J Pest Sci (2016) 89:749–759
DOI 10.1007/s10340-016-0739-6

ORIGINAL PAPER

An invader supported by a parasite: Mistletoe berries as a host for food and reproduction of Spotted Wing Drosophila in early spring

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Received: 11 November 2015 / Revised: 24 January 2016 / Accepted: 1 February 2016 / Published online: 11 February 2016

Abstract The Spotted Wing Drosophila (SWD), *Drosophila suzukii* Matsumura, an invasive pest species in Europe and the Americas, is able to feed and reproduce on numerous fruit crops and a wide range of wild host plants. SWD is thought to overwinter outside of agricultural fields in forests and hedges. To identify overwintering sites and early spring oviposition hosts, traps were installed in forests. In spring 2015, traps in the canopy of pine trees parasitized by mistletoe, *Viscum album* subsp. *laxum,* captured significantly more SWD than traps in pine trees without mistletoe. We found SWD females with ripe eggs coinciding with ripening and ripe mistletoe berries. We investigated whether mistletoe may serve as a host for SWD. Under laboratory conditions, SWD developed from egg to adult in mistletoe berries. More adults emerged from wounded berries. Females were observed to feed on berries and survived up to eight days without other food. A few adults emerged from wild mistletoe berries. To understand the attraction of SWD to parasitized trees, we analyzed the volatile organic compounds (VOCs) collected from the headspace of mistletoe berries by GC–MS and identified the main components. Thirty-two VOCs were found. Wounded and unwounded berries differed significantly in the quantity of 11 VOCs emitted. The odor spectrum showed many similarities to other typical berry odors. The combination of field surveys and laboratory assays identified a new reproduction host for SWD in spring. This host plant may help SWD to withstand the bottleneck period for survival in winter and spring.

Keywords Alternative host · Overwintering · Invasion biology · Volatile compounds · Reproductive status · Population dynamics

Key message

- Mistletoe berries support SWD nutrition and reproduction.
- SWD abundance in the canopy of pine trees parasitized by *V. album* was higher than in the canopy of *P. sylvestris* without *V. album* or at lower heights in the vegetation.
- The odor spectrum showed many similarities to other typical berry odors.
- Adult SWD emerged from field-collected mistletoe berries indicating that mistletoe is one of the first reproductive hosts for SWD in Central Europe.

Introduction

The invasive Spotted Wing Drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a worldwide pest species that recently became established in Europe (Cini et al. 2012, 2014). After its discovery in the

Communicated by A. Biondi.

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USA (California) and Southern Europe (Italy & Spain) in 2008, it rapidly dispersed and is now present in many regions of North America and most European countries, and since 2013 in Brazil (Calabria et al. 2012; Deprá et al. 2014; Asplen et al. 2015). In Germany, it was recorded for the first time in 2011 (Heuck 2012; Vogt et al. 2012a, b) and spread rapidly throughout the country. Since the summer 2014, it has been found in all Federal States of Germany (Asplen et al. 2015; Köppler and Vogt 2015; Vogt and Briem 2015).

As a highly polyphagous pest species, SWD infests a broad range of wild hosts and cultivated fruits. Its complete host range has not yet been determined. It infests economically important berry crops (e.g., raspberries and blackberries: *Rubus* spp.), stone fruits (e.g., cherries: *Prunus avium*, *P. cerasus*, plums: *Prunus domestica*), as well as many wild host plants like *Sambucus* sp., *Prunus serotina*, or *Cornus* sp. (Hauser 2011; Cini et al. 2012; Poyet et al. 2014; Lee et al. 2015). The female fly penetrates the skin of ripening and ripe fruits with its sclerotized saw-like ovipositor and lays its eggs underneath the fruit skin (Mitsui et al. 2010; Hauser 2011; Cini et al. 2012; Bellamy et al. 2013). Deposited eggs are detectable by their respiration filaments that protrude from the fruit.

According to current knowledge, SWD is overwintering as adult. It shows phenotypic plasticity with darker and bigger individuals in winter that can survive several months at 1 °C (Shearer PW, personal communication). Winter morphs are the most cold-tolerant life stage, but are chill intolerant. Thus, adults must avoid extreme cold temperatures to survive, e.g., by hiding in sheltered sites (Stephens et al. 2015; Jakobs et al. 2015). In addition, there is no egg production from fall to spring (Mitsui et al. 2010; Gerdeman and Tanigoshi 2012; Zerulla et al. 2015, and own observations, unpublished), suggesting a reproductive diapause. The findings of Ometto et al. (2013) about the low rate of molecular evolution in SWD support this as it could be due to its reduced rate of generations per year compared with its relatives. In temperate climates, SWD is a winter-active species when mean temperatures allow flight activity. Although exact data on temperature-dependent flight are not available, seasonal catches from diverse regions during winter indicate that flight activity seems to be still possible when mean daily temperatures are around 5 °C (Harris et al. 2014; Wiman et al. 2014; Briem et al. 2015; Shearer, pers. communication). During winter, SWD occurs especially in forest edges and interiors, a finding which supports the assumption of fly migration over larger distances away from orchards to suitable overwintering sites (Hamby et al. 2014; Briem et al. 2015). Furthermore, its ability for a fast recovery and survival potential at low temperatures and during short freezing periods by feeding on fruit dropped on the ground or leftover after harvest could enable successful overwintering (Dalton et al. 2011; Jakobs et al. 2015; Stephens et al. 2015).

During the mild winter 2013/2014, SWD adults were caught continuously in traps installed in forests at a standard height of 1.5 m above the ground as well as in the canopy (~18–20 m) of trees (Briem et al. 2015). In 2014, following an extraordinarily warm spring, reproduction of the overwintered population started earlier than in previous years. The shortened generation time due to the high ambient temperature allowed the population to increase rapidly causing high infestation levels in stone and soft fruits early in the season. As population growth in spring depends on winter survival as well as on early available reproduction hosts, identifying wild host plants where SWD can feed and oviposit in early spring might help provide additional knowledge about its population dynamics. Alternative host plants, such as *Lonicera* sp. or mistletoes (*Viscum* sp.), can supply sugar sources to SWD in winter (Lee et al. 2015) and early spring which might increase the survival rate of SWD. Within our landscape monitoring, traps in pine trees infested by mistletoes captured more SWD in spring than traps in pine trees without mistletoes. We hypothesized that mistletoe might serve as an early food source and host for reproduction. Thus, we conducted a study to investigate the suitability of mistletoe berries as food and host for SWD reproduction.

Methods and materials

SWD rearing and colony maintenance

Female SWD used for no-choice assays were obtained from a laboratory colony maintained at the Julius Kühn-Institut (JKI) Dossenheim, Germany since October 2013. The culture was started with offspring of adults that emerged from fruit (blackberry, raspberry, and cherry) sampled at the experimental fields of the JKI Dossenheim. Adult SWD were kept in cages (Bugdorm-1, Megaview, Taiwan) provided with a sugar-water source (5 % sucrose) and dry sugar and brewer's yeast mix (1:1). For rearing offspring JKI standard diet (30 g sugar, 142 g cornmeal, 20 g soy flour (Reformhaus, Germany), 34 g brewer's yeast (Diana, Germany),11.2 g agar and 5 g vitamin mixture (Vanderzant, MP Biomedicals, USA), 9.4 ml propionic acid, 1748 ml water) was used, based on a recipe from Fondazione Edmund Mach, San Michele, Italy (pers. communication G. Anfora). For oviposition, cups (125 ml, Huhtamaki, Finland) filled with JKI standard diet were placed in the cages. After 2–3 days, cups were removed and closed with a perforated lid. These oviposition substrates were stored under the same conditions as above until adults emerged. Then lids were removed and cups were placed in rearing cages. Rearing cages were stored in an environmental chamber at 23 °C, 60 % relative humidity (RH), and a photoperiod of 16 h:8 h (L:D).

Monitoring SWD adults

Transparent plastic cups (Jokey JETB 850, 870 ml, 12 cm high, 10 cm in diameter) closed with a lid and prepared with 20 holes ($d = 2.5$ mm) in the upper half were used as traps for monitoring the abundance of SWD in the field. As bait, 200 ml of naturally clouded apple cider vinegar (K-Classic, Kaufland, Neckarsulm, Germany) was mixed with water (ratio 2:3) and 0,025 % detergent (Ultra Sensitive, dm, Karlsruhe, Germany). The traps were distributed among pine trees in a forest (Dossenwald, N49.418949, E8.564489; 100–114 m a.s.l) between Mannheim and Schwetzingen (Baden-Württemberg, Germany) and in a second forest near Dossenheim (N49.450382, E8.691917; 290–330 a.s.l., Baden-Württemberg, Germany).

The Dossenwald is a nature reserve near Mannheim situated in the plain of the Rhine valley (Breunig and Demuth 2000). It is characterized by a high proportion of Scots pine, *P. sylvestris,* and several other deciduous forest tree species (e.g., *Robinia pseudoacacia, Fagus sylvatica, Prunus avium, P. padus*, *P. serotina, Quercus robur*). The forest near Dossenheim is part of the low mountain range named Odenwald. We chose sites on the western mountain side bordering the Rhine valley. Wild and cultivated host fruits (*Prunus* sp., *Rubus* sp., *Sambucus* sp., *Vitis* sp.) are found along its edges. The main part of the forest is a mixed stand with beech (*F. sylvatica*) and oak trees (*Quercus* sp.). In the sun-exposed parts, *P. sylvestris* are common; moreover, wild cherry trees (*P. avium, P. padus*), maple (*Acer* sp.) and spruce (*Picea abies*) are also present.

At each forest site, traps were installed at a height of about 20 m at the canopy of five *P. sylvestris* (one trap per tree) using a bow and arrow: first a thin string was shot to the treetop, then a thicker string was drawn up with the help of the thin one resulting in an infinite loop. Finally, the trap was fixed to the thick string and pulled to the canopy. For changing the trap, it was lowered with the help of the string loop. In addition, one trap was placed at standard monitoring height ($\sim$1.5–1.8 m) in each of the five trees. These trees were distanced by 50–100 m. Throughout the entire year, traps were biweekly exchanged and numbers of male and female SWD captured were determined under a stereo microscope (M3Z, Wild Heerbrugg, Switzerland). Female flies captured between March and May 2015 were stored in 70 % ethanol for further evaluation of ovarian development.

Ovigeny assessment

A total of 522 female individuals caught during March and May 2015 were dissected to evaluate ovarian development. Ovarian development was categorized into five categories (Fig. 1) (King et al. 1956; Zerulla et al. 2015):

1. Indiscernible ovarioles,
2. Unripe ovarioles,
3. Maturing eggs in ovarioles visible,
4. Mature eggs with filaments, and
5. Old eggs.

To determine the developmental stage of the ovaries, the abdomen of each fly was cut with a pair of tweezers and held open between the 3rd and 4th segments with another pair of tweezers. By doing this, the internal organs in the abdomen were exposed and ovarioles could be categorized. Photographs of representative developmental stages of eggs were taken with a photomacroscope (M400, Wild Heerbrugg, Switzerland).

Oviposition preference and development

No-choice trials were conducted to investigate the suitability of mistletoe berries for oviposition and development, and to determine the effect of berry wounding. Berries of *Viscum album* subsp. *laxum* were collected from pine trees in the forest site near Mannheim (N49.418949, E8.564489) at April 14th and 29th 2015. Branches were cut

Fig. 1 Developmental stages of SWD eggs (magnification: ×32). Photographs: J. Just & A. Frank, JKI Dossenheim

from the canopy of *P. sylvestris* at an approximate height of 15–18 m using a manlift (Nifty 150-T, Niftylift LtD, Germany). Fruits were stored at 18 °C until use. Six subsets of 10 berries which were not exposed to female SWD were macerated to determine Brix (% soluble solids) and pH.

The experimental unit was a small cage assembled with a Plexiglas ring ($d = 10$ cm, 3 cm height) and closed at both sides with glass plates (11 × 11 cm). The Plexiglas ring had five ventilation holes ($d = 1.3$ cm) covered with fine gauze and was equipped with a sugar-water source (5 % sucrose). In each of these cages, 10 wounded or 10 unwounded berries, respectively, were offered to ten 11-day-old gravid females. Ten replicates were performed for each treatment. Field-collected berries were checked for intactness by examining each berry for any cracks using a stereomicroscope. Intact berries were used for the treatment "unwounded," whereas for the treatment "wounded," intact berries were slit with a scalpel over a length of ~3–4 mm. The berries were arranged on small plastic lids to ensure that the slit remains on the upper side during the trials. These cages were stored in an environmental chamber at 23 °C, 60 % relative humidity (RH), and a photoperiod of 16:8 (L:D). After 48 h of exposure, females were removed from the cages and the number of eggs laid in berries of *V. album* subsp. *laxum* was counted by checking for egg filaments using a stereo microscope. The berries were then transferred to clear rearing cups (pint-sized Bugdorm 360 ml/12 oz, Megaview, Taiwan) with mesh lids and kept for 18 days in the environmental chamber and emerged adults were counted regularly. The trials were repeated three times, on April 15th, 22nd, and May 4th, 2015.

Survival rate

No-choice trials were conducted to determine the effect of intact or artificially wounded mistletoe berries on the survival rate of female SWD. The experimental unit for the assay was the same as described above, equipped with a water source and a small petri dish ($d = 6$ cm), filled with a moistened cotton pad and water instead of sugar-water. On the cotton pad, as described previously, 10 wounded or 10 unwounded berries per cage were offered to ten 11-day-old gravid females. Ten replicates were conducted per treatment. A third treatment offering only water served as a control. Dead females were counted once per day.

Initial infestation of mistletoe

To determine the infestation rate under natural conditions, a random sample of 100 berries (*V. album* subsp. *laxum* growing on *P. sylvestris)* collected on April 14th in Dossenwald and about 1,000 (*V. album* subsp. *album* growing on *Malus domestica*) on May 19th from apple trees of a meadow orchard near Heddesbach (N49.474216, E8.834986, Odenwald, Germany) were used. The berries were stored in small rearing tents (Bugdorm-2120, Megaview, Taiwan) in the environmental chamber until June 16th and daily checked for emerged adult SWD. As described previously, SWD abundance was assessed in this orchard with one trap in each of four apple trees from May 19th to June 16th 2015.

Sampling of volatile organic compounds (VOCs) emitted by mistletoe berries

To compare the profile of volatile chemical compounds emitted by wounded and unwounded berries of *V. album* subsp. *laxum*, berries (50 g) were put in clean glass petri dishes (without lids) and placed in 0.7-l desiccators continuously flushed with charcoal-filtered air. For each treatment six replicates were sampled. A quantitative 5-channel sampling device according to Rid et al. (2016) was used. Headspace of berries was sampled for 100 min (flow 100 ml/min) in an environmental chamber (18–20 °C). Volatiles were trapped in stainless steel sample tubes (Tenax TA 60/80, PerkinElmer, USA).

GC–MS analysis

Samples were analyzed using a thermal desorber (TurboMatrix ATD 650, PerkinElmer) connected to a gas chromatograph coupled mass spectrometer system (Clarus 680, PerkinElmer). Tubes were desorbed for 10 min at 250 °C. Volatiles were collected in a cold trap (Tenax TA) at −20 °C and transferred to the GC–MS system (99 °C/s to 250 °C, hold 1 min). A nonpolar Rxi-5 ms capillary column (Restek, Germany) was used for volatile separation. Splitless injection was employed using helium as a carrier gas (flow rate 5 ml/min, column head pressure 5 bar). The initial oven temperature of 40 °C was held for 1 min, followed by a gradient (40–180 °C at a rate of 5 °C/min, and a rate of 20 °C/min from 180 to 280 °C, final temperature was held for 6 min). The quadruple mass detector was operated in electron impact (EI) mode at 70 eV. Full-scan mass spectra were collected within the range of 35–350 m/z. Volatile compounds were identified by comparing fragmentation patterns with data from mass spectral libraries (NIST 08 Mass Spectral Library, National Institute of Standards and Technology, Wiley; JKI-OW Library). Peak retention times were compared with standards according to Weintraub and Gross (2013). Relative proportions of selected compounds were calculated from peak areas and the sum of the selected compounds was set at 100 %.

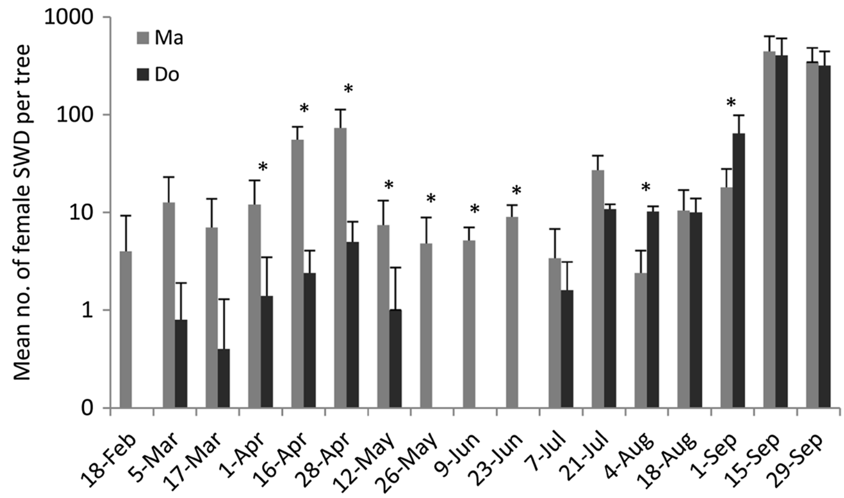

Fig. 2 Flight activity of female SWD monitored in pine trees at Dossenwald (Ma) and Dossenheim (Do) in 2015. *Bars* = mean number of female flies per tree. Log scale with standard deviation (SD) ($n = 5$; 2 traps per tree, i.e., standard height and canopy summarized); statistically significant differences are indicated by * ($p < 0.05$)

Statistical analysis

A linear mixed effect model was fitted (Pinheiro and Bates 2000) to assess the effect of trap height, forest site, and sample date on abundance of female SWD. Using trap ID nested within tree ID as a random factor, this model accounts for non-independent errors that may occur due to repeated sampling of trees. Data were log transformed to achieve normality of residuals. Generalized linear models (GLMs) were used to analyze the effect of treatment and replicate and their interaction on egg number and hatch rate of adults. The GLM was performed using quasi-Poisson family for count data due to the observed overdispersion. Significance of terms was tested using F-test and the function drop1. Both models were simplified by removing nonsignificant interactions ($p > 0.05$) and nonsignificant factors. Factors that figured in significant interactions were kept in the model (Crawley 2002). Post hoc comparisons between treatments and dates were obtained from least-square means and confidence intervals from statistical models using the function lsmeans. *P* values were adjusted using the method of Hochberg (1988). A Mann–Whitney *U* test was performed for the comparison of relative volatile amounts released by unwounded and wounded berries. Significance level was set at $p < 0.05$. All analyses were performed using R (R Development Core Team 2015) with packages lsmeans (Lenth 2015) and nlme (Pinheiro et al. 2015).

Results

Monitoring SWD adults

At the location Dossenheim (Do), no females were caught in February and from mid-May to end of June, while at Dossenwald (Ma) on trees parasitized by *V. album* subsp. *laxum* the first females were trapped in February and occurred continuously throughout the season. Numbers of trapped individuals were significantly greater ($p < 0.05$) at Ma than at Do at most occasions between February and the end of July. Only on August 4th and September 1st numbers were significantly higher ($p < 0.05$) in traps located at Do (Fig. 2).

On several dates from March to May, the abundance of female SWD was higher in traps at both heights in trees with *V. laxum* at Ma when compared with Do where no mistletoe was growing in the canopy, with significant differences observed in April (Fig. 3a, b; $p < 0.05$). More individuals were caught in the canopy than at the standard height at both forest sites, with significant differences on April 28th (Fig. 3c, d; $p < 0.05$).

Ovigeny development

All female SWD captured in Ma and Do from March 18th to April 28th were dissected ($n = 522$). Numbers of females caught in this period were about 10-fold higher in Ma ($n = 480$) than in Do ($n = 42$). Individuals captured before April 1st did not have maturing or mature eggs. Females with mature eggs (9.6 %) were found for the first time in canopy traps from Ma covering the collection period from April 1st to April 16th (Table 1). In the following period, from April 16th to April 28th 71.7 % of the females from the canopy in Ma carried mature eggs, compared with only 26.1 % from traps at the standard height. In Do, 75 % of the captured females in the canopy carried mature eggs. First “old eggs” appeared in females on the April 28th collection date. During the observation period, more individuals with mature eggs were captured in the canopy of Ma ($n = 120$) compared with standard height ($n = 35$) and Do ($n = 18$) (Table 1).

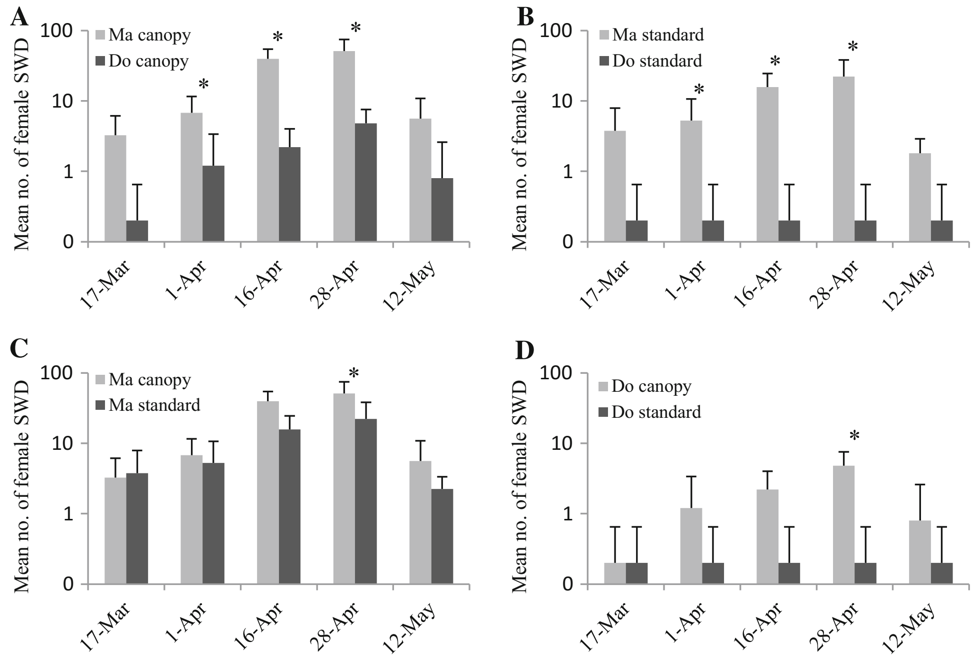

Fig. 3 Female SWD captured at Dossenwald (Ma) and Dossenheim (Do) comparing the sites and the heights (mean of five traps at each height, respectively; log scale, with standard deviation (SD). Statistical differences are indicated by *($p < 0.05$)

Table 1 Status of ovarioles and eggs in females caught in spring 2015 in Dossenwald (Ma) and Dossenheim (Do) in the canopy (C) of pine trees and at standard height (S)

Date	Site	*N*	Indiscernible ovarioles (%)	Unripe ovarioles (%)	Maturing eggs (%)	Mature eggs (%)	Old eggs (%)
01. Apr	Ma-C	17	41.2	58.8	–	–	–
	Ma-S	21	4.8	95.2	–	–	–
	Do-C	6	50	50	–	–	–
	Do-S	–	–	–	–	–	–
16. Apr	Ma-C	115	3.5	61.7	25.2	9.6	–
	Ma-S	41	–	90.2	9.8	–	–
	Do-C	11	–	92.3	7.7	–	–
	Do-S	0	–	–	–	–	–
28. Apr	Ma-C	152	–	12.5	15.1	71.7	0.7
	Ma-S	134	0.7	27.6	45.5	26.1	–
	Do-C	24	8.3	12.5	4.2	75	–
	Do-S	1	–	–	100	–	–

The numbers of dissected females and the percentage per category are given

Performance and survival

In no-choice assays, a higher number of eggs were laid in wounded (17.2 ± 10.7 SD) than in unwounded (6.8 ± 9.9 SD) berries. The model stresses the influence of wounding on egg laying success (Fig. 4a; df = 1; F value = 22; $p < 0,05$; $r^2 = 47.4$ %). The number of emerging adults (9–15 days after oviposition) was higher in wounded (8.6 ± 4.4 SD) than in unwounded (2.0 ± 2.7 SD) berries. The model stresses the influence of wounding on successful development into adults (Fig. 4b; df = 1; F value = 72; $p < 0.05$, $r^2 = 65.6$ %). Model shows that egg numbers and adult emergence vary among sampling dates (eggs: df = 2, F value = 14; $p < 0.05$; hatch: df = 2; $F = 17$; $p < 0.05$). The pH of the berries was 6.35 and degree brix was 20.3°. No female died during the 48-h exposure period. In cages without berries first individuals died at 48 h. All individuals were dead by the fifth day. In cages equipped with unwounded or wounded berries, all individuals survived for 8 days. Flies were

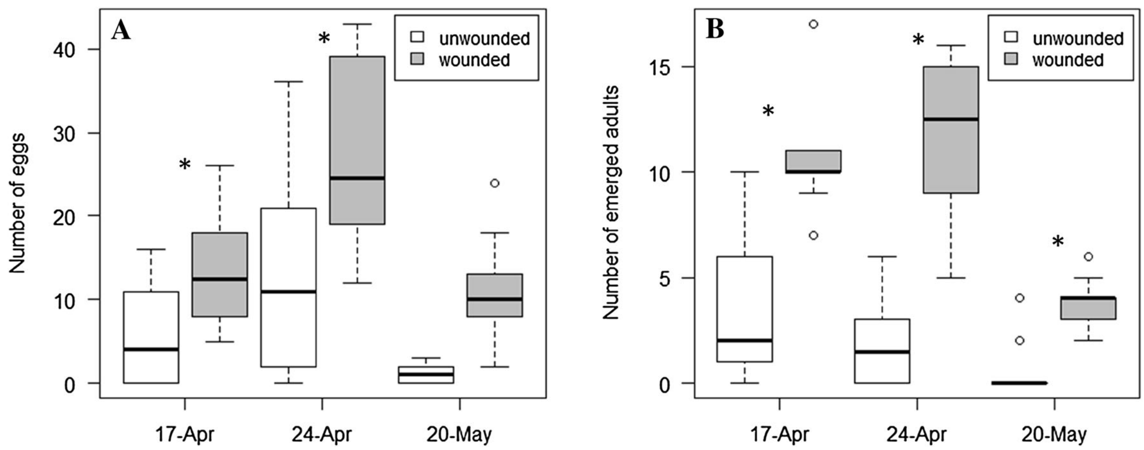

Fig. 4 Boxplots of eggs laid (**a**) and emerged adults (**b**) in no-choice assays. The *bottom* and *top* of the *boxes* represent the lower and upper quartiles, respectively, and the *line* divides the *box* into two parts the median. The ends of the whiskers represent the standard deviation (SD); the dots above the boxplot represent the outliers. In each replicate ($n = 10$), 10 berries of *V. album* subsp. *laxum* were exposed to 10 gravid females for 48 h. Statistical differences are indicated by *($p < 0.05$)

observed feeding on the surface of unwounded and wounded berries.

Field infestation of mistletoes

After four weeks of incubation, no adult SWD emerged out of 100 *V. album* subsp. *laxum* berries collected from Ma (sampling date: April 14th). However, 8 adults (7 ♂, 1 ♀) emerged from the field-collected *V. album* subsp. *album* berries from Heddesbach on May 19th. The monitoring traps in Heddesbach caught 3 male and 2 female SWD in the specified four weeks of trapping.

VOCs emitted by mistletoe berries

In the gas chromatographic analysis of the headspace of both unwounded and wounded berries, 32 peaks of VOCs were detected in the chromatograms (Fig. 5). Twenty-four peaks were selected for a statistical comparison between VOCs emitted by unwounded and wounded berries (Table 2). Of those 19 were identified. While we could not detect any qualitative differences, 11 peaks showed significant differences ($p < 0.05$) in proportional amounts of each compound between unwounded and wounded berries. Out of those benzaldehyde, octanal, methyl salicylate, and farnesene (the isomer could not be precisely determined) could be identified. Seven of the statistically different volatile compounds detected were found in higher amounts in wounded berries (e.g., benzaldehyde and octanal), whereas four volatile compounds were present in higher amounts in unwounded berries (methyl salicylate and farnesene) (Table 2).

Discussion

This study identified mistletoe (*V. album* subsp. a*lbum* and *V. album* subsp. *laxum*) as a new host for SWD in early spring in Central European temperate forests. Adult SWD were successfully reared from *V. album* berries collected from apple trees on a meadow orchard near Heddesbach. Laboratory assays demonstrated that SWD can successfully complete its life cycle in mistletoe berries. SWD laid more eggs in artificially wounded berries compared with undamaged berries. This effect is also known from other fruit crops, e.g., cranberries and grapes (Steffan et al. 2013; Ioriatti et al. 2015). This was more likely due to easier access to the fruit pulp in wounded fruits than to the emitted volatiles, because we could not detect qualitative differences between intact and damaged fruits. The quantitative differences in volatiles measured in our experiments should not have played a role due to the no-choice experimental design. In contrast to cranberries, where SWD can only develop within wounded decaying fruit (Steffan et al. 2013), this study showed for the first time that SWD can develop from egg to adult stage in both unwounded and wounded mistletoe berries.

Furthermore, we showed that adult female SWD were able to subsist on unwounded and wounded berries of mistletoes for at least eight days. As no female died during exposure to berries during the assay, we assume that they could survive even for a longer time on mistletoe as only food source. The flies obviously fed on the surface of berries as they were observed grazing. The microflora on the berry surface seems to offer nutritional components (e.g., microorganisms like yeast, fungi, and bacteria). After

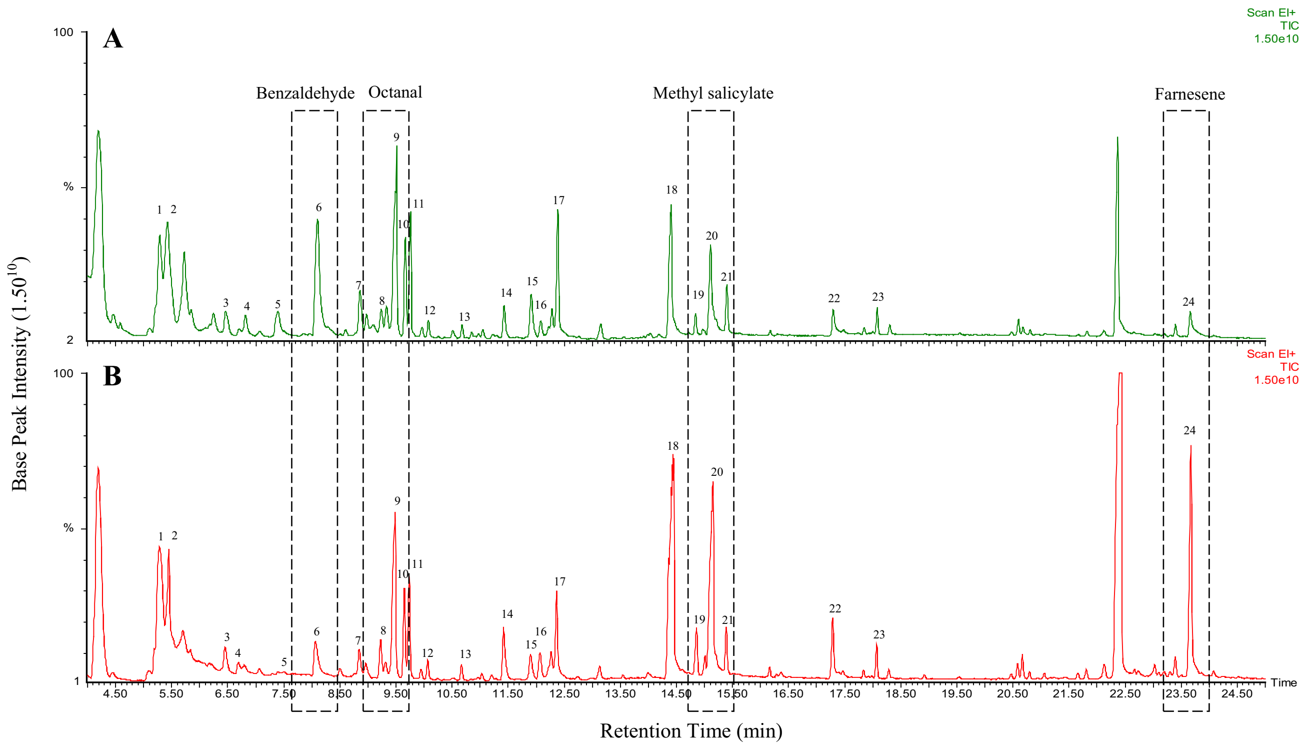

Fig. 5 Total ion current chromatograms obtained from GC–MS analysis of odor spectra of wounded (=A) and unwounded (=B) *V. album* subsp. *laxum* berries. Peaks selected for statistical analysis are indicated by numbers. *Dotted boxes* indicate identified compounds with significant differences ($p < 0.05$)

oviposition, fully intact berries are incised and females can feed on sap or fruit flesh (Walsh et al. 2011; Poyet et al. 2014). Besides, there might be microcracks that can be used for oviposition and feeding as well. A temporal extension of the feeding assay was not possible due to deterioration of the berries and growth of different microorganisms which reduced the differences between wounded and unwounded berries. Further studies could focus on these microorganisms to determine if they have any special attraction to SWD.

SWD has been observed to feed on leftover fruits like overripe and damaged persimmon, figs, and fallen rotting apples during winter (Lee et al. 2015). Additionally, Ioriatti et al. (2015) expected that SWD can utilize grapes wounded by cracking, disease, and/or bird damage in autumn as a nutrient resource, which may result in increased longevity, fecundity, and reproduction. Shearer (pers. communication) reported that winter morphs of SWD were able to survive at 1 °C for several months. We showed that SWD winter survivors can use mistletoe in spring as nutrient and reproduction host. These circumstances support SWD winter and early spring survival success and subsequently its population growth in spring.

The monitoring traps captured greater numbers of SWD in *P. sylvestris* canopies when associated with ripe *V. album* subsp. *laxum* berries. Additionally, traps captured more SWD in the upper canopy of a forest in the presence of mistletoe when compared with traps at standard height. The parasitic mistletoe is always located in the canopy and never at lower heights. Moreover, abundance of SWD captured in traps revealed greater numbers of SWD when *V. album* subsp. *laxum* berries were ripe. These significant differences in the distribution of SWD in forest habitats measured by monitoring traps may be due to a higher attraction of SWD to volatiles produced by mistletoe berries.

Sampling the headspace of mistletoe berries revealed that some volatiles ((E)-2-hexenal and (Z)-3-hexen-1-ol, benzaldehyde, octanal, methyl salicylate, farnesene) were emitted in high amounts. Thus, these chemicals might help SWD moving through the forest canopy to locate potential host fruits. The detected compounds include volatiles typical for ripening fruits (Abraham et al. 2015; Revadi et al. 2015), e.g., benzaldehyde and octanal, and others considered common green leaf volatiles. The volatiles may lure the flies to pine trees parasitized by mistletoe. It was recently shown that SWD is not only more responsive to fruit volatiles than the closely related *D. melanogaster*, but also attracted to green leaf odors (Keesey et al. 2015).

Hexyl acetate and octanal are known from the headspace of ripe strawberries (Keesey et al. 2015). Benzaldehyde is a compound regularly emitted by several berry fruits

Table 2 Volatile organic compounds selected from unwounded and wounded berries of mistletoes

#	RT	Compounds
1	5.28	(E)-2-Hexenal
2	5.45	(Z)-3-Hexen-1-ol
3	6.45	Not ident*
4	6.80	Not ident*
5	7.49	Not ident*
6	8.06	Benzaldehyde*
7	8.85	Not ident*
8	9.31	Octanal*
9	9.48	Cis-3-Hexenyl acetate
10	9.65	Hexyl acetate
11	9.74	Trans-2-Hexenyl acetate
12	10.06	Limonene
13	10.67	Ocimene
14	11.42	Not ident*
15	11.90	Not ident*
16	12.07	Not ident*
17	12.36	Pelargonaldehyde
18	14.44	Ethyl benzoate
19	14.86	Cis-3-Hexenyl butyrate
20	15.15	Methyl salicylate*
21	15.40	Decanal
22	17.29	Ethyl salicylate
23	18.07	Tridecane
24	23.63	Farnesene*

[#] Peak number

Statistical differences are indicated by * based on Mann–Whitney *U* tests at $p < 0.05$

RT Retention time (Rxi-5 ms capillary)

(Abraham et al. 2015), and a common constituent of leaf odors of many green plants (Steck et al. 2012). (E, E)-α-farnesene is the main constituent from flower volatiles of several subspecies of *V. album* (Bungert et al. 2002). Interestingly, another isomer β-farnesene has only been reported from *V. album* a parasite exclusive to *P. sylvestris* (Bungert et al. 2002). With our analytic equipment, it was not possible to distinguish between the different isomers of farnesene. Thus, further analysis is necessary to identify the isomer of farnesene produced by mistletoe berries. Methyl salicylate was emitted in significantly lower amounts by artificially wounded berries than by intact fruits. This organic ester is derived from the shikimic acid pathway (Dicke et al. 2009) and increases in the headspace of plants after attack by herbivores with chewing feeding behavior (Heil 2007; Van Poecke et al. 2001), and in plants infested by pathogens (Mann et al. 2012; Rid et al. 2016). However, methyl salicylate is naturally produced by many species of plants and has been reported from numerous floral scents (Knudsen et al. 1993). It can also be found in the headspace of fruits from several *Rubus* sp. and cherries (Keesey et al. 2015). It was recently shown that methyl salicylate can be detected by olfactory sensory neurons of SWD (Keesey et al. 2015).

Brix (20.6°) and pH (6.35) levels of berries used in our study are comparable to other susceptible hosts, e.g., grapes (Ioriatti et al. 2015). It was shown that higher pH and Brix levels increased the number of eggs laid from which a higher percentage of adults emerged (Lee et al. 2011). In contrast, Lee et al. (2015) could not identify any trend in wild and non-crop ornamental fruits in relations of pH and Brix suggesting that other fruit characteristics are affecting susceptibility of SWD, e.g., fruit firmness and fruit skin characteristics. Additionally, we assume that wounded berries (e.g., by birds or severe storms) increased the susceptibility of mistletoe to SWD.

No infestation by SWD of field-collected berries at Ma from April 14th was observed. Dissections of females captured at this time revealed few or no mature eggs. Unfortunately, it was not possible to sample further mistletoe berries at Ma. For this reason, we collected further berries (*Viscum album* subs. *album*) in May at Heddesbach from scattered apple trees. After four weeks, seven males and one female emerged out of several hundred berries. SWD was the only species that emerged from these fruits. Thus, we showed for the first time that SWD is able to lay eggs and complete its development entirely within mistletoe berries at sites where berries and gravid females occur at the same time.

The newly identified wild host, *V. album,* may increase reproductive success when ripening and ripe berries coincide with gravid females bearing mature eggs. We observed such a scenario during April 2015. The dissection of field-captured SWD females showed that the majority of the females had ripe eggs in their ovaries in the second half of April. At that time the mistletoe berries were fully ripe. No-choice assays of unwounded and wounded berries showed that both are accepted by SWD for oviposition. However, more eggs were laid in wounded berries yielding a higher percentage of emerged adults. Thus, susceptibility of *V. album* to attack by SWD may increase due to severe storms, feeding birds, and rain cracking of berries. This study indicates that SWD can feed, oviposit, and fully develop to adults on mistletoe berries in early spring. Further studies on this newly reported host should provide more information on initial infestation at different sites and different years. Besides fruit availability, other aspects like microclimate or light conditions may explain why SWD is more abundant in the canopy of *P. sylvestris* when compared with SWD abundance at the standard height. One next step is to investigate the abundance of SWD in other tree species compared. More knowledge about wild hosts is needed in order to better understand the population dynamics of SWD, especially with regard to the starting point for seasonal built-up of populations. Such information can be used to develop forecasting models to estimate the potential threat to fruit production.

Author contribution

FB, JG, and HV conceived and designed the study relating to monitoring, survival, and reproduction; FB and JG conducted headspace sampling and GC–MS analyses of mistletoe volatiles. FB and AE conducted the oviposition experiments. FB, JG, and HV analyzed the data. HV supervised the study. FB wrote the first draft of the manuscript, which was supplemented by all authors. All authors approved the final version of the manuscript.

Acknowledgments The authors thank Jürgen Just and Anja Frank (JKI) for technical support in rearing of SWD, monitoring SWD phenology, taking photographs of developmental stages of eggs, and dissection of females. We thank Michael Breuer (State Institute for Viticulture and Enology (WBI), Freiburg, Germany) for briefing us in installing monitoring traps in the canopy of trees. We thank Michael Herwig (forestry commission Mannheim, Germany) for the permit to collect mistletoes in the Dossenwald as well as Peter Burger (JKI) for sampling mistletoes in the meadow orchard near Heddesbach and communicating the contact to the owner family Falter, Heddesbach, Germany. We thank Kai Lukat, Tobias Schneider, Michael Papke (all JKI), and Anna-Maria Baumann (WBI) for assistance in the mistletoe sampling. We thank Svenja Stein and Kai Lukat (JKI) for support in headspace sampling. We are grateful to Doreen Gabriel and Jannicke Gallinger (JKI) for statistical advice. The authors wish to thank Peter Shearer (Oregon State University, USA) for the English revision. Suggestions by three anonymous reviewers greatly improved the manuscript.

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