Entomological Research

Entomological Research **45** (2015) 354–359

SHORT COMMUNICATION

Oviposition and development potential of the spotted-wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), on uninjured Campbell Early grape

Min Jee KIM[1], Jong Seok KIM[1], Jeong Sun PARK[1], Deuk-Soo CHOI[2], Jinyoung PARK[3] and Iksoo KIM[1]

[1] Department of Applied Biology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Republic of Korea
[2] Department of Plant Quarantine, Animal and Plant Quarantine Agency, Anyang 430-016, Republic of Korea
[3] Department of Nature Survey, National Institute of Ecology, Seocheon 325-813, Republic Korea

Correspondence
Iksoo Kim, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea.
Email: ikkim81@chonnam.ac.kr

Received 6 August 2015;
accepted 4 October 2015.

doi: 10.1111/1748-5967.12142

Abstract

The spotted-wing drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), originally distributed across a few Asian countries including South Korea, has invaded North America and Europe but is absent from Australia. In order to export the South Korean grape cultivar Campbell Early to Australia, its potential to serve as an oviposition and development medium for SWD must first be determined. In this study, we determined the oviposition and development potential of SWD on Campbell Early, after elucidating the SWD life cycle and establishing an artificial diet-based mass-culturing system. An investigation of the life cycle under five temperature regimes (16, 19, 22, 25 and 28°C) showed that the durations of the egg, larval and adult stages were shortened when temperature was increased from 16, 19, 22, 25 and 28°C, but pupal duration was shortest at 25°C and extended again at 28°C. A test of oviposition and development potential of SWD on Campbell Early grape clusters showed oviposition of 30.8 ± 6.8 eggs per cluster of injured grapes and 157.7 ± 16.2 eggs on a culture dish of artificial diet. However, in a similar experiment using uninjured grape clusters, only a single egg was deposited on the grape skin, which soon dried. In light of these results, newly harvested grapes left at vineyards during daily harvests are unlikely to serve as an oviposition and development medium for SWD, as long as the grapes remain uninjured.

Key words: Campbell Early grape, *Drosophila suzukii*, quarantine, spotted-wing drosophila.

Introduction

The spotted-wing drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), is economically damaging because of its ability to develop on a variety of both cultivated and wild soft-skinned fruits, including the wine and table grapes grown in both the native and invaded ranges of SWD (Kanzawa 1935, 1939; Cini *et al*. 2012). SWD is more damaging than most other drosophiline species because the females possess a serrated ovipositor that can pierce most thin-skinned, healthy, unwounded and ripening fruits, thereby causing high fruit damage leading to economic losses (Mitsui *et al*. 2006; Walsh *et al*. 2011). Furthermore, the oviposition scar exposes the fruit to secondary attack by pathogens and other insects (Hauser *et al*. 2009).

SWD is native to several Asian countries, including Korea, Japan, China and India (Toda 1991; Hauser *et al*. 2009), but has invaded the USA, British Columbia, Italy, France and Spain (Toda 1991; Hauser *et al*. 2009; Hauser 2011; Lee *et al*. 2011). After its first reported sighting in Hawaii in 1980 (Kaneshiro 1983), SWD continued to expand its range across the USA, and an economic loss of $US100 million was subsequently reported (Lee *et al*. 2011).

Since its first detection in Japan in 1916, the country has recorded severe damage to grapes and cherries due to SWD (Kanzawa 1939). However, no severe damage due to SWD has been reported in Korea, although the incidence of SWD on grape, linden, apple, peach, pear, plum, persimmon and loquat has been reported in early Korean literature (Nagayama & Okamoto 1940).

The table grape variety Campbell Early is the primary grape cultivar produced in Korea, accounting for 73% of grapes produced. Attempts to export table grapes from Korea to Australia began in 1990, leading Australia to conduct a pest risk assessment of the cultivar. As a result, several pest risk management measures and phytosanitary procedures have been proposed, with the aim of reducing the risk of introducing SWD to Australia (Biosecurity Australia 2011). The goal of one of the requested trials was to demonstrate that Campbell Early has a low level of susceptibility to SWD. Typical harvesting procedures in Korea include storing the harvested grapes in the shade, in order to keep them cool during daily harvests prior to transfer to the packing house. This initial storage period may provide SWD females with an oviposition opportunity. Typically, damaged, low-quality grapes are excluded from exportation, and so it is highly unlikely that such grapes, which may contain SWD eggs, are exported to Australia. However, if SWD females tend to oviposit on undamaged grapes by piercing the skin with their serrated ovipositors, there is high potential for SWD invasion in Australia.

In this study, we investigated the egg deposition and development potential of SWD on both injured and uninjured Campbell Early grapes. For this, we first examined the life cycle of SWD on an artificial diet and established a mass-culture system by using the artificial diet.

Materials and methods

Collection of SWD

The collection of SWD was mainly focused on individuals in the egg stage. In the field, female SWD individuals were allowed to oviposit on fruits (banana, apple, kiwi, etc.) in round plastic bottle traps (diameter 6.5 cm, height 13.5 cm) baited with apple cider vinegar (Bragg, Santa Barbara, CA, USA). Alternatively, fallen fruits (blueberry, wild mulberry and wild strawberry) were collected in the field and incubated at room temperature in the laboratory until SWD individuals reached the pupal stage. SWD pupae have characteristic SWD-specific features: they are about 3 mm long, brown, and football-shaped with small finger-like projections on two stalks at the head end (Johnson & O'Neill 2013; Toševski *et al*. 2014). Thus, the pupal stage was used to identify SWD pupae. Some field-collected SWD individuals were further identified by sequencing the DNA barcoding region and comparing it to the GenBank-registered *D. suzukii* barcoding sequences (e.g. GenBank accession numbers HM803277, HM803279 and HQ646999). Comparisons of the SWD barcoding sequences among Korean and foreign populations will be presented in detail elsewhere, along with the methodology performed in this study. Once the adults emerged, male SWD individuals were further examined for the black/grey spot on the end of each wing that is a characteristic of the species (Johnson & O'Neill 2013; Toševski *et al*. 2014).

SWD culture

All SWD rearing and experimentation was conducted at 22 ± 1°C, relative humidity 70–80% and a photoperiod cycle of 16 h light : 8 h dark (LD 16:8). Larvae were reared on the cornmeal–malt medium described by Dalton *et al*. (2011). The artificial diet was prepared as described by Dalton *et al*. (2011), with an additional liter of boiled distilled water. To culture the SWD populations, nine insect breeding dishes (diameter 10 cm, height 4 cm) lined with artificial diet (2.5 cm deep) were set in an insect cage (32.5 × 32.5 × 32.5 cm; Bug-dorm, MegaView Science, Taichung, Taiwan). Cotton swabs soaked in 10% sugar solution were placed in a plastic dish (diameter 4 cm at the bottom, height 7 cm), then in the cage. Every 3–4 weeks, one bug-dorm cage was connected to another in order to transfer SWD adults to a new cage with a container of the fresh artificial diet. The sugar solution was replaced every two weeks. Thus, 24 bug-dorm insect cages, each with approximately 1500 flies, were maintained.

Life cycle

In order to investigate the duration of different developmental periods and variations in the adult lifespan under different temperature regimes, SWD females were allowed to oviposit for 4 h on fresh artificial diet in the insect breeding dishes. Individual eggs, along with a paucity of circumjacent artificial diet, were transferred to plastic cups (diameter 5 cm, height 1 cm) lined with artificial diet (60 mm in depth). Thirty to 50 newly oviposited eggs were subjected to each temperature regime. The growth of the SWD in the plastic cups was checked every 12 h. Larvae, pupae and adults were scored as dead if they made no detectable movement when touched with a brush. The relative humidity and photoperiod were fixed to 70–80% and LD 16:8, and temperatures were set to 16, 19, 22, 25 and 28 ± 1°C. All experiments were conducted in triplicate. The standard deviation was calculated using the program SAS v8 (SAS Institute, Cary, NC, USA).

Oviposition and development potential test

To investigate the oviposition and development potential of SWD on Campbell Early, grapes of the highest available

Figure 1 Uninjured and injured grape clusters used for the test of oviposition and development potential. (A) Injured Campbell Early cluster, (B) uninjured Campbell Early cluster.

quality were purchased from the local fruit market. Of these, the individual grape clusters with no visible injury and higher than 15° brix were used for the experiment.

Two experiments were performed: (i) testing individual grapes; and (ii) testing the efficacy of full clusters as oviposition and development media.

The individual grape experiment allowed for the observation of egg deposition traces, egg hatching and larval growth, because the individual grapes were not tightly clustered. In the individual test, four experimental groups were prepared: (i) a control using an artificial diet as the oviposition and development medium: (ii) injured grapes as the oviposition and development medium; (iii) uninjured grapes as the oviposition and development medium; and (iv) a preference test between injured and uninjured individual grapes as oviposition and development media. Individual grapes were prepared by detaching them from the grape cluster with their stalks still attached, and placing them in plastic cups (diameter 4 cm, height 4 cm).

As a control, an artificial diet was provided in the plastic cups at a depth of about 1.5 cm (diameter 4 cm, height 4 cm). To test the efficacy of individual injured grapes as an oviposition and development medium, two 0.5 cm-long injuries were generated per individual grape using a gimlet to mimic bird pecks, after which the individual grapes were placed in the plastic cups, whereas uninjured grapes were selected from the grape cluster. Each two SWD females were allowed to mate with four males for 24–48 h, then allowed to oviposit for 24 h on each control, injured and uninjured group. In the preference test, each injured and uninjured grape was placed in a plastic cup without a cap, after which the cups were placed at each end of custom-made insect cages (35 × 35 × 39.5 cm) at a distance of 30 cm. A single mated female was used in this test. All experiments included six replicates. Every 24 h, the individual grapes were examined by the naked eye and examined further under a stereomicroscope (6.5–50×; Zeiss Stemi 2000, Edmund Optics Inc., Barrington, NJ, USA). Because of the small size and internal feeding behavior of the SWD eggs and larvae, obtaining an accurate count of the number of premature stages is very difficult. Thus, the number of F_1 (eggs, larvae, pupae and adults) individuals were counted 14 d after oviposition. The adults that emerged during this period were removed to eliminate any potential for confusing the F_1 and F_2 offspring.

In the grape cluster experiments, another four experimental groups were prepared: (i) a control using an artificial diet as an oviposition and development medium; (ii) injured grape clusters used as an oviposition and development medium; (iii) uninjured grape clusters as an oviposition and development medium; and (iv) two additional grape varieties. As a control, an artificial diet was provided to the individuals in the culture cage, as described in the SWD culture section. To create injured grape clusters, twenty 0.5 cm-long injuries were inflicted per grape cluster (Fig. 1A), but grape clusters in the uninjured treatment were not subjected to injury (Fig. 1B). In each treatment group, 20 SWD females were allowed to mate with 40 males for 24–48 h and then allowed to oviposit for 24 h. Along with Campbell Early, two other grape varieties, Gerbong (Kyoho grape) and Moru (Korean wild grape, Muscat Bailey A), which are the next most popular in Korea after Campbell Early, were also tested as oviposition and development media. All experiments were replicated six times. This test was also performed in the bug-dorm insect cage (32.5 × 32.5 × 32.5 cm). The remaining experimental methods and observations were conducted as described for the individual grape test.

The standard deviation of the mean was computed using the formula STDEV in Microsoft Excel (Microsoft office Excel 2013; Microsoft Corp., Seattle, CA, USA).

Results and discussion

Life cycle

The investigation of the life cycle under five temperature regimes (16, 19, 22, 25 and 28°C) showed that the duration of the egg, larva and adult periods decreased as temperature increased up to 28°C (2.7 to 1.1 d for eggs, 8.3 to 4.1 d for larvae, and 59.7 to 20.7 d for adults), but the pupal period decreased by temperatures of only up to 25°C (9.8 to 4.2 d) (Table 1).

SWD adults survive substantially longer under laboratory conditions (38.6 ± 7.38 d at 22°C). A previous investigation of the adult longevity of different *Drosophila* species has

Table 1 Developmental periods of *Drosophila suzukii* under different temperature regimes

Temperature (°C)	*n*	Duration (days ± SD)				Accumulative duration (days ± SD)
		Egg	Larva	Pupa	Adult	
16	100	2.7 ± 0.91 (94)	8.3 ± 1.11 (86)	9.8 ± 0.76 (83)	59.7 ± 12.55 (71)	80.3 ± 12.27 (71)
19	100	2.3 ± 0.70 (92)	6.7 ± 1.03 (87)	7.3 ± 0.54 (69)	51.5 ± 6.35 (69)	67.8 ± 6.37 (69)
22	120	2.2 ± 0.42 (119)	4.5 ± 0.59 (107)	5.6 ± 0.53 (104)	38.6 ± 7.38 (96)	50.4 ± 7.39 (96)
25	120	2.1 ± 0.60 (119)	4.2 ± 0.74 (105)	4.2 ± 0.47 (96)	24.9 ± 6.10 (90)	35.2 ± 6.18 (90)
28	150	1.1 ± 0.33 (143)	4.1 ± 0.59 (120)	5.3 ± 0.78 (117)	20.7 ± 5.47 (79)	31.3 ± 5.26 (79)

n, numbers of individuals used for experiment.

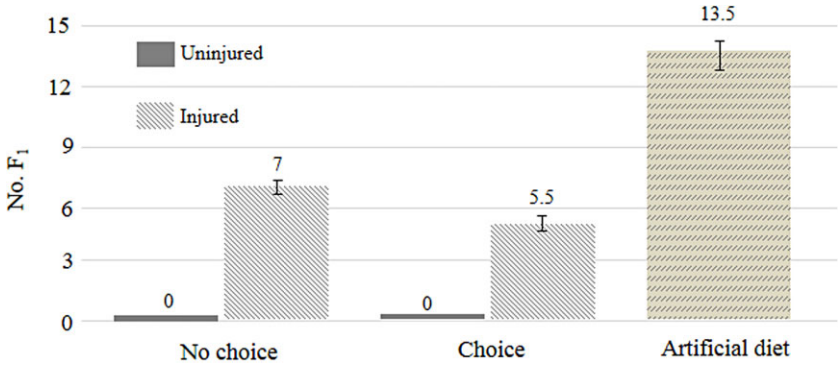

Figure 2 Mean number of F_1 individuals observed on individual Campbell Early grapes 14 d after oviposition. Vertical bars represent the standard deviation of the mean value.

shown that the duration of the adult period is approximately 70.8 d in *D. virilis*, 68.2 d in *D. lummei*, 47.4 d in *D. texana*, 42.3 d in *D. americana* and 46.9 d in *D. novamexicana* under laboratory conditions at 23°C. Thus, the adult period varies substantially from species to species (Durbin & Yoon 1986), but is somewhat shorter in SWD than in other *Drosophila* species. Furthermore, a substantial intraspecific difference among some species has been reported depending on the species' geographic origin (Durbin & Yoon 1986). For example, the adult longevity of *D. americana*, from Anderson, Indiana, was 58.5 d in males and 71.4 d in females, but it was 24.2 d in males and 34.3 d in females originating from Chinook, Montana, USA. It is supposed that many factors (e.g. temperature, humidity, photoperiod, natural enemies, pathogens and food sources) may affect the longevity of adults (Zwaan *et al*. 1991; Partridge *et al*. 2005).

Oviposition potential test with individual grapes

In order to determine whether Campbell Early can serve as an oviposition and developmental medium, traces of egg deposition, egg hatching, larval development, the number of pupae, and adult emergence were examined in detail for two weeks in both uninjured and injured individual grapes (Fig. 2). No egg deposition traces were found on the uninjured grape individuals, although SWD females continuously wandered around the uninjured grapes while repeatedly contracting and expanding their ovipositors. Conversely, all injured grapes served as oviposition and development media, resulting in seven F_1 individuals per grape (counted at 14 d after oviposition). They comprised 18.61% adults, 11.62% live pupae, 9.30% dead eggs, 37.21% dead larvae and 23.26% dead pupae. Egg death occurred when the SWD female deposited eggs on the grape skin, causing them to dry in 3 d. Pupal and larval death appeared to occur because of the inability to respire because of the release of juice from the site of injury. In contrast, 13.5 F_1 individuals were observed per grape in the artificial diet treatment, 65.4% of which emerged during the 2-week observation period (Fig. 2). A preference test comparing the uninjured and injured grapes also showed that no egg and deposition traces were found on the uninjured grapes, whereas 5.5 F_1 traces were found per grape in the injured grapes. Collectively, the uninjured Campbell Early grapes did not serve as oviposition and egg development media, whereas injured grapes can serve as an oviposition medium.

Test of oviposition potential with grape clusters

To further verify whether the uninjured Campbell Early grapes can serve as oviposition and development media, the

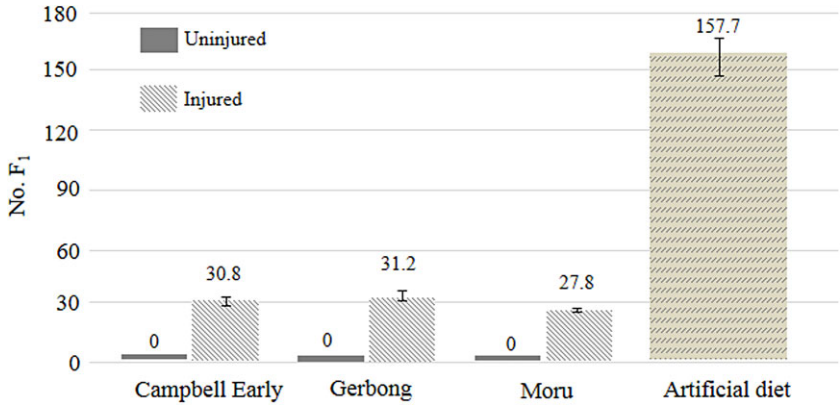

Figure 3 Mean number of F_1 individuals observed on grape clusters of Campbell Early, Gerbong, and Moru 14 d after oviposition. Vertical bars represent the standard deviation of the mean value.

same experiment was carried out, but using grape clusters instead of individual grapes. In addition, 20 SWD females were used instead of two. No egg or deposition traces were detected on the uninjured grape clusters of Campbell Early (Fig. 3). The Gerbong and Moru varieties also did not display egg or deposition traces on their uninjured clusters. A single egg was found on an uninjured Campbell Early grape skin, but it was unable to develop. On the injured grape clusters, 30.8 F_1 individuals were observed per cluster (counted at 14 d after oviposition). Similarly, 31.2 F_1 and 27.8 F_1 individuals per cluster of injured Gerbong and Moru grapes were observed, respectively, but no statistically significant difference was noted among grape varieties. When the artificial diet was used as a control, 157.7 F_1 individuals were observed per plastic cup. Collectively, the uninjured grapes of Campbell Early and the other two varieties did not serve as oviposition and development media, whereas injured grape clusters served as oviposition and development media; the results are consistent with those of the individual grape experiment.

Previously, Lee *et al*. (2011) evaluated the susceptibility of blackberries, blueberries, cherries, grapes, raspberries and strawberries to *D. suzukii* at various ripeness stages and in various cultivars. Among grape cultivars, intact Chardonnay, Merlot, Pinot gris and Pinot noir were nearly free of eggs in the choice test (>0.8 individual), and 0–9% of the eggs deposited under those conditions developed in each cultivars (Lee *et al*. 2011). In addition, a no-choice test of intact individual grapes of the Flame and Merlot cultivars in plastic cups showed eggs (Maiguashca *et al*. 2010). However, an individual grape test of uninjured Campbell Early and Riesling resulted in no eggs deposited during no-choice tests where five adult female SWD were added to the plastic cups (Maiguashca *et al*. 2010). This result is completely consistent with those of the current study of individual Campbell Early grapes and the grape cluster study that uninjured Campbell Early grapes do not serve as oviposition and egg development media. One grape skin was found to harbor a single egg, but this differs from typical egg depositions, as the egg was deposited on the surface of the grape skin rather than inside the grape. Furthermore, this egg dried completely after a few days, without developing further. The skin of the Campbell Early variety appears to be too thick for the SWD females to puncture, even though they continuously wandered around the surface of the uninjured grapes and repeatedly contracted and expanded their ovipositors. As indicated previously (Maiguashca *et al*. 2010), injury might be the greatest factor influencing the success of oviposition by SWD females and the successful hatching of maggots in Campbell Early grapes. On the basis of these results, newly harvested grapes that are left in vineyards until transfer to the packing house are not an adequate medium for the oviposition and development by SWD females, as long as the grapes remain uninjured.

Acknowledgements

This research was supported by a grant (22143383400) from the Animal and Plant Quarantine Agency, South Korea.

References

Biosecurity Australia (2011) *Final Non Regulated Import Risk Analysis Report for Table Grapes from the Republic of Korea*. Department of Agriculture, Fisheries and Forestry, Canberra.

Cini A, Ioriatti C, Anfora G (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology* **65**: 149–160.

Dalton DT, Walton VM, Shearer PW, Walsh DB, Caprile J, Saacs R (2011) Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. *Pest Management Science* **67**: 1368–1374.

Durbin EJ, Yoon JS (1986) Longevity in the *Drosophila virilis* species group. I. The *D. virilis* Phylad. *Ohio Journal of Science* **86**: 14–17.

Hauser M (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest Management Science* **67**: 1352–1357.

Hauser M, Gaimari S, Damus M (2009) *Drosophila suzukii* new to North America. *Fly Times* **43**: 12–15.

Johnson DT, O'Neill J (2013) *Biology, Identification and Management of Spotted Wing Drosophila*. Cooperative Extension Service, University of Arkansas, US Department of Agriculture, and County Governments, Little Rock, AR.

Kaneshiro KY (1983) *Drosophila* (*Sophophora*) *suzukii* (Matsumura). *Proceedings Hawaiian Entomological Society* **24**: 179.

Kanzawa T (1935) *Research into the fruit-fly* Drosophila suzukii *Matsumura (preliminary report)*. Yamanashi Prefecture Agricultural Experiment Station, Kofu.

Kanzawa T (1939) *Studies on* Drosophila suzukii *Mats*. Yamanashi Prefecture Agricultural Experiment Station, Kofu.

Lee JC, Bruck DJ, Dreves AJ, Ioriatti C, Vogt H, Baufeld P (2011) In focus: spotted wing drosophila, *Drosophila suzukii*, across perspectives. *Pest Management Science* **67**: 1349–1351.

Maiguashca F, Ferguson H, Bahder B, Brooks T, O'Neal S, Walsh D (2010) Injured and ripening fruit may become more attractive: monitoring strongly recommended. Washington State University Extension, Spotted Wing Drosophila Grape Update, October 4, 2010.

Mitsui H, Takahashi HK, Kimura MT (2006) Spatial distributions and clutch sizes of *Drosophila* species ovipositing on cherry fruits of different stages. *Population Ecology* **48**: 233–237.

Nagayama S, Okamoto H (1940) List of fruits insect pests in Korea. *Annals of Agriculture Experimental Station of Governor-General of Chosen* **12**: 195–247.

Partridge L, Piper MDW, Mair W (2005) Dietary restriction in *Drosophila*. *Mechanisms of Ageing and Development* **126**: 938–950.

Toda MJ (1991) Drosophilidae (Diptera) in Myanmar (Burma) VII. The *Drosophila melanogaster* species-group, excepting the *D. montium* species sub-group. *Oriental Insects* **25**: 69–94.

Toševski I, Milenković S, Krstić O *et al*. (2014) *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae), a new invasive pest in Serbia. *Zaštita bilja* **65**: 99–104.

Walsh DB, Bolda MP, Goodhue RE *et al*. (2011) *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management* **1**: 1–7.

Zwaan BJ, Bijlsma R, Hoekstra RF (1991) On the developmental theory of ageing. I. Starvation resistance and longevity in *Drosophila melanogaster* in relation to pre-adult breeding conditions. *Heredity* **66**: 29–39.