PREDICTED AND ACTUAL DISTRIBUTION OF THE INVASIVE SPECIES *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE) IN BULGARIA

Olia Karadjova[1]*, Zhenya Ilieva[1], Elena Petrova[1], Mariana Laginova[2]

[1]Institute of Soil Science, Agrotechnology and Plant Protection, Agricultural Academy
7 Bansko Shose Str., Sofia 1331, Bulgaria
[2]Central Laboratory for Plant Quarantine, Bulgarian Food Safety Agency
120 Nikola Mushanov Blvd., Sofia 1330, Bulgaria

***E-mail: oliakaradjova@abv.bg**

Abstract

In 2008, the spotted wing drosophila, *Drosophila suzukii* 1931 (Diptera: Drosophilidae) was detected for the first time in Europe (Spain). Currently the species is distributed in most Balkan countries and in 2014 it was also found in Bulgaria. The host plants of *D. suzukii* include cherry, strawberry, raspberry, blackberry, blueberry, peach, grape, plum, apricot and many others. In 2011 a climatic model was developed to determine the potential for permanent establishment and spread of the invasive species in Bulgaria. Specialized software (CLIMEX v. 3) was used to create maps of the ecoclimatic indices (EI). The model predicts that under the current climatic conditions *D. suzukii* can be established on the whole territory of the country and develop 3–7 generations per year.

In 2014 and 2015 a total of 670 adult individuals were trapped. The sex ratio was 3.5 males to 1 female. The greatest numbers of *D. suzukii* adults were captured using Tephri-traps, followed by specialized Riga cup traps. The high male to female ratio and the low number of flies trapped in spring were likely due to the development of transient populations of the species or insufficient effectiveness of the traps. No damages by *D. suzukii* were observed on fruit crops.

In the region of Plovdiv the number of trapped flies was significantly higher in comparison with Blagoevgrad and Kjustendil. The developed predictive model indicates up to 7 generations per yearfor the regionof Plovdiv. This prognosis was confirmed by the monitoring data: *D. suzukii* was established at the highest number of sites and with greatest abundance in the area.

Key words: *Drosophila suzukii,* predictive model, monitoring, distribution.

INTRODUCTION

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is a species of the temperate and subtropical climatic zones(Mitsui et al., 2010). It originates from Southeast Asia and has spread to many Asian countries (Calabria et al., 2012) and North America (Lee et al., 2011). In Europe *D. suzukii* was first detected in 2008 from Spain (Calabria et al., 2012). For several years the species spread to most countries on the continent (Cini et al., 2012; Asplen et al., 2015; Mazzetto et al., 2015).

The females of *D. suzukii* lay eggs in healthy ripening fruit on branches (Mitsui et al., 2010). The characteristic large, robust and serrated ovipositor of the fly allows it to place its eggs inside the fruit (Hauser et al., 2011). The main damages are caused by the larvae feeding on the fleshy tissues. In several days the fruit deform, soften and become unfit for the market (Grassi et al., 2009). *D. suzukii* is particularly harmful to fruit production in Bulgaria and worldwide due to the following features: high reproductive potential and a short life cycle with up to 13 generations per year (Kanzawa, 1939; Sasaki and Sato, 1995 a, b; Walsh et al., 2011; Walsh, WSUER); high biological plasticity and tolerance to a wide range of climatic conditions(Walsh et al., 2011;Cini et al., 2014; Asplen et al., 2015); significant potential for spread mainly through infested fruit (Calabria et al., 2012; Cini et al., 2014; Asplen et al., 2015); wide host range including over 90 cultivated and wild plant species, many of which are present in Bulgaria (Kanzawa, 1939; Lee et al., 2011; Calabria et al., 2012; Asplen et al., 2015; CABI, 2015); potential to cause significant economic losses on stone fruit and berries.

In 2010 France and Italy registered 80% yield losses from *D. suzukii* on strawberries and raspberries (EPPO, 2010). The losses from an outbreak on 400 ha of berries in Trento Province, Italy have been evaluated at € 500 000 for 2010 and € 3 million for 2011 (De Ros et al., 2013). The species has been reported from Greece, Bosnia and Herzegovina, Romania, Slovenia, Serbia, Croatia, Montenegro and other countries adjacent to Bulgaria. Most of the countries in the region have not registered significant losses (Asplen et al., 2015).

The aim of the present study is to: i) evaluate the potential for establishment of *D. suzukii* in Bulgaria; ii) predict the number of generations for the different regions in the country and iii) compare the outputs of the predictive model with the current distribution of *D. suzukii* in Bulgaria.

MATERIALS AND METHODS

Potential for establishment. For the purposes of evaluating the potential for establishment and spread of *D. suzukii* in Bulgaria, a model characterizing the ecological niche of the species with respect to climatic and some physiological factors was developed. Maps with ecoclimatic indices (EI) measuring the suitability of different regions for survival of the species were prepared using specialized software (CLIMEX v. 3) (Sutherst et al., 2007). The model was developed using information on the local climate, the distribution and some biological parameters of *D. suzukii.*

Endangered zone. The data on production areas for the main cultivated hosts of *D. suzukii* in Bulgaria for the period 2010–2014 were obtained from the Department of Agrostatistics at the Ministry of Agriculture and Food. They were used for evaluation of the potential for transfer of the pest to a suitable host.

Monitoring. The observations were carried out in the framework of a monitoring programme of the Bulgarian Food Safety Agency for *D. suzukii* (2012–2015) in the following regions of the country: Blagoevgrad, Burgas, Vidin, Vratsa, Varna, Dobrich, Kardzhali, Kyustendil, Pazardzhik, Plovdiv, Pernik, Razgrad, Sliven, Stara Zagora and Haskovo.

The traps and lures used in 2014 were, as follows: a formulation of the Central Laboratory for Plant Quarantine (CLPQ) using red wine or vinegar as attractants; universal traps for fruit flies Tephritrap (85 pcs) and Jackson traps (26 pcs). The Tephri-trap includes the three-component attractant Biolure (thrimethyl-amine, ammonium acetate and putrescine) and DDVP insecticide (strips with 2,2 – dichlorodimethyl phosphate). The Jackson trap (delta) is set with a trimedlure capsule as attractant. This parapheromone attracts mainly males and females of *Ceratitis capitata* (Wiedemann), as well as other species of fruit flies. The attractant was replaced every two months.

The traps set in 2015 included Tephri-traps (62 pcs), Jackson traps (33 pcs) and Riga traps (32 pcs). The Riga traps include a food-based lure and are specific to *D. suzukii.* The traps were set in the regions of Blagoevgrad, Kyustendil and Plovdiv, where *D. suzukii* was detected in 2014. Throughout the course of the monitoring program results were recorded from May to November.

RESULTS AND DISCUSSION

Potential for establishment and spread. Maps showing the ecoclimatic indices (EI) and the potential number of generations per year on the territory of Bulgaria were prepared using CLIMEX software (Fig. 1 and 2). EI helps determine the territories where the climatic conditions are suitable for development and possible overwintering of the pest. The higher the EI is for a particular area, the more favourable are the conditions for development of the species. Values of 10 and above signify that the insect may be able to overwinter in the area (Sutherst et al., 2007).

The values of EI for parts of Northwestern and Central Bulgaria (Fig. 1) show that the climatic conditions are suitable for establishment of *D. suzukii* in the country. The possibility for development of transient populations with 3–7 generations per year capable of causing significant yield losses cannot be excluded even for areas where the conditions do not favour survival during the winter (Fig. 2).

Kanzawa (1939) reports that the adult is the only overwintering life stage of *D. suzukii*. At low temperatures in autumn mature females stop laying eggs until next spring. In Japan *D. suzukii* has been captured in areas with mean winter and summer temperatures of -5.1°C and 28°C, respectively (Kimura 2004), and has been reported from the island of Hokkaido where the mean winter temperatures are in the range of -12°C to -4°C (Walsh et al., 2011). Dean (2010) reports a low temperature threshold for development of 8.9°C, optimal activity at 20°C and a high temperature threshold of 31.7°C. The low and high temperature thresholds determined by Coop (2010) are 10°C and 30°C, respectively. Coop et al. (2012) develop a model showing that the most favourable territories for overwintering of *D. suzukii* in the Pacific Northwest are the coastal and southern areas where survival has been estimated at 25 to 100 per 10 000 individuals.

Damus (2009) reports that the distribution of *D. Suzukii* in Asia is limited by cold winters, low humidity and very high temperatures to the south. Van Steenwyk et al.(2012) indicate that the fly prefers high air humidity and moderate temperatures (around 24°C). According to EPPO (2010) *D. suzukii* does not tolerate high temperatures when air humidity is low and in the southern Mediterranean region the pest can survive only on irrigated crops. Calabria et al. (2012) note that in Europe and the western parts of North America *D. suzukii* is expanding its area of distribution to the north. The authors consider that their model corresponds to the ecological simulations of Hauser et al. (2009) which show that dry Mediterranean climate is not preferred by *D. suzukii.*

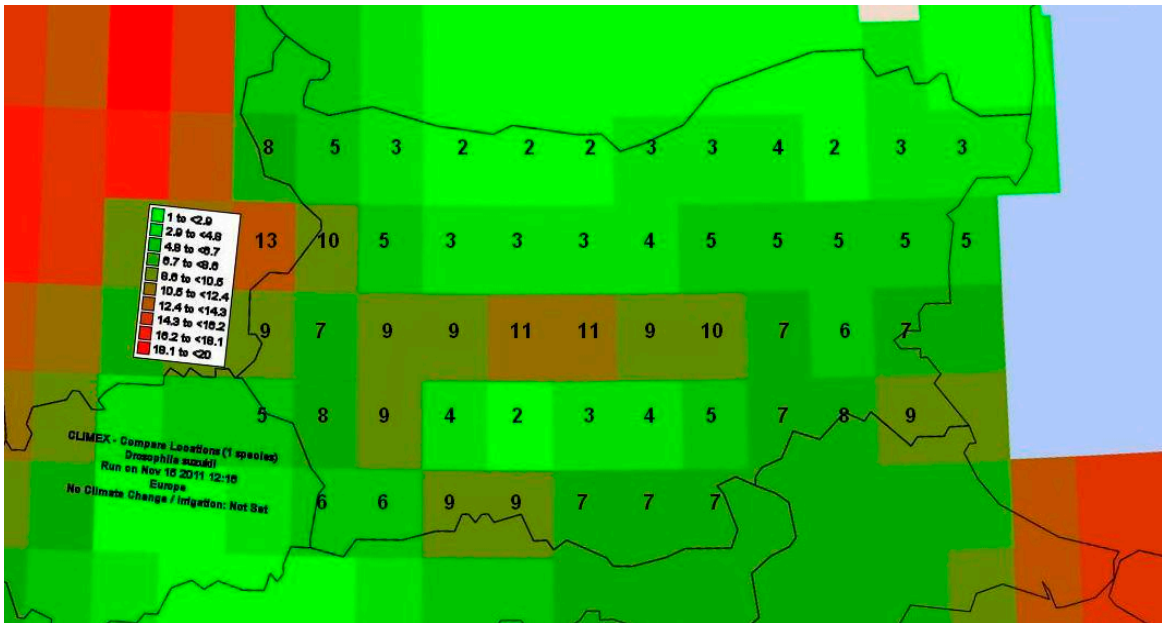

Fig. 1. *Climatic suitability of different regions in Bulgaria for D. suzukii based on ecoclimatic indices (EI)*

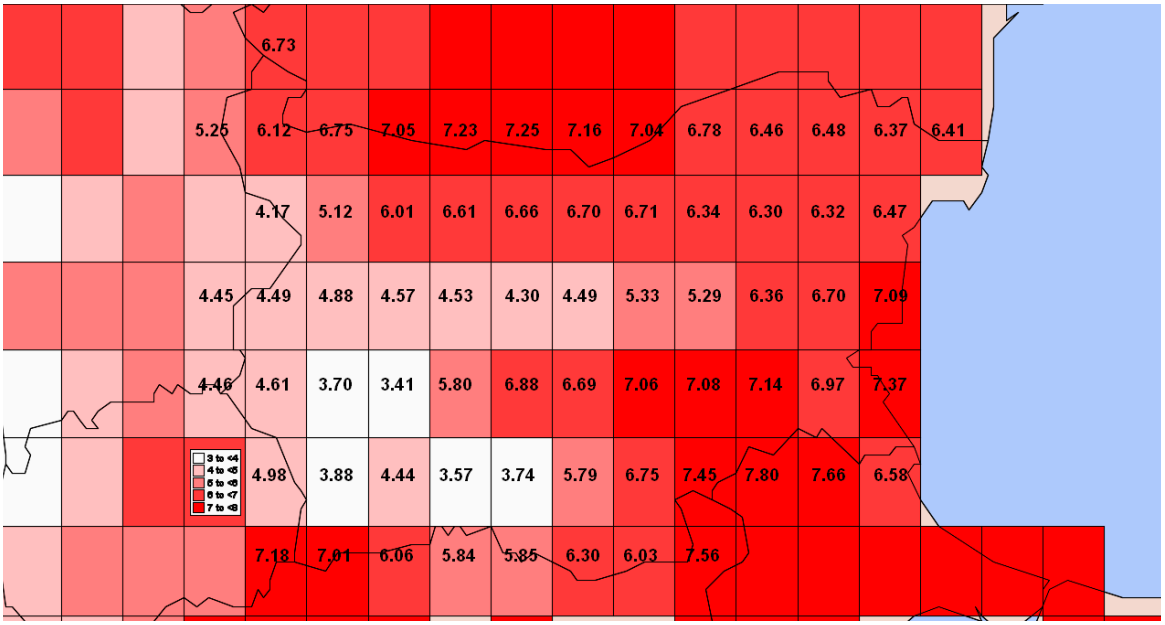

Fig. 2. *Potential number of generations of D. suzukiiper year in different regions in Bulgaria*

Despite of the extensive research on the subject it is difficult to determine the capability of *D. suzukii* to overwinter at low temperatures because of various factors influencing its cold hardiness which may contribute to increased tolerance. In eastern Asia the area of distribution of *D. suzukii* to the north includes locations where winter temperatures reach -35°C (EPPO, 2010).

The host range of the species includes wild and cultivated fruiting plants of which the most economically important crops are cherries, strawberries, raspberries, blackberries, *Vaccinium* spp., peaches, plums, grapes and apricots. Only damaged pear and apple fruits are prone to infestation. The presence of many host species in Bulgaria facilitates establishment. In 2014 the cropping area for fruit tree hosts

of *D. suzukii* in the country was 47 269 ha or 46% of the total area for fruit tree cultivation (Table 1). The total areas of cultivated host plants are largest in the Southwestern, Southeastern and South Central regions of Bulgaria. Adults can fly from fruit waste, warehouses and transport vehicles to a suitable host. The fist high-risk month with respect to the spread of *D. suzukii* is May when early varieties of cherries ripen. In June and July the available suitable hosts are some berries (strawberries, raspberries) and in July – August these are *Vaccinium* spp., figs, peaches and apricots. At the end of summer *Vaccinium* spp. continue to ripen along with *Aronia* spp. and grapes. Until the end of September – beginning of October suitable hosts are different varieties of grape, blackberries, remontant varieties of raspberries and strawberries, etc. The suitable hosts differ depending on the time of the year but are available throughout the whole vegetation period.

The most important wild host of *D. suzukii* is blackberry which is distributed on the whole territory of the country. It grows in plains, along roads and fences, on dry hills and rocky outcrops. Another common species is *Prunus machaleb* L. which rarely dominates in phytocenoses but is present in various shrub and forest communities up to 1100 m. a.s.l. (Gusev and Tsonev, 2011). The three *Vaccinium* spp. present in Bulgaria are preferred hosts of *D. suzukii* and are mainly distributed in wild plant communities. *Vaccinium uliginosum* L. is found in all high mountains from 1700 to 2200 m a.s.l. *V. myrtillus* L. is distributed in all Bulgarian mountains, excluding Strandzha, at elevations from 1000 to 2200 m a.s.l. *Vaccinium vitis-idaea* L. grows at 1600–2200 m a.s.l. (Vitakova and Rusakova, 2011). In July and August when temperatures in Bulgaria increase and humidity decreases, mountain strawberries, raspberries, blackberries and *Vaccinium* spp. can provide sufficient resources for feeding and reproduction of *D. suzukii.*

Kanzawa (1939) observed the seasonal population dynamics of adult fruit flies on cherries and grapes, determining high abundance in June and July, followed by a rapid decrease in August, increase in September and October and gradual decrease in the beginning of winter. The author notes that the two peaks in abundance correspond to the period of ripening of cherries and grapes in Japan.

Multiple *D. suzukii* females can lay a total of up to 60 eggs in one fruit, each laying no more than 2 – 3 eggs. Thus a single fruit may contain a sufficient number of non-related individuals to establish a new population without inbreeding which may affect their adaptive potential (Damus, 2009).

After establishment, short-distance spread of the species (from orchard to orchard) can occur through active flight. Although research on the topic is lacking for the specific pest, studies on related species of family Drosophilidae show that fruit flies from one generation can actively spread at distances of up to 45 km (Johnston and Heed, 1976). Coyne et al. (1987) evaluated the spread potential of three species of *Drosophila* in desert conditions and established that released flies were able to cover the large distances from oasis to oasis. In the same study *D. pseudoobscura* was found 26 km away from the release site. Mitsui et al. (2010) investigate the migration of *D. suzukii* from low to high altitudes but do not report exact distances.

D. suzukii can also be carried by wind. Long-distance spread occurs mainly through transport with infested fruit of host plants.

Monitoring. In Bulgaria *D. suzukii* has been monitored since 2012 but the first individuals were trapped in June, 2014 (Table 2) in an orchard in Blagoevgrad region using a lure with red wine. All trapped individuals were males. Then in October, at the market of the village of Parvenets near Plovdiv, more males were trapped with the same type of lure.

Table 1. Main cropping areas of some main hosts of *D. suzukii* in Bulgaria in 2014 (ha) (Agrostatistics, 2014)

Area/Crop	North-western	North Central	North-eastern	South-western	South Central	South-eastern
Raspberries	164	114	428	197	213	75
Strawberries	124	309	38	76	110	15
Cherries	33	327	230	1 868	1 122	2 676
Peaches and nectarines	91	262	246	622	273	1 645
Grapes	2 973	1 970	2 541	3 300	10 319	10 789
Plums and cherry plums	575	947	542	328	1 643	84

Adult males and females were captured using fruit-fly Tephri-traps (with Biolure attractant) in the regions of Blagoevgrad, Kiustendil and Plovdiv. During the period November 20–27 adults of *D. suzukii* were captured in a warehouse for storage of fruit imported from Turkey, Greece and Poland using Jackson traps with a trimedlure attractant. In 2014, a total of 76 adult individuals were trapped at four monitored sites in the country. The sex ratio was 3.22 males to 1 female.

The monitoring continued through 2015. In June, in the region of Blagoevgrad, one male individual was trapped using apple cider vinegar (Table 2).

Table 2. Results of the monitoring of *D. Suzukii* in Bulgaria in the period 2014–2015

№	Date	Crop/Market	Settlement/ Region	***Trap type	Number of sampling	Number of traps	♂♂ Number of males	♀♀ Number of females
2014								
1	19-VI	Cherries and peaches	Blagoevgrad	RW	1	1	2	0
2	IX	Peaches and apples	Belo Pole Blagoevgrad	TEP	1	1	1	
3	IX	Plums, cherries, peaches and apples	Sheytanitsa Blagoevgrad	TEP	2	1	2	2
4	IX	Plums	Tarnovlag Kiustendil	TEP	2	1	2	0
5	7-XI	Market 1*	Prvenetsh Plovdiv	RW	10	1	1	0
6	X	Apples	Kalekovets Plovdiv	TEP	4	3	7	3
7	X	Apples and peaches	Voyvodinovo Plovdiv	TEP	4	3	39	6
8	20-27-XI	Market 2**	Varna	JA	2	2	4	7
9	27-XI	Market 2**	Varna	JA	1	1	0	2
Total numbers of adult individuals captured in 2014: 58 males and 18 females								
2015								
10	10-VI	Cherries	Blagoevgrad	ACV	1	1	1	0
11	1-IX	Plums	Rupite Blagoevgrad	JA	1	1	2	1
12	1-X	Apples	Voyvodinovo Plovdiv	TEP	1	1	0	1
12	17-VI	Apples	Kalekovets Plovdiv	TEP	1	1	1	0
13	25-VIII	Apples	Kalekovets Plovdiv	TEP	1	1	1	0
14	1-X-	Apples	Kalekovets Plovdiv	TEP	1	4	296	45
15	1-X	Raspberries	Tsalapitsa Plovdiv	RIG	1	2	165	81
Total numbers of adult individuals captured in 2015: 466 males and 128 females								

**(Market 1: fruits imported from Greece, Italy, Macedonia, Poland and Turkey, – apple, apricot, pear, fig, kiwi, citruses)*

***(Market 2: fruits imported from Greece, Poland and Turkey, – lemon, orange, grape, apple, pear)*

*******Traps:*** *RW – trap with attractant red wine, ACV – trap with attractantapple cider vinegar;*

TEP – Tephri-Trap with Biolure attractant; JA –Jackson delta trap with trimedlure attractant; RIG – Riga trap

Adults were also captured using Jackson traps, Tephri-traps and Riga traps in the village of Rupite and three locations in Plovdiv region (Voivodinovo, Kalekovets and Tsalapitsa). A total of 594 adult individuals of *D. suzukii* were trapped at five monitored sites. The ratio between the sexes was 3.6 males to 1 female.

In the two years of observation, a total of 670 adults were collected. Males were 3.5 times more abundant than females. The highest number of captured adults of *D. suzukii* was recorded for Tehpri-traps, followed by the specialized Riga traps. In the region of Plovdiv the number of trapped flies was significantly higher in comparison to Blagoevgrad and Kyustendil. The highest number of individuals was recorded in the village of Kalekovets in apple orchards (Table 2). Apple is not a preferred host of *D. suzukii* and only damaged fruit are infested. In the village of Tsalapitsa adults were captured using Riga traps in plantations of raspberries which are preferred hosts. The high established ratio between male and female individuals and the low number of trapped flies in spring suggest that the traps and their method of use are not sufficiently effective.

Reliable methods for detection and monitoring of *D. suzukii* have not yet been developed (Cini et al., 2012). It has not been established whether the species produces pheromones to attract partners and there are no specific pheromone traps for detection (Walsh et al., 2011). Different fruits and fruit products have been tested for their effectiveness in trapping the fly, including a mixture of brown sugar, alcohol, vinegar and water, ripe bananas, strawberry puree, apple cider, baker's yeast, apple vinegar, sugar and water (Wu et al., 2007; EPPO, 2010; Burrack, 2011; Isaacs et al., 2011; Ohrn & Dreve, 2011). For the purposes of monitoring of *D. suzukii,* Skinkis (2009) recommends the use of apple cider vinegar or sweet white wine in combination with yellow sticky traps.

The evaluation of trap effectiveness should also include other factors such as production price (Lee et al., 2012; Mazzetto et al., 2015). Currently it is recommended to use apple cider vinegar as a relatively cheap and effective attractant (Wu et al., 2007; Kleiber et al., 2011; Mazzetto et al., 2015).

Wu et al. (2007) establish that the effectiveness of the lure containing brown sugar, alcohol and vinegar improves when it is hanged next to a red ball. Kleiber et al. (2011) perform a multiple-year experiment to compare the effectiveness of differently coloured traps in attracting *D. suzukii.* Eventhough the attractiveness of the colours varies, red traps prove to be most attractive and transparent traps are least attractive for the flies. Other authors (Basoalto et al., 2013; Lee et al., 2013) also establish high effectiveness for red traps, while the studies of Palese (2014) and Mazzetto et al. (2015) do not confirm such dependence. It may be that the wavelength of the red colour also influences the attractiveness of the trap.

Various reports suggest that the number of trapped individuals of *D. suzukii* does not reflect the actual abundance of flies in orchards (BCMAL, 2010; Wilson, 2011). Kleiber et al. (2011) prove that the current method for monitoring is ineffective at low degrees of infestation and producers may observe damages while the set traps remain empty. Traps positioned in a cherry orchard and three strawberry plantings with infested fruit captured few or no individuals (OSU, 2010; Wilson, 2011).

Further research is necessary to develop highly sensitive traps that reflect the actual abundance of the species (Lee et al., 2013). The lack of effectiveness of the traps in areas with infested fruit is due to poor trap setting, non-compliance to the protocols for trapping and the presence of aromas of large quantities of ripe fruits diminishing the attractiveness of the lure.

CONCLUSIONS

1. The analysis of the results of the climatic model shows that the pest can establish on the territory of Bulgaria and develop 3–7 generations per year. Seven generations could develop in the northern parts of the Danubian Plane, the Black Sea coast, the southern parts of the regions of Blagoevgrad, Haskovo and Yambol and parts of Stara Zagora and Plovdiv regions.

2. Currently there are no effective means for detection and monitoring of *D. suzukii* and it is likely that the results of the monitoring programme do not reflect the actual distribution of the species in the country. In the two years of observation, a total of 670 adults were collected. Captured males were 3.5 times more abundant than females. The highest number of captured adults of *D. suzukii* was recorded for Tehpri-traps, followed by the specialized Riga traps. The high male to female ratio and the ineffective trapping in spring are probably due to the development of transient populations of the species or low effectiveness of the traps. No damages from *D. suzukii* on fruit crops in the country were established.

3. The number of individuals captured in Plovdiv region was significantly higher in comparison to Blagoevgrad and Kiustendil. The developed predictive model for Plovdiv region indicating up to 7 gene-rations per year was confirmed by the monitoring data. There the pest was established at the highest number of monitored sites and with highest abundance. In the following years the monitoring programmes should use traps specialized for *D. suzukii* and a variety of lures.

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