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PEST MANAGEMENT

Population Dynamics of *Drosophila suzukii* (Diptera: Drosophilidae) in Berry Crops in Southern Brazil

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Abstract

Drosophila suzukii (Matsumura) is an invasive species originating in Southeast Asia and considered a severe pest in berry crops in several countries of the Northern Hemisphere and Europe. In South America, the species was first detected in 2013. The objective of the study was to monitor the seasonal activity of *D. suzukii* in commercial crops of blackberry, strawberry guava, surinam cherry, blueberry, and strawberry during two consecutive harvests and in three properties in the Southern region of Brazil during the 2015/2016 and 2016/2017 harvests, with the aid of traps baited with apple cider vinegar. The highest population peaks were observed during late spring to mid-fall in all areas and plant species studied. It was verified that temperature is the factor that most influenced the seasonal activity of *D. suzukii* in the field, promoting low catches of the species during winter. However, even during periods of low temperatures (winter period), the presence of *D. suzukii* in the crops was verified, demonstrating the species' ability to stay in place from year to year, surviving in alternative hosts such as Eriobotrya japonica, a common species in the region. The information on the time of the highest occurrence of the pest in different hosts presented in this study provides the basis for decision-making in relation to the management of *D. suzukii*, to avoid further economic damage.

Introduction

Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae), commonly referred to as spotted-winged drosophila (SWD), is an exotic species that attacks soft-skinned fruits. It is considered to be the main invasive insect pest in several European countries (Calabria *et al* 2012), North America (Lee *et al* 2011), South America, mainly Brazil (Andreazza *et al* 2017), Uruguay (González *et al* 2015) and Argentina (Santadino *et al* 2015), and with potential for adaptation and establishment in Oceania and Africa (Dos Santos *et al* 2017).

Factors related to the high biotic potential (Tochen *et al* 2014), the short period between generations (Emiljanowicz *et al* 2014), high polyphagia (Asplen *et al* 2015), and the presence of a serrated ovipositor (Lee *et al* 2015) have resulted in estimated losses of millions of dollars per year in crops of strawberries, blueberries, cherries, blackberries, and raspberries in Canada, USA, and Europe (De Ros *et al* 2015).

To reduce economic losses, it is of primary importance to understand the population dynamics of this species in the field (Burrack et al 2015). Monitoring the locations where the insects occur using traps baited with food based on

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fermented products, such as wine, vinegar, and yeast, has been recommended for determining the fluctuation and density of populations of the pest in crops over time and to facilitate the development of management strategies (Frewin *et al* 2017, Tonina *et al* 2017). As well, fruit sampling can be used to detect the presence of larvae or the emergence of *D. suzukii* from the fruits collected (Van Timmeren *et al* 2017). However, these methods may provide resistance by producers in collecting fruits, apparently not damaged by the pest.

However, local environmental conditions may have a direct influence on the bioecological behavior of insects in the population and interfere with the management strategies according to the region (Zerulla *et al* 2017), mainly, *D. suzukii* that presents high dispersion capacity, and can migrate to favorable habitats in adverse conditions (Mitsui *et al* 2010) and does not have host fidelity (Diepenbrock *et al* 2016). Thus, local observations of the *D. suzukii* occurrence pattern are recommended to delineate management strategies.

In Brazil, given that *D. suzukii* is a new species affecting agricultural crops (Santos 2014), its behavior in the field is poorly understood, although there are records of its occurrence in several regions of the country (Andreazza *et al* 2017). Thus the objective of this study was to investigate the population dynamics of *D. suzukii* during the 2015/2016 and 2016/2017 harvests in berry crops in the Southern region of Brazil.

Material and Methods

Samples sites

The population dynamics of *D. suzukii* in the field was evaluated by monitoring populations during the 2015/2016 (harvest 1) and 2016/2017 (harvest 2) harvests in different berry crops (i.e., blackberry, strawberry guava, surinam cherry, blueberry, and strawberry). Each crop was considered a treatment and was distributed in three distinct locations in the Southern region of the Rio Grande do Sul state, Brazil. Area 1 (Cascatinha, "C"), in Pelotas city (31°38′24″S and 52°30′55″W), was cultivated with three host species, blackberry (*Rubus* spp.; 0.2 ha), yellow and red strawberry guava (*Psidium cattleianum* Sabine; 0.3 ha), and surinam cherry (*Eugenia uniflora* L.; 0.5 ha). There was a maximum distance between orchards of approximately 100 m. Area 2 (Rincão da Caneleira, "RC"), in Morro Redondo city (31°32′36″S and 52°34′26″W), was cultivated with two vegetal species, blackberry (0.5 ha) and blueberry (*Vaccinium myrtillus* L.; 1.0 ha), which were approximately 100 m apart. Area 3 (Cerrito Alegre, "CA"), belonging to Pelotas city (31°35′12″S and 52°25′15″W), was cultivated with strawberry (*Fragaria* × *ananassa*; 0.6 ha) in a semi-hydroponic system. In all three areas, the cultivation system adopted throughout the crop cycle was based on the organic production of fruits.

For all sample sites (crops), the adjacent landscape in the three localities was formed by remnants of semi-deciduous seasonal forest, interspersed with fields and shrub forests, in addition to the sporadic occurrence of fruit species, both native (surinam cherry), and exotic [loquat, *Eriobotrya japonica* (Thumb.) Lindley] (Andreazza *et al* 2017) in the region. According to the Köppen system, the region has a Cfa climate, which is a subtropical climate characterized by high humidity (ranging from 75 to 85% in all seasons), precipitation in all months of the year and temperature higher than 22°C during summer (usually January) and above 3°C during winter (usually July) (Kottek 2006).

Monitoring of ***D. suzukii***

To determine the population dynamics of *D. suzukii* in the different areas and in different crop species, traps made from plastic containers (500-mL volume) (Wollmann *et al* 2017) and baited with undiluted apple cider vinegar (pH 4.40; 150 mL per trap), to which was added 1 mL of neutral detergent to break the surface tension of the attractant liquid (Walsh *et al* 2011), were used. Three traps were placed for each plant species cultivated in each study area. These were hung in branches in the inner part of the crop row at 1 m above the soil surface and spaced approximately 10 m from each other. The experimental design was completely randomized with three replicates (traps) and six treatments (host species or cultivars). Each week, the attractant solution in each trap was replaced with a fresh solution, and the captured insects were removed and packed in labeled (date, host, and collection site) plastic bottles (200-mL volume). The bottles were taken to the laboratory for sorting of the contents. Males and females of *D. suzukii* were counted and identified according to specific taxonomic characters with the aid of a binocular stereomicroscope (40×). After separation and identification, all individuals were packed in 10-mL glass tubes containing 70% alcohol.

To examine the possible influence of the presence of fruits in the orchards on the capture of adults in the traps, the periods of fruiting were recorded (horizontal bars in Figs 1, 2, and 3), in which only the presence of ripe or ripening fruits was considered for all areas. In the same way, monthly data on the meteorological variables of mean temperature (in °C), maximum temperature (mean high temperature in °C), minimum temperature (mean low temperature in °C), total precipitation (mm), and relative humidity (mean UR in %) were collected from the Embrapa Clima Temperado Meteorological Station, Pelotas, RS, a maximum of 40-km distance from the sample sites. These data were used to determine the existence of a relationship between the

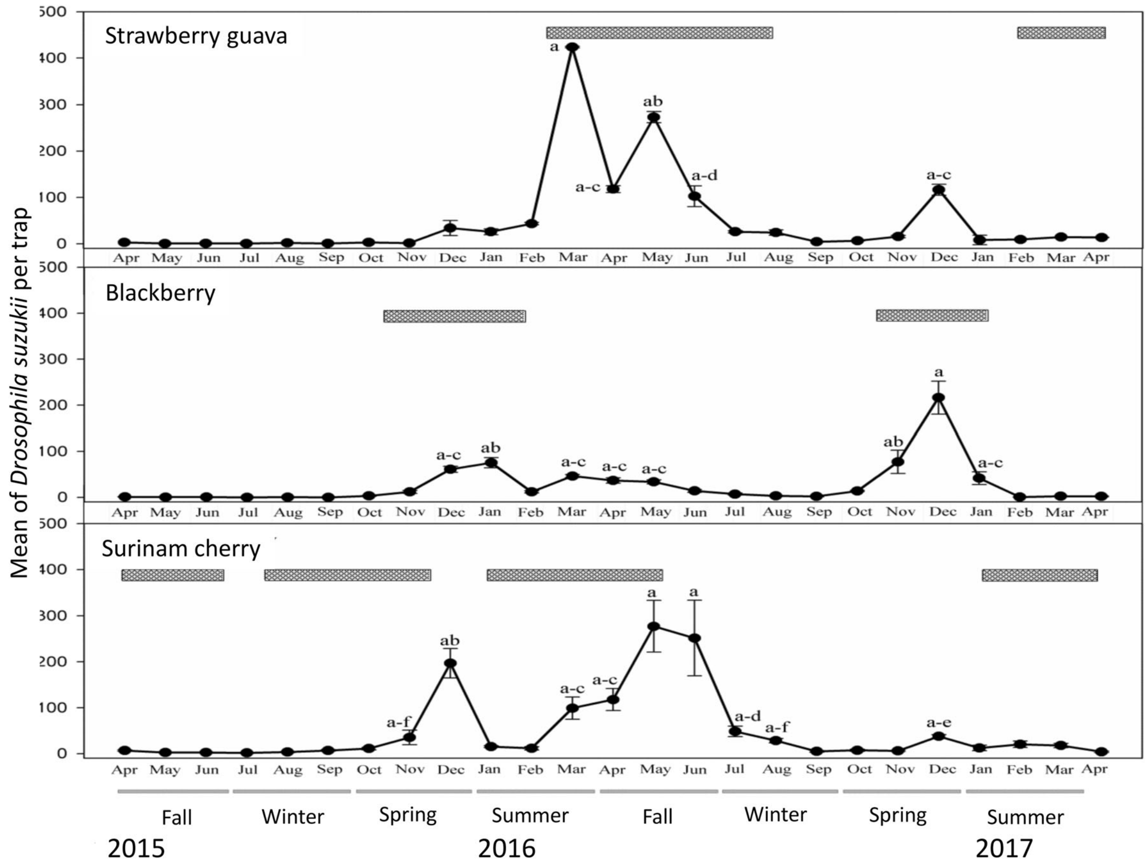

Fig 1 Seasonal catch numbers of *Drosophila suzukii* in blackberry, strawberry guava, and surinam cherry crops during the period 2015 to 2017, Cascatinha, RS, Brazil. Upper gray bars indicate the beginning of the fruiting period in the monitored crops. The lines followed by the same letter within each cultivar did not differ by the Tukey test ($P < 0.05$).

dependent variable (SWD catch) and the independent variables (meteorological elements) according to each season of the year (fall, winter, spring, and summer).

Statistical analyses

The total number of flies captured per month was analyzed separately for each fruit species, comparing the months (fixed effect) with the respective fly catches (variable effect). SWD capture data were transformed into log complement to meet the normality assumptions, using the Shapiro-Wilk test, and homogeneity, using the Barlett test. Subsequently, the mean values were submitted to analysis of variance (ANOVA) using the F-test ($P \leq 0.05$) and, when statistically significant, the mean values were compared by the Tukey test ($P \leq 0.05$). Due to the dissimilarities relating to the crops studied (combined and semi-hydroponic cultivation, fruit species) and the local characteristics, no comparisons were made between the areas. To investigate the effects of climatic independent variables (mean, maximum and minimum temperature, relative humidity, and precipitation) on the *D. suzukii* catches (dependent variable) in all sites studied, the monthly average values of each meteorological element were calculated to obtain the average for each quarter within each period studied (seasons). With quarterly averages for each of the periods, fall (≈ 21 March to 21 June), winter (≈ 21 June to 23 September), spring (≈ 23 September to 22 December), and summer (≈ 22 December to 21 March), multiple regressions were performed by means of a stepwise selection procedure, observing the coefficient of determination (R^2) with significance lower than 5% ($P < 0.05$). For all analyses performed, RBio version 2017 statistical software was used (Bhering 2017).

Results

A total of 13,935 *D. suzukii* adults (males and females) were captured. There were 9488 (68.1%) in Area 1 (Cascatinha) in blackberry, strawberry guava, and surinam cherry, 2648 (19.0%) in Area 2 (Rincão da Caneleira) in blackberry and blueberry crops, and 1799 (12.9%) in Area 3 (Cerrito Alegre) in cultivated strawberry crops. Throughout the period of evaluation, it was verified that there was a significant variation in the number of *D. suzukii* adults captured monthly in Area 1 [(blackberry: $F = 25.72$; $df = 24, 50$; $P < 0.001$;

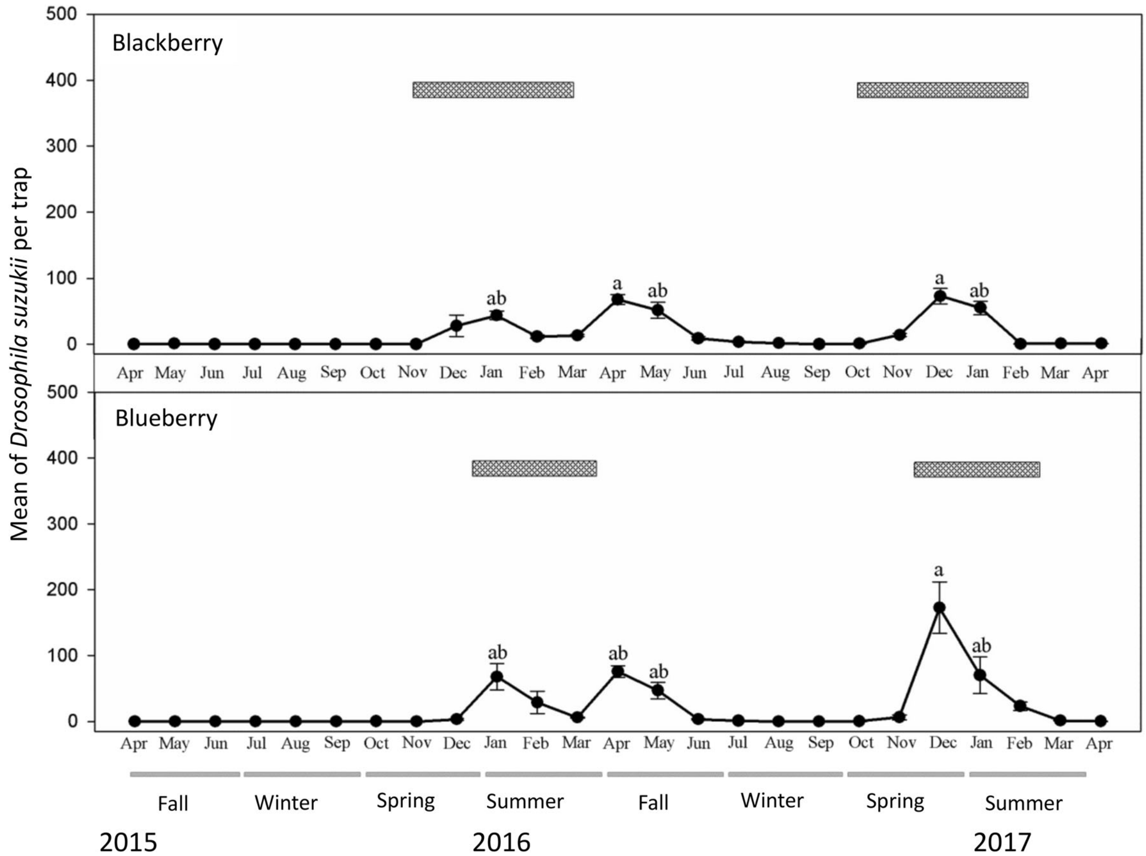

Fig 2 Seasonal catch numbers of *Drosophila suzukii* in blackberry and blueberry crops during the period 2015 to 2017, Rincão da Caneleira, RS, Brazil. Upper gray bars indicate the beginning of the fruiting period in the monitored crops. The lines followed by the same letter within each cultivar did not differ by the Tukey test ($P < 0.05$).

strawberry guava: $F = 26.54$; $df = 24, 50$; $P < 0.001$; and surinam cherry: $F = 10.42$; $df = 24, 50$; $P < 0.001$)] (Fig 1), Area 2 [(blackberry: $F = 30.95$; $df = 24, 50$; $P < 0.001$ and blueberry: $F = 26.25$; $df = 24, 50$; $P < 0.001$)] (Fig 2), and Area 3 [(strawberry: $F = 28.23$; $df = 24, 50$; $P < 0.001$)] (Fig 3). However, the peak population of *D. suzukii* adults captured was similar in all areas evaluated according to the fruiting period of monitored plant species and season (Figs 1, 2, and

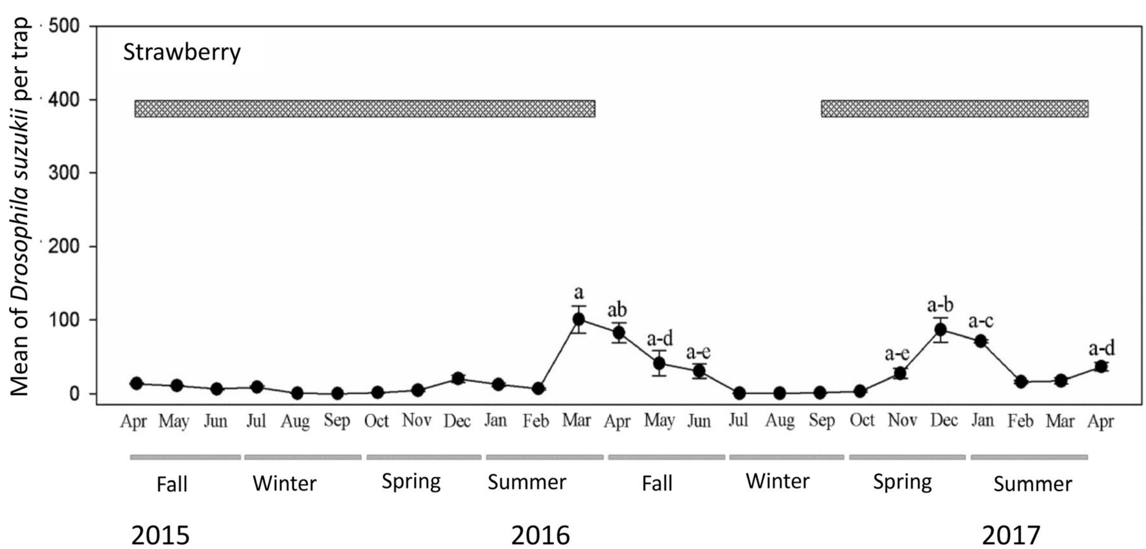

Fig 3 Seasonal catch numbers of *Drosophila suzukii* in strawberry crops during the period 2015 to 2017, Cerrito Alegre, RS, Brazil. Upper gray bars indicate the beginning of the fruiting period in the monitored crops. The lines followed by the same letter within each cultivar did not differ by the Tukey test ($P < 0.05$).

3). The highest peaks were observed between late spring to mid-fall, a period associated with the fruiting of plant species. However, in the other study areas and plant species during this same period, low numbers of insects were recorded in *D. suzukii* catches (Figs 1, 2, and 3).

Using the multiple regression model of the densities of *D. suzukii* as a function of the climatic variables of temperature (minimum, medium, and maximum), relative humidity, and precipitation, a significant effect on the number of insects captured during winter (2016), spring (2015/16), and summer (2016/17) was observed (Table 1). Catches were inversely related to the minimum temperature in winter (14%), while this variable had a positive effect in the spring (29% and 56%) of both years. In the summer of the first year of monitoring, catches of *D. suzukii* adults were inversely related to the minimum temperature (17%). In the other evaluated periods, fall (2015) and winter of the first year (2016), analysis of the abiotic factors evaluated did not show an influence on the abundance of *D. suzukii* caught in the traps.

Discussion

In this study, the activity of *D. suzukii* populations in the Southern region of the Rio Grande do Sul was recorded and showed great variation in the occurrence of the *D. suzukii* over time in all evaluated areas and plant species. The fact that there were few catches during the 2015/16 harvest, with a population peak occurring only in strawberry crops (CA), may be associated with the adaptation phase of the *D. suzukii* population to the new environment, with the first record in the culture in the 2013/2014 harvest (Deprá *et al* 2014). Acclimatization of the species is a long-term response and, in the field, can be induced by seasonal changes (temperature or photoperiod) that act directly on the population behavior of the pest and on the adaptation process of the species on site (Teets & Denlinger 2013).

The pattern of occurrence and population increases of *D. suzukii* demonstrated during 2 years of evaluations is similar to that observed for fly populations in regions producing berries of the USA during the fall and spring (Harris *et al* 2014, Wang *et al* 2016). Through the application of multiple regression models, it was verified that temperature was the factor that had the most significant influence on population behavior of *D. suzukii* in the field over time (Table 1). By affecting reproduction and survival, fluctuating temperatures may alter the pest population levels in the field (Tochen *et al* 2014). The increase in temperature during the spring favored the activity of *D. suzukii*. This period also culminated with the beginning of the fruiting in most host plants evaluated, where the presence of the fly in the orchards increases.

In the following summer, rising average temperatures (average variation from 27 to 30°C) favored an increase in the *D. suzukii* population, being in the ideal range (temperature between 25 and 28°C) for the biological development (eggs to adults) of the species (Tochen et al 2014, Ryan et al 2016). The highest population peaks recorded in some crops occurred during this period; however, a significant decrease in seasonal activity was observed at temperatures close to 30°C, which is considered the upper temperature limit for the development of *D. suzukii*, being that in this temperature range, the oviposition by adults is dramatically impacted (Wiman *et al* 2014).

Insects subjected to this thermal condition have been reported to present a reduction in survival, reproductive rate, and pre-imaginal development (Zerulla *et al* 2017), which may have led to a lower population density during the warmer period. Other local factors may contribute to variations in *D. suzukii* populations, such as the availability of hosts and the existence of alternative hosts nearby, as well as interactions between biotic factors such as symbionts and parasitoids (Lee *et al* 2015, Hamby *et al* 2016).

Low catches of *D. suzukii* were recorded in the winter during the second year of monitoring, when the mean

Table 1 Multiple regression of densities of *Drosophila suzukii* adults as a function of the meteorological elements of temperature, relative humidity, and precipitation in the different seasons of the year in all monitored areas during the 2015/2016 and 2016/2017 harvests.

Season	Year	R^2	Descriptive equation	Statistical parameters
Winter	2016	0.1447	$y = 2.72102 - 0.17783_x$	$F_{1,\ 35} = 5.92$ $P = 0.0202$
Spring	2015	0.2964	$y = -1.105878 + 0.13941_x$	$F_{1,\ 29} = 12.22$ $P = 0.0015$
	2016	0.5645	$y = -0.83349 + 0.15172_x$	$F_{1,\ 40} = 51.85$ $P = < 0.0001$
Summer	2016	0.1732	$y = 5.\ 30{,}844 - 0.20580_x$	$F_{1,\ 52} = 10.90$ $P = 0.0017$
	2017	0.4135	$y = 1.58216 + 0.48311_{x1} - 0.14829_{x2} - 0.01150_{x3}$	$F_{1,\ 35} = 5.92$ $P = 0.0202$

Minimum temperature (*x*); maximum temperature (*x*1), relative humidity (*x*2), and precipitation (*x*3). $P < 0.05$

No statistical significance for the fall of 2015, 2016, and 2017, and winter of 2015 thermal variation was between 9 and 10.7°C. Although occurring in low densities, the number of *D. suzukii* adult males and females captured during this period demonstrates the ability of insects to remain active in the environment during cold periods. Although, no insects were captured in the northeastern region of Rio Grande do Sul, where the cold season is more pronounced during this period (Klesener *et al* 2018).

The survival capacity of *D. suzukii* at low temperatures has been reported in numerous studies, which is observed in an adaptation phenotype, called the "winter morph" (Hamby *et al* 2016), demonstrating the seasonal phenotypic plasticity that *D. suzukii* developed through the adaptive process of surviving during the unfavorable periods of biological development (Stephens *et al* 2015). Although the cold season in the South of Brazil is less significant in relation to several regions of North America and Europe, we observed the occurrence of this phenotype in insects captured during the winter period until the beginning of spring, with insects that were larger and more sclerotized and pigmented compared to insects captured during the summer period, similar to that observed in *D. suzukii* populations collected in North America (Hamby *et al* 2016). Another factor that may have contributed to keeping *D. suzukii* in the field during low temperatures is the predominance of alternative hosts, such as loquat (*E. japonica*), on the edges of the orchard or in the interior of the native forest (Andreazza *et al* 2017). This plant species has a fruiting period during the winter and may serve as a refuge for SWD, providing food to enable their survival during critical periods and until the re-establishment of the main culture in the field (Lee *et al* 2015).

Through this study, we have demonstrated the behavioral plasticity of *D. suzukii* in one of the main crops producing berries in the temperate climate of Brazil. In view of the climatic characteristics of the region, as well as the availability of hosts, the permanence of the species in agroecosystems is favored throughout the year, presenting a greater risk of damage during fruiting of the crop of interest. Investigation of the patterns of occurrence of SWD in new areas invaded by *D. suzukii* broadens understanding of the insect and enables improvement of appropriate forms of management, with both crops susceptible hosts already reported in the literature (strawberry, blackberry, and blueberry), such as native host (strawberry guava and surinam cherry), which serve as alternative sources for survival in the off-season periods.

Since it is a new pest in Brazil and some other countries in South America, understanding the seasonal behavior of *D. suzukii* in our agricultural crops will provide information which can be used to improve new management strategies for farmers, such as mass capture with monitoring traps, removal of alternative hosts, cleaning of orchards to avoid initial outbreaks of the insect during the off-season (Lee *et al* 2016), and improvements in the use of toxic baits (Schlesener *et al* 2017). These alternative management practices will facilitate the permanence of natural enemies, such as the parasitoids *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault) (Hymenoptera: Figitidae) and *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae), which are recurrent insects in all study areas (Wollmann *et al* 2016). This fact, together with the results from this study on peaks of occurrence in the different host crops, propitiates the design of appropriate management strategies to mitigate the damage caused by the pest in a region made up of many small family farms with significant savings invested in fruit production.

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