

# Single and combined effects of *Drosophila suzukii* and *Drosophila melanogaster* on sour rot development in viticulture

Wiebke Entling  | Christoph Hoffmann

Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Siebeldingen, Germany

## Correspondence

Wiebke Entling, Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Geilweilerhof, 76833 Siebeldingen, Germany.  
Email: wiebke.entling@julius-kuehn.de

## Abstract

Sour rot is a disease complex that causes serious damage in viticulture. The common vinegar fly *Drosophila melanogaster* (Diptera: Drosophilidae) is associated with sour rot in overripe or otherwise damaged grapes. *Drosophila suzukii* (Diptera: Drosophilidae) is an invasive species, which is suspected to induce sour rot in previously undamaged grapes due to the flies' ability to infest healthy, undamaged soft fruits with its serrated ovipositor. As a consequence, infection of healthy grapes by *D. suzukii* may facilitate the colonization by *D. melanogaster*. We investigated the single and combined effects of *D. suzukii* and *D. melanogaster* on sour rot development by measuring volatile acidity under near-natural conditions in the vineyard, along with laboratory experiments under controlled climate. In 2017, the combined field and laboratory experiments suggested that the presence of *D. suzukii* and *D. melanogaster* increased the volatile acidity levels at a similar rate. In 2018, the field experiments showed an only marginal increase in sour rot development in treatments with both *Drosophila* species. Under more favourable laboratory conditions, the presence of *D. suzukii*, but not *D. melanogaster* triggered sour rot emergence. A facilitating effect of *D. suzukii* infestation for *D. melanogaster* was not detectable. These findings suggest that *D. suzukii* does in fact have the potential to trigger sour rot, but will probably rarely do so under field conditions in the vineyard, at least in the studied region. Instead, our study showed that *D. melanogaster* can have a similar impact on sour rot development as *D. suzukii*, emphasizing the need of comparative studies.

## KEYWORDS

grape, invasive pest insect, spotted wing *Drosophila*, vinegar fly, vineyard, volatile acidity

## 1 | INTRODUCTION

In viticulture, sour rot is a well-known disease complex, which causes serious damages in years with high daytime temperatures, pre-harvest rain and humid conditions after veraison (McFadden-Smith & Gubler, 2015). Various yeasts and acetic acid bacteria contribute to its development by decomposing the juice of infected

berries (Barata, Malfeito-Ferreira, & Loureiro, 2012; Barata, Santos, Malfeito-Ferreira, & Loureiro, 2012; Hall, Loeb, Cadle-Davidson, Evans, & Wilcox, 2018). This induces the development of acetic acid in grapes, which is volatile and responsible for the typical vinegar smell. As volatile acidity causes sensorial problems in wine even in small amounts, there are legal limits for winemaking, creating a high pressure to avoid sour rot (Lemperle, 2007).

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Vinegar flies (*Drosophila* spp.) are known to trigger sour rot development (Barata, Malfeito-Ferreira, et al., 2012; Barata, Santos, et al., 2012; Hall, Loeb, Cadle-Davidson, et al., 2018) and recent research showed that vinegar fly regulation with the help of insecticides can reduce disease severity (Hall, Loeb, & Wilcox, 2018). Different scenarios of *Drosophila* spp. contributing to sour rot development are proposed. First, the flies are able to vector the yeasts and bacteria associated with the disease (Barata, Santos, et al., 2012; Ioriatti et al., 2018). More importantly, a recent study with axenic flies demonstrated that flies also play a non-microbial role, presumably through the developing larvae, which themselves trigger the decomposition of berries and thereby reinforce sour rot development (Hall, Loeb, Cadle-Davidson, et al., 2018).

However, the common vinegar fly, *Drosophila melanogaster* (Diptera: Drosophilidae) is able to oviposit only in overripe, decaying or in other way damaged fruit (e.g., cracked grapes after rain or insect feeding). In contrast, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive vinegar fly species from Asia, introduced to North America and Europe since 2008, is able to infest healthy, undamaged fruit with its serrated ovipositor (Asplen et al., 2015; Atallah, Teixeira, Salazar, Zaragoza, & Kopp, 2014; Hamby & Becher, 2016; Ørsted & Ørsted, 2019; Schetelig et al., 2018; Walsh et al., 2011). It is a major pest of cherries, raspberries and blueberries, but can also infest some, primarily soft-skinned, grape varieties (Entling, Anslinger, Jarausch, Michl, & Hoffmann, 2019; Ioriatti et al., 2015; Kehrl, Linder, Cahenzli, & Daniel, 2017; Lee et al., 2011; Shrader, Burrack, & Pfeiffer, 2019). In viticulture, it is still debated whether *D. suzukii* is able to induce sour rot disease. Rombaut et al. (2017) confirmed this ability in laboratory experiments. Moreover, they hypothesize that *D. melanogaster* in turn is able to oviposit into berries damaged by *D. suzukii*, thus reinforcing disease severity. Thus, oviposition by *D. suzukii* may facilitate *D. melanogaster* access, leading to a larger damage by the combination of both species than would be expected by their single effects. However, experiments were performed with sterilized berries, which were afterwards dipped in sour rot extract and placed in cages in the laboratory. Thus, these results are not necessarily transferable to the field situation.

The aim of this study was to investigate the single and combined influence of *D. suzukii* and *D. melanogaster* on sour rot development under near-natural conditions in the vineyard, along with laboratory experiments under controlled climate. For that, we performed a manipulative experiment with gauze-bagged grapes comprising four different treatments: (a) *D. suzukii*, (b) *D. melanogaster*, (c) both *Drosophila* species combined and (d) without flies as control. We determined the sour rot development by measuring the volatile acidity levels and checked for differences between the treatments.

We expected to find higher levels of sour rot damage in treatments with vinegar flies than in the control. As *D. suzukii* is able to attack also undamaged grapes, we hypothesized that the treatments comprising this species would be infested heavier than the treatment with only *D. melanogaster*. If *D. melanogaster* is able to

use the pre-damaged grapes from *D. suzukii* oviposition, we finally expected the combined treatment (with both species) to have even higher volatile acidity levels than the treatment with only *D. suzukii*.

## 2 | MATERIALS AND METHODS

*Drosophila suzukii* flies were laboratory reared at JKI in Dossenheim, Germany, and originated from the collection of wild specimens close to this research institute (49°26'57.6"N 8°38'21.7"E) during October 2013. *Drosophila melanogaster* flies were obtained from a laboratory rearing of the RLP AgroScience GmbH. We maintained the flies in rearing cages with mesh side panels (30 × 30 × 30 cm, Bugdorm-1; Megaview) with *Drosophila* cornmeal diet (1.2 L water, 25 g agar, 30 g wheat germ, 25 g corn meal, 25 g brewer's yeast, 22.5 g apple pulp, 50 g sugar, 2.5 g ascorbic acid, 2.5 g Wesson's salt, 1.125 g Vanderzant vitamin mixture, 0.75g methyl-4-hydroxybenzoate and 0.75 g benzoic acid) as described by Bellutti et al. (2018). The climatic chamber was set to 23°C, 75% relative humidity and a photoperiod of L16:D8h.

Field experiments were conducted in a vineyard planted with the vitis vinifera cv. Dornfelder close to the JKI in Siebeldingen, Germany (49°12'55.7"N 8°02'09.2"E). The vineyard was organically managed using standard procedures of soil tillage, fertilization and plant protection and had 18 rows with 25 plants each. In both years, field experiments were set up in a randomized block design. Blocks comprised four different treatments (with *D. suzukii*, with *D. melanogaster*, with both fly species together and with no fly as control), repeated 10 times and randomly distributed over the vineyard. Each sample of each treatment contained one grape cluster wrapped in a 28 × 60 cm gauze-sleeve with mesh size <1 mm, which was fixed at the stem of the grape cluster and closed at the open end with wire cords generating a gauze bag that locks the added flies in place and prevents other arthropods from accessing the cluster. Gauze-bags were established when the grapes began to blush to preclude preceding infestation. In 2018, we additionally performed the experiment with one grape cluster per treatment in plastic cages in the laboratory, using grape clusters from the same experimental vineyard.

Field and laboratory experiments were performed around the harvest date starting with addition of the flies (control: no flies, *D. suzukii* treatment: 50 individuals, *D. melanogaster* treatment: 50 individuals, combined treatment with both fly species: 50 individuals of *D. suzukii* and 50 individuals of *D. melanogaster*). The flies used in the experiment were between 1 and 60 days old, reflecting the variable age that can be found in natural populations. As sex determination in *D. melanogaster* is relatively time-consuming and would stress the experimental animals, we decided to add the individuals of both species without sex determination, but in sufficient numbers to ensure egg-laying. Retrospective sampling from our cultures revealed a mean number of 26 females per 50 individuals, with no significant difference in the sex ratio or its variability

between species (95% confidence interval: 20–32 females per sample). Both species are able to lay at least five eggs per female per day (Emiljanowicz, Ryan, Langille, & Newman, 2014) and each female had several days for egg-laying. Thus, the flies should have enough possibilities to oviposit and induce damage. To reduce any influence of random variation in the number of females added in the single trials, we used the high number 10 replicates per treatment. At the end of the experiment, we performed the must analysis for determination of volatile acidity levels (Barata, Pais, Malfeito-Ferreira, & Loureiro, 2011). For that, the experimental grape clusters were blended and centrifuged and their juice was analysed (Sigma 6K15; Sigma Laborzentrifugen GmbH; 10 min, 20°C, 14,087 g). From the supernatant, we determined the volatile acidity (g/L) by Fourier-transform infrared spectroscopy (FTIR; WineScan FT 120; FOSS). As more tightly clustered grapes have a higher potential to crack by compression within expanding bunches (McFadden-Smith & Gubler, 2015), we stated the relationship between bunch density and the weight of the grape cluster in the used cultivar. Besides, we checked for the influence of the grape cluster weight in all experiments.

In 2017, we performed semi-field experiments with two experimental repetitions starting at 30.8.2017 and 6.9.2017 and ending 3 weeks later at 20.9.2017 and 25.9.2017 by determination of the volatile acidity of the experimental grape clusters. One week after the addition of flies, gauze-sleeves and flies were removed, grape clusters were cut off, weighed and individually placed in plastic boxes with a gauze lid (11.5 × 15.5 × 12.5 cm). The plastic boxes with individual grape clusters were incubated for 2 weeks in the laboratory and flies were removed every 2 days and stored in ethanol based on the individual samples. Later, the flies were identified and counted under a stereo microscope (Stemi 2000; Carl Zeiss AG).

In 2018, the field experiments took place directly in the vineyard. Again, two experimental repetitions were performed in the field starting at 24.8.2018 and 31.8.2018. After 3 weeks, the gauze-sleeves and flies were removed and the experimental grape clusters were cut off, weighed and individually transported in plastic boxes to the laboratory where volatile acidity was determined as an indicator of sour rot at 13.9.2018 and 20.9.2018, respectively. In addition to the field experiments, we performed two laboratory repetitions starting at 29.8.2018 and 4.9.2018. Therefore, we randomly collected grape clusters from the same vineyard where the field experiment took place. In order to control the condition of the experimental grape clusters, we removed any damaged berries from the grape clusters prior to the experiments. We individually weighed the grape clusters, placed one grape cluster per sample in a plastic box with a gauze lid (11.5 × 15.5 × 12.5 cm) and added flies according to the treatment. We chose the same experimental set up as in the field experiments with four treatments repeated 10 times and distributed them randomly in the climatic chamber with 23°C, 75% relative humidity and a photoperiod of L16:D8h. After 2 weeks, we determined volatile acidity of the grape clusters at 12.9.2018 and 29.9.2018, respectively.

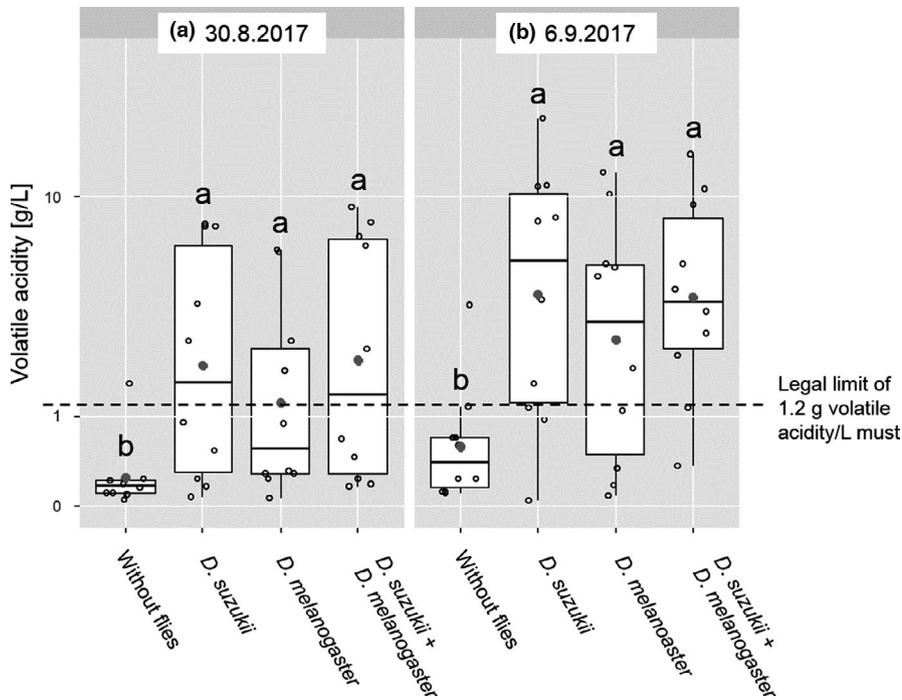
## 2.1 | Statistics

We performed all analyses using the open-source program R (R Core Team, 2017). For all experiments, we used  $n = 40$  samples (four treatments × 10 replicates) to check for differences in volatile acidity levels between the treatments performing variance analyses followed by post hoc tests. We checked and controlled for a potential influence of the grape cluster weight by adding it as an additional depended variable in the model. However, as the effect of grape cluster weight was only significant in the 2017 data set, we did not consider grapes' weight in the 2018 data sets. We used diagnostic plots to estimate the quality of the model. If diagnostics plots were not optimal, we rank transformed the volatile acidity data (combined field and laboratory data 2017, laboratory data 2018). We checked for model robustness with permutation tests using the *pgirmess* package (Giraudoux, 2017). For illustration of the different reproductive success of *D. suzukii* and *D. melanogaster*, we displayed boxplots with number of eclosed *D. suzukii*/*D. melanogaster*. As the numbers of flies were highly skewed (especially in *D. melanogaster*), we  $\log_{10}(x + 1)$  transformed them. Moreover, we fitted linear models to investigate the relationship between volatile acidity and number of eclosing flies after oviposition in the three different treatments comprising *D. suzukii*/*D. melanogaster*. As volatile acidity data were not normally distributed, we rank transformed them and performed permutation tests to check for the robustness of the model.

## 3 | RESULTS

In both repetitions of the combined field and laboratory experiments in 2017, volatile acidity levels were significantly higher in all treatments with flies than in the control without flies (Figure 1). However, the fly treatments showed no significant differences among them, *D. melanogaster* triggered volatile acidity development as strongly as *D. suzukii* and both species together. Interestingly, the variance in the number of eclosing adults ( $\log_{10}(x + 1)$ -transformed) was 3–10 times higher in *D. melanogaster* than in *D. suzukii*. Thus, the number of eclosed adults was highly variable in the treatments with *D. melanogaster* (e.g., 0–172 in the first repetition of the *D. melanogaster* treatment), but much lower and relatively constant in the treatments with *D. suzukii* (e.g., 2–16 flies in the first repetition of the *D. suzukii* treatment; Figure 2). Moreover, in four of six treatments with flies, volatile acidity increased significantly with the number of emerging flies (Figure 3). Interestingly, the slope of the regression lines was steeper in the *D. suzukii* treatments, indicating that a *D. suzukii* individual had a higher effect than one of *D. melanogaster*.

In the 2018 field experiment, 3 weeks after adding the flies, volatile acidity levels were far below the legal limit of 1.2 g volatile acidity/1 L must (Lemperle, 2007) in all samples and all treatments (Figure 4). Moreover, only in one of the two repetitions the treatments showed slight differences in volatile acidity levels



**FIGURE 1** Effects of *Drosophila suzukii* and *Drosophila melanogaster* on sour rot development in semi-field experiments 2017 in a Dornfelder vineyard. Grapes were exposed to flies in gauze-sleeves in the field, followed by an incubation in the laboratory. Presence of flies and grape weight increased volatile acidity in both subsequent repetitions (a) set up 30.8.2017, ANOVA:  $n = 40$ ; treatment:  $F = 5.02$ ,  $p = .005$ ; grape weight:  $F = 4.60$ ,  $p = .039$  and (b) set up 6.9.2017, ANOVA:  $n = 40$ ; treatment:  $F = 7.44$ ,  $p = .0006$ ; grape weight:  $F = 25.79$ ,  $p < .0001$ ). Different letters indicate significant differences ( $p < .05$ ) in volatile acidity between the treatments at the 5% level following the Tukey post hoc test

with having significantly more volatile acidity in the combined treatment than in the control (Figure 4a). However, the picture was completely different when we conducted the experiment in the laboratory. Under favourable conditions for the flies in the climatic chamber, flies were able to survive, oviposit, and start larval development resulting in elevated volatile acidity levels in the treatments containing *D. suzukii* (Figure 5). However, the volatile acidity levels in the treatments containing *D. melanogaster* did not differ significantly from the control in any of the three approaches.

#### 4 | DISCUSSION

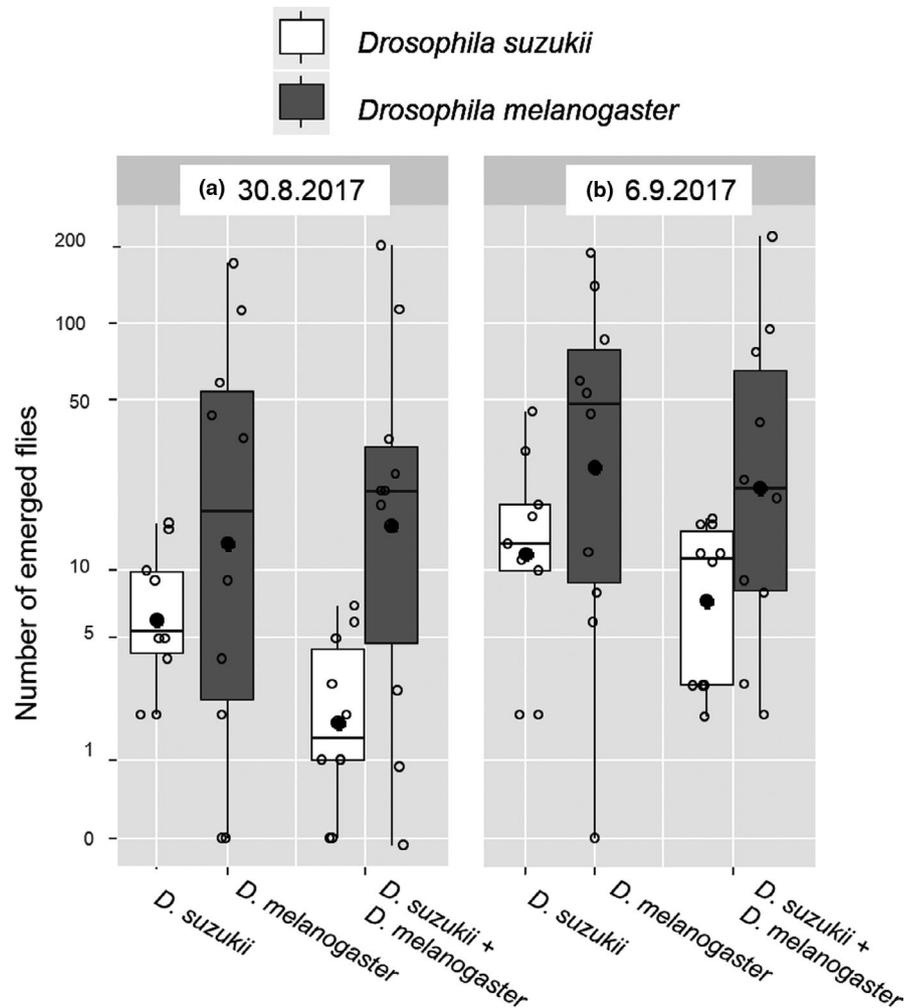
Our experiments demonstrate that *Drosophila* spp. in general is able to trigger sour rot development in grapevine. These findings are in line with the previous research to this topic (Barata, Santos, et al., 2012; Hall, Loeb, Cadle-Davidson, et al., 2018; Hall, Loeb, & Wilcox, 2018). However, the mechanisms behind sour rot induction by *Drosophila* spp. are less clear. It is widely accepted that *Drosophila* spp. has a microbial contribution as vector for the microorganisms involved in sour rot development (Barata, Santos, et al., 2012). Previous studies even showed that this transfer can take place without egg-laying solely through contact (Ioriatti et al., 2018). However, our findings from 2017 showed that the flies are not only crawling over the berry surface, spreading the agents involved in sour rot, but additionally were able to oviposit, develop through larval stages and emerge as adults (Figure 2). Recent experiments with axenic flies showed that *Drosophila* spp. also has a non-microbial contribution in sour rot development. The authors conclude that especially the larval development of *Drosophila* spp. leads to the loss of berry integrity and catalyses the decaying

process (Hall, Loeb, Cadle-Davidson, et al., 2018). Our experiments support a role of developing *Drosophila* larvae on sour rot by showing an increase in volatile acidity with increasing numbers of emerging flies (Figure 3).

However, the finding that *D. melanogaster* had a similar effect on sour rot development as *D. suzukii* in our experiments in 2017 was unexpected as the common vinegar fly is thought to infest only pre-damaged fruits. Emergence rates showed that *D. melanogaster* was nevertheless able to oviposit—at least in some grape clusters—and therefore provoked sour rot just as intensively as *D. suzukii* (Figure 2). In fact, we observed cracking in large compact grape clusters presumably because of compression within expanding bunches. This observation is reflected in the data, as grape clusters were on average heavier in 2017 and the variability in grape weight was also higher in 2017 than in 2018 (2017:  $\bar{X}$  406 g [range: 196–724 g]; 2018:  $\bar{X}$  167 g [range: 75–315 g]) and high grape cluster weight enhanced the development of volatile acidity in 2017 (Figure 1), but not in 2018. Thus, the different effects of *D. melanogaster* in 2017 and 2018 indicate that this species is in fact more dependent on the physical conditions of grapes. However, as cracking is frequent in thin-skinned and tight-clustered varieties, *D. melanogaster* may pose a larger threat to these varieties in relation to sour rot development than *D. suzukii*.

Our experiments from 2018 expand the experiences from 2017. This time we created a “worst case scenario” in the laboratory with favourable conditions for the flies and the rot agents and a “natural scenario” with the given conditions in the vineyard. Unfortunately, the weather was exceptionally hot and dry during the ripening period in 2018 in our region, which is generally known to reduce reproductive success of *D. suzukii* (Hamby et al., 2016; Ryan, Emiljanowicz, Wilkinson, Kornya, & Newman, 2016). Indeed, field studies showed that *D. suzukii* is able to hide in cooler areas

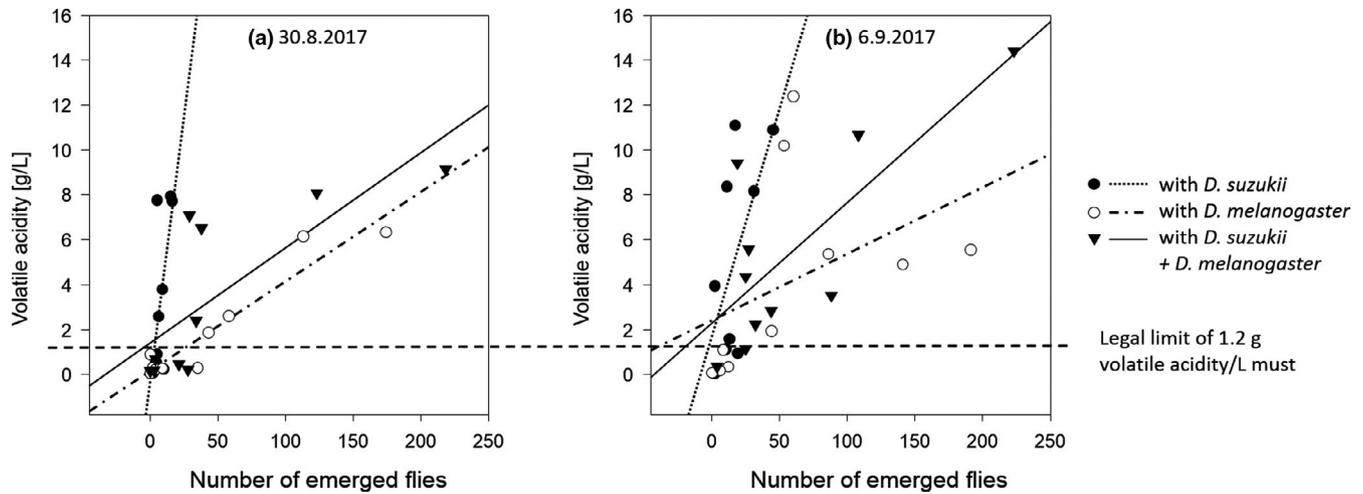
**FIGURE 2** Reproductive success of *Drosophila suzukii* and *Drosophila melanogaster* in three different treatments in 2017 in two subsequent repetitions (a) set up 30.8.2017 and (b) set up 6.9.2017 performed in a Dornfelder vineyard



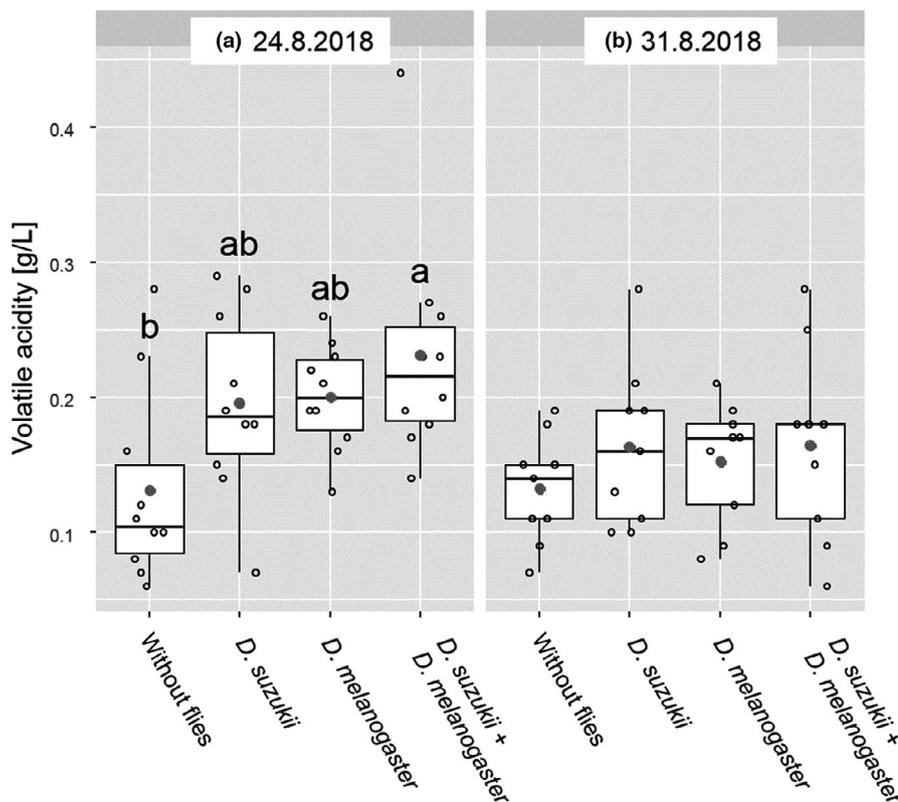
at daytime and becomes active during dusk/dawn avoiding unfavourable, potentially lethal conditions (Evans, Toews, & Sial, 2017; Jaffe & Guédot, 2019; Shaw, Fountain, & Wijnen, 2018). However, as the flies in our experiments were caged in the experimental bags, they were not able to escape and as a result presumably not able to oviposit at all. For future research it will be interesting if *D. suzukii* is more damaging in the field in more temperate summers. In the laboratory, *D. melanogaster* did not increase volatile acidity levels, diverging from the 2017 results. As we used only intact grape clusters without any cracking in this experiment, it seems that *D. melanogaster* is not able to reproduce and cause any damage under these conditions. However, as we did not check egg-laying and emergence rates, we can only speculate about the exact processes.

Interestingly, the combined effect of *D. suzukii* and *D. melanogaster* was not higher than the effect of the single treatments. Rombaut et al. (2017) hypothesized that grape damage induced by *D. suzukii* facilitates *D. melanogaster* oviposition leading to a synergistic induction of sour rot. As we added twice as many flies, we expected at least twice as much volatile acidity development. Moreover, if development is possible because of pre-damaged

grapes, sour rot damage should be more extensive in *D. melanogaster* than in *D. suzukii*, as its oviposition rate is up to ten times higher than in *D. suzukii* (Asplen et al., 2015; Emiljanowicz et al., 2014). We can imagine different explanations for the lack of such facilitation. Firstly, the injuries produced by the *D. suzukii* egg-laying may be too small to provide access for *D. melanogaster*. In addition, *D. melanogaster* could also avoid oviposition in grapes already occupied by *D. suzukii* in order to prevent competition. However, predatory cannibalism seem to be a functional behaviour in *D. melanogaster* larvae and next-generation experiments on raspberries showed that *D. melanogaster* emergence was not affected by previous egg-laying of *D. suzukii*, which is in contrast to what we would have expected (Shaw, Brain, Wijnen, & Fountain, 2018; Vijendravarma, Narasimha, & Kawecki, 2013). Another explanation could be that the time may be too short for synergisms to develop, as *D. suzukii* had to oviposit before *D. melanogaster* could benefit from the resulting injuries to the grape skin. In this regard, Rombaut et al. (2017) showed in laboratory experiments that sour rot, but not the simple presence of *D. suzukii* larvae induced increased oviposition by *D. melanogaster* females. Thus, a facilitating effect of *D. suzukii* on *D. melanogaster* oviposition could be



**FIGURE 3** Relationship between volatile acidity and number of flies eclosing after oviposition in three different treatments with *Drosophila suzukii* (filled circles, dotted line), with *Drosophila melanogaster* (open circles, dot-dashed line), with *D. suzukii* and *D. melanogaster* (filled triangles, solid line) in 2017. Volatile acidity increased with increasing number of eclosed flies in both subsequent repetitions (a) with *D. suzukii*:  $n = 10$ ,  $t = 2.60$ ,  $p = .032$ ; with *D. melanogaster*:  $n = 10$ ,  $t = 4.05$ ,  $p < .004$ , with *D. suzukii* + *D. melanogaster*:  $n = 10$ ,  $t = 3.42$ ,  $p = .009$  and (b) with *D. suzukii*:  $n = 9$ ,  $t = 1.61$ ,  $p = .151$ ; with *D. melanogaster*:  $n = 10$ ,  $t = 2.15$ ,  $p = .063$ , with *D. suzukii* + *D. melanogaster*:  $n = 10$ ,  $t = 2.49$ ,  $p = .037$



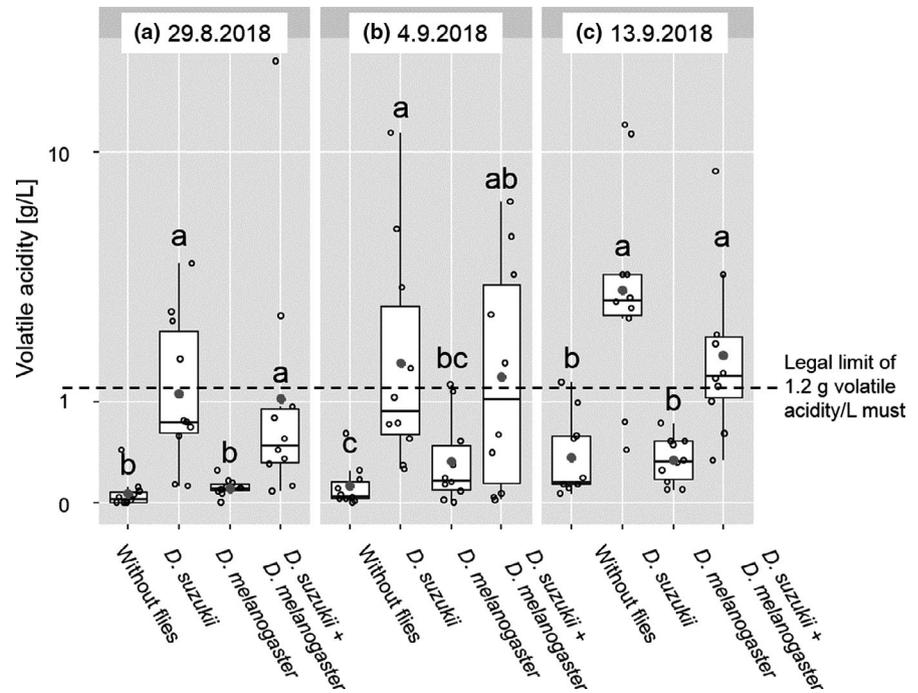
**FIGURE 4** Effects of *Drosophila suzukii* and *Drosophila melanogaster* on sour rot development in field experiments 2018 in a Dornfelder vineyard. Presence of flies increased volatile acidity only in one of two repetitions, still remaining far below the legal limit of 1.2 g/L: (a) set up 24.8.2018, ANOVA:  $n = 40$ ,  $F = 3.82$ ,  $p = .0179$  and (b) set up 31.8.2018, ANOVA:  $n = 36$ ,  $F = 0.65$ ,  $p = .59$ . Different letters indicate significant differences ( $p < .05$ ) in volatile acidity between the treatments at the 5% level following the Tukey post hoc test

expected as soon as the *D. suzukii* already induced sour rot, which will take at least a few days. Because *D. suzukii* pre-infestation may be the pre-condition for the subsequent infestation of *D. melanogaster* in undamaged vineyards as described in the introduction, future research should include different timelines regarding the introduction of *D. suzukii* relative to *D. melanogaster*. Additionally, as our results indicate a diverging potential of the two fly species to provoke sour rot development (with lower numbers of *D. suzukii*

having the same effect as higher numbers of *D. melanogaster*), it would be worthwhile to investigate this point in more detail.

Overall, our study shows that *D. suzukii* has the potential to lead to sour rot development under favourable conditions in the laboratory. These results should be interpreted with care, as they may not hold under less ideal conditions in the field, as the experiments of 2018 showed. In fact, *D. suzukii* was associated with considerable damages in viticulture only in one single year (2014) since its

**FIGURE 5** Effects of *Drosophila suzukii* and *Drosophila melanogaster* on sour rot development in laboratory experiments with grapes from a Dornfelder vineyard. Treatments including *D. suzukii* developed increased volatile acidity levels in all subsequent repetitions (a) set up: 29.8.2018, ANOVA:  $n = 40$ ,  $F = 19.7$ ,  $p < .0001$  (b) set up: 4.9.2018, ANOVA:  $n = 40$ ,  $F = 7.35$ ,  $p = .0006$  (c) set up: 13.9.2018, ANOVA:  $n = 40$ ,  $T = 18.55$ ,  $p < .0001$ . Different letters indicate significant differences ( $p < .05$ ) in volatile acidity between the treatments at the 5% level following the Tukey post hoc test



appearance in Germany in 2011, and it is still unclear to what degree *D. suzukii* was contributing to the severe sour rot development, and in how far it was merely benefitting from already high grape damage and humid weather conditions (C. Hoffmann, JKI Siebeldingen, unpublished data). Thus, the risk of *D. suzukii* for viticulture appears minor, at least in our region. Instead, our study shows that the common vinegar fly *D. melanogaster* is able to induce sour rot as well, but in contrast to *D. suzukii*, it is dependent on grapes' physical conditions. *Drosophila melanogaster* is a well-known amplifier of this disease and winegrowers keep an eye on it at all times (Hall, Loeb, Cadle-Davidson, et al., 2018; Hall, Loeb, & Wilcox, 2018). Thus, it requires further investigation to determine under which circumstances *D. suzukii* can really increase grape damage beyond the damage levels that *D. melanogaster* can cause. Moreover, we think it is important to keep investigating the combined effect of *D. suzukii* and *D. melanogaster*. We could not detect a synergistic induction of sour rot, but we suspect that the experiment was too short for a combined effect to develop. Future research should investigate if this synergistic induction may be possible over longer time periods.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHOR CONTRIBUTION

WE and CH designed research. WE conducted the experiments, analysed data and wrote the manuscript. WE and CH edited and approved the manuscript.

#### DATA AVAILABILITY STATEMENT

When accepted, the data that support the findings of this study will be openly available at [https://www.openagrar.de/receive/openagrar\\_mods\\_00050596](https://www.openagrar.de/receive/openagrar_mods_00050596)

#### ORCID

Wiebke Entling  <https://orcid.org/0000-0002-7210-8323>

#### REFERENCES

- Asplen, M. K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K. M., ... Desneux, N. (2015). Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): A global perspective and future priorities. *Journal of Pest Science*, 88(3), 469–494. <https://doi.org/10.1007/s10340-015-0681-z>
- Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., & Kopp, A. (2014). The making of a pest: The evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proceedings of the Royal Society B: Biological Sciences*, 281(1781), 20132840. <https://doi.org/10.1098/rspb.2013.2840>
- Barata, A., Malfeito-Ferreira, M., & Loureiro, V. (2012). The microbial ecology of wine grape berries. *International Journal of Food Microbiology*, 153(3), 243–259. <https://doi.org/10.1016/j.ijfoodmicro.2011.11.025>
- Barata, A., Pais, A., Malfeito-Ferreira, M., & Loureiro, V. (2011). Influence of sour rotten grapes on the chemical composition and quality of grape must and wine. *European Food Research and Technology*, 233(2), 183–194. <https://doi.org/10.1007/s00217-011-1505-x>
- Barata, A., Santos, S. C., Malfeito-Ferreira, M., & Loureiro, V. (2012). New insights into the ecological interaction between grape berry microorganisms and *Drosophila* flies during the development of sour rot. *Microbial Ecology*, 64(2), 416–430. <https://doi.org/10.1007/s00248-012-0041-y>

- Bellutti, N., Gallmetzer, A., Innerebner, G., Schmidt, S., Zelger, R., & Koschier, E. H. (2018). Dietary yeast affects preference and performance in *Drosophila suzukii*. *Journal of Pest Science*, 91(2), 651–660. <https://doi.org/10.1007/s10340-017-0932-2>
- Emiljanowicz, L. M., Ryan, G. D., Langille, A., & Newman, J. (2014). Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet. *Journal of Economic Entomology*, 107(4), 1392–1398. <https://doi.org/10.1603/EC13504>
- Entling, W., Anslinger, S., Jaraus, B., Michl, G., & Hoffmann, C. (2019). Berry skin resistance explains oviposition preferences of *Drosophila suzukii* at the level of grape cultivars and single berries. *Journal of Pest Science*, 92(2), 477–484. <https://doi.org/10.1007/s10340-018-1040-7>
- Evans, R. K., Toews, M. D., & Sial, A. A. (2017). Diel periodicity of *Drosophila suzukii* (Diptera: Drosophilidae) under field conditions. *PLoS ONE*, 12(2), e0171718. <https://doi.org/10.1371/journal.pone.0171718>
- Giraudoux, P. (2017). *pgirmess: Data analysis in ecology*. R package version 1.6.7. Retrieved from <https://CRAN.R-project.org/package=pgirmess>
- Hall, M. E., Loeb, G. M., Cadle-Davidson, L., Evans, K. J., & Wilcox, W. F. (2018). Grape sour rot: A four-way interaction involving the host, yeast, acetic acid bacteria, and insects. *Phytopathology*, 108(12), 1429–1442. <https://doi.org/10.1094/PHYTO-03-18-0098-R>
- Hall, M. E., Loeb, G. M., & Wilcox, W. F. (2018). Control of sour rot using chemical and canopy management techniques. *American Journal of Enology and Viticulture*, 69(4), 342–350. <https://doi.org/10.5344/ajev.2018.17091>
- Hamby, K. A., & Becher, P. G. (2016). Current knowledge of interactions between *Drosophila suzukii* and microbes, and their potential utility for pest management. *Journal of Pest Science*, 89(3), 621–630. <https://doi.org/10.1007/s10340-016-0768-1>
- Hamby, K. A., E. Bellamy, D., Chiu, J. C., Lee, J. C., Walton, V. M., Wiman, N. G., ... Biondi, A. (2016). Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. *Journal of Pest Science*, 89(3), 605–619. <https://doi.org/10.1007/s10340-016-0756-5>
- Ioriatti, C., Guzzon, R., Anfora, G., Ghidoni, F., Mazzoni, V., Villegas, T. R., ... Walton, V. M. (2018). *Drosophila suzukii* (Diptera: Drosophilidae) contributes to the development of sour rot in grape. *Journal of Economic Entomology*, 111(1), 283–292. <https://doi.org/10.1093/jee/tox292>
- Ioriatti, C., Walton, V., Dalton, D., Anfora, G., Grassi, A., Maistri, S., & Mazzoni, V. (2015). *Drosophila suzukii* (Diptera: Drosophilidae) and its potential impact to wine grapes during harvest in two cool climate wine grape production regions. *Journal of Economic Entomology*, 108(3), 1148–1155. <https://doi.org/10.1093/jee/tov042>
- Jaffe, B. D., & Guédot, C. (2019). Vertical and temporal distribution of spotted-wing drosophila (*Drosophila suzukii*) and pollinators within cultivated raspberries. *Pest Management Science*, 75(8), 2188–2194. <https://doi.org/10.1002/ps.5343>
- Kehrli, P., Linder, C., Cahenzli, F., & Daniel, C. (2017). Susceptibility of various grape cultivars to *Drosophila suzukii*. *Schweizer Zeitschrift Für Obst-und Weinbau, Wädenswil*, 153(14), 10–12.
- Lee, J. C., Bruck, D. J., Dreves, A. J., Ioriatti, C., Vogt, H., & Baufeld, P. (2011). In focus: Spotted wing drosophila, *Drosophila suzukii*, across perspectives. *Pest Management Science*, 67(11), 1349–1351. <https://doi.org/10.1002/ps.2271>
- Lemperle, E. (2007). *Weinfehler erkennen. Bestimmen - Vermeiden - Abhilfe*. Stuttgart, Germany: Eugen Ulmer KG.
- McFadden-Smith, W., & Gubler, W. D. (2015). Sour rot. In W. F. Wilcox, W. D. Gubler & J. K. Uyemoto (Eds.), *Compendium of grape diseases, disorders, and pests* (pp. 87–90). St. Paul, MN: The American Phytopathological Society (APS-Press).
- Ørsted, I. V., & Ørsted, M. (2019). Species distribution models of the spotted wing drosophila (*Drosophila suzukii* Diptera: Drosophilidae) in its native and invasive range reveal an ecological niche shift. *Journal of Applied Ecology*, 56(2), 423–435. <https://doi.org/10.1111/1365-2664.13285>
- R Core Team (2017). *R: A language and environment for statistical computing*. Retrieved from <https://www.R-project.org/>
- Rombaut, A., Guilhot, R., Xuereb, A., Benoit, L., Chapuis, M. P., Gibert, P., & Fellous, S. (2017). Invasive *Drosophila suzukii* facilitates *Drosophila melanogaster* infestation and sour rot outbreaks in the vineyards. *Open Science*, 4(3), 170117.
- Ryan, G. D., Emiljanowicz, L., Wilkinson, F., Kornya, M., & Newman, J. A. (2016). Thermal tolerances of the spotted-wing drosophila *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, 109(2), 746–752. <https://doi.org/10.1093/jee/tow006>
- Schetelig, M. F., Lee, K.-Z., Otto, S., Talmann, L., Stoekl, J., Degenkolb, T., & Halitschke, R. (2018). Environmentally sustainable pest control options for *Drosophila suzukii*. *Journal of Applied Entomology*, 142(1–2), 3–17. <https://doi.org/10.1111/jen.12469>
- Shaw, B., Brain, P., Wijnen, H., & Fountain, M. T. (2018). Reducing *Drosophila suzukii* emergence through inter-species competition. *Pest Management Science*, 74(6), 1466–1471. <https://doi.org/10.1002/ps.4836>
- Shaw, B., Fountain, M. T., & Wijnen, H. (2018). Recording and reproducing the diurnal oviposition rhythms of wild populations of the soft and stone- fruit pest *Drosophila suzukii*. *PLoS One*, 13(10), e0199406. <https://doi.org/10.1371/journal.pone.0199406>
- Shrader, M. E., Burrack, H. J., & Pfeiffer, D. G. (2019). *Drosophila suzukii* (Diptera: Drosophilidae) oviposition and adult emergence in six wine grape varieties grown in Virginia. *Journal of Economic Entomology*, 112, 139–148. <https://doi.org/10.1093/jee/toy305>
- Vijendravarma, R. K., Narasimha, S., & Kawecki, T. J. (2013). Predatory cannibalism in *Drosophila melanogaster* larvae. *Nature Communications*, 4, 1789. <https://doi.org/10.1038/ncomms2744>
- Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., ... Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*, 2(1), G1–G7. <https://doi.org/10.1603/IPM10010>

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