

## FIRST RECORD OF THE SPOTTED WING DROSOPHILA *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE) IN ROMANIA

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**Abstract:** The paper presents the results on the first identification of the Spotted Wing Drosophila, *Drosophila suzukii* (Matsumura 1931) (Diptera: Drosophilidae) in Romania. In September 2013, seven adults were captured in a Tephri trap (used for the Mediterranean fruit fly *Ceratitidis capitata*) placed on wild blackberry (*Rubus caesius* L.) bushes in a site from the Northern part of Bucharest city (Southern Romania). In 2014, using bottle traps baited with red grape wine, the presence of *D. suzukii* was reported in different ecological habitats, again on wild blackberry fields as well as in gardens in urban, suburban and rural locations from Bucharest, Ilfov and Giurgiu Counties (Southern Romania). Accordingly, we managed to perform the population dynamics of adults captured in red wine bottle traps on *R. caesius* under weather climatic conditions of Bucharest area. The *D. suzukii* flight activity took three months, i.e. from September to November. Our investigations confirmed that the wild blackberry (*R. caesius*) and pokeweed (*Phytolacca americana*) fruits were harmed by *D. suzukii*, for which they were hosts.

**Key words:** invasive species, population dynamics, *Rubus caesius*, Spotted Wing Drosophila

### INTRODUCTION

The spotted wing Drosophila, *Drosophila suzukii* Matsumura 1931 (Diptera: Drosophilidae) is an invasive vinegar fly originating from Japan and the countries from the South-East of Asia and which invaded Europe (Spain and Italy), as well as North America (California) in 2008 (Calabria et al., 2012). Since then the fly has had a rapid expansion, so that new findings on its presence have been reported in many important fruit production countries from Western Europe and Mediterranean Basin (Cini et al., 2012; Mortelmans et al. 2012; Kiss et al., 2013) and, recently, in South America (Depra et al., 2014). This paper is the first reference made to this species and its first evidence results in Romania.

Currently, the fly is known as the Spotted Wing Drosophila (SWD), whilst the name of cherry vinegar fly (CVF) was formally used in early references. Nowadays, *D. suzukii* is widely considered as one of the most destructive pest of many fruit crops due to its preference to attack ripening healthy fruits. Females have a characteristic serrated ovipositor used to insert the eggs in perfect healthy skin of fruits at various ripeness stages, and then, the larvae feed inside them (Lee et al., 2011). Holed fruits are exposed to phytopathogen infections (fungi and bacteria) that may cause premature deterioration of fruits and additional losses (Seljiak, 2011). This way of feeding itself with the healthy fruit, makes *D. suzukii* distinct from most of the species that belong to genera *Drosophila* and which prefer the fallen overripe and decomposed fruit.

*D. suzukii* is described as an extremely polyphagous pest, its damages being associated with a huge range of wild and cultivated fruit plants. Exotic species such as *Actinidia spp.*, *Diospyros kaki*, *Ficus carica* and temperate climate fruit trees *Malus domestica*, *Morus rubra*,

*Prunus avium*, *P. domestica*, *P. persica*, *P. persica* var. *nucipersica*, *Pyrus pyrifolia* are in the host plants range of the fly. Berry crops such as *Fragaria ananassa*, *Rubus idaeus*, *Rubus* spp., *Vaccinium* spp. and *Vitis vinifera* seem to be very vulnerable (Lee et al., 2011; Seljiak, 2011). The wild berries are also among the hosts mostly preferred by the fly, as it results from the percentage of 70% infested fruits that have been found in wild *Rhamnus alpina* ssp. *fallax* (Seljiak, 2011).

Like for many other invasive species, the timely information about the presence and abundance of *D. suzukii* fly generated by early detection and monitoring programs has a crucial importance in defining the pest control to protect the vulnerable crops.

Within this research paper, we present the results of the first monitoring of *Drosophila suzukii*, concerning its presence and abundance in some fruit habitats, as well as the dynamic of the population density located in the South part of Romania.

## MATERIALS AND METHODS

In September 2013, the spotted wing *Drosophila D. suzukii* was detected for the first time in Romania, in a Tephri trap (designed for *Ceratitis capitata* Wiedemann 1824) placed on wild blackberry (*Rubus caesius* L. 1753) bushes in a site from Northern part of Bucharest city (Southern Romania). The *C. capitata* monitoring was carried out within the framework of a regional project under the International Atomic Energy Agency Vienna (IAEA) to evaluate the presence of exotic invasive fruit flies of the family Tephritidae.

In 2014, to reconfirm the presence of the fly and eventually to evaluate the size and dynamics of adults' population in the same site, three traps with red grape wine used as attractant to *D. suzukii* flies, were set up at the beginning of August. The wild blackberry bushes climbed some apple trees, previously cultivated and later abandoned. The traps consisted in clear plastic bottles of 0.5ml each filled with 150 ml red grape wine and to which six 5mm holes were made at the top area. Another six similar traps (one trap per site) were placed in private garden habitats with mixture of seasonal fruits (figs, apples, grapes) in urban (1), suburban (2) and rural (2) sites in Bucharest city, Ilfov (IF) and Giurgiu (GR) Counties, for short periods of time, during September and November months. All habitats were ecologically distinct, no protection measures, disposed in a perimeter of one hundred square kilometres in the Southern Romania. The traps were hanged 1.5m in the plant canopy and they were marked using global position system. The three traps on wild blackberry (*R. caesius*) were verified for insect presence and the bait was replaced weekly from August to November. They were maintained on the same position for the entire collecting period. The collected insects were counted and used to accomplish the population dynamics for this plant species and area. The adults' population density was presented as number of flies per trap per day (FTD). The other six traps were checked and the insects were collected at the end of the each period.

To perform the species identification, the adults of *D. suzuki* found in the traps were analysed under a stereomicroscope following the descriptions and diagnostic protocol given in OEPP standard (OEPP/EPPO, 2013). We differentiated the individuals of *D. suzuki* from other species of *Drosophila* group, considering the morphological characters of adults: the evident dark spot at the top of the wings and the two rows of combs on the fore tarsi in case of males; the serrated ovipositor and uninterrupted bands on abdominal tergites in case of females. After identification, we separated them into males and females and their proportion was estimated for each site and sampling date in order to evaluate the structure of autochthonous natural population. Likewise, other *Drosophila* species caught along with *D.*

*suzukii* were analyzed. The flies, preserved in 70% alcohol, may be found at the Research-Development Institute for Plant Protection Bucharest.

Two berry samples of wild blackberry (*R. caesus*) from the infested site and the American pokeweed (*Phytolacca americana* L. 1753) near this were collected in October 2014, to detect the presence of *D. suzukii* in fruits. Some fruits were immersed in saline solution to get larvae and others held in controlled environment chambers until the pupae and adults emerged.

A Metrilog weather station installed in the nearby surveyed area provided us with detailed weather information, i.e. daily precipitation totals (mm) and maximum, average and minimum atmospheric temperatures (°C), during the trapping period of 2014.

## RESULT AND DISCUSSIONS

In this work we present results on the first record of *D. suzuki* in Romania. Seven adults (3 males and 4 females) were unexpectedly collected on 6 September 2013 in a Tephri trap (specific for *Ceratitis capitata*) on wild blackberry (*Rubus caesius*) bushes situated in an area of abandoned apple trees from the Northern part of Bucharest city (Southern Romania). This fact created the opportunity to extend in 2014 our observation and survey to other fruit species in sites from the Southern country. Table 1 provides the flies catching results in 2014 along with data on sites, composition of seasonal fruits present in investigated habitats, and geographic position of traps.

**Table 1.** Abundance and species composition of Drosophilidae flies caught in habitats investigated for *D. suzukii* presence in 2014

GPS coordinates of traps	Habitat/site/fruit species availability	Period of flies trapping	<i>Drosophila suzukii</i>		Others Drosophila	<i>Chymomyza amoena</i>	
			females	males		females	males
44.501866N/ 26.069593E 44.502458N / 26.068990E 44.501461N / 26.068297E (89m)	Urban/Bucharest/ wild blackberry fruit, apples	8 Sep – 24 Nov	982	1588	632	11	10
44.469860N/ 26.069557E	Urban/Bucharest figs, jujube fruit	1- 31 Nov.	62	31	28	0	0
44.709724N / 26.169481E (112m)	Suburban/Snagov (IF) fruit trees, no fruits	17-21 Nov.	2	0	3	0	0
44.527002N/ 26.011199E (137m)	Suburban/Mogoșoaia (IF) apples, grapes	27 Oct.-17 Nov	13	21	14	0	0
44.150365N / 25.766486E (70m)	Rural/Naipu (GR) grapes, figs, apples	27 Sept.- 25 Oct.	115	57	278	0	0
44.155714 N/ 26.297974E (38m)	Rural/Izvoarele (GR) grapes	28 Oct.-13 Nov.	0	1	9	1	0

Our results in table 1, showed the fact that *D. suzukii* was present in traps placed in all habitats comprised in the monitoring study. During the entire catching period, from September to November 2014, we captured 3822 adult individuals belonging to Drosophila

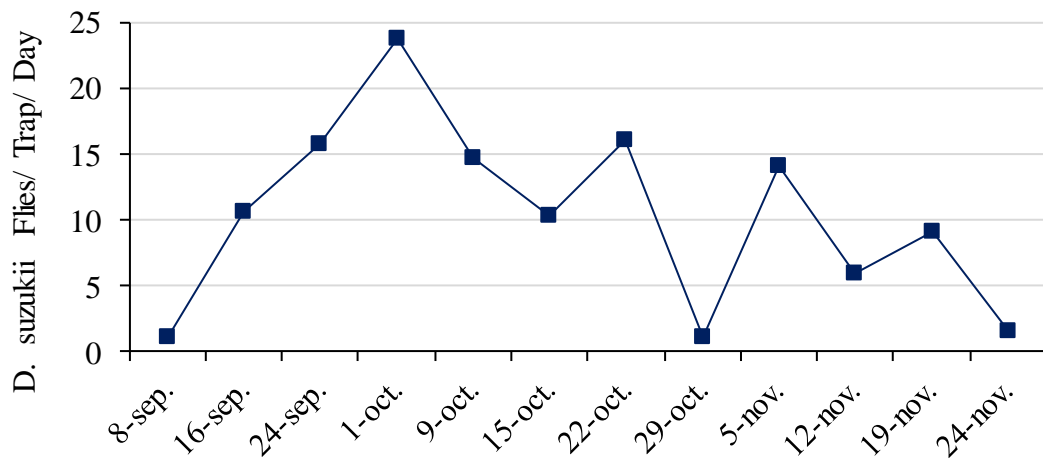
genus, in all traps, both in the field of wild blackberry and private gardens. Out of these, the most abundant species was the Asian fruit fly, *D. suzukii*, with 2872 individuals (75.14%). The proportion of 24.86% was assigned to individuals in the category 'other *Drosophila*' found in association with *D. suzukii* captures. In this material, we recognized individuals belonging to *Drosophila melanogaster* (Meigen 1830), *D. immigrans* (Sturtevant 1921) and *D. simulans* (Sturtevant, 1919) and others species unidentified. The three species are described as components of *Drosophila* community which operates together on common feeding substrate, and *D. melanogaster* and *D. simulans* are described as sister fruit fly species (Pavkovic-Lucic & Kekic, 2014). In table 1 we also showed the females and males number in natural population of *D. suzukii* captured for each location.

Additionally to flies of genus *Drosophila*, flies of other drosophilid genera were also captured, out of which, 22 flies (10 males and 12 females) belonged to genus *Chymomyza*, species *Chymomyza amoena* (Loew, 1862). *C. amoena* is also an invasive fruit fly, originating from North American continent, present in Europe since 1975 when it was first detected by Maca and Bachli (1997) in the former Czechoslovakia. It has been reported in Central and Southern European countries, including Romania (Ceianu, 1989; Band et al., 2005) as well as in Northern countries (de Jong, 2003; Escher et al., 2004, 2006). The adults of this species were recognised following their distinct pattern with two transverse bands on the wings. In Romania, *C. amoena* was recorded as new species in the country's fauna by Ceianu (1989) who caught one male in 1988 and another one in 1989 in the spruce forest environment in the North-Eastern part of the country. The fact that we found *C. amoena* species in new habitats situated in the plain areas in Southern part of the country reveals the fly's well ability to spread in different ecological and climatic zones in the territory of Romania. The locations where we found this are at a considerable distance and opposite, in term of geographical position, to the forest zone where the first two specimens were identified in 1988-89. The findings of Band et al. (2005) pointed out that *C. amoena* prefers the fruits (apples, plums) and nuts, previously infested by other major pests, as breeding substrates.

Table 1, showing collecting data in 2014, indicates the fact that the greatest captures of *D. suzukii* were associated with the wild blackberry *R. caesus* in Bucharest area. The traps in the garden from Giurgiu, with a mixture of season fruits, figs, grapes and apples attracted many flies of *D. suzukii*, too. Figs and grapes as well as apples are described as preferred hosts for *D. suzukii* mainly in late season (Doris et al., 2013). The figs and jujube fruit in garden from Bucharest, lead to modest fly catches. In the other garden habitats, the captures were almost absent or at a very low level.

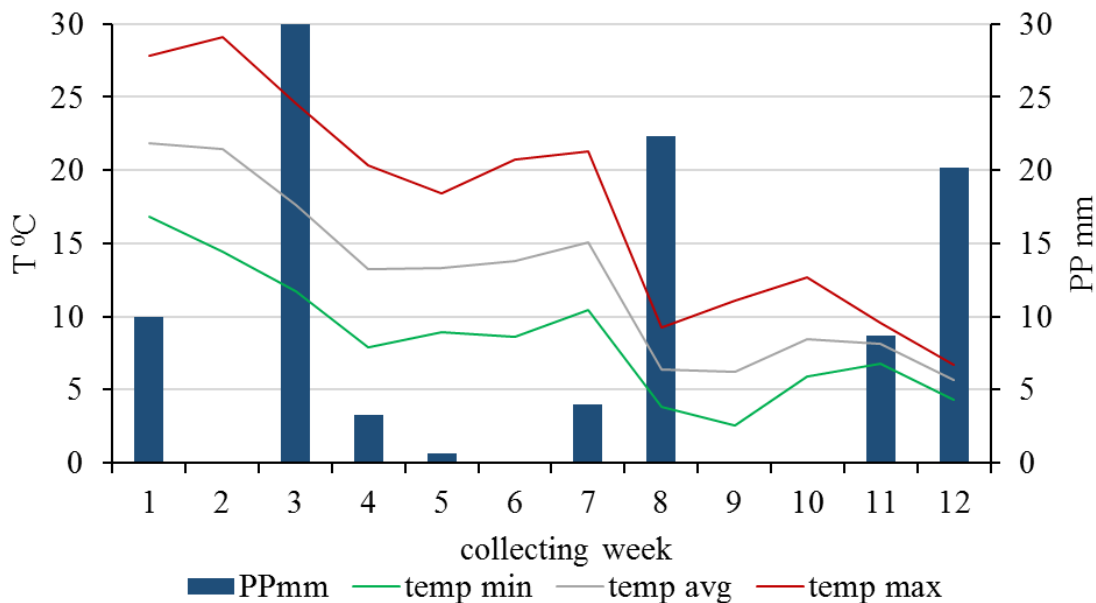
Out of the total captured flies of *D. suzukii* in 2014, 2570 flies (67.24%) corresponded to traps on wild blackberry bushes and 1252 flies (32.76%) to the habitats in gardens (table 1). The abundance of flies' population was clearly more numerous in the wild blackberry and was positively related to the number of simultaneously active traps and their uninterrupted activity during the trapping period. The three red grape wine-baited traps, placed from August to November in the wild blackberry, caught flies for 12 week, from September to November, while the traps in gardens were placed and worked only for a short period of time during October-November.

We evaluated the dynamics of the fly population as well as density through the 12 weekly sampling dates, based on the number of *D. suzukii* adults trapped in the *R. caesus* field. The adults' density expressed as adults per trap per day and their population fluctuations on *R. caesus* are given in Figure 1.



**Figure 1.** Population dynamics of adults *D. suzukii* (Flies/Trap/Day) in 3 red wine bottle traps placed on wild blackberry (*R. caesus*) in Bucharest area in 2014

The course of weekly weather parameters (temperature and precipitation) for study area during the trapping period, from September to November is offered in Figure 2.



**Figure 2.** Weekly precipitation (mm) and temperature (°C) in Bucharest area during capturing of *D. suzukii* in September-November 2014

Results of weekly surveys, shown on graph in figure 1, indicated that *D. suzukii* have been found in monitoring traps only during the autumn months, from early-September until late-November. The dynamics of flight activity for *D. suzukii* exhibited in this figure fluctuated with the weather conditions in the surveyed area. Generally, the weather modifications determined a series of changes in number of adults in traps. The first captures of *D. suzukii* in 2014 detected in the traps were made on 8 September, 2 days later than in the

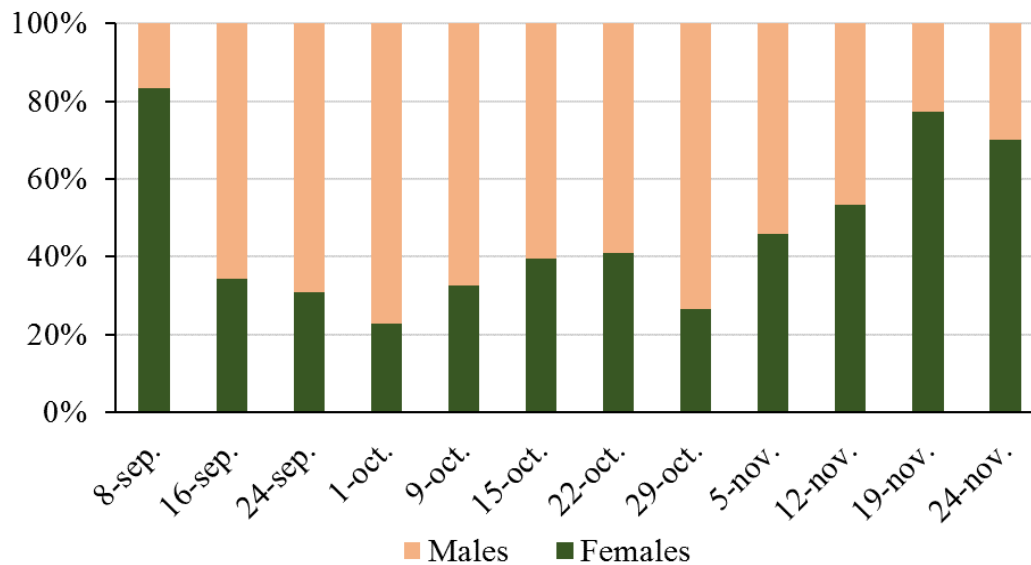
previous year of capturing 2013. According to weather data, the winter 2013-2014 was cold in area of the study, the average air temperature got down below  $-6^{\circ}\text{C}$  in January-February (minimum temperature dropped even to  $-16^{\circ}\text{C}$ ). Nevertheless, the fly has developed an abundant population in autumn months 2014 in this site. This situation would indicate the fact that the fly tolerated negative temperatures during the winter and it had also a good potential to overwinter in Southern Romania's cold winter climate.

From the very beginning, the number of trapped adults has been substantially increased during September, when the maximum weekly temperatures have passed  $25^{\circ}\text{C}$ . On October 1<sup>st</sup>, there was recorded the highest number of flies in a day (24 flies per day) during the whole 2014 season. For 2 weeks, namely in mid-October, we noticed a decline in population, presumably as a consequence of decreased air temperatures and rich precipitations time (Figure 2). After only one week of increasing catches, a drastic sharp drop occurred. In that period, there were precipitations, predominantly rain which turned into sleet and snowfall on 25 October, both in Bucharest and Southern Romania, which severely affected the population dynamics of *D. suzukii* during 22-29 week of October. As soon as temperatures had arisen, a rapid numerically increase in population occurred in the first week of November; there was a decreasing and then an increasing of captures in mid-November in relation to temperature and precipitation variations. The last captures occurred on 24 November 2014, during a few days period of freezing temperatures (minimum temperatures ranged to  $-0.9$  and  $-1.7^{\circ}\text{C}$ ).

The population dynamics of *D. suzukii* reached three peaks, possibly due to temperature variations and precipitation falling during monitoring period. One peak was more pronounced in mid-September when the maximum weekly temperature exceeded  $29^{\circ}\text{C}$  (Figure 2). Our results are in line with relevant studies which highlighted the importance of weather parameters as well as environment characteristics on intensity of *D. suzukii* captures in traps (Dalton et al., 2011; Cini et al., 2012; Ohn et al., 2012; Harris et al., 2014).

In surveyed area, *R. caesus* produced an abundant and constant quantity of fruit over late summer and autumn seasons, providing a good nutrition source for the fly during flight activity. The first *D. suzukii* trap captures corresponded with the presence of blackberry fruit at stage of ripeness. Also, the scattered apples in the abandoned apple trees in area could be used by the fly to complete its food needs. According to current literature, the wild soft-skinned berries are ranked among the most attractive fruits for the spotted wing drosophila (Selijak, 2011). Elsensohn and Hesler (2013) revealed that wild blackberries of the *Rubus* genus offer a suitable consistent food substrate for *D. suzukii*, mainly late in the season.

The total captures of *D. suzukii* in red wine baited traps included in Table 1 showed that females in natural populations represented 38.2% on *R. caesus* and between 38.2-66.9% in the other trapping sites. We found that the ratio between sexes (m/f) for the entire period of flies captures was in males' favour (1.61) on *R. caesus* in Bucharest site and in one of garden habitats, and in females' favour (0.5) in two of garden habitats. The results on the fluctuations of females and males proportion in *D. suzukii* population on *R. caesus* throughout the monitoring period are given in Figure 3.



**Figure 3.** Male and female *D. suzukii* caught in red wine bottle traps on wild blackberry (*R. caesus*) during the monitoring period September-November 2014

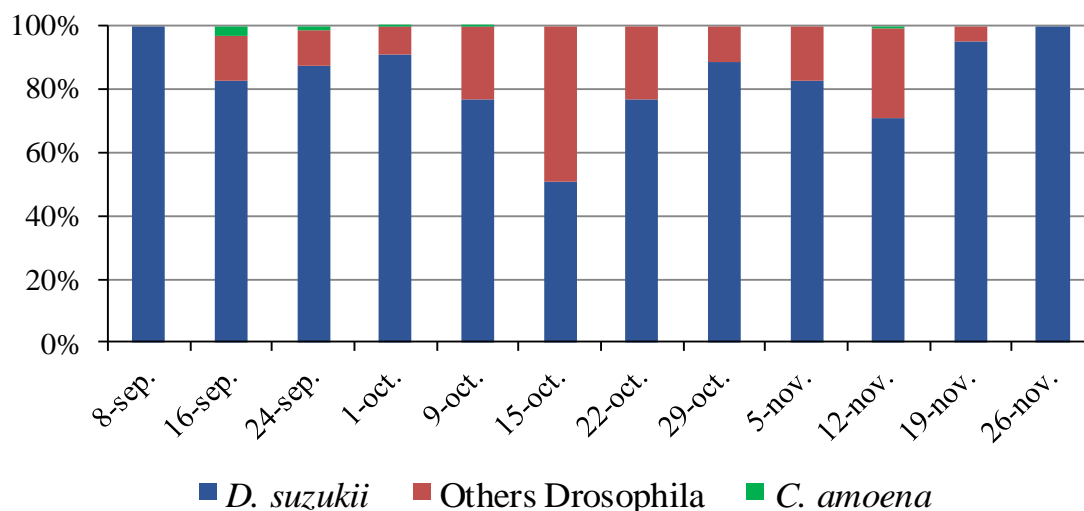
Although generally there were fewer females in traps, they showed more peaks of abundance than males during the flight activity. According to literature, variations in female and male captures in traps can be caused either by the type of traps and attractants, or collecting period in the growing season. The apple cider vinegar traps, alone in grapevine, or in combination with red wine and sugar in sweet cherry, evidently attracted more females in Italy (Armanasco, 2013; Gargani et al., 2013), whereas in late season, the apple cider vinegar traps attracted more males in raspberry crops in America (Hamby et al., 2014).

Further to the data regarding the activity of *D. suzukii*, the captures of flies in red wine traps offered us important information on others drosophilids living together with *D. suzukii* in investigated habitats. The comparative analysis of the fly collections in table 1 showed a number of *D. suzukii* adults superior to those of other drosophilids. The total catches of *D. suzukii* were 4 times higher than those of other *Drosophila* spp. Few adults of *C. amoena* were found. The proportion of these is showed in figure 4.

In case of testing fruits for *D. suzukii* larval infestation, our observations confirmed that drosophila larvae have resulted from fruits of *R. caesus* and *P. americana* in salt water solution but they were not identified into *D. suzukii* or other drosophilids. In the fruits trials in rearing room, our observations confirmed that fruit of *R. caesus* and *P. americana* were infested by *D. suzukii* larvae, this result being supported by the adults and pupae which have been obtained.

We also found symptoms of the spotted wing drosophila attack on fruits of *R. caesus* and *P. americana*. The wild blackberry *R. caesus*, a perennial weed with semi woody port, is frequently found in plane regions of our country (Manea 2011), including in the Southern parts of the Romanian Plain where we performed the *D. suzukii* monitoring. *P. americana* is also a weed commonly found in the southern parts of the country, spontaneous or cultivated, used for phytotherapeutic purposes (Tănăsescu & Gacea, 2004). Both weeds are invasive for Europe. Fruits of *P. americana* and wild blackberries of *Rubus* genus are reported as important hosts to *D. suzukii* (Maier, 2012; Elsensohn & Hesler, 2013), which are alternative hosts to cultivated berry species. As for the wild blackberry of *R. caesus* species, as host of

the wing spotted drosophila *D. suzukii*, no information or citations in the specialized literature have been found yet.



**Figure 4.** Captures of *D. suzukii*, other Drosophilidae and *C. amoena* in red wine bottle traps on wild blackberry (*R. caesus*) in September-November 2014

Despite the fact that no damage on cultivated fruits has been reported, but considering important the results in this work, which showed that *D. suzukii* possesses a great potential of spreading in the Southern parts of Romania, this could become a serious pest for autochthonous fruits production. Of great importance are further researches in the field to evaluate the potential risk for the fruits in the summer season and to understand the late presence of *D. suzukii* in the autumn season. Our findings bring a significant contribution to the defining of the spreading zone for this species in Europe, mainly in South-Eastern Europe.

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