Spread of Two Invasive Flies (Diptera: Drosophilidae) Infesting Commercial Fruits in Southeastern Brazil

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Spread of two invasive flies (Diptera: Drosophilidae) infesting commercial fruits in southeastern Brazil

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Drosophila suzukii Matsumura (Diptera: Drosophilidae) is among the most recent invasive insect pests in southern Neotropical countries such as Argentina, Brazil, Chile, and Uruguay (Andreazza et al. 2017). This species is highly polyphagous, and attacks thin-skinned fruits such as blackberry (Rubus spp.), blueberry (Vaccinium spp.), raspberry (Rubus idaeus L.), and strawberry (Fragaria × ananassa Duchesne) (Bel-lamy et al. 2013), as well as many non-crop hosts (Kenis et al. 2016). The larvae hatch from eggs laid under the fruit skin and feed on the fruit, causing it to become unmarketable (Asplen et al. 2015). The first record of D. suzukii in the Neotropics was in 2013 in southern Brazil, and was associated with a 30% loss in strawberry production (Deprá et al. 2014; Santos 2014). Despite the widely reported damage capacity of D. suzukii, the infested area was not placed under quarantine, and insecticides were not registered for its management in Brazil. This likely contributed to its spread from southern Brazil to Rio de Janeiro (Bitner-Mathé et al. 2014) and Minas Gerais States (Andreazza et al. 2016) in southeastern Brazil (Fig. 1). Zaprionus indianus Gupta (Diptera: Drosoophilidae) is another invasive fly pest of fruits in South America (Vilela 1999; Fartyal et al. 2014; Bernardi et al. 2017). It has recently been detected in D. suzukii monitoring traps in a large number of crops in Canada, Mexico, and the United States (Renkema et al. 2013; Joshi et al. 2014; Lasa & Tadeo 2015). Laboratory studies indicated that Z. indianus attacks undamaged strawberries, with increased oviposition associated with previous D. suzukii oviposition (Bernardi et al. 2017).

Drosophila suzukii is currently expanding its range in the Neotropics and threatens economically valuable fruit crops in invaded areas. Espírito Santo State, Brazil, borders areas invaded recently by D. suzukii in southeastern Brazil (Fig. 1). It has a diverse range of climates, which makes possible the cultivation of both subtropical and tropical fruits, including some of the principal D. suzukii hosts and potential Z. indianus hosts, such as blackberry and strawberry. Climate-based prediction models indicate there is potential for D. suzukii to invade this Brazilian state (Benito et al. 2016). Therefore, an increase in knowledge of the distribution and hosts of both species in southeastern Brazil is needed. To determine the spread of D. suzukii in this region, fruit samples were collected in Espírito Santo from blackberry (n = 76), and strawberry (n = 577) fields in the highlands (> 900 masl), papaya (Carica papaya L.) (n = 2) and cattley guava (Psidium cattleianum Afz. ex Sabine) (n = 2) at sea level, and robusta coffee (Coffeea canephora Pierre ex A. Froehner) (n = 1,085) at intermediate altitudes (100–300 m asl). Fruits were randomly collected from a blackberry field (n = 1) (Field 1: 20.3716667°S, 41.0641667°W, 970 m asl), and several strawberry fields (n = 8) ([Field 2: 20.2541667°S, 40.9905556°W, 1,026 m asl]; [Field 3: 20.3052778°S, 41.0294444°W, 912 m asl]; [Field 4: 20.3763889°S, 41.0305556°W, 1,080 m asl]; [Field 5: 20.1386111°S, 41.0025000°W, 1,050 m asl]; [Field 6: 20.1608333°S, 40.9191667°W, 1,036 m asl]; [Field 7: 20.2194444°S, 40.9850000°W, 1,110 m asl]; [Field 8: 20.0819444°S, 40.8907778°W, 851 m asl]; [Field 9: 20.1744444°S, 40.8563889°W, 962 m asl]) in the municipalities of Domingos Martins and Santa Maria de Jetibá from Jul 2016 to Feb 2017 (Fig. 1). To monitor adult emergence, the fruits were placed on a layer of paper towels inside plastic boxes (20 × 30 × 17 cm) with vents covered with fine mesh, at the Laboratory of Entomology, Federal University of Viçosa (UFV) at 25 ± 2 °C, 50 ± 5% RH and 12 h (L:D) photoperiod. Adult flies were collected daily over a 14 d period, and preserved in 96% alcohol for species identification. Additional samples were collected directly from strawberry canopies using an aspirator. Two collections of flies associated with papaya and P. cattleianum fruits that were roting on the ground in a small garden in the municipality of Serra, Espírito Santo, ([Field 10: 20.2038889°S, 40.1972222°W, 3 m asl]; [Field 11: 20.2038889°S, 40.1975000°W, 3 m asl]) were made in Aug 2017 to determine the presence of D. suzukii in a coastal area of the state (Fig. 1). Collections were made directly by quickly placing a large, open, transparent plastic bag over fruit infested with flies and quickly closing the bag as the flies flew upward into the bag, which was then lifted away from the fruit. After being anesthetized inside a freezer, the flies were killed and preserved in 96% alcohol for later identification. Mature berries of robusta coffee were collected in the municipalities of Governador Lindenberg (Field 15: 19.2083333°S, 40.5008333°W, 376 m asl); Linhares (Field 16: 19.3669444°S, 40.4472222°W, 142 m asl); Marilândia (Field 12: 19.4047222°S, 40.5405556°W, 97 m asl); (Field 13: 19.4016667°S, 40.5391667°W, 127 masl); (Field 14: 19.4044444°S, 40.5408333°W, 93 m asl); and Sooretama (Field 17: 19.2194444°S, 40.0819444°S, 61 m asl) (Fig. 1), and maintained in plastic containers as described previously for blackberry and strawberry fruits to collect adult flies that developed in the fruit. Drosophila suzukii and Z. indianus specimens were identified according to Vilela & Mori (2014), and

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Van der Linde (2010), respectively. Specimens of *D. suzukii* collected in papaya and *P. cattleianum* were sent to the California Department of Food and Agriculture for molecular identification.

In the first yr of collecting (i.e., 2016), *Drosophila* was the most common genus collected (n = 485) in strawberry fields, followed by *Z. indianus* (n = 16). However, no *D. suzukii* specimens were collected during 2016. *Drosophila suzukii* was collected directly only in Feb 2017, as adults (28♂, 8♀) from blackberry fruits in the municipality of Domingos Martins, and this is the first report of *D. suzukii* in Espírito Santo. A total of 965 *D. suzukii* adults (54% females) emerged from the blackberry fruits (n = 76) demonstrating a high infestation in this region (Table 1). From these blackberry fruits, an additional 458 *Z. indianus*, and 21 *Drosophila* spp. adults also emerged (Table 1). *Drosophila suzukii* emerged from fruits of 6 of the 8 strawberry fields surveyed in 2017 (Table 1), but it was collected directly as adults only from 1 field. *Zaprionus indianus* and *Drosophila* spp. were present in all strawberry fields sampled (Table 1). The former species was first recorded in Espírito Santo in Sep 2004 associated with ripe papaya used as bait to attract flies (Culik 2004). Other species collected in these soft fruits were not identified. In this study, 5 fly specimens associated with papaya and *P. cattleianum* fruit rotting on the ground were collected and identified as *D. suzukii* (Table 1) based on species-specific taxonomic characters.
including the distinct black spot on the wings, and tarsal combs on the legs (Hauser 2011). Also, the 2 specimens sent to the California Department of Food and Agriculture were confirmed as D. suzukii based on morphological characteristics and CO1 molecular analysis [collection data: Manguinhos, Serra, Espírito Santo, Brazil; 5 Aug 2017; col. MP Culik; ex. Carica papaya fruit, mature or rotting on ground (1 male); Manguinhos, Serra, Espírito Santo, Brazil; 12 Aug 2017; col. MP Culik; ex. C. papaya and P. cattleianum fruit, mature or rotting on ground (1 male)]. Six samples of robusta coffee berries were infested by drosophilid flies, but none with D. suzukii or Z. indianus; Drosophila ananassae Doleschall (3 ♂, 4 ♀) was associated with this coffee species in Governor Lindenberg, and Drosophila melanogaster Meigen (14 ♂, 26 ♀) was found in all coffee fields sampled (Table 1). However, these species associated with robusta coffee have been of low agricultural importance, and are frequently associated with post-harvest fruits and advanced ripening. They are cosmopolitan species of wide geographic distribution found more frequently in domestic environments and rarely in natural habitats (Bock & Parsons 1978; Singh & Yadav 2015).

Results of this study confirm the continued spread of the D. suzukii in the Neotropics. Its current distribution is verified now in the highlands and coastal areas of Espírito Santo with high levels of infestation found in blackberry fruits, a known host of D. suzukii (Bellamy et al. 2013; Burrack et al. 2013). Although strawberry has been reported as a host of D. suzukii in the Neotropics (Bernardi et al. 2017), the low infestation level found in this study may indicate that D. suzukii is in an initial stage of invasion in this crop. Zapriónus indianus has previously been shown to oviposit in undamaged strawberry fruits under laboratory conditions (Bernardi et al. 2017), but has not previously been reported infesting undamaged blackberries. Zapriónus indianus not only was present in high numbers in the present study, but also has been recently noted under field conditions, and in traps from several other regions, including Asia and North America (Fartyal et al. 2014; Joshi et al. 2014; Lasa & Tadeo 2015). Thus, the infestation abilities of Z. indianus in undamaged or D. suzukii-damaged blackberry fruits should be further investigated in laboratory oviposition studies, and in more extensive field surveys. Other D. suzukii fruit hosts, including grapes, loquats, and raspberries were not evaluated in the present study, but they also are cultivated economically in Espírito Santo, and they are hosts of D. suzukii. Tropical fruits such as papaya also are important economically in this Brazilian state, so laboratory susceptibility tests with tropical fruit species and other potential new hosts also should be done. Such research efforts will facilitate the ability to predict and delay further dispersal of D. suzukii and similar invasive pests throughout this and other Neotropical areas.

Findings from this survey also highlight the continued lack of effective mitigation actions to stop D. suzukii spread within Brazil. Even after being demonstrated by Vilela & Mori (2014) that the fresh market of blueberries allowed D. suzukii flies to be transported across several states in Brazil, its spread and damage capacity apparently remain neglected. The pest presence in the region, coupled with the development of appropriate management methods, should be widely reported to soft fruit growers, who can increase the monitoring ef-

<table>
<thead>
<tr>
<th>Fly species/Host Plant</th>
<th>Host Plant</th>
<th>Field code</th>
<th>Sample date</th>
<th>Field code</th>
<th>Fruits evaluated (N)</th>
<th>Specimens identified (N)</th>
<th>Infestation rate (flies/fruit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila suzukii</td>
<td>Coffea canephora</td>
<td>1</td>
<td>Feb/2017</td>
<td>76</td>
<td>445 ♀, 520 ♀</td>
<td>12.70</td>
<td></td>
</tr>
<tr>
<td>Drosophila suzukii</td>
<td>Coffea canephora</td>
<td>2</td>
<td>Feb/2017</td>
<td>74</td>
<td>5 ♀, 10 ♀</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Drosophila suzukii</td>
<td>Coffea canephora</td>
<td>3</td>
<td>Feb/2017</td>
<td>110</td>
<td>4 ♀, 1 ♀</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Drosophila suzukii</td>
<td>Coffea canephora</td>
<td>4</td>
<td>Feb/2017</td>
<td>63</td>
<td>1 ♀, 2 ♀</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Drosophila suzukii</td>
<td>Coffea canephora</td>
<td>5</td>
<td>Feb/2017</td>
<td>61</td>
<td>0 ♀, 2 ♀</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Drosophila suzukii</td>
<td>Coffea canephora</td>
<td>6</td>
<td>Feb/2017</td>
<td>79</td>
<td>2 ♀, 1 ♀</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Drosophila suzukii</td>
<td>Coffea canephora</td>
<td>7</td>
<td>Feb/2017</td>
<td>66</td>
<td>1 ♀, 4 ♀</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Zapriónus indianus</td>
<td>Papaya</td>
<td>10</td>
<td>Aug/2017</td>
<td>1</td>
<td>3 ♀</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>Zapriónus indianus</td>
<td>Papaya and P. cattleianum</td>
<td>11</td>
<td>Aug/2017</td>
<td>NC</td>
<td>2 ♀</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Note: Each row in the table corresponds to a sampled field. *fruits rotting on the ground; *NC = fruits not counted; *this species was not sexed.

Table 1. Field infestation rates of Drosophilidae occurring in diverse host plants in Espírito Santo State, Brazil.
forts through the active daily scouting of the fields, for example, and take early management actions for its control. This improvement in the communication among producers, researchers and extension agents should, at least partially, reduce the spread and the economic losses caused by these invasive species.

**Summary**

Two invasive fruit flies, *Drosophila suzukii* Matsumura and *Zaprionus indianus* Gupta (Diptera: Drosophilidae), recently have become established, and are expanding their range in the Neotropics. This research documents the range expansion of these species in southeastern Brazil, where they were found infesting cultivated blackberries and strawberries in the highlands of Espírito Santo, Brazil. *Drosophila suzukii* also was found associated with rotting *Psidium cattleianum*, and for the first time associated with papaya at sea level. *Drosophila suzukii* and *Z. indianus* may further affect establishment and production of important soft fruit crops and papaya in this region of Brazil. Management methods for fruit production in this Brazilian region need to be improved to avoid damage and further spread of *D. suzukii*.

Key Words: blackberry; *Drosophila suzukii*; invasive pests; papaya; strawberry; *Zaprionus indianus*

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