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Journal of Pest Science (2021) 94:251–259
https://doi.org/10.1007/s10340-021-01330-1

RAPID COMMUNICATION

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Detection of the spotted wing drosophila, *Drosophila suzukii*, in continental sub-Saharan Africa

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Received: 5 October 2020 / Revised: 21 December 2020 / Accepted: 5 January 2021 / Published online: 23 January 2021
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Abstract

The spotted wing drosophila, *Drosophila suzukii* Matsumura, is an insect pest of soft-skinned fruit, native to Eastern Asia. Since 2008, a world-wide dispersal of *D. suzukii* is seen, characterized by the establishment of the pest in many Asian, American and European countries. While the potential for invasion of continental Africa by *D. suzukii* has been predicted, its presence has only been shown for Morocco in Northern Africa. Knowledge about a possible establishment in other parts of the continent is needed as a basis for pest management. In 2019, we carried out a first survey in three counties in Kenya to monitor for the presence of *D. suzukii* using traps baited with a blend of apple cider vinegar and red wine. A total of 389 *D. suzukii* flies were captured in a fruit farm at Nakuru county, with more female flies being trapped than males. We confirmed the morphological identification of *D. suzukii* using DNA barcoding. In 2020, we performed a follow-up survey at 14 locations in six counties to delimit the distribution of *D. suzukii* in the main berry growing zones in Kenya. The survey indicated that so far *D. suzukii* is restricted to Nakuru county where it was initially detected. This is the first study to provide empirical evidence of *D. suzukii* in continental sub-Saharan Africa, confirming that the pest is expanding its geographic range intercontinentally. Given the high dispersal potential of *D. suzukii*, a concerted effort to develop management strategies is a necessity for containment of the pest.

Keywords *Drosophilidae* · Fruit fly · Global · Invasive pest · Soft fruits ·

Key message

- Despite the global spread of *Drosophila suzukii*, its distribution in Africa lacks clarity.
- We carried out a monitoring study in Kenya by using traps and investigating fruit samples.
- *Drosophila suzukii* was captured in raspberry, strawberry blueberry and pomegranate plants.
- Morphological identification was confirmed using DNA barcoding.
- The findings confirm the presence of *D. suzukii* in continental sub-Saharan Africa.

Communicated by Antonio Biondi.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-021-01330-1.

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Introduction

Native to Asia, the spotted wing drosophila (SWD) *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) was first reported as a pest in wild blueberries and cherry orchards in Shioyama Town, Japan, in 1916 (Kanzawa 1939). Since then, *D. suzukii* has become known as a pest of soft and stone fruits which inflicts serious economic loss to the fruit industry (Asplen et al. 2015). The loss is attributed to the serrated ovipositor of the female with which it pierces the skin of ripening fruits. In addition to the direct damage through oviposition and subsequent larval feeding, damaged fruits also get exposed to secondary infestation by other drosophilids and opportunistic pathogens (Rombaut et al. 2017).

Drosophila suzukii has nowadays been reported in many parts of the globe outside Asia. In 1980, it was reported in Hawaii and later in 2008 in the Pacific coast of California as an invasive pest (Bolda et al. 2010; Goodhue et al. 2011). Subsequently, *D. suzukii* has been found in other parts of the Americas (Hauser 2011; Walsh et al. 2011) as well as across European countries (Calabria et al. 2012; Ørsted and Ørsted 2019). Comparisons between selected European and North American *D. suzukii* populations reveal that invasions occurred through different routes as the latter populations were suggested to originate from southeast China while the former were traced to northeast China (Rota-Stabelli et al. 2020). Overall, *D. suzukii* shows rapid and ongoing inter-and intracontinental dispersal. Recently, *D. suzukii* was confirmed from samples collected in Morocco during 2017 and 2018, demonstrating presence in Northern Africa (Boughdad et al. 2020). Observations of *D. suzukii* are reported in Réunion island (IPPC 2017) and in the Comoros archipelago (Hassani et al. 2020). However, it was unknown if *D. suzukii* is present in continental sub-Saharan Africa.

Distribution models predict the establishment of *D. suzukii* in numerous sub-Saharan countries (Dos Santos et al. 2017) which would likely affect fruit production similar as seen in other regions. In sub-Saharan Africa, fruits generally are produced through irrigated, urban-based or highland mixed-rainfed farming systems (Costa et al. 2013; Dixon et al. 2001). While irrigated and urban-based farming systems mainly target foreign markets, rain-fed farming systems mostly supply local markets (Costa et al. 2013; Dixon et al. 2001). Moreover, unlike rain-fed farming systems which are characterized by indigenous skills and knowledge, irrigated and urban-based farming systems apply modern farming technologies, including integrated pest management (De Bon et al. 2014; Pretty and Bharucha 2015).

Generally, the global spread of *D. suzukii* is aided by international trade (Cini et al. 2014). Sub-Saharan Africa is trading commercial commodities such as fruits and vegetables, on the international market (European Union 2019; Legge et al. 2006). For example, South Africa, Cameroon, Kenya, Ghana and Ethiopia are exporting off-season and tropical fruits to both regional and international trade partners (Hoffmann and Vossenaar 2008).

Kenya, as a strategically located country, provides a bridge, through its port of Mombasa, that allows for cross-border movement of commodities to the neighboring landlocked countries including Uganda, Burundi, Rwanda and South Sudan (Sebuny 2015). Consequently, Kenya and the neighboring partner countries are exposed to a high risk of invasion by economically significant insect pests. A prime example of such an invasion is represented by *Bactrocera* species, such as *Bactrocera dorsalis* (Hendel) (Diptera; Tephritidae), which originates from Asia and was first recorded in Kenya with subsequent reports of its establishment in other Eastern, Central and Southern African countries (Drew et al. 2005; Lux et al. 2003). As of 2018, Kenya was importing strawberries, raspberries, blackberries, gooseberries, cherries and plums, which all are known as *D. suzukii* host fruits (International Trade Centre 2019). Of the fruit-importing countries, South Africa, Italy, Egypt, Spain, Chile and India the four non-African countries have confirmed the presence of *D. suzukii* (International Trade Centre 2019; CABI 2019; Kenis et al. 2016). Introduction of *D. suzukii* might lead to a permanent invasion and dispersal of the pest in sub-Saharan Africa. Through inductive modeling, Dos Santos et al. (2017) previously showed that southwestern Kenya and bordering countries (Uganda and Tanzania) have suitable abiotic conditions that would support *D. suzukii* establishment.

Evidently, the above-mentioned factors provided a backdrop on which we hypothesized that *D. suzukii* might already have been introduced into continental sub-Saharan Africa and be present in soft and stone fruit cultivating regions. To address this hypothesis, we carried out a monitoring survey during 2019 and 2020. We report the detection but apparently restricted distribution of *D. suzukii* in Kenya. Species identity was confirmed using morphological features together with DNA barcoding of the mitochondrial cytochrome oxidase subunit I (*COI*).

Material and Methods

Detection survey in 2019

To monitor for potential invasion of *D. suzukii* in Kenya, a survey was carried out during the dry season, from 13th to July 20th, 2019, in three counties; Nairobi county (farm: Duduville campus of the International Centre of Insect Physiology and Ecology (*icipe*), 1° 22′S, 36° 89′E, about

1, 600 m above sea level, hereafter masl); Kiambu county (farm: Sasini estate 1° 16′S, 36° 89′E, about 1,700 masl) and Nakuru county (farm: Longonot farm 0° 83′S, 36° 38′E, about 1,935 masl). We used the Leaflet package in R v 2.0.2 (Cheng et al. 2018) to show the locations on a map (Fig. S1). Brief descriptions of *icipe,* Sasini estate and Longonot farm are given in the supplementary material. In each of the farm, 10 traps (1 trap/site, see supplementary for details; Fig. S2) of 60-mL volume filled with 40 mL of a blend of apple cider vinegar and red wine (20:80 v:v) were used (Huang et al. 2017; Landolt et al. 2012). At *icipe* and Sasini, we monitored crops cultivated in a mixed-cropping system. At Longonot, we sampled raspberries (*Rubus idaeus*), strawberries (*Fragaria ananassa*), blueberries (*Vaccinium corymbosum*) and pomegranates (*Punica grantum*) (Table S1) grown in separate tunnels (Fig. S3). Two traps were placed per tunnel. At the time of the survey, fruit ripening was more advanced in raspberries than in the other crops. After a week, the captured insects were collected and inspected for the presence of *D. suzukii* by morphological features using a Zeiss microscope (Göttingen, Germany) fitted with an Axiocam 105 color microscope camera (Göttingen, Germany). Generally, the adults were about 2–3 mm long, having red eyes with brown thorax and abdomen, with unbroken bands. The males were characterized by a dark spot on each wing (Fig. S4a) and a sex comb on the first and second segment of the foreleg with 3–6 teeth running parallel to the feet (Fig. S4b). Females were identified based on the distinctive ovipositor with a pronounced serration that is different from *D. subpulchrella* (Atallah et al. 2014; Vlach 2010) (Fig. S4c).Through a weather information provider (Visual Crossing 2020), daily data on temperature, relative humidity and precipitation were extracted from nearest accessible weather stations in Nairobi, Kiambu and Naivasha (Nakuru) representing the respective survey sites (Table S3). In a follow-up survey in 2020 (see below), weather data were recorded in Laikipia, Murang'a, Nyandarua and Nyeri (Table S4).

Follow-up survey in 2020

As it was unclear whether or not the pest had established and dispersed since its detection in Longonot in 2019, we carried out a follow-up survey between May 20 and June 11, 2020. A total of 11 farms and 3 roadsides distributed in major berry growing areas including Longonot farm (Nakuru county), Kiambu, Nyandarua, Murang'a, Nyeri and Laikipia counties were surveyed (Fig. S1 and Table S2). Roadsides have earlier been shown as suitable sites to detect *D. suzukii* (Lengyel et al. 2015). Baited Drososan traps (Koppert Biological Systems, the Netherlands) were set up at 25 sites (one trap per site) at the 14 locations (Table S2). Fruits monitored at the farms were cultivated in tunnels. After 5–7 days, traps were collected for sorting and identification at i*cipe*. The number of caught flies was computed as daily catches of flies per trap. Like in the 2019 survey, traps were set up and collected once.

Samples of *D. suzukii* from both the 2019 and 2020 surveys are stored in 70% ethanol at *icipe* and are accessible for future reference, through the code SWD-Longonot-07-2019.

Survey on infestation of berries by *D. suzukii*

To assess host infestation, we sampled ripe raspberries, strawberries, blackberries (*Rubus fruticosus*) and blueberries grown at 11 farms at different locations. The specific types of fruit grown and investigated at different farms are provided in Table S2. For each type of fruit investigated at a respective farm, at least 0.2 kg of berries was sampled. Each sample consisted of berries that were handpicked from the plants and berries that had fallen to the ground. With the exception of *R. idaeus* and *V. corymbosum* at Longonot, which were collected at two dates, fruits at other farms were collected once. Collected berries were kept separately by type in 2-L plastic containers (Kenpoly Manufacturers Ltd, Nairobi, Kenya), covered with lids fitted with fine netting material. Samples were transported to the laboratories at the Animal Rearing and Containment facility at *icipe*. Sample weight was measured using an electrical balance (KERN PCB 2500, KERN & Sohn GmbH Ziegelei Balingen, Germany). Developing larvae were protected from drowning in excess juice by lining each container with four layers of Velvex premium kitchen towels (Chandaria Industries Ltd, Nairobi, Kenya) which were exchanged twice a day.

Pupae were collected, transferred into a Petri-dish lined with moistened paper towel and kept in a Perspex cage ($0.2 \times 0.2 \times 0.2$ m) fitted with a fine mesh sleeve. Emerging adults of *D. suzukii* were counted and recorded as flies per kilogram of fruit.

Molecular identification

We extracted the genomic DNA from eight flies ($n = 4$ per sex) caught in 2019 using phenol-chlorofom (Sambrook and Russell 2006) and from three flies (2 males and 1 female) that emerged from incubated berries in 2020 using Isolate II Genomic DNA kit. For polymerase chain reaction (PCR) amplification, we used *D. suzukii* specific primers to amplify the barcode region of the cytochrome oxidase I (*COI*) for 2019 samples and general primers (Hajibabaei et al. 2006) for 2020 samples. Amplified fragments were purified and bidirectionally sequenced at Macrogen Europe BV (Meibergreef, Amsterdam, the Netherlands). Following visual inspection and manual trimming of the DNA sequence electropherograms, we aligned forward and reverse sequences using ClustalW algorithm (Bioedit v. 7.2.5) (Hall 1999). The representative sequences were aligned to reference sequences from the National Center for Biotechnology Information (NCBI). Additionally, we compared the sequences with those of closely related species and generated a maximum likelihood phylogenetic tree (Fig. S5) with 1000 bootstraps using MEGA X v 10.0.5 (Kumar et al. 2018). To generate the tree, we aligned the sequences using the default settings of MUSCLE algorithm in MEGA X. The resultant alignment was used to predict the best model for constructing a phylogenetic tree using Tamura–Nei model (Tamura and Nei 1993) which had the lowest Bayesian Information Criterion (BIC = 4561.79). All the sequences submitted to GenBank can be accessed through: accession MN689051-MN689058 for 2019 samples, and accession MT966718, MT975699 and MT981339 for 2020 samples. More details are provided in the supplementary material.

Results

Out of the 30 sites at the three locations monitored in the 2019 survey, *D. suzukii* was detected at ten sites at Longonot farm, Nakuru county. Flies were first identified by morphological features. We confirmed the morphological identification of *D. suzukii* through *COI* DNA fragment amplification from genomic DNA, followed by direct sequencing and BLAST searches. The resulting sequences (Table S5a) were aligned to reference sequences from NCBI compared with publicly available data on GenBank and yielded a similarity score of 100% with *D. suzukii* samples from Switzerland (Accession number MG605095.1), Turkey (Accession number MK435596.1) and Italy (Accession number KJ671599.1) (Kuyulu et al. 2019) (Fig. S5). A total of 389 *D. suzukii* flies were captured in the 7-day period of the survey, in which females accounted for 62.0% (95% confidence interval of 56.9–66.8%, for binomial distribution) of the total catches. In detail, with the two traps per plot, 130 and 127 *D. suzukii* flies (80 and 78 females, respectively) were trapped in the two raspberry plots, 60 flies (47 females) in the strawberry plot, 37 flies (15 females) in the blueberry plot and 35 flies (21 females) in the pomegranate plot (Fig. 1). Other species of Diptera, Coleoptera and Hymenoptera were captured at Longonot, Sasini and *icipe;* these specimens were stored but not further identified. In Longonot, *D. suzukii* represented 60.8% of all the captured *drosophilids.*

In the follow-up survey in 2020, *D. suzukii* was detected in Nakuru county but only at Longonot farm where we had trapped flies already in 2019. From the monitoring traps, 358 *D. suzukii* flies were captured, in which 257, 63 and 38 flies were caught in raspberries, blueberries and strawberries, respectively. Moreover, 799 adults of *D. suzukii* emerged from incubated fruit, of which raspberries had the highest number of flies (782, compared to 14 and 3 flies that emerged from blueberries and strawberries, respectively). *Drosophila suzukii* was neither observed in traps nor emerged from incubated fruits collected at berry growing areas in Kiambu, Nyandarua, Murang'a, Nyeri and Laikipia counties. We further confirmed the identity of *D. suzukii* captured in the 2020 survey through morphological and molecular

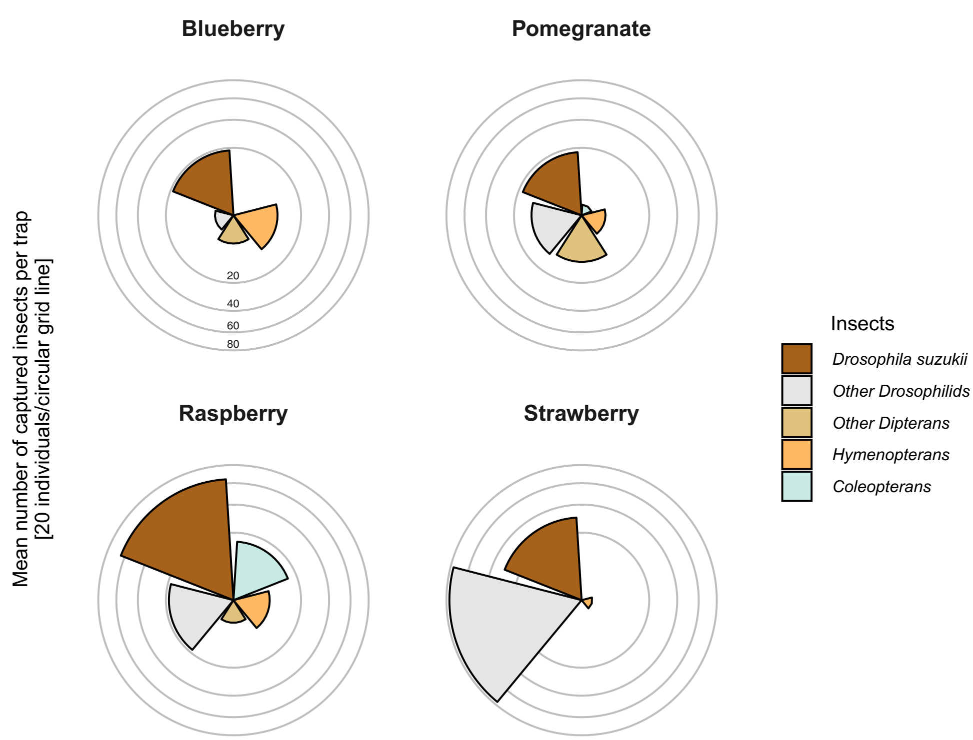

Fig. 1 *Drosophila suzukii* monitoring results from a fruit farm in Kenya. Radial plots illustrate the mean number of *D. suzukii,* other drosophilids, other Dipterans, Hymenopterans and Coleopterans per trap caught across four different fruit crops in 2019. The circular grid lines of each radial plot represent 20 captured individuals belonging to different insect categories listed above

identification. The resulting sequences (Table S5b) gave a similarity score of 94–100% with reference sequences in GenBank (MK435596.1 and MK801757.1).

Discussion

In the last decade, *D. suzukii* has spread from its native region in Asia to America and Europe, and the possibility for further dispersal to continental Africa was predicted (Dos Santos et al. 2017). Recently, the presence of *D. suzukii* was reported in Morocco, North Africa (Boughdad et al. 2020). Through monitoring, we show the presence but apparently restricted distribution of *D. suzukii* in Kenya, sub-Saharan Africa. Morphological examination and molecular confirmation by *COI* barcoding were used to prove identification at the species level.

During 2019, we captured *D. suzukii* in Longonot, a commercial farm growing blueberries, strawberries, raspberries and pomegranates, fruits which are known to support the development of the flies (Arnó et al. 2016; Wang et al. 2019). *Drosophila suzukii* was captured in plots of all different fruits. The establishment of invasive *D. suzukii* at Longonot farm was confirmed in 2020 where the pest was found both in monitoring traps and sampled fruit. Traps in raspberry tunnels caught most *D. suzukii* in 2019 and raspberries were more infested than blueberries and strawberries in 2020. This suggests that *D. suzukii* infestation differs across fruits and that raspberry is the most susceptible crop to *D. suzukii* at Longonot farm. Oviposition and infestation are known to differ among host species with raspberries representing one of the preferred fruits (Burrack et al. 2013; Lee et al. 2011). Moreover, more ripe berries were observed in plots with raspberries compared to plots with other types of fruits. *Drosophila suzukii* prefers ripe to unripe berries (Karageorgi et al. 2017; Keesey et al. 2015) and captures of *D. suzukii* across orchards in close proximity are known to fluctuate depending on fruit phenology (Lee et al. 2015; Wang et al. 2016). In the 2019 survey, we did not investigate the infestation of fruit. However, damage by unspecified insect pests was occurring in the farm as was reported by the field manager. The detection of *D. suzukii* since 2019 and the emergence of the pest from incubated fruits collected during the 2020 survey provide a likely explanation for the reported insect damage. At Longonot, the mixed production of different crops with successive availability of ripe fruits throughout the season, as well as the practice of drip irrigation and cultivation in tunnels, might provide most suitable microhabitats facilitating *D. suzukii* establishment at the farm (Diepenbrock and Burrack 2017; Khaliq et al. 2014; Toxopeus et al. 2016).

Despite the availability of host fruits at the farms that represent 11 out of the 14 locations surveyed in 2020, *D. suzukii* was only found at Longonot farm. This, in consequence, does not allow conclusions about the presence or absence of the pest apart from Longonot. Repetitive detection of *D. suzukii* at Longonot proves the validity of our monitoring technique and suggests that the fly would have been detected if present. *Drosophila suzukii* has been shown to disperse over long distances (Tait et al. 2018). The establishment at Longonot implies the possibility of an area-wide dispersal if *D. suzukii* is not contained. In the recent past, invasive pest species of the genus *Bactrocera* have caused substantial losses to Africa's fruit industry (Drew et al. 2005; Ekesi et al. 2016; Lux et al. 2003). In Europe and America, *D. suzukii* infestation has caused huge yield losses and costs exceeding $100 million per fruit per year (De Ros et al. 2013; Mazzi et al. 2017; Walsh et al. 2011), and therefore, Kenya and other African countries are likely to experience similar economic impact by the newly found pest. There has been a growing interest amongst small-scale farmers in Kenya, to grow soft fruits such as plums (Mbora et al. 2008) and berries. In the neighboring country, Ethiopia, strawberry cultivation is on the rise with small- and medium-scale growers adopting the practice. The detection and establishment of *D. suzukii* are a threat to the potential expansion of berry cultivation and trade in the region. Therefore, our findings serve a two-front wake-up call to stakeholders of the Kenyan and regional fruit industry. It calls for continuous region-wide monitoring to establish the extent of *D. suzukii* distribution, from which management programs can be defined and executed (Kenis et al. 2016).

In addition to monitoring, other measures have been proved as important tools to control *D. suzukii* and should support the development of integrated management practices (IPM) in Kenya and other African countries. Informing growers about the appearance of *D. suzukii* and how to recognize specific damage and the pest itself is a most urgent task. Self-made traps similar to that applied in our study (Fig. S2) could facilitate on-farm monitoring. Directed sanitary measures on farms should be implemented to reduce development of *D. suzukii*, e.g., in decaying fruit left on the plant or fallen to the ground (Haye et al. 2016). Further, adjustments in cultivation practice such as selection of less susceptible varieties or harvesting at an early stage of ripening before berries become soft and more likely to be infested should be considered for the coming growing seasons (Hampton et al. 2014; Lee et al. 2016). Another measure that could be applicable to farmers is the use of netting to reduce the entering of *D. suzukii* into tunnels or to cover and protect crops such as blueberries (Del Fava et al. 2017). Insecticides have been intensively studied and are applied in conventional and organic production (Van Timmeren and Isaacs 2013). For example, spinosad is one of the widely used insecticides for control of *D. suzukii* and even used in organic production (Bruck et al., 2011).

Recent studies increase the general concern about insecticide resistance in *D. suzukii* advocating the application of sustainable pest control methods (Gress and Zalom 2019). Biological control is a promising route to reduce *D. suzukii* infestation using natural enemies and insect pathogens (Becher et al. 2018; Biondi et al 2020; Daane et al. 2016; Gabarra et al. 2015; Lee et al. 2019; Wolf et al. 2020). Findings on the specificity of parasitoids with respect to *Drosophila* host species, different fruit species as well as fruit ripeness give grounds for hope toward the development of biological control (Girod et al 2018; Wolf et al. 2020; Biondi et al 2020; Seehausen et al 2020). In our trap captures, non-targeted Coleopterans and Hymenopterans were recorded but not identified. Previously, predatory Coleopterans and parasitic Hymenopterans were reported in *D. suzukii* traps and monitoring (Wang et al. 2016; Wolf et al. 2018). The possible existence of natural enemies in the area warrants follow-up studies. Generally, *D. suzukii* is not limited to sites of fruit production but is often found in forests or unmanaged areas which emphasizes the need for area-wide and long-term control strategies exploiting approaches such as biological control or the sterile insect technique (Haye et al. 2016; Seehausen et al. 2020; Nikolouli et al. 2020).

The detection of *D. suzukii* at only one location indicates that the distribution of *D. suzukii* in Kenya still is restricted and the invasion is at an early stage. New pest invasions are characterized by an arrival and an establishment phase that generally allow management to interfere with pest dispersal (Kolar and Lodge 2001; Liebhold and Tobin 2008). Overall, the combination of different measures such as monitoring, chemical, biological and cultural management, combined with preventive cultural practice, provides a toolbox to develop a sustainable IPM approach at local and landscape scale (Cini et al. 2012; Del Fava et al. 2017; Haye et al. 2016), targeting *D. suzukii* as a new pest in sub-Saharan Africa.

Dos Santos et al. (2017) had predicted that Tanzania offers a larger region suitable for *D. suzukii* establishment than Kenya, suggesting that while this is the first record of the pest in continental sub-Saharan Africa, a region-wide monitoring program is required to obtain a better overview on the current status of *D. suzukii* occurrence. Previous studies established multiple invasions in other infested countries (Carvajal and Markow 2010; De la Vega et al. 2020; Lavrinienko et al. 2017). It would be intriguing to study population genetics of the Kenyan population and establish the possible nature of the invasion. Clearly, more information is needed about the distribution and phenology of *D. suzukii* in Africa, potential host fruits on farms and in the wild, and the occurrence of natural enemies. Cost estimations on the economic impact and control programs in Kenya and other African countries would be helpful to guide future strategies for adequate management. Altogether, understanding the population dynamics of *D. suzukii* in the local ecological, environmental, landscape and horticultural context will provide a benchmark for successful management.

In conclusion, by using traps we showed the presence of *D. suzukii* at Longonot farm (Nakuru county) in Kenya. Through host incubation in the laboratory, we confirmed infestation of the berries collected from the farm. We have through morphological examination and DNA barcoding confirmed the identity of the collected flies. Altogether, *D. suzukii* is present in Kenya but was only detected in one out of six monitored counties suggesting that the pest is at an early phase of invasion and might be absent in the main host-fruit production areas of Kenya. Our findings add to the growing list of countries recently invaded by *D. suzukii*, illustrating the exceptionally fast and ongoing geographical range expansion of this pest insect. *Drosophila suzukii* has a high potential of dispersal, which necessitates quick and concerted efforts to prevent further distribution in the region.

Author contribution

CAK, IK, GR and PGB conceived the idea. CAK, IK, GR, SN, MK, SAM, PN and PGB designed the experiments. CAK, SN, SAM, PWN, MK, MKG and GGK conducted the survey. CAK and SN did morphological identification. LAO, FMK, SR and HMGL did molecular identification and analysis. MK assayed fruit incubation. CAK and GR analyzed the trap capture results. CAK and PGB wrote the manuscript with contributions by SR, SN, SAM and FMK. All authors read and approved the manuscript.

Acknowledgements We thank Sunday Ekesi, Ylva Hillbur and Segenet Kelemu for their advice and support to accomplish this study and Robinson Okoth Kisero and Evans Odhiambo for their support during the field work. We are also thankful to Levi Odhiambo Ombura for assistance with molecular characterization of the flies reared from fruits, Mary Guantai from KEPHIS JKIA and Joseph Mulwa from KALRO Kabete for insect identification, Sebastian Larsson-Herrera and Adam Flöhr (statistics@slu) for assistance in R and Sharon Hill for comments and language edits. We are grateful to berry famers and the Longonot management for collaboration to access their farms. This work was supported by the Gösta och Anna-Birgit Henrikssonss foundation, the Swedish Research Council Formas (Grant 2015-1221) and the Wallenberg Foundation (travel grant), the SLU Platform Plant Protection and the SLU Centre for Biological Control (CBC); the Norwegian Agency for Development Cooperation, the Section for research, innovation, and higher education (Grant No. RAF-3058 KEN-18/0005) and the Government of Kenya through the Horticulture Research Fund (Grant to KALRO). We would also like to acknowledge the core financial assistance to *icipe* by UK's Foreign, Commonwealth and Development Office (FCDO); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors.

Funding Open Access funding provided by Swedish University of Agricultural Sciences.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This study did not involve any human or animal (vertebrate) subject.

Informed consent Informed consent was obtained from all individual participants included on the study.

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