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First observations of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) suggest that it is a transient species in Finland

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Drosophila suzukii, known as the spotted wing drosophila (SWD), is a major pest of berries and stone fruits. In 2014 it was recorded for the first time in the Nordic countries. In this study, we report the first observations of D. suzukii in Finland. The first trap catches of SWD were observed in 2019 in North Savo at a berry farm that handles imported berries. In 2020–2021, no adult flies were trapped at the farm, but in August 2022, adult SWDs were caught at the same farm. Observed SWD adults in 2022 probably originated from a new introduction rather than an overwintered population, because no SWDs were observed during the two preceding seasons. In 2023, one female of the same haplotype as the flies in previous year was found in mid-July, but no further population growth was observed. This suggests that some overwintering events may happen, but lack of overwintered males may hinder the population growth. In 2021–2022, larvae and eggs of SWD were found in several batches of imported berries. Our results suggest that there is a pathway of introduction for SWD up to the farm level. Identification of *D. suzukii* specimens was confirmed with PCR and sequencing of the COI region, and four SWD haplotypes were found.

Key words: berries, introduction pathways, invasive pests, PCR, haplotype

Introduction

The spotted wing drosophila *Drosophila suzukii* (Matsumura 1931) (Diptera: Drosophilidae) is an invasive pest endemic to Southeast Asia (Kanzawa 1939, Asplen et al. 2015). It first invaded Hawai'i in the 1980's and reached mainland North America and Europe in 2008 (Hauser 2011, Calabria et al. 2012, Asplen et al. 2015). SWD has a very wide host range among berries and stone fruits (Lee et al. 2011, Cini et al. 2012, Poyet et al. 2015, Olazcuaga et al. 2019). It is one of very few drosophilids that can oviposit in healthy, ripe fruits, and thus, this species can cause considerable damage to crops (Mitsui et al. 2006, Bolda et al. 2010, Lee et al. 2011). In Europe, SWD was first found in Italy and Spain in 2008, and it has spread rapidly to most European countries (Calabria et al. 2012, Cini et al. 2012, Asplen et al. 2015, Kiss et al. 2016, Lavrinienko et al. 2017, Briem et al. 2018).

According to pest risk analysis by the EPPO, the climate in most parts of Finland is not suitable for SWD: the threshold of 500 degree days counted on a base of 10 degrees is reached only along the southern costal line of Finland and the favorable area is also restricted to the southern parts of Sweden and Norway (EPPO 2010). Likewise, according to species distribution models generated using Maximum Entropy algorithms by Ørsted and Ørsted (2019), Finland would not have a suitable climate for *D. suzukii* if the data is based on the current European distribution of *D. suzukii*. However, if the global distribution is used as base data, climatic suitability for *D. suzukii* could reach central Finland with a low probability. In summer 2014, SWD was recorded for the first time in the Nordic region, in Sweden, and a few months later also in Denmark (Manduric 2017). In Norway, SWD was found in 2016 (Nina Trandem, personal communication).

In this paper, we report the first field observations of *D. suzukii* in a berry farm that also handles imported berries in Finland. We also report the introduction pathways for SWD larvae and eggs via imported berries. In these observations the presence of *D. suzukii* was confirmed with molecular techniques.

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Material and methods

Field trapping

A preliminary survey of the possible occurrence of SWD was carried out in southern and central Finland in summer 2019. A total of five field plots were chosen from the provinces of Uusimaa (Karkkila), Tavastia Proper (Jokioinen, Janakkala), and North Savo (Suonenjoki). Raspberry (*Rubus idaeus* L.) was the cultivated crop in all fields except in Tavastia Proper where blueberry (*Vaccinium corymbosum* L.) was also cultivated (Fig. 1). The field plot sizes varied from 0.5 to 2.25 ha and all the plots were bordered by a forest edge on at least one side, except for one plot that was bordered by a small bog. Four traps were placed in the peripheral raspberry rows of each plot, where possible close to the forest edge, at the height of the unripe berries protected from the sun. In Tavastia Proper, two traps were placed in raspberry field plots as described above, and two in bushes at the edge of the blueberry field.

Fig. 1. A map of trapping locations in Finland in 2019–2023

In the period 2020–2023, field trapping was conducted at the farm where the first observation was made in 2019. In 2020, there were 5 traps for 9 weeks at the edge of the raspberry field where the original SWD observation was made. In 2021, there were a total of 15 traps on the farm, with 3 situated in the trees surrounding the fields, 6 in the raspberry field, and 6 in the strawberry fields and their surroundings. In 2022, there were 6 traps on the farm, with 3 in the raspberry field, and 3 in the strawberry field and its surroundings (Table 1). Next to the strawberry field, there is a cold storage building where imported berries are stored at the beginning of the season before they are transported to marketplaces. In June 2023, there were 5 yeast baited traps of which 3 were situated in the forest patch near the raspberry field and 2 were near the strawberry field. Later there were a total of 9 yeast baited traps on the farm, with 6 traps in raspberry fields, 3 traps in the surrounding vegetation of strawberry fields, and 2 traps in another location baited with SuzukiiTrap® liquid.

In 2021, field trapping was conducted at two additional places in Tavastia proper (Jokioinen, Janakkala), and in 2022 at other locations in North Savo (Suonenjoki), South Savo (Mikkeli), and Uusimaa (Hyvinkää) (Fig. 1). The field plot sizes in 2020–2023 varied from 0.12 to 4.46 ha and all the plots were bordered by forest edge on at least at one side, except for one plot that was 73 m away from the nearest forest patch. Farms handling imported berries during the growing season were selected as trapping locations, as well as farms located near to a highway or railway, since highways have been shown to facilitate the spread of SWD (Kiss et al. 2016). The aim of these studies was to find out whether SWD occurs at the berry farms at risk of introduction.

Table 1. Number of trapping locations in different areas, number of traps in these areas in each year and the host plants where the traps were located. The traps where *Drosophila suzukii* was found on one farm in North Savo, are marked with an asterisk.

Year	Number of traps/location				Number of traps/host plants									
	North Savo	South Savo	Tavastia Proper	Uusimaa	raspberry	strawberry	bird cherry	honeysucle	taxus	blueberry	black currant	apple	forest patch/ road verge	traps/year
2019	8/2		8/2	4/1	4*/18					2				20
2020	5/1				5									5
2021	15/1		6/2		7	4	1	1		3	1	1	3	21
2022	8/2	2/1		4/1	3*	1*	2*/3		2		3		2	14
2023	11/2				1*/6		2	1*					2	11

Traps

In 2019 and 2020, DROSAL® Pro traps (Andermatt Biocontrol AG, UK) filled with a liquid SuzukiiTrap® lure (Bioiberica, Spain), were used. The SuzukiiTrap® lure was selected because it performs well in trapping SWD females on raspberry (Frewin et al. 2017), which is the most produced berry in Finland in August. In 2021 and 2022, DROSAL® Pro traps were replaced by DrosoTraps® because the red cup used in the trap construction performs better than transparent cups (Lasa et al. 2017). In addition, DrosoTraps® have a good trapping efficiency without the killing agent (Van Kerkvoorde et al. 2020), which is essential because traps with a killing agent are not yet registered in Finland. The liquid lure in the traps was changed on a mostly weekly basis from early July to late August (2019, 2023) or late September (2020–2022). In June 2021 and 2023, a yeast bait (Frewin et al. 2017) was used in the DROSAL® Pro traps in the forest patch. This trapping technique was aimed at catching unmated females at the beginning of the season in the case that some SWD would have managed to overwinter near to the raspberry field where the first observation was made. The yeast bait was also changed weekly. The trap catches were extracted from the liquid lure or yeast bait by rinsing the insects in water and storing them in 75% ethanol until inspection under a stereo microscope (Olympus SZX-16, Olympus corporation, Tokyo, Japan). Morphological identification of *D. suzukii* was carried out according to the key by EPPO (EPPO 2013) and thereafter identification of some specimens was confirmed by molecular methods.

Inspection of imported berry samples

Berries were inspected using a filtration method described in Van Timmeren et al. (2017). If the sample size exceeded 300 g (2–3 kg boxes of cherries), the samples were divided into subsamples of 250 g. In 2021 and 2022, 128 and 30 imported berry samples, respectively, were inspected for SWD. The samples were obtained from retailers, supermarkets, marketplaces, and berry growers selling berries imported mainly from other European countries. In 2021, the inspected berry samples were blueberries (84%), cherries (7%) and grapes (9%). Of these samples, 4 blueberry samples (4% of blueberry samples) and one cherry sample (11% of cherry samples) were infested with *D. suzukii*. In 2022, the majority of the samples were raspberries (83%) and the rest of the samples were cherries (10%) and blueberries (7%). Of these samples, 7 raspberry samples (28%) and 1 cherry sample (33%) were infested with *D. suzukii*. However, it should be noted that cherry samples were much bigger than other berry samples and divided into several subsamples. Only 3% of the inspected subsamples were infested.

Molecular identification of SWD

Genomic DNA was extracted from adult individuals of *D. suzukii*, and larvae or eggs found in berry samples, using DNeasy Blood and Tissue kit (Qiagen). *D. suzukii*-specific primers Dro-suz-S390 (TTGAACTGTTTACCCACCTCTT) and Dro-suz-A380 (GGTATTCGGTCTAATGTAATACCC) (Wolf et al. 2018) targeting part of the mitochondrial cytochrome c oxidase subunit 1 gene (*COI*) were used in PCR to produce a 171 bp fragment. In addition, the COI gene was amplified and sequenced using the universal primer pairs LCO1490 and HCO2198 (Folmer et al. 1994), and/or C1-J-1718F and C1-N-2191 (Simon et al. 1994). Each 25 µl PCR reaction contained 0.4 µM of each primer, 0.2 mM of each dNTP, 1 U Taq Polymerase and 1x reaction buffer (Thermo Scientific) and 1–2 ng of template DNA. The PCR program used was as follows: 94 °C for 1 min, then 35 cycles of 94 °C for 30 s, primer annealing for universal primers at 52 °C and for SWD specific primers at 63 °C for 30 s and 72 °C for 30 s, followed by 72 °C for 5 min. PCR products were purified using QIAquick PCR Purification kit (Qiagen) and sequenced using an automated sequencer at the Natural Resources Institute Finland, Jokioinen. Consensus sequences were created with Sequencher v. 5.4.6, and ClustalW (Larkin et al. 2007) with the neighbour-joining method (Saitou and Nei 1987) used for making sequence alignments.

Results

Trap catches

In August 2019, three male and two female SWDs were found in the traps on raspberry bushes from North Savo. According to a key by EPPO (2013) the males possessed a distinct apical wing spot on the forewing. On the first and second tarsomere of the foreleg two parallel sex combs were seen, the first with six spines and the second with three spines. In the ovipositor of the female, the dark saw-like serrations were easily seen (Fig. 2). The identification of one female and one male was confirmed by molecular methods.

Fig. 2. A female (on left) and a male (on right) *Drosphila suzukii* from the first trap catches in 2019. Photo: Anne Nissinen

In 2020 and 2021, no SWDs were found in the traps, but in 2022, 54 adult SWDs were caught on the same farm where the first observation was made in 2019. Fourteen of the adult SWDs were females and 40 males. They were trapped between 4 August and mid-September. The first catch was made in the trap nearest to the cold storage unit where imported cherries were stored at the beginning of the season. Within two weeks, the first two males were also caught in one of the traps in the raspberry field on the other side of the hill. In the middle of September, highest number of SWDs were caught on the farm: on average 4.2 ± 2.3 per each of the six traps. In total, identification of 14 adult SWDs was confirmed by molecular methods in 2022. In 2023, one female was caught in the raspberry field at the edge of the forest patch on 13 July and one male was caught on the other side of the hill near the strawberry field on 26 July. No further adults were caught on this farm by mid-August 2023. In the provinces of Uusimaa, Tavastia Proper, and South Savo no SWDs were caught during the study.

Imported berry samples

In 2021, 4% of the 128 filtered berry samples yielded between one and four SWD larvae. In 2022, in 27% of the 30 filtered berry samples, one to nine *D. suzukii* larvae were found. All the sampled larvae (22) were confirmed to be *D. suzukii* by PCR analysis. Furthermore, 23% of berry samples contained eggs. Of these samples 57% also contained larvae. Of the eggs, 50% were confirmed to be the eggs of *D. suzukii*, but the species could not be verified for the remaining eggs.

In total, 39 *D. suzukii* specimens were sequenced to confirm the species identification and to study genetic diversity of SWD specimens found in Finland. The PCR fragments produced by SWD specific primers were sequenced from 25 specimens, and the ones produced by the universal COI primers from 18 specimens, in four specimens (included in the aforementioned numbers) both of the PCR fragments were sequenced. COI sequence data revealed four genetic haplotypes (Table 2). The haplotypes Ds2 and Ds83 were only detected in one sample. Three of the sequenced specimens were of the haplotype Ds18, whereas all the other studied specimens were of the haplotype Ds1. In their study, Lavrinienko et al. (2017) categorized the haplotypes from S1 to S10. The haplotypes of this study: Ds1, Ds2, Ds18 and Ds83 are identical to the haplotypes S4, S9, S5 and S8 of Lavrinienko et al. (2017), respectively.

Table 2. Different COI haplotypes detected from *Drosophila suzukii* (SWD) specimen found in Finland either from the traps (adult SWDs) or from imported berries (larvae and eggs) in the period 2019–2023. All COI sequencies are 658 bp in length.

Haplotype	Source	Origin	Year	GenBank accession number	Single nucleotide polymorphisms (SNPs)				
					site 214	site 263	site 271	site 407	site 646
Ds1	Adult SWD Larvae, eggs	Trap Imported berries	2019, 2022 2021, 2022	OQ913863	A	C	T	C	T
Ds2	Adult SWD	Trap	2019	OQ913864	G	C	C	C	T
Ds18	Larvae Eggs	Imported berries Imported berries	2021 2022	OQ913865	A	C	C	T	T
Ds83	Adult SWD	Trap	2023	OR462790	A	T	T	C	C

Discussion

Field trapping from 2019–2023 was executed in the same field where the first SWD observation was made in 2019. However, no further observations were made in 2020 or 2021. This suggests that SWD did not overwinter on the farm. In 2022, the flies were found again on the same farm. The first observation was made in the trap nearest to the cold storage unit on strawberry. Along with the apparent increase in population size, SWD adults were also caught on the other side of the hill in the raspberry field. However, the maximum trap catches were still low, which also supports a new introduction. Using the filtration method (Van Timmeren et al. 2017), four SWD larvae were found in one 2 kg sample of imported cherries in June 2021. These results show that there was an introduction pathway up to the farm level in 2021. It is possible that a similar introduction happened in 2022 and that some of the larvae managed to pupate, for example in the wooden boxes used for cherry importation, and emerged as adults as the boxes were discharged. The adult emergence presumably happened at the beginning of July. The mean temperature in North Savo was 17.6 °C in July 2022 (Finnish Meteorological Institute). According to the life table data by Tochen et al. (2014), at 18 °C the development time of a female from egg to adult is 20.1 days on blueberry. This suggests that the first observation on 4 August could be from the first population developed in the strawberry field. By mid-September, observations of the SWD were made in all 6 traps at the farm, which suggests that a second generation had developed. The mean temperature in August in Kuopio was 17.2 °C (Finnish Meteorological Institute), which enabled nearly as rapid development as in July. In September, the temperature declined rapidly to an average 8.6 °C. The development time of a female from egg to adult was over 77 days at 10 °C (Tochen et al. 2014). This suggests that development of a 3rd generation in the field in North Savo was quite unlikely because in November the mean temperature had already dropped to –0.4 °C. The first observation of SWD in 2019 was made in September which suggests a similar kind of population development as in 2022.

Trapping carried out early in 2023 revealed a female of the same haplotype as the flies caught in 2022, which suggest a possible overwintering event. However, it apparently did not result in population growth. The second adult found in 2023, a male at the edge of the strawberry field, was of a different haplotype, suggesting a new introduction. No berries were imported by the farmer, thus, the new import probably originated from household biowaste from the yard between the raspberry and strawberry fields. No further observations of adults were made by 24 August in the fields of this farm, which suggests an asynchrony in the time of the introduction and the occurrence of possible overwintered females. The newest observations in Finland resemble the situation in Canada, where Champagne-Cauchon et al. (2020) suggested that some females might be able to overwinter in suitable microclimatic conditions at forest edges, but no males were observed in early summer. Thus, lack of overwintered males may have hindered the population growth during the 2023 season in Finland.

The results from 2019–2022 suggest that the SWD population in 2022 was a new introduction. In 2023, the observation of one female of the same haplotype as detected on the farm in 2022 suggests that some occasional overwintering events may happen, but SWD is still transient in Finland. Our observations support the prediction model by Ørsted and Ørsted (2019) utilizing the global distribution of *D. suzukii*, which suggests climatic suitability even in central Finland with a low probability. Population establishment in Finland is most likely limited by the harsh climate. According to Tait et al. (2021) a debate has been going on whether SWD can overwinter in northern Italy and the Great Lakes regions of the United States. In Germany, spread of SWD was considered to be facilitated by a mild winter 2013/2014 when no days with temperatures under 0 °C occurred (Briem et al. 2018). A recent modeling study showed that accumulated degree days below 5 °C in the preceding month caused a linear decline in trap catches, which in turn suggests that cold weather periods will significantly decrease the abundance of SWD (Ørsted et al. 2021). In central Finland during winter, the time period with temperatures lower than 5 °C often reaches 150 days. In North Savo (Kuopio) mean temperatures during five winter months (November–March) varied between –1.1 and –7.5 °C in 2021–2022 and in April the mean temperature was under 5 °C (Finnish Meteorological Institute). The long duration of the cold period may be the reason why no further observations of adult flies were made at the first observation site in the two subsequent years 2020–2021. However, we cannot ignore the possibility that the species may be behaviorally adapted to overwinter in man-made protected habitats (Dalton et al. 2011, Zerulla et al. 2015), which could facilitate population establishment in the harsh northern climate.

Based on genetic research, introductions of SWD to the USA and Europe are different demographic events, and introduction to Europe may have narrowed the genetic pool of the population (Adrion et al. 2014). Indeed, Rota-Stabelli et al. (2020) showed that European and American strains of SWD have genetic differences, and further differences in hatch rate, generation time, and parasitoid susceptibility, which in turn may affect their fitness. Rota-Stabelli et al. (2020) hypothesized that the Italian strain could be preadapted to colder climates, because this strain showed increased reproduction capability. Cold periods shorten the reproductive season, and increased reproduction capability during the warmer periods may be an adaptation to local conditions.

Our study revealed four different *COI* haplotypes among the different SWD introductions to Finland. Interestingly, the two flies from the first observations in 2019 represented two different haplotypes (Ds1 and Ds2). In the light of the results from imported berry samples in 2021 and 2022, SWD larvae enter Finland in berries originating from several different European countries and therefore two different introduction events during one season on the same farm is possible although it may be quite a rare event. All four haplotypes have previously been observed in Europe based on the sequence data in the GenBank and different reports (Calabria et al. 2012, Lavrinienko et al. 2017). The haplotype Ds1**,** which was the most common haplotype in this study, and also the only haplotype among adult flies tested in 2022**,** is the most common European *D. suzukii* haplotype. Ds2 was only found in one sample of this study, but this haplotype has been reported from USA, Canada, Japan, South Korea and Serbia**.** Ds83 was also found only in one sample of this study. This haplotype has been reported only from the USA and Ukraine. Haplotype Ds18 is found only in the European countries and Turkey.

Conclusions

We have observed that a probable introduction pathway of SWD to the farm where the first observation was made is via imported cherries and that the pest pressure on the introduction pathway is frequent, but that the degree may differ from year to year. Since berries are imported every year during the growth season from areas where SWD is present, it is likely that multiple introduction events have happened since SWD was first observed in Europe in 2008. So far, severe infestation on the Finnish berry farms have not been observed. This may be due to a long wintertime, and partly due to the genetic pool of the European SWD population, which seems to be less diverse than among North American populations and thus limiting SWD acclimatization to extreme conditions.

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