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SHORT COMMUNICATION

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Laboratory evaluation of nine highbush blueberry cultivars susceptibility to *Drosophila suzukii* (Matsumura, 1931) in the Southwestern Spain

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Abstract

Aim of study: To determine how susceptible the most used Southern highbush blueberry (SHB) cultivars were to the spotted wing Drosophila (SWD), *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) as well as those recently introduced to Southwestern Spain.

Area of study: Southwestern Spain (Huelva province).

Material and methods: Nine of the SHB cultivars which were recently introduced in Southwestern Spain and the most used ones were selected: 'Arana', 'Camellia', 'Kirra', 'Mayra', 'Misty', 'O'Neal', 'Sharpblue', 'Star' and 'Ventura'. In order to determine how susceptible the cultivars were to SWD, no-choice tests were performed under laboratory conditions. In addition, berry size, berry firmness, ºBrix, and pH were recorded in order to assess what influence these variables had on oviposition preference by SWD.

Main results: Mean clutch size and mean number of emerged adults in 'Star' were significantly higher than in the other tested cultivars. 'Mayra', 'Camellia' and 'Ventura' received the lower clutch sizes and mean number of emerged adults. Mean developmental time (egg to adult) differed significantly among tested cultivars and were highest in 'Camellia' than in the other tested cultivars. Only firmness and pH were correlated with SWD infestation as females tend to oviposit more eggs in softer fruits than in firmer fruits. Results also showed that a higher pH increased the emergence of adults and shortened the egg to adult developmental time.

Research highlights: Our results showed significant differences in the susceptibility of SHB to SWD. This information may help design IPM programs and in making recommendations for blueberry crops as planting of low-chill cultivars expands.

Additional keywords: spotted wing Drosophila; invasive pest; berry crops; pest-resistant; integrated pest management; sustainable agriculture.

Abbreviations used: GLMs (generalized linear models); SHB (Southern highbush blueberry); SWD (spotted wing drosophila).

Authors' contributions: Designed and performed the tests: JMM and LA. Wrote the paper: JMM and SPG. All authors contributed to the statistical analyses, read and approved the final manuscript.

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Introduction

The spotted wing Drosophila (SWD), *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae), is an invasive frugivorous pest native to South East Asia, which was first detected in Southern Europe (Spain and Italy) and continental USA in 2008, and currently affects a wide range of important crops in Europe and America, especially berries (Asplen *et al.,* 2015). Unlike other *Drosophila* species, SWD infests healthy ripening fruits, and inserts eggs with its serrated ovipositor (Walsh *et al.,* 2011) so larvae feed and develop inside the fruits, which then become unmarketable, resulting in dramatic reductions in fruit production and economical losses in berry production in Europe (Cini *et al.,* 2012) and North America (Goodhue *et al.,* 2011). Among the wide range of SWD hosts, blueberries are one of the most susceptible crops (Cini *et al.,* 2012; Gargani *et al.,* 2013) and 30%–40% of production is lost.

Southern highbush blueberry cultivars (SHB) are tetraploid interspecific hybrids developed in the USA by adding genes from several blueberry species to the Northern highbush blueberry (*Vaccinium corymbosum* L.). As it has lower chilling requirements and ripens earlier, SHB can be planted in drier and hotter climates (Lang, 1993; Brevis *et al.,* 2008). SHB cultivars are planted in significant amounts at present in the Southern states of North America (Florida, Georgia, and California), Mexico, Ecuador, Peru, Chile, Argentina, Australia, Morocco and Spain (Bañados, 2009; Lobos & Hanckonck, 2015; Scalzo *et al.,* 2016). In Southwestern Spain (Huelva province), SHB cultivars were introduced on a commercial scale in the 1990s. Currently, crop production is well established there and increasing as the local environment is suitable for them and they are highly profitable (Bañados, 2009). They occupy an area of approximately 3,410 ha and total production was 42,522 tons in 2017 (AGAPA, 2018). Hence, blueberries are an important socio-economic driver in the region.

The use of pest-resistant or tolerant varieties and hybrids in plant production is fundamental to Integrated Pest Management (IPM) programs, can significantly reduce the need for insecticides, and helps environmentally-friendly crop management. Selecting resistant crop cultivars or ones with a low susceptibility to pests is still a major agri-environmental concerns and a preventive method for reducing the cost of pesticide control and the negative effects of using synthetic products (Sharma & Ortiz, 2002). Several authors have analyzed the susceptibility of berries and other crops cultivars (blueberries, raspberries, blackberries, strawberries, cherries and grapes) to SWD (Lee *et al.,* 2011; Linder *et al.,* 2014; Ioriatti *et al.,* 2015; Gong *et al.,* 2016; Hemer *et al.,* 2016; Baser *et al.,* 2018). There has been little research into the susceptibility of blueberries to SWD. Lee *et al.* (2011) showed no significant differences among California blueberry cultivars in terms of number of eggs laid and developing SWD, although there were variations in development percentage (number of developing SWD/number of eggs laid) and these were higher in 'Star' than in 'Misty' and 'O'Neal' cultivars. Kinjo *et al.* (2013) showed softer fruits cultivars were more vulnerable to SWD females than firmer ones. Recently, Stringer *et al.* (2017) determined the range of resistance or non-preference available in sundry sources of blueberry germplasm and detected antibiosis in some of the blueberry hosts tested. In the berry market where new varieties are constantly developed and sold, it is crucial to know how resistant the crop cultivars that grow in a given region are when developing an IPM program, especially for crops in different geographical areas with varying soil types, climatic conditions and agronomic methods (Sharma & Ortiz, 2002). Thus, in this study there was an assessment of how susceptible the most used SHB cultivars were to SWD as well as those recently introduced to Southwestern Spain, with a no-choice test under laboratory conditions. Additionally, blueberry traits (fresh weight, firmness, pH and sugar content) were recorded in order to assess what influence these variables had on oviposition preference by SWD.

Material and methods

Insects and berries

The SWD adults used in the bioassays came from an experimental colony established in the IFAPA Las Torres Laboratory of Entomology (Alcalá del Río, Seville; Spain) from larvae collected from infested raspberry fields in Huelva (Southwestern Andalusia). The colony was reared on berry fruits (blueberries and raspberries). Individuals from naturally infested fruits (also collected in Huelva) were introduced into the colony several times during rearing to prevent endogamy, and ensure genotypic diversity. The insects were kept at 22 ± 1 Cº, 65% RH, and 16:8 h (L:D) photocycle in 0.3 m^3 cages (BugDorm® 1; Bio-Quip Products Inc., Rancho Rodríguez, CA, USA), and provided with 5% w/v brewer's yeast/dH_2O, and 10% w/v sugar-dH_2O as food sources. Nine of the SHB cultivars which were recently introduced in Southwestern Spain and the most used ones were selected: 'Arana', 'Camellia', 'Kirra', 'Mayra', 'Misty', 'O'Neal', 'Sharpblue', 'Star' and 'Ventura'. Berries from 'Camellia', 'O'Neal', 'Misty', 'Sharpblue', and 'Star' were collected from plants growing at the IFAPA 'El Cebollar' Experimental Station (Moguer, Huelva). Commercial berries from 'Arana', 'Kirra', 'Mayra', and 'Ventura' (FresDoñarosa; Superexport Cia. Agraria S.L.; Huelva, Spain) were used in the bioassays. Finally, fruit ripe for consumption from the nine cultivars were used in the bioassays.

Laboratory assays

In order to assess how susceptible the cultivars were to SWD, no-choice tests were performed under laboratory conditions. Berries were rinsed with distilled water, air dried, and examined before testing with a stereomicroscope (x20, Leica MZ6, Leica Microsystems, Germany) to ensure there were no bruises or SWD infestation. In each test, 8 berries were exposed for 24 hours to

5 female and 4 male SWD in a 0.3 m^3 cage (BugDorm® 1; Bio-Quip Products Inc., Rancho Rodríguez, CA, USA). After exposition, the berries were removed from the cage, and the number of eggs per berry (clutch size) were counted. Each fruit was separately placed in a polystyrene tube with a piece of moistened foam at the bottom and kept under laboratory conditions (22 ± 1 ºC, 60 ± 5% RH, and a 16:8 h (L:D) photocycle) for 21 days, recording the number of emerged adults. Clutch size, number of adults emerged, sex and the time from egg to adult development (days) were also recorded. A total of six replicates per cultivar was established.

Fruit samples (12-20 per cultivar) from the same batches in the no-choice test were analysed to measure weight (g fresh weight), firmness (skin penetration force, g mm^{-2}), pH and sugar content (ºBrix). Fruit firmness was measured in grams force (gf) using a manual penetrometer (Effegi® tr FDP 500 g; Italy) fitted with a 1 mm Ø blunted needle. For each blueberry, two measurements in the middle of the berry were recorded and averaged. Sugar content was determined by puncturing and squeezing individual fruits and placing a drop of juice on a portable refractometer (Eclipse®, Bellingham & Stanley Ltd., UK). After sugar content was recorded, juice was obtained by crushing the berries two by two, and pH was measured using a Crimson pH-meter basic 20 (Alella, Spain).

Statistical procedures

Since clutch size, number of adults emerged, and days of development, did not fulfil normality conditions nor linearity of residuals, generalized linear models (GLMs) were ran to test the effects of different cultivars using the R v. 3.1.3 software package. GLMs were carried out separately, including total number of eggs and total number of adults as dependent variables, and cultivars as the factor fitted to a Poisson distribution with a log link function. GLMs with interaction terms were also performed including cultivars and adult sex as factors, and days of development as the dependent variable fitted to a Poisson distribution with a log link function. Where differences were detected by GLM, multiple comparisons, post-hoc Tukey HSD tests (p<0.05) were performed using the "glht" function in the "multcomp" package. GLM procedures used the Wald statistic ("z") value and Pr([|z|) to analyse the effects each factor had on the response variable to test the hypothesis that the corresponding parameter (regression coefficient) was zero (Crawley, 2005).

Given that fruit weight, firmness, pH, and sugar content of berries matched normality and linearity of residuals, a one-way ANOVA (p<0.05) was used to determine differences in berry traits among cultivars. When statistical differences were detected, their means were separated by Tukey´s honestly significant difference (HSD) test (p <0.05) The correlation between berry traits and SWD infestation data were conducted using a non-parametric Spearman's rank correlation coefficient (ρ).

Results

Significant differences in mean clutch size and emerged adults were detected among cultivars (z=7.6, p< 0.001 and z=7.5, p<0.001 respectively; Fig. 1). Post-hoc tests showed that mean clutch size in 'Star' (64.7 ± 5.7 eggs per berry) was significantly higher than in the other tested cultivars (z=11.5, 13.7, 8.9, 10.9, 7.9, 11.7, 6.5 and -13.7 respectively; p<0.001 in all cases). 'Sharpblue' and 'Kirra' also showed high clutch sizes (30.3 ± 5.0 and 28.5 ± 9.3 eggs per berry, respectively) but there were no significant differences between them (z=0.5; p>0.05). Berries from 'Mayra', 'Camellia' and 'Ventura' had the lowest mean clutch size (4.3 ± 2.0, 7.0 ± 2 and 7.0 ± 1.6 eggs per berry, respectively; Fig. 1). A similar pattern was found with the emerged adults. A significantly higher number of flies emerged from 'Star' berries (39.7 ± 4.0 adults per berry) with respect to the other cultivars tested (z=9.7, 10.2, 7.9, 7.6, 5.7, 9.2, 4.9 and -10.7 respectively; p<0.001 in all cases). Once again, 'Mayra', 'Camellia' and 'Ventura' displayed the lowest mean number of emerged adults (2.3 ± 1.9, 2.2 ± 1.2 and 3.7 ± 1.0 adults per berry, respectively) with no significant differences between them (z= 1.5; p>0.05; Fig. 1).

Mean developmental time (egg to adult) was 14.4±0.1 days; with significant differences among cultivars (z=-2.4, p<0.05). Developmental time was higher in 'Camellia', and significantly different to that in the other cultivars (z=3.4, -3.8, -4.6, -3.9, -4.2, -3.3, -4.8 and -3.4 respectively; p<0.05 in all cases). Egg to adult developmental time was slightly shorter in males (14.3±0.1 days)

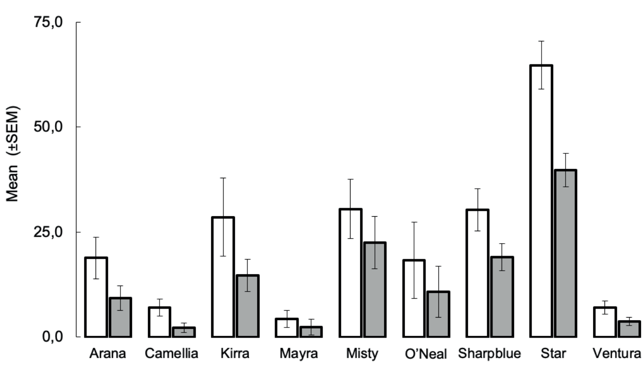

Figure 1. Mean number of eggs laid and adults emerged of *Drosophila suzukii* per berry from nine Southern highbush blueberry obtained in the no-choice assays after exposing of 8 berries to 5 females and 4 males of *D. suzukii* over 24 hours. Error bars indicate standard error of the mean.

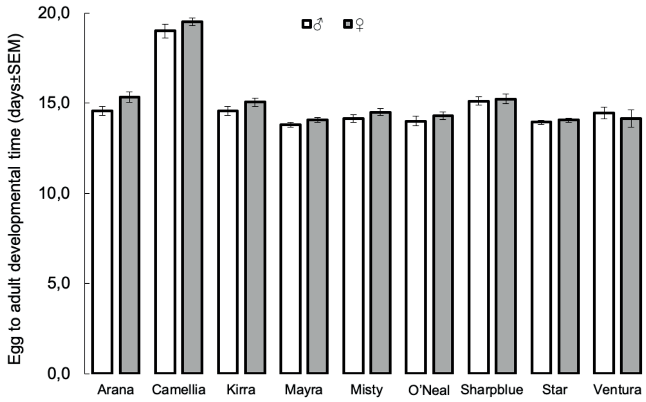

Figure 2. Mean developmental time (egg to adult) of males and females *Drosophila suzukii* from nine Southern highbush blueberries in the no-choice tests. Error bars indicate standard error of the mean.

than in females (14.6±0.1 days), but there were no significant differences between sexes and their interaction with the cultivars (z=0.8 and -0.6 respectively; p>0.05 in both cases; Fig. 2).

Berries from the different cultivars varied in their quality attributes (Table 1). Berry size (expressed as g fresh weight) differed significantly among cultivars ($F_{8,99}$=33.5; p<0.001) with 'Camellia' showing the highest value and 'O'Neal' the lowest. Firmness also differed significantly ($F_{8,99}$=49.3; p<0.001) with the minimum, 105.6±2.1 g mm^{-2}, for 'Sharpblue', and the maximum, 294.8±4.5 g mm^{-2}, for 'Kirra'. Results also showed significant differences in their sugar content ($F_{8,99}$=3.9; p<0.001). 'Mayra' and "Misty" had the highest and lowest sugar contents respectively. Similarly, there were significant differences in their pH values ($F_{8,45}$=7.3; p<0.001), with the lowest ones in 'Camellia', and the highest in 'Arana'.

In general, no correlation between berry attributes, clutch size and number of emerged adults was found. Results only showed a negative correlation between clutch size and firmness (Spearman's ρ=-0.205; p<0.05) and a positive one between number of adults emerged and pH (ρ=0.512; p<0.05). Furthermore, a negative correlation was found between egg to adult developmental time and pH (ρ=-0.582; p<0.05).

Discussion

To date there has been little research into the susceptibility of SHB to SWD. Overall, the results obtained herein showed that the nine SHB cultivars in this study were susceptible to SWD oviposition, and suitable for larval development although there were some differences among them. Few studies have identified pest-resistant SHB cultivars, which suggests that commercially-available blueberry cultivars lack meaningful genetic resistance to SWD (Stringer *et al.,* 2017; Rodríguez-Saona *et al.,* 2018; 2019). Results showed that when the 'Star' cultivar was exposed to SWD, significantly more eggs and emerged adults were detected with respect to the other cultivars. In contrast, Lee *et al.* (2011) found no significant differences in the number of eggs laid among 12 California SHB cultivars including 'Star', Misty', 'O'Neal' and 'Sharpblue' (also included in this paper). However, they were found significant differences in the development percentage, the highest of which were in 'Star' and 'Sharpblue'. Our results also showed that 'Mayra', 'Camellia' and 'Ventura' were less susceptible to SWD, with the lowest values for clutch size and number of emerged adults. In fact, out of all the cultivars 'Camellia' showed the longest developmental time (egg to adult) compared to the remaining cultivars in this study. Currently, 'Star' and 'Ventura' are among the most frequently used blueberry cultivars in Southwestern Spain (http://www.estrategiaprovincial-huelva.com/). Laboratory results obtained herein indicate

Table 1. Berry traits (mean ± SE) measured from nine Southern highbush blueberries. Size (ANOVA; $F_{8,99}$=33.5, p<0.01), Firmness ($F_{8,99}$=49.5, p<0.01), Sugar content (ANOVA, $F_{8,99}$=3.9, p<0.01) and pH ($F_{8,45}$=7.3, p<0.001). Letters denote significance differences by Tukey's HSD (p<0.05).

Cultivars	Size (g fresh weight)	Firmness (g mm^{-2})	Sugar content (ºBrix)	pH
Arana	2.2±0.2 b	261.7±5.1 ab	12.5±0.3 ab	3.8±0.2 a
Camellia	3.4±0.2 a	176.9±6.4 e	12.1±0.4 ab	2.6±0.0 c
Kirra	2.1±0.1 bc	294.8±4.5 a	12.5±0.6 ab	3.5±0.1 ab
Mayra	1.7±0.1 bcde	226.0±18.2 bc	13.5±0.6 a	3.5±0.3 ab
Misty	1.3±0.1 de	187.7±7.5 de	10.4±0.8 b	3.3±0.1 ab
O'Neal	1.1±0.0 e	176.0±3.1 e	11.1±0.5 ab	3.2±0.1 ab
Sharpblue	1.6±0.1 cde	105.6±2.1 f	10.7±0.6 b	3.2±0.1 ab
Star	1.7±0.1 bcd	172.7±7.8 e	11.5±0.7 ab	3.4±0.1 ab
Ventura	1.6±0.1 cde	215.0±2.8 cd	13.5±0.6 a	3.1±0.1 bc

that there could be substantial differences in their susceptibility to SWD in contrast to previous results obtained by Lee *et al.* (2011). However, further research and field observations are required to confirm these differences in susceptibility and any potential practical applications. Berry traits in SHB cultivars are largely determined by genetics, modified not just by selection and breeding (Ehlenfeldt & Martin, 2002; Rodríguez-Saona *et al.,* 2018), but also by cultural practices (Forney, 2001; Stückrath *et al.,* 2008; Angeletti *et al.,* 2010, Lee *et al.,* 2015; Little *et al.,* 2018). Blueberry farming is a highly dynamic sector, in constant growth and adapted to meeting consumer demand. Consequently, new varieties of SHB cultivars, are constantly being produced and tested; therefore, to reduce SWD damage, the cultivars grown should be constantly compared and new ones screened, especially before they are planted in any specific area.

In the susceptibility to SWD infestation analysis, links were found to several physical and/or chemical fruit traits and berry firmness played a primary role in limiting SWD infestation (Lee *et al.,* 2011; Kinjo *et al.,* 2013). According to our results, there was a negative correlation between number of eggs laid and fruit firmness, thus indicating that SWD females tended to oviposit more eggs in softer fruits than firmer ones. Consistent with this finding, Ioratti *et al.* (2015) and Baser *et al.* (2018) found a negative correlation between the number of eggs laid and berry skin penetration force when they analyzed the susceptibility of grape varieties to SWD. In addition, other factors were significant. Stringer *et al.* (2017), working with blueberry genotypes and cultivars, reported a positive correlation between SWD clutch size and berry weight. Gong *et al.* (2016) also found variations in the emergence of SWD among strawberry accessions, which correlated with fruit diameter. Our results found no correlation between clutch size, number of emerged adults and berry fresh weight. So, other factors may then be more influential in determining differences in clutch size and emerged adults among the blueberry cultivars tested herein. Previous studies on the effects fruit sugar content has on SWD oviposition have yielded variable results. Lee *et al.* (2011, 2016), and Stringer *et al.* (2017) reported a positive correlation between oviposition and °Brix of fruits. In contrast, Little *et al.* (2017), and Rodríguez-Saona *et al.* (2018) showed a preference for fruits with low sugar content. Results obtained herein showed the sugar content of the nine SHB cultivars bore no relation to SWD infestation, in line with previous results by Pelton *et al.* (2017) on grapes. Therefore, further laboratory and field research is required to determine the real effects sugar content has on SWD infestation.

A positive correlation was found between number of adults emerged and pH. Additionally, egg to adult developmental time and pH were negatively correlated. In general, previous studies showed a positive relationship when pH increased on SWD infested blueberries (Lee *et al.,* 2016; Little *et al.,* 2017; Rodríguez-Saona *et al.,* 2018). In contrast, Pelton *et al.* (2017) found no correlation between pH and SWD performance in grapes. Our results are coherent with a general pattern in which less acidic blueberry cultivars were more susceptible to SWD infestation.

In summary, our results showed that the nine SHB cultivars tested in this study are susceptible to SWD oviposition, with significant differences in the number of eggs laid and emerged adults between 'Star' and the other cultivars, mainly 'Camellia' and 'Ventura'. However, it must be stressed that blueberry cultivars should be selected on the basis of an analysis of many other relevant factors of an agricultural, socio-economic nature, and whose results do not necessarily need to concur with those reported herein. Among the fruit traits analyzed, only firmness and pH were correlated with SWD infestation. According to previous research, SWD females tend to oviposit more eggs in softer fruits than firmer ones. In addition, the higher the pH, the more adults emerged and the shorter the larval development time. Given the dynamic evolution of SHB crops in Southwestern Spain, more laboratory and field studies in this area are required and an analysis of how susceptible blueberry cultivars are to SWD would help design IPM programs.

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