Population dynamics of spotted wing drosophila 
(\textit{Drosophila suzukii}) in orchards in the Zagreb area

Dinamika populacije octene mušice ploda (\textit{Drosophila suzukii}) u voćnjacima na području Zagreba

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ABSTRACT

The invasive species \textit{Drosophila suzukii} (Matsumura, 1931), the spotted wing drosophila, was first recorded in Croatia in 2010. Since then, it has spread on Croatian territory but its presence has not yet been confirmed in the City of Zagreb. In this research population dynamics of \textit{D. suzukii} was investigated in three orchards in Zagreb (Maksimir, Jelenovac, Zelenjak) in the period from 20\textsuperscript{th} of March to 27\textsuperscript{th} of November 2017. Feeding traps with apple vinegar were used for catching \textit{D. suzukii}. The flight began in the period from 27\textsuperscript{th} of June to 11\textsuperscript{th} of July and lasted until 27\textsuperscript{th} of November. The total pest catches were 874 specimens (Jelenovac 187, Maksimir 232 and Zelenjak 455). From September to November there was a continuous flight and a large increase in the number of caught flies, suggesting the presence of more overlapping generations. Besides the host plants, the development of the pest in this area is also enabled by suitable climatic conditions. The present population in the City of Zagreb County poses a danger to fruit producers in the Zagreb County. The results of this research are a contribution to better understanding of the population and the spread of the pest in Croatia.

Key words: catch dynamics, City of Zagreb, \textit{Drosophila suzukii}, perennial crops

SAŽETAK

Invazivna vrsta \textit{Drosophila suzukii} (Matsumura, 1931.), prvi put je zabilježena u Hrvatskoj 2010. godine. Od tada proširila se na području Hrvatske, ali njezina prisutnost još nije potvrđena u gradu Zagrebu. U ovom istraživanju dinamika populacije vrste \textit{D. suzukii} istraživana je u tri voćnjaka u Zagrebu (Maksimir, Jelenovac, Zelenjak) u razdoblju od 20. ožujka do 27. studenoga 2017. godine. Za praćenje vrste korištene su hranidbene lovke na bazi jabučnog octa. Let štetnika započeo je u razdoblju od 27. lipnja do 11. srpnja i trajao je do 27. studenog 2017. godine. Ukupno su ulovljena 874 primjerka vrste (Jelenovac 187, Maksimir 232 i Zelenjak 455). Od rujna do studenog zabilježen je neprekidni let i velik porast broja ulovljenih muha, što pretpostavlja prisutnost više generacija štetnika na području istraživanja. Osim pogodnih biljaka...
INTRODUCTION

The spotted wing drosophila, *Drosophila suzukii* (Matsumura, 1931) is an invasive pest originating in eastern Asia (Asplen et al., 2015) where it was first recorded and described in Japan in 1916 (Asplen et al., 2015). For the last decade it has spread to European, as well as South and North American countries (Zerulla et al., 2015). *D. suzukii* is polyphagous species; its larvae feed on more than 100 cultivated and wild hosts from more than 25 families (Sampson et al., 2016). This allows successful spread and retention on a large geographic area (Poyet et al., 2015). First record of *D. suzukii* in Europe was during 2008 in parts of France, Italy and Spain (Baser et al., 2015). In Croatia it was first recorded in 2010 (Masten Milek et al., 2011). The key to its great invasion is the ability to adapt to various climatic conditions and wide range of hosts, having large number of overlapping generations, limited number of natural enemies and high female fertility (Cini et al., 2014; Zerulla et al., 2015; Klick et al., 2016). So far, economic damages in Europe have been recorded in Italy, France, Spain (Asplen et al., 2015), Switzerland (Mazzi et al, 2017) and in Croatia (Pajač Živković et al., 2016). The wide and fast spread has been enabled by the global trade in fruit and plant material (Klick et al., 2016).

The development from egg to adult lasts 8 to 14 days and as adult forms they are able to live for 3 to 9 weeks, forming up to 13 generations per year (Calabria et al., 2012). Females lay eggs on stone and soft fruits with their sclerotized ovipositor (Sampson et al. 2016); after developing from eggs inside fruits, feeding larvae cause fruit decay (Pajač & Barić, 2010). During one oviposition, the female can lay up to 3 eggs (Masten Milek et al., 2013). Inside one fruit there can be eggs from more than one female (Masten Milek et al., 2013), but the overall number of developing larvae is set by the size of the fruit (Poyet et al., 2015). Oviposition is mainly happening in the phase of fruit color change (Lee et al., 2011). Females visually locate the host using color contrast; green versus red and blue versus yellow (Little et al., 2019). Damage is done by larvae feeding inside fruit causing the fruit to become soft to the touch, mesocarp changes color and the location of the sting is subject to the attack of
secondary pests and pathogens (Pajač & Barić, 2010). Such fruit is reduced in quality, unsuitable for consumption in fresh state and for industrial processing (Zerulla et al., 2015).

Although a complete protection plan has not yet been developed (Masten Milek et al., 2015), it is assumed that insecticide treatments would be effective at the time of fruit ripening (Walsh et al., 2011), that is, when the fruit changes color (Kleiber et al., 2014). Recent research has shown that *D. suzukii* is more attracted to traps made of mixture of red wine and apple cider vinegar, especially in mass trapping (Marjanović & Tanasković, 2019). Methods using natural enemies have not had much effect so far; *D. suzukii* appears to react differently with parasites and parasitoids depending on the environmental conditions of the area in which it is located (Bohinc & Trdan, 2014). Also, it has been proven that *D. suzukii* detects and avoids the parasitoid wasp *Leptopilina boulardi* by sensing its pheromone compounds (Ebrahim et al., 2015). Research in the UK has shown efficacy of spinosad at all stages of *D. suzukii*, even on the larvae inside the fruit (Cuthbertson et al., 2014).

The presence and flight dynamics of *D. suzukii* have not yet been studied for the City of Zagreb. Such research would have an impact on better knowledge of the population and the spread of the pest in orchards in this area. It is assumed that *D. suzukii* is already present in this area due to its presence in neighboring counties and also because of favorable climatic conditions and wide range of hosts. The assumption is that the pest develops several overlapping generations in the mentioned area. Furthermore, the presence of *D. suzukii* in the city can lead to losses in extensive fruit production, but could also make Zagreb a focal point for further spread on intensive orchards existing in the area of Zagreb County.

**MATERIALS AND METHODS**

The monitoring of *D. suzukii* population dynamics was carried out during the vegetative season of 2017 in three orchards in Zagreb: Jelenovac (45°49'28"N, 15°57'28"E), Maksimir (45°49'43"N, 16°01'44"E) and Zelenjak (45°49'11"N, 15°59'37"E).

Orchards Jelenovac and Zelenjak are extensive orchards on private property and the orchard Maksimir is one of the experiment stations of the University of Zagreb, Faculty of Agriculture. In all three orchards there are several species of stone, soft and pome fruits. In Jelenovac there are plums, apricots, cherries, apples, pears, red currants, blackberries, and raspberries. This orchard is located near the forest park Jelenovac, a mixed forest of oak, beech, hornbeam, and acacia (Zg Portal, 2013).
For the insight into the meteorological conditions in Zagreb, data of the Croatian Meteorological and Hydrological Service for 2017 were used (DHMZ, 2018). For this study, data referring to the mean monthly air temperatures and total monthly precipitation rates from the nearest meteorological station Maksimir were processed (Table 1). The lowest average monthly air temperature was recorded in January (-3.2 °C) and the highest in July (24 °C). During the year 2017, the average annual air temperature amounted to 12.6 °C while the total annual precipitation amounted to 897 mm. The lowest total monthly precipitation amounted to 19.8 mm and was recorded in March, while the highest was recorded in September amounting to 239.6 mm.

Table 1 Mean monthly air temperature (°C) and total monthly precipitation (mm) for City of Zagreb in 2017.

| 2017. | I   | II  | III | IV  | V   | VI  | VII | VIII | IX  | X   | XI  | XII |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Mean monthly air temp. (°C) | -3.2 | 5.2 | 10.0 | 12.4 | 17.7 | 22.5 | 24.0 | 23.7 | 15.4 | 11.9 | 7.3 | 4.0 |
| Mean annual air temp. (°C) | 12.6 |

| 2017. | I   | II  | III | IV  | V   | VI  | VII | III | IX  | X   | XI  | XII |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Total monthly precipitation (mm) | 34.3 | 41.4 | 19.8 | 44.3 | 35.2 | 107.8 | 58.0 | 39.1 | 239.6 | 72.0 | 113.2 | 92.3 |
| Total annual precipitation (mm) | 897.0 |

The flies were sampled by using feeding traps made from plastic bottles of 500 ml volume filled with apple vinegar. In each orchard one feeding trap was set on 30th of March 2017 and samples were collected every 15 days. The species population monitoring lasted until 27th of November 2017. During the monitoring period, a total of 54 samples of flies was collected. The collected samples were stored in 70% alcohol. Determination of the samples was performed in the entomological laboratory of the Department for Agricultural Zoology by using the binocular stereo microscope and following the OEPP / EPPO Diagnostic Protocol PM 7/115 (1) for D. suzukii type (OEPP / EPPO, 2013).
RESULTS AND DISCUSSION

The presence of *D. suzukii* flies was found in all three explored orchards. Altogether 874 individuals were caught, out of which 421 were males (48% of the total catch) and 453 were females (52% of the total catch). Since the proportion of males and females in the population should be the same, the predominant share of females is a prerequisite for a successful population expansion and the spread of flies in a given area (Kovačević, 1959).

As shown in Figure 1., the highest catch was recorded in the orchard Zelenjak where 455 flies (52%) were caught (233 males and 222 females). First flies were caught between 11th and 24th of July. The flight increased slightly during August and September, and in October it increased rapidly. Number of caught females during that period (52 individuals) was lower in comparison to males (83 individuals). By the end of the monitoring period, i.e. until 27th of November, the maximum number of individuals (140 males and 164 females) was recorded. The orchard Zelenjak had the highest number of present flies probably because of the widest range of fruit tree hosts which were grown there.

![Graph showing the number of *Drosophila suzukii* caught in three orchards during the monitoring period.](image)

**Figure 1** Relation between numbers of *Drosophila suzukii* caught in three orchards during monitoring period in 2017 in the Zagreb area.

**Slika 1. Odnos između brojnosti vrste *Drosophila suzukii* ulovljene u tri voćnjaka tijekom perioda praćenja u 2017. godini na području Zagreba.
Second to the highest catch was recorded in the orchard Maksimir with 232 caught flies (27%) (99 males and 133 females) (Figure 1). The flight started in the period from 8th to 21st of August. By the end of the monitoring period, the number of males increased in October and November. Female catch dynamics peaked during October, and began to decline during November.

The lowest catch was recorded in the orchard Jelenovac where 187 flies were caught (21%) (89 males and 98 females) (Figure 1). The flight began in the period from 27th of June to 11th of July. The first period of flight lasted until middle of August. A re-catch was recorded in October, from when the flight lasted until the end of the monitoring period (until 27th of November). In the last monitoring period, a maximum number of adult specimens of the species (99 individuals; 40 males and 59 females) was caught. In this orchard the decrease in the number of caught males was observed in the period from July to August. Also, from October until the end of the monitoring period the males' catch in relation to the females' was lower. Several more female flies (98 individuals) were caught in comparison to male flies (89 individuals).

In all three researched orchards an increase in caught *D. suzukii* flies by the end of monitoring period (October and November) was observed (Figure 1). The population began to increase in September and a sharp increase in catches was recorded during October when more males were caught in relation to females. The trend of catches continued in November. Depending on sex and date of captured individuals, it is assumed that males started to fly two weeks before females, but a greater number of females were active until the end of November or in colder days. This difference corresponds to the literature data on the increased number of overwintering females (Zerulla et al., 2015).

The pest was sporadically present at the beginning of the monitoring period which is why it is assumed that *D. suzukii* individuals did not overwinter in these three orchards and were attracted by apple vinegar traps. The increase in later period (mid-September) was probably due to mixed fruit varieties at different stages of ripening which also had an impact on population maintenance and growth until the end of monitoring period. This pattern with low capture period during summer has also been observed in Italy, Switzerland, Germany, Spain, Belgium and in the United States (Clymans et al., 2019).

Females have host preferences throughout the year and primarily are in search of oviposition sites, unlike the winter females, which are in search of overwintering sites and are much more attracted by fermentation compounds in traps (Clymans et al., 2019). Trap counts only start increasing in late summer, to reach peak levels in autumn (Clymans et al., 2019).
The survival and population maintenance of *D. suzukii* is not only limited by hosting plants but also by meteorological conditions such as average annual air temperature, minimum air temperature and annual precipitation (dos Santos et al., 2017).

These flies require an average annual air temperature of 5 to 20°C (dos Santos et al., 2017) and for active flight minimum air temperature of 10°C (Asplen et al., 2015). According to DHMZ (2018), average annual air temperature for Zagreb in 2017 amounted to 12.6°C. The average daily air temperature required for *D. suzukii* flight is 10°C and was achieved throughout the day in July, August and September, whereas in October it fell below 10°C for 6 days and in November for 19 days (Figure 2). Those other days in October and November when the air temperature was over 10°C allowed *D. suzukii* to keep on searching for a source of food and place for overwintering.

An annual precipitation that favors survival of *D. suzukii* in given area is between 500 and 2500 mm (dos Santos et al., 2017). An annual rainfall in Zagreb area in 2017 was 897 mm which is suitable for the *D. suzukii* development (Table 1). The highest monthly precipitation was recorded in September (239.6 mm), when 28 adult individuals were caught. In November, when the highest number of individuals (501 individuals) was caught, the average monthly precipitation amounted to 113.2 mm (Figure 2).

The later appearance of adult flies, although the temperature conditions and precipitation rates were suitable throughout most of the monitoring period, is probably the result of later fruit ripening; in the first monitoring period there was no suitable substrate for females to lay their eggs in. Despite low temperatures, the maximum catch was recorded during the last monitoring period. It is assumed that in these last two months flight occurred during those days when mean daily temperatures were 10°C or higher; in October there was a total of 25 such days, and in November 11 days (Figure 2). Also, as there were no rippening fruits available during that last period, the apple vinegar traps were the last source of food before the overwintering.
Figure 2 Relation between the total number of *Drosophila suzukii* and climatic conditions during the monitoring period in 2017 in the orchards of the City of Zagreb.

Slika 2. Odnos ukupnog broja vrste *Drosophila suzukii* i klimatskih uvjeta tijekom razdoblja praćenja u 2017. godini u voćnjacima na području Zagreba.

Considering the catch dynamics results of *D. suzukii* in Zagreb's orchards and the decrease in total male catches during October in the Jelenovac and Maksimir orchards, it is assumed that *D. suzukii* develops several generations per year which, with favorable climatic conditions, overlap each other.

CONCLUSION

The research on the catch dynamics of *D. suzukii* populations confirmed presence of *D. suzukii* in Zagreb area. Since 874 specimens of *D. suzukii* were found in three orchards, out of which 52% were females and 48% were males, the population of this pest has a potential of further spread. Also, climatic conditions in the researched area favor the development of *D. suzukii*, enabling flies to develop several generations per year that overlap. Private yards and surrounding forest vegetation in the City of Zagreb offer food sources and shelter for this pest which makes Zagreb a potential focal point for further spread to industrial orchards in the Zagreb County, especially because there is still no designed protection program for the territory of the Republic of Croatia which would include owners of small private city orchards.
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