Variation in the susceptibility of blackberry varieties
to Drosophila suzukii infestation

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Abstract

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is an invasive polyphagous species, that is frequently found in blackberry culture, affecting the commercial value of fruits. Between 2020-2021, in the blackberry collection of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (Romania), were carried out observations on susceptibility of five cultivated varieties (‘Thornless’, ‘Loch Ness’, ‘Thornfree’, ‘Thornless Evergreen’ and ‘Navaho’) to D. suzukii. In 2020, the attack frequency in the whole collection was 78.2%. ‘Thornfree’ had the highest attack frequency, 89% while ‘Loch Ness’ was the least susceptible with a frequency of attack of 71%. In 2021, the overall proportion of damaged fruits in the collection was 59.6%. The most susceptible was ‘Thornless’ with an attack frequency of 66% and ‘Thornless Evergreen’ with only 49%. The incidence of infestation by the spotted-wing Drosophila suzukii is influenced by the thermal characteristics of the year, with temperatures higher than 30 °C impacting the fertility of females and the viability of eggs. In the year 2020, the average number of larvae per fruit collected was 6.4, while the average number of larvae in the ‘Loch Ness’ cultivar was 9.4, with a maximum of 48 larvae per fruit. In 2021, an average of 3.4 larvae per fruit were observed, with variations ranging from 2.8 larvae in the ‘Thornfree’ cultivar to 4.2 larvae in the ‘Navaho’ cultivar.

Keywords: blackberry; Drosophila suzukii; pest; varieties

Introduction

Invasive alien species are accidentally introduced outside their natural range and then spread, resulting in negative impacts on the new ecosystem (Roy et al., 2011). The penetration of invasive alien species on the European continent is also facilitated by climate change, especially global warming (Hrubik and Kollár, 2007). These species can sometimes even cause the decrease of biodiversity (Bellard et al., 2016; Paini et al., 2016).
The genus *Drosophila*, contains over 1600 species (O’Grady and DeSalle, 2018) and most of them feed on degraded fruit (Swoboda-Bhattarai and Burrack, 2020).

Compared to other dipteran species that are of interest for their beneficial role in ecosystems (Macavei *et al*., 2020), the spotted wing drosophila, *Drosophila suzukii* Matsumura (SWD) is an invasive species that causes severe economic losses in fruit production worldwide (Rego *et al*., 2017). SWD native to eastern and southeastern Asia (Walsh *et al*., 2011), subsequently the pest spread to other continents. In Europe, the first record of the species was in 2008 in Spain, in the Bellaterra area, near Barcelona (Grassi *et al*., 2009). In September 2013, it was detected for the first time in Romania, in a Tephri trap located on wild blackberry (*Rubus caesius* Linné) in the northern part of Bucharest (Chireceanu *et al*., 2015).

The blackberry fruit, owing to its intricate and high-quality chemical composition, is widely esteemed by consumers for its manifold beneficial impacts on human health, including the reduction of cardiovascular disease risk, immune system reinforcement, cancer prevention, and alleviation of diabetes, as well as the maintenance of neurological health, strengthening of bones, protection of ocular tissues, and purgative effects, among others (Zafra-Rojas *et al*., 2018; Jara-Palacios *et al*., 2019).

The cultivation of blackberry is on the rise throughout Romania, both on agricultural land and in private gardens. Given its polyphagous nature and rapid spread, early detection of the presence of the pest is crucial to ensure timely and effective intervention.

The present study investigates the susceptibility of various blackberry cultivars to infestation by SWD and quantifies the number of larvae that may be found within a single fruit.

**Distribution of *Drosophila suzukii* M.**

SWD is a rapidly spreading pest that causes severe economic losses, making it a matter of great concern (Cini *et al*., 2012). Since 2010, the species has been included in the A2 List of quarantine pests (Lee *et al*., 2011), with the primary mode of introduction being the importation of fruits (Anfora *et al*., 2012). The worldwide trade in fresh fruit, coupled with the ability of the larvae to remain hidden within the fruit until after transportation, promotes the expansion of this pest (Gerdeman and Tanigoshi, 2011).

The danger of spreading is constantly increasing due to the free movement of goods within the European Union and the reduction of customs controls at border crossings (Tomov and Trencheva, 2013; Minkov *et al*., 2017). Calabria *et al.* (2012), estimated that SWD can spread approximately 1400 km annually, with international transport along highways playing a key role in its dissemination, facilitating the spread of approximately 320-390 km per year (Lengyel *et al*., 2015). Research using marked specimens has shown that adults can fly up to a distance of approximately 9 km (Tait *et al*., 2018).

Adults have a high power of adaptability. They are found in Spain and the Alpine regions of Japan. However, this tolerance is still unclear, whether physiological or adaptable to climatic conditions (Kimura, 2004).

**Description of *Drosophila suzukii* M.**

Females have a body of yellow or brownish colour. Their size is between 3.2-4 mm but does not exceed 4 mm (Calabria *et al*., 2012).

The antennae are short and round with branched aristles. The eyes are red. The wings are transparent without having portions enclosed in colour around the ribs. The last abdominal segment has a series of uninterrupted transverse bands. It does not show spurs on the legs. The ovipositor is sharp in the form of a saw with teeth darker in colour than the rest of the ovipositor (Hauser, 2011).

The male has a body length of about 2.6-2.8 mm and a yellow or brownish colour (Calabria *et al*., 2012). The eyes are red. On the wings, it presents a dark spot located towards the apex, centred on the leading wing edge. Males can also appear in populations without this stain. The last abdominal segment shows a series of uninterrupted transverse bands of a blackish colour.
Many of the morphological characteristics of this species can be found in others (*D. ashburneri* Tsacas, *D. immaculares* Okada, *D. lucipennis* Lin, *D. mimetic* Bock et Wheeler, *D. oshimai* Choo et Nakamura, *D. subpudrella* Takamori and *D. unipectinata* Duda). For identification, are used the male’s spurs on the pretars, the shape and length of the female’s ovipositor, and the most significant is the analysis of mitochondrial DNA (Naserzadeh et al., 2020).

The egg is oval, translucent, glossy white, 0.6 mm long, and 0.2 mm wide, and in the subapical part, two filaments of white colour can be seen (Toševski et al., 2014).

The larva is white, cylindrical, with the parts of the buccal apparatus black. The body is conical in the anterior part with prominent respiratory stigmas. Three larval ages vary between 0.7-3 mm in length. The internal organs are visible, especially after the larva feeds (Calabria et al., 2012).

The pupa is about 2-3 mm long and 1 mm wide. At first, it has a greenish colour, then brownish, and in the end, it becomes yellowish (Walsh et al., 2011).

**Biological cycle of *Drosophila suzukii* M.**

The species is polyvoltine. The number of generations it can develop in a year is between 3 and 15, depending on climatic conditions (Cini et al., 2012).

SWD winters as an adult in hidden places, but under proper conditions, the species is active all year round (Dalton et al., 2011). Adults start their activity at temperatures higher than 10 °C and are very active at temperatures between 20-25 °C. Temperatures above 30°C determine the reduction of egg laying and the rate of hatching of eggs, the process of pupation of the larvae and the appearance of new adults (Kinjo et al., 2014; Tochen et al., 2014; Seljak et al., 2015).

The adults make their appearance in the spring, although some of them can also be active during warm winter days. After coupling, the female begins laying eggs. Unlike other species of the Drosophilidae family, SWD prefers laying eggs on ripe and healthy fruits instead of very ripe or rotten fruits (Belien et al., 2014; Swoboda-Bhattarai and Burack, 2020). The prolificacy of females is between 200 and up to 400 eggs (Baroffio et al., 2014; Tochen et al., 2014), and takes place from April to November, once the fruits have begun to colour (Minkov et al., 2018). The egg is deposited in the perforation made in the fruit by the female with the ovipositor (Iglesias and Liburd, 2017).

Eggs hatch in 1-3 days, the larvae reach maturity in 3-13 days. Larvae cannot replenish their development in fermented fruits (Tochen et al., 2014).

Pupation of the larvae occurs within the fruit, although a portion of the larvae may also undergo pupation in the soil originating from fallen fruits. The duration of the pupation period ranges from 4 to 43 days (Tochen et al., 2014).

**Host-plants and damages**

SWD is a polyphagous species that attacks a wide range of cultivated and spontaneous plants, preferring soft fruits. After Kenis et al., 2016, the fly develops on 84 species of plants belonging to 19 families, and Minkov et al., 2018, show that it attacks 90 species of plants belonging to 23 families.

Mainly the host plants belong to the following families: Actinidiaceae, Caprifoliaceae, Cornaceae, Ebenaceae, Elaegnaceae, Ericaceae, Grossulariaceae, Lauraceae, Moraceae, Myricaceae, Myrtaceae, Phytolaccaceae, Rhamnaceae, Rosaceae, Rutaceae, Solanaceae and Vitaceae (Mitsui et al., 2010; Grassi et al., 2011; Bellamy et al., 2013; Saguez et al., 2013; Yu et al., 2013; Poyet et al., 2015; Lee et al., 2015; Nagy et al., 2020).

This species causes severe damage to blackberries and raspberries, fruits that have high antioxidant activity due to anthocyanin and phenolic compounds (Bowen-Forbes et al., 2010), nevertheless, it is considered
a key pest for other several fruit productions (Asplen et al., 2015) such as plums, peaches, cherries, strawberries, blueberries or grapes (Grassi and Pallaro, 2012; Ros et al., 2013).

Depending on the ripening of the host plants, the species migrates from one crop to another if they are nearby (Cahenzli et al., 2018; Weißinger et al., 2019). In addition, a population migration also occurs from wild shrubs near the crop plant (Diepenbrock et al., 2016).

As previously mentioned, the larvae of SWD cause damage to various plants, with a preference for soft and undamaged fruit (Keesey et al., 2015). However, in the absence of suitable fruit, the pest may attack decomposing or damaged fruit (Lee et al., 2011). Infestation of unripe fruit by the pest is a rare occurrence (Arnó et al., 2012).

As a consequence of the infestation, the fruit is exposed to microorganisms that cause damage, rendering it unsuitable for commercial use (Goodhue et al., 2011).

**Materials and Methods**

The studies regarding the attack produced by *Drosophila suzukii* Matsumura on different blackberry varieties, were carried out in the blackberry collection of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania. The geographical coordinates of the area are 46°46′0″N, 23°35′0″E.

In the vicinity of the blackberry collection is located according to the geographical orientation: to the east - the Botanical Garden of the university; to the west - the university orchard with collections of apple, pear, cherry and vine; in the north - a forest with various species of deciduous, resinous and wild shrubs; in the south - the dendrological park of the university.

The blackberry varieties taken under study were: Thornless, Loch Ness, Thornfree, Thornless Evergreen and Navaho. All varieties are devoid of thorns.

To determine the timing of the emergence of SWD adults within the blackberry cultivars, food bait traps were employed. The food bait was composed of apple cider vinegar (75%), Merlot wine (25%), brown sugar, and liquid apple soap (Iglesias et al., 2014), and was installed one month prior to fruit ripening (Mazzetto et al., 2015; Lasa et al., 2017). The food bait used is known to exhibit a high degree of attractiveness (Cha et al., 2012; Landolt et al., 2012). Food bait traps were set on June 1 each year and read weekly. In 2020, on August 3, the first adults were reported and in 2021, on August 16, the first adults were reported.

Further, for each of the five blackberry cultivars, the incidence of infestation and number of larvae per infested fruit were assessed. Three sets of 100 fruits were randomly harvested and subsequently analysed in the Entomology laboratory at the University. The number of larvae present within each infested fruit was also recorded. On September 17, 2020, 100 attacked fruits of each variety were harvested in 3 repetitions. On September 29, 2021, 100 attacked fruits of each variety were harvested in 3 repetitions.

No chemical treatments were applied during the two experimental years.

Finally, the data collected was statistically processed with the statistical softwares ANOVA followed by LSD test.

**Results**

*The frequency of the attacked fruits*

In 2020, the first detection of adults in the blackberry cultivars were made on August 3, during which the fruits were in the first phenophase for ripening (Figure 1). After that, females were observed laying eggs on blackberry fruits (Figure 2). Infested fruit typically exhibits a scar at the location of egg deposition, and may
also undergo deformations as a result of the infestation, thus, the fruit sampling for analysis was conducted only in September 17.

![Figure 1](image1.png)

**Figure 1.** Capture of *Drosophila suzukii* Matsumura adults by traps: a) food bait trap b) captured adults (Cluj-Napoca, 2020)

![Figure 2](image2.png)

**Figure 2.** Female of *Drosophila suzukii* Matsumura laying eggs on blackberry fruits (Cluj-Napoca, 2020)

In 2021, vegetation development was delayed in comparison to the preceding year, owing to prevailing weather conditions. In this case, the first instances of capture were observed during the latter half of August, with a mass emergence of SWD adults within the blackberry collection occurring between September 6 and 13. On September 7, fruits at varying stages of ripeness, including both unripe and fully ripened fruits, were found to be infested with SWD larvae, with most of the larvae present in the fully ripened fruit. The fruit samples were collected for analysis on September 29.

Figure 3 shows the frequency of the attacked fruits in the five varieties during the two years of monitoring.
In 2020, the highest level of infestation of fruits was reported in the ‘Thornfree’ variety; the average was 89% (with oscillations between 85% and 91%), while the ‘Thornless Evergreen’ variety, the attack frequency was between 76% and 85%, with an average of 82%. The Least susceptible was the ‘Loch Ness’ variety. The average was 71% (with oscillations between 61% and 76%), followed by the ‘Navaho’ variety with an average of 72% (oscillations between 64% and 82%). The overall average frequency of fruits attacked this year was 78.2% of the entire blackberry collection.

In 2021, the level of infestation was much lower than the previous year for all varieties (Figure 3). On the entire blackberry collection, the frequency of attack this year was 59.6%, a decrease of 18.6 percentage points, which represents a reduction of 23.8%. Among the varieties, the average frequency of the attacked fruits was between 49% for the ‘Thornless Evergreen’ variety (with oscillations between 42% and 61%) and 66% for the ‘Thornless’ variety (with oscillations between 61% and 73%). It is found that this year there were several differences compared to the previous year in terms of the frequency of attack on the blackberry varieties.

Compared to the previous year, the attack frequency decreased by 11.1% for the ‘Navaho’ variety, 14% for the ‘Thornless’ variety, 22.5% for the ‘Loch Ness’ variety and 28.1% for the ‘Thornfree’ and by 40.2% for the ‘Thornless Evergreen’ variety.

However, from a statistical point of view between the five varieties of blackberry analysed, there are no significant differences in the frequency of the attack produced by Drosophila suzukii Matsumura (F=0.9503, df = 4, p > 0.05) (Figure 4).
Figure 4. Statistical difference between the five varieties of blackberry concerning the attack frequency (Cluj-Napoca, 2021)

There are very significant differences between the two experimental years (F= 29.6779, df = 1, p < 0.001) (Figure 5).

Figure 5. Statistical difference between the five varieties of blackberry concerning the attack frequency (Cluj-Napoca, 2021)
The second objective of this study was to analyze the number of larvae reported in a fruit (Figure 6).

![Figure 6. Larvae of Drosophila suzukii Matsumura in blackberry fruit (Cluj-Napoca, 2020)](image)

In 2020 there were identified eight groups of fruit infestation level per variety (Table 1). The group with 1-5 larvae/fruit had the most fruits, their proportion being between 43.2% ('Thornfree' variety) and 69.5% ('Thornless evergreen' variety), and the average within this group was 54.4% of the attacked fruits. A high percentage was also reported in fruits with 6-10 larvae/fruit, which accounted for 27.7%. A lower proportion of fruits containing more than 21 larvae per fruit was observed, with this phenomenon being more prevalent in the 'Loch Ness', 'Thornfree', and 'Navaho' varieties.

<table>
<thead>
<tr>
<th>No of larvae/fruit</th>
<th>'Thornless'</th>
<th>'Loch Ness'</th>
<th>'Thornfree'</th>
<th>'Thornless Evergreen'</th>
<th>'Navaho'</th>
<th>% attacked fruit/variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>66.2</td>
<td>43.7</td>
<td>43.2</td>
<td>69.5</td>
<td>51.4</td>
<td>54.4</td>
</tr>
<tr>
<td>6-10</td>
<td>28.6</td>
<td>19.7</td>
<td>33.0</td>
<td>20.8</td>
<td>34.7</td>
<td>27.7</td>
</tr>
<tr>
<td>11-15</td>
<td>3.9</td>
<td>18.3</td>
<td>17.1</td>
<td>8.5</td>
<td>11.1</td>
<td>11.9</td>
</tr>
<tr>
<td>16-20</td>
<td>1.3</td>
<td>7.1</td>
<td>3.4</td>
<td>1.2</td>
<td>1.4</td>
<td>2.8</td>
</tr>
<tr>
<td>21-25</td>
<td>-</td>
<td>5.6</td>
<td>1.1</td>
<td>-</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>26-30</td>
<td>-</td>
<td>2.8</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
<td>0.8</td>
</tr>
<tr>
<td>31-35</td>
<td>-</td>
<td>1.4</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>45-50</td>
<td>-</td>
<td>1.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.3</td>
</tr>
</tbody>
</table>

During the year 2021, the incidence of attacks had decreased compared to the previous year, only five groups or infestation level per variety were identified (as indicated in Table 2). Among these, the group with 1-5 larvae per fruit was the most prevalent, constituting between 69.9% (for the 'Navaho' variety) and 88.9% (for the 'Thornless' variety), with an overall average of 80.7% of fruits being affected. Additionally, a relatively high percentage of fruits with 6-10 larvae per fruit (16.3%) were also observed. Fruits with more than 11 larvae per fruit were comparatively rare.
Table 2. Percentage of blackberry fruit rendered according to the number of spotted wing drosophila larvae contained/attacked fruit (Cluj-Napoca, 2021)

<table>
<thead>
<tr>
<th>No of larvae/fruit</th>
<th>% fruit</th>
<th>% attacked fruit/variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>80.0</td>
<td>83.3</td>
</tr>
<tr>
<td>6-10</td>
<td>18.5</td>
<td>14.8</td>
</tr>
<tr>
<td>11-15</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>16-20</td>
<td>-</td>
<td>1.9</td>
</tr>
<tr>
<td>21-25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1-5</td>
<td>80.0</td>
<td>83.3</td>
</tr>
<tr>
<td>6-10</td>
<td>18.5</td>
<td>14.8</td>
</tr>
<tr>
<td>11-15</td>
<td>1.5</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 7 shows the maximum number of larvae that have been reported in a fruit. In 2020, this parameter was between 17 larvae in the ‘Thornless’ variety and 48 larvae in the ‘Loch Ness’ variety, and in 2021, between 11 larvae in the ‘Thornfree’ variety and 21 larvae in the ‘Navaho’ variety.

Assessment of attacked fruits
We also proceeded to determine the average number of larvae in an attacked fruit (Figure 8).
In 2020, the average number of larvae/fruit was between 4.4 larvae in the 'Thornless' variety. In the 'Loch Ness' variety the average number was 9.4 larvae, varying between 7.6 and 11, being the same variety, where the maximum number of larvae in one fruit - 48 larvae, was reported. In the whole blackberry collection, this parameter was 6.4 larvae/fruit attacked.

In 2021, the average number of larvae/fruit was between 2.8 in the 'Thornfree' variety and 4.2 in the 'Navaho' variety. In the whole blackberry collection, this parameter was 3.4 larvae/fruit attacked. Compared to the previous year, the average number of larvae/fruit generally decreased as follows: in the 'Thornless' variety with 20.5%, in the 'Navaho' variety with 26.3%, in the 'Thornless Evergreen' variety with 32.7%, in the 'Thornfree' variety with 63.6%, in the 'Loch Ness' variety with 66% and on the overall average with 46.9%. However, from a statistical point of view between the five varieties of blackberry analysed, there are no significant differences in the average number of larvae of SWD observed per one fruit (F=1.1036, df = 4, p > 0.05) (Figure 9).
Figure 9. Statistical difference between the five blackberry varieties concerning the average number of larvae of *Drosophila suzukii* Matsumura/attacked fruit (Cluj-Napoca, 2021)

There are very significant differences between the two experimental years ($F = 25.4246$, $df = 1$, $p < 0.001$) (Figure 10).

Figure 10. Statistical difference between the two experimental years concerning the average number of larvae of *Drosophila suzukii* Matsumura/attacked fruit (Cluj-Napoca, 2020-2021)
Discussion

In 2020, the unusually high frequency of attacks can be attributed to two factors. Firstly, the 'Thornfree' and 'Thornless Evergreen' varieties were situated within the plantation, and it is known from literature that the frequency of attacks is usually greater in the central region of the plot, where atmospheric humidity tends to be higher compared to the edges (Diepenbrock and Burrack, 2017). Secondly, these varieties are characterized as late ripening, which increases the likelihood of attack due to the population growth of pests throughout the year. This phenomenon has been reported in blackberries, blueberries and raspberries (Baroffio et al., 2014). The 'Thornfree' variety ripens in a staggered season from the end of July to September. 'Thornless Evergreen' is a late variety in which ripening begins from the second decade of August and lasts until October.

The 'Loch Ness' and 'Navajo' varieties are the earliest to ripen. 'Loch Ness' variety is medium ripening, starting from mid-July, and in the 'Navaho' variety, the ripening period is from the last decade of July to September. Moreover, the dissimilarities in the frequency of attacks observed in these varieties may have alternative explanations. The selection of a host plant, including the choice of a specific variety within a species, can be influenced by various factors such as fruit firmness (with softer fruits being more susceptible to infestation compared to firmer ones) and the soluble sugar content (Burrack et al., 2013).

As observed by other researchers (Diepenbrock et al., 2016; Cahenzli et al., 2018; Weißinger et al., 2019), the placement of a particular variety can facilitate the movement of adult pests from other neighbouring host plants. In the case of SWD, the cherry, raspberry, and vines grown in close proximity to the blackberry collection are among the preferred host plants. As the blackberries ripen, the pest population tends to migrate towards these fruit bushes that ripen later. Additionally, the pest population may also migrate from wild shrubs in the forested area and the dendrological park of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca.

The decrease in attack frequency in 2021, demonstrates that subtle variations in ecological factors can have a determining character in the bioecology of a species. The climatic features of 2021, especially the temperature factor, were unfavourable to the pest, as other authors have shown (Kinjo et al., 2014; Tochen et al., 2014; Seljak et al., 2015). The high temperatures in August and September affected the population of Diptera, both in the parcels adjacent to the cultivated variety and inside the plantation. For example, in the first decade of August, there were six consecutive days in which the temperature between 26-30 °C was for 8 to 12 hours of the day and in the second decade, there were eight consecutive days with such a temperature.

In the present study it was observed that fruits with a high number of larvae are susceptible to mold due to the installation of microorganisms, rendering them unsuitable for marketing. This phenomenon has been observed to occur annually and has been documented by other authors (Goodhue et al., 2011) as well.

Conclusions

The level of infestation by SWD in the blackberry collection at the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca (Romania) in 2020 was 78.2%, and in 2021 59.6%, with oscillations between the varieties. The most susceptible variety in 2020 was reported in the 'Thornfree' variety and in 2021 in the 'Thornless' variety. The average number of larvae/fruit attacked was 6.4 in 2020 and 3.4 in 2021. The maximum number of larvae reported in an attacked fruit in 2020 was 48 larvae and in the year 2021, 21 larvae respectively. 'Loch Ness' and 'Thornless Evergreen' were found to be the least affected varieties, and therefore, it is strongly recommended to utilize them in crop production, as they have demonstrated favourable productive performance.
Authors’ Contributions


Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research and the APC was funded by Doctoral School of Agricultural Engineering Sciences of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (Romania). The first author Ionuț-Bogdan Hulujan (I.B.-H.) is PhD candidate coordinated by prof. Teodora Florian (T.F.) and this study is a part of his thesis.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References


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