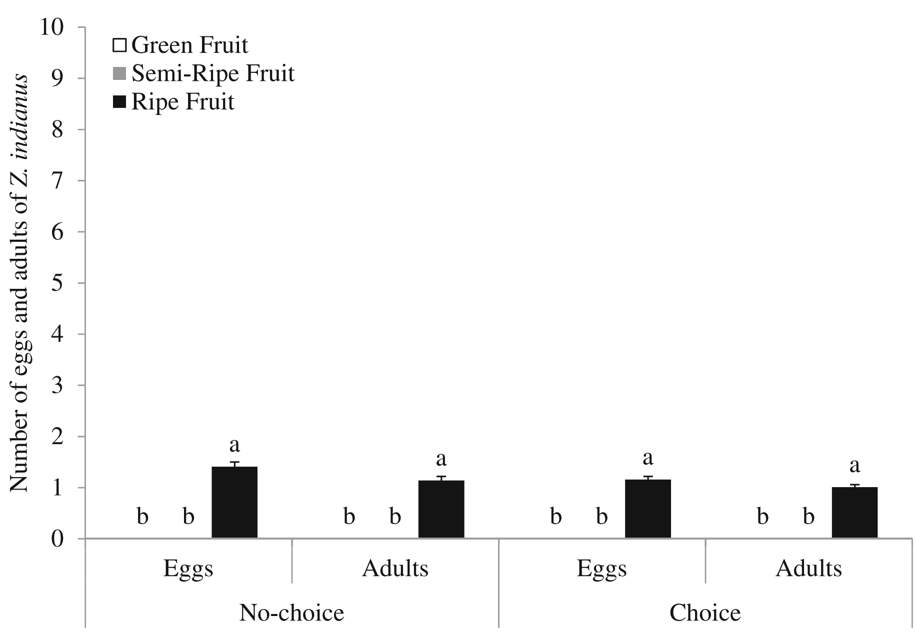

Fig 2 Number of *Zaprionus indianus* eggs and adults emerged per fruit/female (mean ± SE) in strawberry fruit cv. Albion at different ripening stages in choice and no-choice bioassays. Note: Different letters indicate significant differences within each group of bars representing the different combinations of insect developmental stage and bioassay type, according to the Tukey test ($P < 0.05$).

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Table 1 Number of eggs and adults per fruit (mean ± SE) and the mean developmental time (days) of *Drosophila suzukii* in strawberry fruits cv. Albion at different ripening stages (G-Green, SR – Semi-ripe and R – Ripe) that were subjected to the presence or absence of mechanical damage.

Treatments	Number of Eggs[a]	Number of Adults[a]	Mean Developmental Time[a]
Green fruit			
Without damage	7.5 ± 0.29 a	6.5 ± 0.28 a	11.3 ± 0.07 a
With mechanical damage	7.7 ± 0.36 a	6.2 ± 0.33 a	11.3 ± 0.11 a
Semi-ripe fruit			
Without damage	6.6 ± 0.40 a	6.1 ± 0.33 a	11.2 ± 0.06 a
With mechanical damage	7.8 ± 0.34 a	6.3 ± 0.36 a	11.2 ± 0.08 a
Ripe fruit			
Without damage	7.9 ± 0.29 a	7.1 ± 0.28 a	11.3 ± 0.06 a
With mechanical damage	8.9 ± 0.37 a	7.0 ± 0.36 a	11.2 ± 0.11 a

[a] Columns followed by the same letter within the same ripening stage do not differ significantly (LSMEANS with Tukey's adjustment; $P > 0.05$).

Z. indianus adults to odours released by ripe fruit, as observed in *D. suzukii* adults and R fruits of raspberry, blackberry, blueberry, cherry and strawberry (Ramniwas *et al* 2012). In the present study, the higher number of eggs and adults of *Z. indianus* found in artificially damaged fruits or previously injured by *D. suzukii* females can be associated with a greater release of volatiles serving as stimuli for orientation, attraction and oviposition. Several studies have shown that insects use volatiles released by plants or fruits for attraction (Bruce & Pickett 2011, Von Arx *et al* 2011, Revadi *et al* 2015), orientation in the search for new hosts for feeding (Lebreton *et al* 2012, Faucher *et al* 2013) and oviposition (Linz *et al* 2013). Fact that may have occurred in the present study in which *Z. indianus* females showed preference for mature fruits or damaged artificially or female of *D. suzukii*. As well as by the fact that females showed no preference for oviposition on intact SR and G fruit or damaged by *D. suzukii* (Burrack *et al* 2013).

The ability of *Z. indianus* to oviposit and generate offspring in healthy R strawberry fruit and benefit from injuries caused by *D. suzukii* or mechanical injuries in R strawberries may contribute significantly to the increase in the incidence of *Z. indianus* in commercial strawberry fields. Similar observations have been done in grape orchards in the United States (Van Timmeren & Isaacs 2013), in sweet orange (*Citrus sinensis* L.) and guava (*Psidium guajava*) cultivation in India (Fartyal *et al* 2014) and Mexico (Lasa & Tadeo 2015), and cattle guava (*Psidium cattleianum* Sabine), Surinam cherry (*Eugenia uniflora* L.) and guava fruit in southern Brazil (Andreazza *et al* 2015). The results from this study help to better understand the behaviour of *D. suzukii* and *Z. indianus* in strawberry crop, allowing for the adoption and improvement of management strategies targeting both species.

Table 2 Number of eggs and adults per fruit (mean ± SE) and the mean developmental time (days) of *Zaprionus indianus* in strawberry fruit cv. Albion at different ripening stages (G-Green, SR – Semi-ripe and R – Ripe) that were subjected to the presence or absence of different types of damage.

Treatments	Number of Eggs[a]	Number of Adults[a]	Mean Developmental Time[a]
Green fruit			
Without damage	0.0 ± 0.00 a	0.0 ± 0.00 a	-
With damage of *D. suzukii*	0.0 ± 0.00 a	0.0 ± 0.00 a	-
With mechanical damage	0.0 ± 0.00 a	0.0 ± 0.00 a	-
Semi-ripe fruit			
Without damage	0.0 ± 0.00b	0.0 ± 0.00 a	-
With damage of *D. suzukii*	0.0 ± 0.00 b	0.0 ± 0.00 a	-
With mechanical damage	0.2 ± 0.03 a	0.2 ± 0.03 a	15.7 ± 0.09
Ripe fruit			
Without damage	1.4 ± 0.09b	1.1 ± 0.08 b	14.1 ± 0.11 a
With damage of *D. suzukii*	1.8 ± 0.11 a	1.2 ± 0.10 a	15.0 ±0.08 a
With mechanical damage	1.8 ± 0.09 a	1.3 ± 0.07 a	15.6 ± 0.08 a

[a] Columns followed by the same letter within the same ripening stage do not differ significantly (LSMEANS with Tukey's adjustment; $P > 0.05$).

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