



# The development of drosophilid species (*Diptera*, *Drosophilidae*) in different strawberry cultivars

I. Pajač Živković<sup>1</sup>, B. Duralija<sup>2</sup>, B. Barić<sup>1</sup>, G. Seljak<sup>1</sup>, D. Lemic<sup>3</sup> and A. Mešić<sup>1</sup>

<sup>1</sup> Department for Agricultural Zoology, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia

<sup>2</sup> Department of Pomology, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia

<sup>3</sup> Agriculture and Forestry Institute Nova Gorica, Nova Gorica, Slovenia

## Summary

**Introduction** – Drosophilids were not considered to be agricultural pests as they usually developed in overripe and fermented fruits. With the arrival of the invasive species *Drosophila suzukii* (Matsamura, 1931) in Europe the pest status of drosophilids changed as the species are able to develop in healthy, unwounded and soft-skinned fruits of commercial value. The first economic damages from *D. suzukii* in Croatia were observed in the commercial production of fresh market strawberry in 2014. The aim of this study was to determine species of drosophilids that can develop in strawberry fruits and to explore their preference between different cultivars in production. **Materials and methods** – The samples of fruits of three strawberry cultivars ('Albion', 'Portola' and 'San Andreas') were collected in 2016 from a greenhouse farm near Zagreb (45°41'24"N, 16°24'0"E). From each cultivar 50 samples of fully ripened fruit were randomly selected and placed into the chamber for insect development at a specific temperature and humidity regime. **Results and discussion** – After development of flies, three drosophilid species: *Drosophila immigrans* Sturtevant, 1921; *D. simulans* Sturtevant, 1919 and *D. suzukii* were identified. An analysis of variance showed statistically more drosophilids in the 'Albion' cultivar compared to the other two cultivars examined. **Conclusion** – Species *D. immigrans* and *D. simulans* can also develop in strawberries and cause economic damage to strawberry production if the fruits were primarily damaged by *D. suzukii*. Finally, drosophilids prefer the 'Albion' cultivar for breeding.

## Keywords

*Drosophila suzukii*, *Drosophila immigrans*, *Drosophila simulans*, *Fragaria × ananassa*, production, damages

## Significance of this study

*What is already known on this subject?*

- Drosophilids were not considered to be agricultural pests as they develop in overripe and fermented fruits until the arrival of invasive species *Drosophila suzukii* into Europe.

*What are the new findings?*

- Apart from *D. suzukii*, other species (*D. immigrans* and *D. simulans*) can also breed in strawberries and cause serious damage to strawberry production.

*What is the expected impact on horticulture?*

- While strawberry is a very important culture in horticultural production, *D. suzukii* has high impact on fruit quality. Deep understanding of its breeding habits which depend on strawberry cultivars can improve their control.

on dumped fruits, including the discarded remains of chored grapes (Alford, 2016).

The genus *Drosophila* contains more than half of the known species and most of these are found in the tropics (Markow and O'Grady, 2006). Roughly, 80 species of fruit flies found throughout the world are actual or potential pests of agriculture (Carey and Dowell, 1989). Two aspects of the fruit fly problem are fairly predictable: (1) future fruit fly introductions are inevitable because of the worldwide distribution and abundance of large numbers of economically important species; and (2) the number of introductions is likely to rise as the number of travellers crossing borders also increases and as commodity imports and exports increase (Carey and Dowell, 1989; Gibert et al., 2016).

The drosophilid flies were not considered to be important pests in European fruit production until the arrival of the invasive species *Drosophila suzukii* (Matsamura, 1931) on the European continent in 2008 in Spain and Italy (Calabria et al., 2012; Cini et al., 2012). It has been found in different localities expanding an altitudinal range from 27 to 1,550 m above sea level. Furthermore, by comparing collections of drosophilids from different European populations distributed along a latitudinal cline it is evident that *D. suzukii* has a high dispersal ability since it spreads approximately 1,400 km in 1 year, either actively or passively through infested fruits (Calabria et al., 2012). Unlike most other *Drosophilidae*, which develop in overripe and fermented fruits, *D. suzukii* lays eggs by inserting the serrated ovipositor into the skin of healthy, unwounded and ripening fruits causing severe economic losses in production (Cini et al., 2012, 2014). Secondary infection

## Introduction

Worldwide, the family *Drosophilidae* has over 4,300 described species (Bächli, 2016) and about 120 species are known in Europe (Kekić et al., 2005). This Family includes small to moderately large flies (1.5–7 mm long), attracted to fermenting juices (Bächli et al., 2004). Small fruit flies (*Drosophila* spp.) imply several species occurring globally in association with overripe or bruised apples, peaches, plums, raspberries, strawberries, grapes and other fruits (Lee et al., 2011; Alford, 2016). Adults of several native species are known as "vinegar flies", and these are frequently abundant

by pathogens via the oviposition wound can further worsen damage (Goodhue et al., 2011; Walsh et al., 2011).

This species is able to develop on a wide range of both cultivated and wild soft-skinned fruits with many host plants in the native and in the invaded areas, with berries being the preferred hosts (Kanzawa, 1939, cit. Cini et al., 2012). The first damages in the commercial production of small fruits were observed in Italy, Trento Province (Grassi, 2009) and today this very productive soft fruit region in Europe is faced with economic losses of several million euros per year (Cini et al., 2012, cit. Ioriatti et al., 2011). It is well known that this pest is spreading rapidly and all of continental part of Europe as well as USA (West and East coast), Canada and Mexico are under treat of its invasion (Cini et al., 2012).

According to FAOSTAT, in 2016 the world's strawberry production was around nine million tonnes, of which more than one million tonnes was produced in the European Union (EU); the main producers are Spain, Poland, Germany, Italy and the United Kingdom. One of the limiting factors for the development of *D. suzukii* is temperature and according to some investigations in the Mediterranean area the peak population can be expected at the end of summer in harvest of neutral day strawberries (fruits harvest in whole vegetation season) (Wiman et al., 2014). Strawberries, mostly for the fresh market, are commercially grown in Croatia on approximately 367 ha with total production of 3,383 tonnes (Statistical Yearbook of the Republic of Croatia, 2016). Neutral day strawberries are becoming popular fruit out of season production in Croatia and significant differences in properties of the fruit such as firmness, colour, total soluble solid etc. between main cultivars have been recently recorded (Šamec et al., 2016).

Economic damages on strawberries have already been recorded in USA (California, Florida), Italy, France, Spain, Brazil (Hauser et al., 2009; Hauser, 2011; Deprá et al., 2012; Calabria et al., 2012; Santos, 2014; Andreazza et al., 2016). Heavy damages and economic losses have been reported in strawberry production in the state of Rio Grande do Sul, Brazil (Santos, 2014), and again in Minas Gerais State, Brazil (Andreazza et al., 2016). A second outbreak in Brazil was observed in only two years and about 1,500 km distant from its first record which confirms the extreme invasiveness of this drosophilid species (Andreazza et al., 2016). In Europe in 2010, losses of up to 80% occurred in strawberry crops in the Alpes Maritimes region of southern France (Reynaud, 2010, cit. Lee et al., 2011) on the 'Fukuba' cultivar (Kanzawa, 1939, cit. EPPO, 2010). Most strawberry production in this area is under integrated pest management. In strawberry production growers only apply plant protection products when they detect the pest and not as a preventive pest management programme from the outset (Lee et al., 2011).

The cultivars that do not support the propagation of *D. suzukii* have not yet been identified in the world, but their development in the future could be an effective alternative to chemical control treatments (Gong et al., 2016).

In Croatia, *D. suzukii* was first recorded in 2010 on raspberry (*Rubus idaeus* L. 1753), peach (*Prunus persica* (L.) Batsch 1801) and grapes (*Vitis vinifera* L.) and since then the pest has spread on new cultivated and wild host plants (Mas-ten Milek et al., 2011; Bjeliš et al., 2015).

In 2016 the first economic damage to the production of fresh market strawberry (*Fragaria* × *ananassa* Duchesne) were observed (Mešić et al., 2017). An early detection of this invasive pest is required for sound management of strawberry production. Till nowadays, detailed research of drosophilid species have not been conducted in Croatia. Detailed knowledge of harmful drosophilid flies development, especially *D. suzukii*, on different strawberry cultivars is crucial for risk assessment and effective preventive pest management programs to succeed. The aim of this study was to determine species of drosophilids which can develop in strawberry fruit and to explore their preference between different cultivars in production.

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## Materials and methods

The trial was conducted at the greenhouse cultivation strawberry farm (45°41'24"N, 16°24'0"E) situated in the north-western part of Croatia (Zelina Breška).

The greenhouse (102.5 m length, 64 m width, and 5 m height) film used was a double polyethylene plastic film with ultraviolet (UV) protection; thickness 200 µm.

'Albion', 'Portola' and 'San Andreas' day-neutral strawberries as the most grown cultivars in Croatia, were planted as frigo plants in plastic bags (0.10 × 0.20 × 1.00 m) filled with coconut coir in 2015 and grown in 2016. Plant density was 15 plants per square meter and a drip irrigation system with spaghetti drip tubes was used. The standard nutrient solution for strawberries had an EC of 1.4–1.6 dS m<sup>-1</sup> and a pH from 5.4 to 5.6. One hive of bumblebees (*Bombus terrestris*) was used for pollination (Koppert B.V., The Netherlands).

During the harvest season of 2016 fruit sampling from three day neutral strawberry cultivars ('Albion', 'Portola' and 'San Andreas') was made. From each cultivar 50 samples (five replications represented with 10 fruits of each cultivar) of fully ripened fruit were randomly selected for possible fly damage from both the field edges and its centre equally. Sampled fruit were taken to the Entomological Laboratory of the Faculty of Agriculture and placed into a chamber for insect development (air temperature of 24°C and relative humidity of 60%), following a standardized procedure (Dean et al., 2013). Each fruit was separated into a 200-mL plastic cup containing a 2-cm layer of coarse Vermiculite®. Cups were sealed inside clear 1-L zip-lock sealed bags perforated with about 50 pin holes to reduce condensation. The containers were set up on October 6<sup>th</sup> into the chamber and checked while all of the flies developed (until October 21<sup>st</sup>). Emergent flies were counted and preserved in 70% EtOH for identification. The identification of the collected drosophilids was based on the standard identification key (Bächli et al., 2004) and OEPP/EPPO diagnostic recommendations (EPPO, 2013).

Data on the reared drosophilid flies were compared among different strawberry cultivars using analysis of variance (MATLAB, 2016); a post-hoc (Tukey's honestly significant test) test was used to further investigate any significant difference found.

## Results and discussion

In the greenhouse cultivation of strawberry, a total of 849 individuals of drosophilid flies were reared from 150 strawberry fruits.

After determination, three drosophilid species: *D. immigrans*, *D. simulans* and *D. suzukii* were specified.

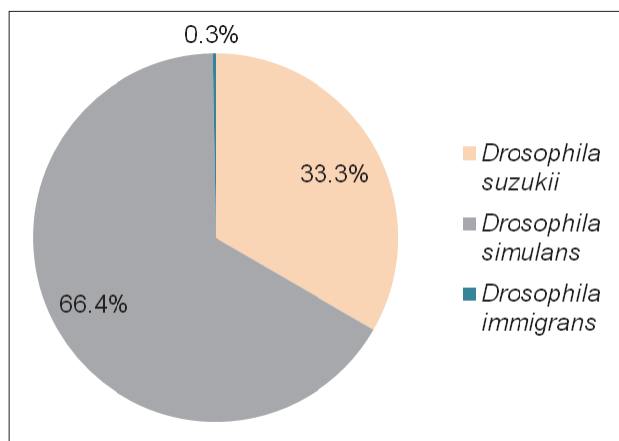
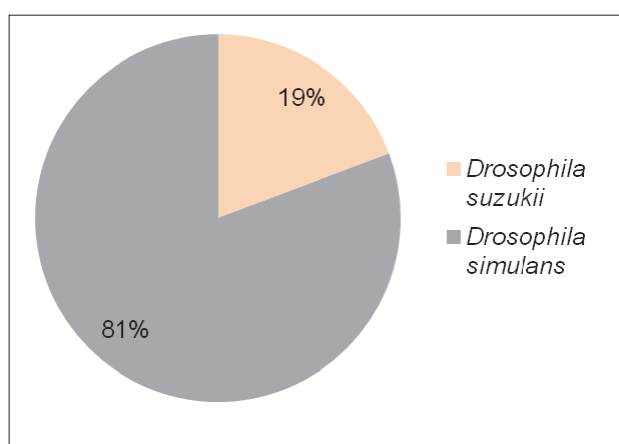
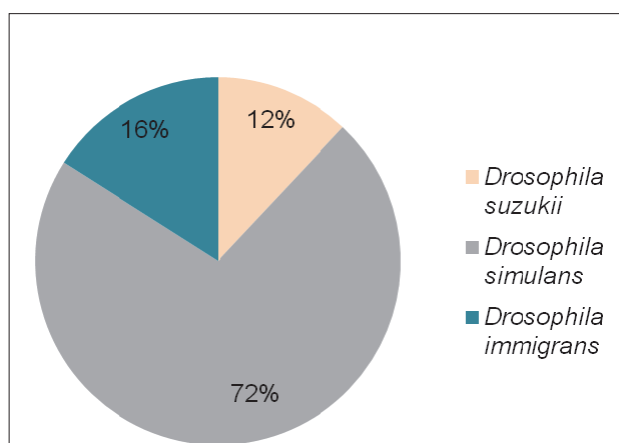
On the 'Albion' and 'San Andreas' cultivars all three drosophilid species developed, while on the 'Portola' cultivar only two species (*D. simulans* and *D. suzukii*) developed.

The majority of drosophilids, with the proportion of more than 80% of the total number, were reared on 'Albion'. On 'Portola' more than 15% of total drosophilids were reared, while less than 2% of flies were reared on 'San Andreas'. The highest number of flies on a single fruit were detected on 'Al-

**TABLE 1.** Average number of drosophilids per fruit sorted by strawberry cultivars.

Cultivar	Average no. of drosophilids/fruit
'Albion'	13.68 <sup>a</sup>
'San Andreas'	0.5 <sup>b</sup>
'Portola'	2.8 <sup>b</sup>

Values followed by the same lowercase letter are not significantly different [ $P > 0.05$ ; Tukey's honestly significant difference (HSD)].

**FIGURE 1.** The abundance of drosophilid species on 'Albion'.**FIGURE 2.** The abundance of drosophilid species on 'Portola'.**FIGURE 3.** The abundance of drosophilid species on 'San Andreas'.

bion' and amounted to 112 drosophilid individuals on a single strawberry fruit, while 24 individuals on 'Portola' and 11 individuals on 'San Andreas' were the highest records.

All of these species belong to the group of synanthropic species and were expected to be found in a semidomestic type of habitat as greenhouse (Kekić, 1985).

The development of two drosophilid species on strawberries, *D. suzukii* and *D. melanogaster*, was reported in 2013 on Florida (Dean et al., 2013). Although *D. melanogaster* was not found in this study, we recorded its sibling species, *D. simulans*, which appears regularly in semidomestic habitats (Kekić, 2002).

Various studies have shown that these two species, although morphologically and genetically very similar, differ in nutritional, reproductive and life-history habits and that the abiotic-biotic characteristics of the observed habitat affect their population (Kekić, 2002).

The ANOVA showed significant variation in total number of drosophilids among different strawberry cultivars ( $F = 22.49$ ). The Tukey post-hoc test showed that there was significant variation at  $P < 0.05$  between 'Albion' and the 'San Andreas' and 'Portola' cultivars which indicates that drosophilid species prefer cultivar 'Albion' for development. The total number of drosophilids on 'San Andreas' and 'Portola' was not significantly different (Table 1).

The most abundant species on all strawberry cultivars (>65% of the total number) was *D. simulans* (Figures 1–3). The species *D. suzukii* represented more than 30% of the total number on 'Albion', almost 20% of 'Portola' and more than 10% of captures on 'San Andreas' (Figures 1–3). The species *D. immigrans* was least represented and the largest number of individuals was recorded on 'San Andreas' (Figure 3). Although two of the three detected species (*D. suzukii* and *D. immigrans*) were characterized as alien to Europe (Skuhravá et al., 2010) the dominance of cosmopolitan and native species, *D. simulans*, indicates that invasive species have not suppressed indigenous species in its habitat. The similar results were not reported in Europe but were reported in 2013 on Florida in which the dominance of indigenous species (*D. melanogaster*) over the invasive one (*D. suzukii*) was confirmed (Dean et al., 2013).

Considering the fact that among determined species only polyphagous species *D. suzukii* is able to lay eggs in healthy, ripening fruit, it is assumed that the other two species (*D. simulans* and *D. immigrans*) have exploited the primary damage caused by *D. suzukii* to lay eggs in strawberry fruits. Finally, they even over-numbered *D. suzukii* population causing economic damage to strawberry production. This study showed that other species from this family are able to breed in healthy, ripening strawberry fruit if the primary fruit attack was made by *D. suzukii*, which can result in additional economic losses to fruit production.

The sex ratio in the majority of drosophilid species seems to be female-biased (>50%) in almost all strawberry cultivars (Table 2). The only exception was the representation of *D. suzukii* on 'Portola' where the sex ratio seems to be male-biased (>50%). The species *D. immigrans* was represented in very low numbers (<10 specimens) on 'Albion' and 'San Andreas' where the sex ratio seems to be equally divided (Table 2).

In general, more female drosophilids (480; 57%) in comparison with males (368; 43%) were representative of the strawberry fly population. The sex ratio in insect populations should be equal (Kovačević, 1959). If males dominate the population, further expansion of the population is threat-



**TABLE 2.** Sex ratio of drosophilids between strawberry cultivars.

Species	Cultivar					
	'Albion' (%)		'San Andreas' (%)		'Portola' (%)	
	♂	♀	♂	♀	♂	♀
<i>Drosophila suzukii</i>	41.67	58.33	33.33	66.67	55.56	44.44
<i>Drosophila simulans</i>	45.81	54.19	33.33	66.67	36.28	63.72
<i>Drosophila immigrans</i>	50.00	50.00	50.00	50.00	-	-

ened, but if females dominate, a population increase can be expected. From a biological standpoint, insect populations where females prevail, have a better biological potential. In future studies, population dynamics should be determined, as well as the sex ratio during the whole growing period of strawberries. By controlling the number of females (SIT methods; suggested by Lanouette et al., 2017), it is possible without using pesticides, to directly influence the new generation pest number, i.e., the height of the future population in a strawberry production.

## Conclusions

The presence of polyphagous, invasive species *D. suzukii* in European habitats opened the space for other drosophilid flies to become economic pests in strawberry fruit production. Among the strawberry cultivars investigated, significant variation in the development of drosophilids was found which suggests that flies prefer cultivar 'Albion' for breeding.

Although drosophilid flies in general were not considered to be economic pests in fruit production (except *D. suzukii*) since they cannot damage ripening fruits, results of this experiment showed that other species (*D. immigrans* and *D. simulans*) can also breed in strawberries and make serious damage to strawberry production, if the fruits are primarily damaged by *D. suzukii*.

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Addresses of authors:

Ivana Pajač Živković<sup>1</sup>, Boris Duralija<sup>2,\*</sup>, Božena Barić<sup>1</sup>, Darija Lemic<sup>1</sup>, Gabrijel Seljak<sup>3</sup> and Aleksandar Mesić<sup>1</sup>

<sup>1</sup> Department for Agricultural Zoology, Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, HR-10 000 Zagreb, Croatia

<sup>2</sup> Department of Pomology, Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, HR-10 000 Zagreb, Croatia

<sup>3</sup> Agriculture and Forestry Institute Nova Gorica, Pri hrastu 18, SI-5 000 Nova Gorica, Slovenia

\* Corresponding author; E-mail: bduralija@agr.hr

Tel.: +385 1 2393726; Fax: +385 1 2393630