Research Article

Received: 29 May 2021 Revised: 25 October 2021 Accepted article published: 29 October 2021 Published online in Wiley Online Library: 17 November 2021

(wileyonlinelibrary.com) DOI 10.1002/ps.6699

Behavioral manipulation of *Drosophila suzukii* for pest control: high attraction to yeast enhances insecticide efficacy when applied on leaves

Guillermo Rehermann,[a] Urban Spitaler,[b,c] Karolina Sahle,[a] Carlo S Cossu,[b] Lorenz Delle Donne,[b,c] Flavia Bianchi,[d] Daniela Eisenstecken,[d] Sergio Angeli,[e] Silvia Schmidt[b] and Paul G Becher[a*]

Abstract

BACKGROUND: The invasive pest, *Drosophila suzukii* attacks fresh soft-skinned fruit. Broad-spectrum insecticides are implemented for control but there is a need to reduce environmental risks and insecticide residues on fruits. *Hanseniaspora uvarum* is a yeast frequently found on ripe fruits and associated with *D. suzukii*. We aim to exploit the ecological association and attraction of *D. suzukii* to *H. uvarum* by developing an attract-and-kill strategy, with spray-application on canopy but not fruit. We therefore investigated *D. suzukii* attraction, egg-laying and mortality when exposed to insecticidal yeast-based formulations.

RESULTS: *Hanseniaspora uvarum* strongly attracted *D. suzukii* when applied on leaves of grapevine, *Vitis vinifera*. Notably, this attractiveness was competitive to ripe grape berries that were susceptible to *D. suzukii* infestation. Moreover, adding *H. uvarum* enhanced the efficacy of insecticidal formulations against *D. suzukii*. Flies exposed to leaves treated with yeast-insecticide formulations showed higher mortality and laid a lower number of eggs compared to flies exposed to insecticide alone. In a wind tunnel, all treatments containing *H. uvarum* alone or in combination with insecticides, caused similar upwind flight and landing at the odor source, which provides evidence that the addition of insecticide did not reduce *D. suzukii* attraction to yeast.

CONCLUSION: *Hanseniaspora uvarum* can be used to manipulate the behavior of *D. suzukii* by attracting flies to insecticide formulations. Yeast attraction is competitive to grape berries and improves insecticide effectiveness, suggesting that sprays covering canopy only, could reduce residues on fruit without compromising management efficacy.

Supporting information may be found in the online version of this article.

Keywords: integrated pest management; semiochemicals; spinosad; spotted wing drosophila; viticulture

1 INTRODUCTION

Integrated pest management (IPM) in cropping systems aim at the optimization of preventive actions to maintain pest pressure below the economic damage threshold, while minimizing the use of chemical pesticides when control is required.[1] Manipulation of insect pest behavior is a management option directed towards the aims of IPM. Behavioral manipulation stimulates or inhibits a behavior, or changes its expression,[2] in order to negatively impact a pest's performance and life cycle, and consequently to reduce crop damage.[3] While for certain crops behavioral manipulation of pests by use of pheromones or plant volatiles is already part of management strategies,[4,5] manipulation methods could in general be further developed and diversified for application in additional cropping systems.

* Correspondence to: PG Becher, Chemical Ecology - Horticulture, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Sundsvägen 14, 23053 Alnarp, Sweden. E-mail: paul.becher@slu.se

a Chemical Ecology - Horticulture, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden

b Entomology Group, Institute for Plant Health, Laimburg Research Centre, Auer (Ora), Italy

c Institute of Plant Protection, Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna, Austria

d Laboratory for Flavours and Metabolites, Institute for Agricultural Chemistry and Food Quality, Laimburg Research Centre, Auer (Ora), Italy

e Faculty of Science and Technology, Free University of Bozen-Bolzano, Bolzano, Italy

Microbial semiochemicals, in addition to plant volatiles and pheromones, affect insect behavior and should therefore be exploited in the development of new pest control methods.[5–8] Yeast volatiles attract insects of several orders, which most likely represents a conserved trait among phylogenetically diverse yeasts.[9] Consequently, yeasts are promising agents for application in insect pest control.

The spotted wing drosophila, *Drosophila suzukii* (Matsumura), is a worldwide spreading pest that can lay eggs in fruit close to harvest.[10] Development of preventive measures[11] and biological control[12,13] contribute to a sustainable pest management, while pesticide application is still common for controlling *D. suzukii* in various crops.[14–17] Despite existing pre-harvest regulations, pesticide residues cause problems with respect to marketing and consumer safety.[18–21] Moreover, application of broad-spectrum insecticides,[14,22,23] together with recent findings on insecticide resistance in *D. suzukii*[16,24,25] have increased the need for alternative methods and improving insecticide efficacy.

A promising approach to meet this need is the use of attractants or phagostimulants to control *D. suzukii*.[26–31] In fruit production, formulations of insecticides with attractants allow application without the need to spray the fruit. One option is to target the canopy only, but little is known about the behavioral response of *D. suzukii* to such treatments (but see[31,32]).

Yeast has potential for being used both as attractant and phagostimulant.[33–35] Interestingly, *D. suzukii* is closely associated with yeasts[36,37] which suggests to exploit the relationship between yeast and fly to lure *D. suzukii* to toxic baits.[34] *Hanseniaspora uvarum* (Niehaus), an apiculate yeast, is the predominant yeast species associated with *D. suzukii*.[36] The presence of *H. uvarum* in infested fruit, larval gut and frass reveals this yeast as a food resource for *D. suzukii*. In addition, a strong olfactory response and attraction of *D. suzukii* towards *H. uvarum* semiochemicals[38] and increased fecundity of females fed with the yeast,[39] suggests a strong and specific ecological relation between *D. suzukii* and *H. uvarum*. A range of field studies confirmed attraction to traps baited with *H. uvarum*.[40–42] Not surprisingly, *H. uvarum* has been suggested for the development of attract-and-kill formulations against *D. suzukii*.[31,35,43] Moreover, *H. uvarum* suppresses plant pathogens[44] and was evaluated with qualified presumption of safety as biological control agent intentionally added to food or feed.[45]

In viticulture, *D. suzukii* causes significant problems in the cultivation of certain *Vitis vinifera* (grapevine) varieties, such as Vernatsch (synonymous Trollinger, Schiava) traditionally grown in parts of Italy, Austria and Germany.[46–49] Saliently, *H. uvarum* is one of the main yeasts associated with *V. vinifera* and is commonly found on the grape-surface and during early stages of wine fermentation.[50–52]

Viticulture is characterized by intensive pesticide use.[53,54] With focus on grapevine, we hypothesized that *H. uvarum* in combination with insecticides will induce *D. suzukii* odor-driven attraction when applied on grapevine leaves and improve insecticide efficacy by increasing fly mortality and reducing fruit infestation. Thus, we assayed the attraction of *D. suzukii* to *H. uvarum* applied on grapevine leaves in comparison to untreated leaves and grape berries. Moreover, testing up-wind flight in a wind tunnel, we investigated the possible change of *D. suzukii* attraction after blending *H. uvarum* with insecticide. Finally, in formulation with yeast, four commercial products were assayed for their insecticidal effects in comparison to formulations without yeast. The obtained results provide a foundation for the development of attract-and-kill strategies to control *D. suzukii* based on the natural association and interaction between insect and yeast.

2 MATERIALS AND METHODS

2.1 Yeast culture and formulation

Hanseniaspora uvarum (strain: LB-NB-2.2; accession number GenBank NCBI: MK567898), was isolated from feeding grooves of *D. suzukii* infested grapes in South Tyrol, province of Bolzano, Italy.[37] Cultures of *H. uvarum* were grown on PDA (Difco, Potato Dextrose Agar: 39 g L^{-1}) plates. For liquid cultures, PDB (Difco, Potato Dextrose Broth: 24 g L^{-1}) was inoculated with single colonies and incubated at 25°C for 24–30 h on an incubator shaker (Stuart Scientific). A freeze-dried stock culture of *H. uvarum* was stored at −80°C and used for generating fresh cultures on PDA.

2.2 Flies

Drosophila suzukii flies from infested fruit collected in South Tyrol in 2019 were used to establish laboratory fly rearings. For attraction assays, flies were reared and tested at the Swedish University of Agricultural Sciences (SLU), Alnarp, and for assaying mortality and egg-laying, flies were reared and tested at the Laimburg Research Center Ora, Italy. At SLU, flies were reared on a Bloomington drosophila cornmeal diet (BDSC Cornmeal Food) at 22–24°C and maintained at 50–65% R.H., under a 12:12 h L:D (light: dark) photoperiod. Adult flies were kept in 30-mL rearing vials on fresh diet, closed with a cotton ball. Mated females were used for testing attraction in cages (two choices between differently treated grapevine leaves) and up-wind flight behavior (wind tunnel). In preparation for behavioral assays, newly emerged flies were anesthetized with CO_2 and sexed. Females and males were kept separate in rearing vials on fresh diet. For obtaining mated females, virgin females and males of similar age were grouped in a rearing vial during the peak hours of their sexual activity[55] within the 2nd and 3rd h of the photophase. Mating couples were isolated and after finishing copulation, females were kept alone or in groups of 10 until the wind tunnel or cage attraction experiment, respectively (see below for further details). Individuals at Laimburg were maintained at similar conditions with slight modifications: flies were reared in insect cages (W47.5 × D47.5 × H93.0 cm, BugDorm – 4 M4590, MegaView Science Co., Ltd., Taichung, Taiwan) on *D. suzukii* cornmeal diet (DSCD(a) containing dry deactivated yeast) with dry baker's yeast (RUF Lebensmittelwerk KG, Quakenbrück, Germany) and additional 5% sugar solution,[37] at 16:8 h L:D photoperiod. To determine the insecticidal activity of yeast-insecticide formulations, *D. suzukii* males and females were kept together from emergence until reaching an age of 5–8 days. Then, groups of 20 males and 20 females were placed together in an insect cage for testing egg-laying behavior and mortality (see below for further details).

2.3 Plant and fruit material

The leaves and grapes used for behavioral assays were of *Vitis vinifera* L., variety Regent, locally grown without pesticide application at the vineyard at SLU, Alnarp (55°39′37.9″N 13°05′07.3″E) in September 2019. Leaves were picked in the morning and transferred to the laboratory about 2 h before the start of the attraction assays. Leaves were chosen to be similar in size and coloration for treatment and control. The berries were picked at the first day of the experimental period and thereafter stored in a fridge for consecutive use during 10 days. The grapes were ripe, firm, undamaged, blue in color and attached to a cluster. Preliminary experiments confirmed that *D. suzukii* was able to infest and develop in Regent and *D. suzukii* flies were found emerging from Regent berries collected at Alnarp in 2020 (for details see Supporting information). Yeast-insecticide formulation efficacy was tested on *V. vinifera* leaves of the variety Vernatsch, from a vineyard cultivated according to the guidelines for integrated fruit production in South Tyrol (46°23′04.8″N 11°17′10.6″E). Non-treated blueberries (*Vaccinium corymbosum*) from organic production were used as substrate for egg-laying assessment.

2.4 Attractiveness of grape leaves sprayed with *H. uvarum*

To evaluate the odor attractiveness of *H. uvarum* when applied on the surface of green plant leaves, a two-choice set-up was designed and mated *D. suzukii* females were exposed to vine leaves sprayed with *H. uvarum* and untreated vine leaves as alternative. For treatment, two fresh *V. vinifera* leaves were sprayed with 250 μL of *H. uvarum* grown in liquid PDB and left to dry for 1.5 h before testing attraction in comparison to two untreated leaves. The experimental arena consisted of a rectangular cuboid (Plexiglas: W66 × D33 × H33 cm). In the top of the cuboid, two closable openings allowed to introduce the green plant material and the experimental flies. In the morning of the experimental day, the leaves were placed with their stems into Erlenmeyer flask (10.5 cm high, 100-mL, VWR) filled with water; the opening around the stems of the leaves closed with cotton wool. The flasks with the treatment and the control leaves were placed in opposite ends of the cage, 5 cm from the sidewall, at equal distance (approximately 16 cm) to the front and the back of the cage. The distance between the treatments was about 46 cm. Ten mated females were tested (n = 18 replicates). For acclimatization, mated females were kept starved in plastic dishes with mesh lids (diameter 10 × H4 cm) inside and at the center of the experimental arena for 18–22 h before the experiments started. The experiments were conducted for 2 h. For the first hour the flies were checked every 10 min and the position of the flies in the cage was observed and recorded (positions: treated leaves, control leaves, elsewhere). After the sixth observation, at 60 min, the flies were left for one more hour and then a final observation was recorded (sketch of experimental set-up, Fig. 1(A)).

In a second experiment, the set-up was slightly modified by adding two clusters of five grape berries (*V. vinifera*., Regent) into the cage, one on each side above the treated and untreated vine leaves, respectively. The clusters were connected to metal wires fastened with paper tape at the roof of the cage (sketch of experimental set-up, Fig. 1(B)). As before, the position of the flies was recorded (treated leaves, control leaves, berries on treatment side, berries on untreated side, elsewhere) every 10 min for 1 h and at the end of the experiment that is, after 2 h (n = 20 replicates).

As supplementary experiment, in order to detangle the attraction of *D. suzukii* females to the formulation of medium and yeast, we tested the preference of groups of 10 mated females (n = 15 replicates) to grapevine leaves sprayed with *H. uvarum versus* PDB. This was conducted with the same procedure as in the first experiment (*i.e.*, with grapevine leaves only), but in smaller cages (W30 × D30 × H30 cm, BugDorm – 1, MegaView Science Co., Ltd., Taichung, Taiwan).

2.5 Efficacy of *H. uvarum* formulations with different insecticides

To evaluate the efficacy of insecticides in formulation with *H. uvarum*, we conducted a mortality and oviposition bioassay by treating freshly picked *V. vinifera* leaves (Vernatsch) and offering (untreated) *V. corymbosum* berries together with water agar as egg-laying substrate. In a first experiment, insecticide-yeast formulations were compared to aqueous insecticide solutions and untreated leaves in separate experimental cages (W30 × D30 × H30 cm, BugDorm – 1, MegaView Science Co., Ltd., Taichung, Taiwan) (n = 5 per treatment). In total, four insecticides toxic for *D. suzukii* and allowed for IPM in Italian viticulture, were tested: (i) 15 mg L^{-1} deltamethrin (Decis EVO, Bayer CropScience S.r.l.), (ii) 100 mg L^{-1} acetamiprid (Epik SL, Sipcam Italia S.p.A.), (iii) 120 mg L^{-1} spinosad (Laser, Dow AgroSciences Italia S.r.l.) and (iv) 720 mg L^{-1} tau-Fluvalinate (Mavrik 20 EW, Adama Italia S.r.l.). The applied doses were established according to the manufacturer's instructions for viticulture in Italy. This allowed to test the efficacy of insecticides in combination with *H. uvarum* culture at the concentrations used in vineyards. For each insecticide-yeast-formulation, 100 μL (10 droplets of 10 μL volume) were applied onto individual leaves and dried at room temperature for approximately 2 h. Similarly, the four different insecticides were applied on leaves at the same dose as before but in 100 μL of distilled water instead of yeast culture, and untreated leaves were used for control tests. For each test, five leaves were placed with their stems into an Erlenmeyer flask filled with water and the opening around the stems was closed with cotton. Leaves were then exposed to groups of 20 female and 20 male *D. suzukii* per experimental cage. A 5% sugar solution supplied on cotton in a small Petri dish (diameter 6 cm) served as water and energy source for the flies. Each cage contained an additional Petri dish with water agar (diameter 9 cm, 15 g L^{-1} agar) on which four blueberries were placed. Egg-laying was quantified from the number of eggs laid on agar and berries together. The blueberries and the agar substrates were removed and replaced by a new set after 24 h. In a second experiment, the four *H. uvarum*-insecticide mixtures were tested like before and in addition *H. uvarum* was tested as pure culture (10 droplets of 10 μL volume) applied on leaves (n = 5 per treatment). For both experiments adult mortality and the number of laid eggs was counted after 24 h and 48 h. Experiments were performed at similar laboratory conditions as insects were reared (*ca*. 22°C, 65 ± 5% R.H., 16:8 h L:D photoperiod).

2.6 Flight attraction behavior to *V. vinifera* leaves with *H. uvarum*-insecticide formulations

To assess odor-mediated flight attraction behavior of *D. suzukii* towards *H. uvarum* blended with insecticides, wind tunnel experiments were conducted with the same equipment (glass wind tunnel system with D100 × H30 × W30 cm flight section), but slightly modified protocol as described earlier.[35,56] Mated females, 4–6 days old, starved 6–8 h prior testing, were released individually at the down-wind end of the tunnel and exposed for 5 min to a main air stream (0.3 m s^{-1}) carrying a plume of stimulus odor. The stimulus was delivered in charcoal filtered air (0.3 L min^{-1}) that was blown through a wash bottle containing the test material described below. The scented airstream was, *via* a Pasteur pipette, vertically injected at the up-wind end into the wind tunnel onto an 18 cm high, 38 mm diameter horizontal platform of aluminum, from which it diffused down-wind as an odor plume.

Three different treatments and two controls were placed into different wash bottles to test their headspace emissions for *D. suzukii* attraction: (1) *H. uvarum* applied on *V. vinifera* leaves, (2) *H. uvarum* blended with spinosad applied on *V. vinifera* leaves, and (3) *H. uvarum* blended with deltamethrin applied on *V. vinifera* leaves. (4) *Vitis vinifera* leaves and (5) *H. uvarum* applied on filter paper were tested to control for leaf and yeast volatiles, respectively. For the insecticide treatments, two leaves were sprayed, each with 250 μL of a formulation of yeast culture and spinosad (5.4 mg L^{-1}, treatment (2), comparable to doses applied in other studies,[31,35]) or deltamethrin (7.5 mg L^{-1}, treatment (3)), respectively. Sprayed leaves were dried for 1.5 h at room temperature prior testing. Similarly, *H. uvarum* was applied on leaves for treatment (1) or on filter paper for control (5). Flies were recorded for upwind flight and landing at the odor source (*i.e.*, on top of the aluminum platform or the tip of the pipette injecting the scented air). In total, 50 individual females were tested for attraction towards each treatment. Experiments were performed at similar conditions as insects were reared, 1.5–2 h prior the onset of the scotophase.

2.7 Data analysis

Analyses were performed using R statistical software.[57] To evaluate odor-driven attraction of *D. suzukii* females to *H. uvarum* applied on plant leaves over time, a mixed linear model (MLM) fitted with a Gaussian error distribution (R software package 'lme4') was performed. The preferred substrate where *D. suzukii* females were observed when exposed to yeast-treated leaves in the presence of grape berries was analyzed with a mixed effects generalized linear model (GLMM) fitted with a binomial error distribution. A Tukey´s contrast test (R software package 'multcomp') was used for pairwise comparison between treated and untreated green leaves and the grapes placed nearby each treatment, respectively. The effects of the treated leaves on the mortality and oviposition of *D. suzukii* adults were evaluated with a GLM fitted with a binomial and Poisson distribution, respectively. A chi-square test (R software package 'car') followed by Tukey's contrast analysis was used to estimate the significance of fixed effects and for pairwise comparison of treatments, respectively. The up-wind flight towards yeast odors in the wind tunnel was modeled with a GLM fitted with a binomial error distribution followed by a Tukey's contrast pairwise comparison between the different treatments. Models were selected, based on Akaike information criterion values. Residuals were analyzed to verify the distribution of the errors. For further details, see supporting information (Table S1 and S2). Figures were drawn using 'Tidyverse' (R software package 'tidyverse').

3 RESULTS

3.1 Attractiveness of grape leaves sprayed with *H. uvarum*

Mated *D. suzukii* females were significantly more attracted to *V. vinifera* leaves sprayed on the surface with *H. uvarum* compared to untreated leaves, and this preference significantly increased along the experimental time (MLM: F = 42.18, df = 231,

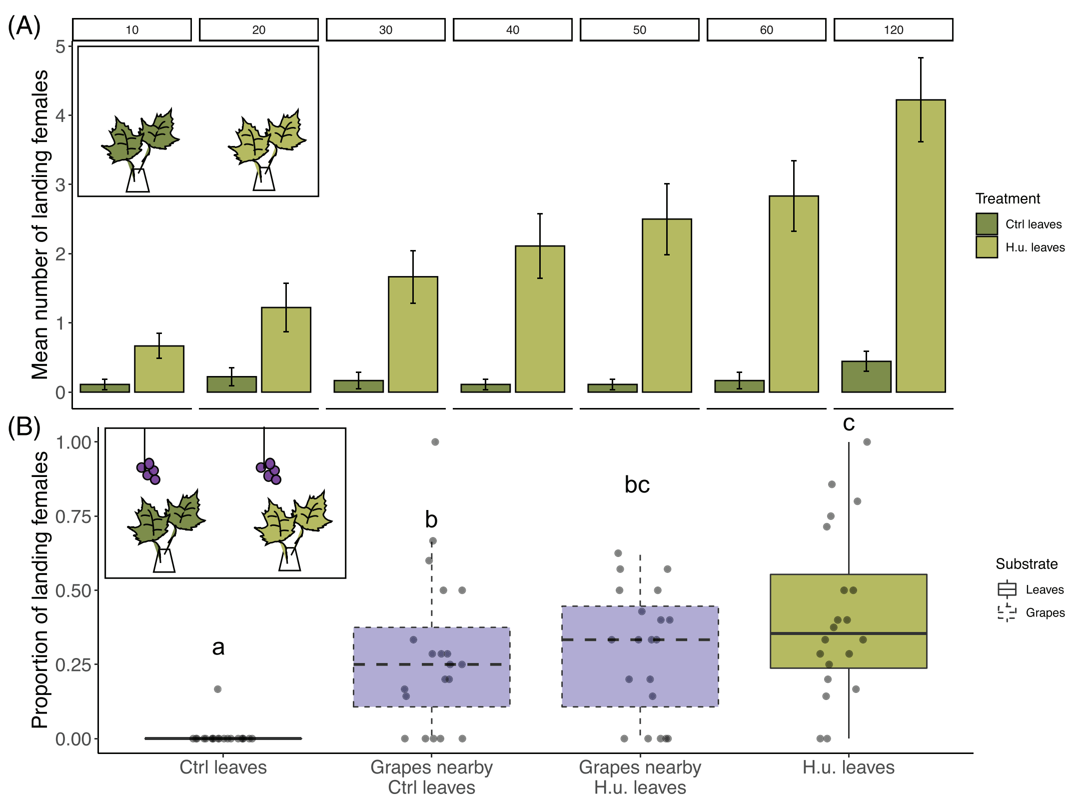

Figure 1. (A) Preference (Mean ± standard error of the mean) of 10 mated *Drosophila suzukii* females (n = 18) when given the choice between *Vitis vinifera* leaves treated with *Hanseniaspora uvarum* yeast (H.u. leaves, light green) or untreated leaves (Ctrl leaves, dark green) along the experimental period of 120 min (after 10, 20, 30, 40, 50, 60 and 120 min). Significantly more females landed on H.u. leaves compared to Ctrl leaves ($P < 0.05$) with a significant increase of preference over time ($P < 0.001$). (B) Preference (after 120 min) of 10 mated *D. suzukii* females (n = 20) when exposed to *V. vinifera* leaves treated with *H. uvarum* (light green), untreated leaves (dark green) and grapes (purple) placed nearby the treated and untreated leaves. Leaves sprayed with yeast were as attractive as the grapes. The boxes represent the interquartile range divided by the median, and whiskers represent the data within 1.5× the interquartile range. Dots represent the data distribution of the individual replicates. Different letters above boxes describe significant difference after multiple comparisons of means. Scheme of the experimental design at the top left corner of each plot illustrating treated and untreated leaves, and grape berries in B.

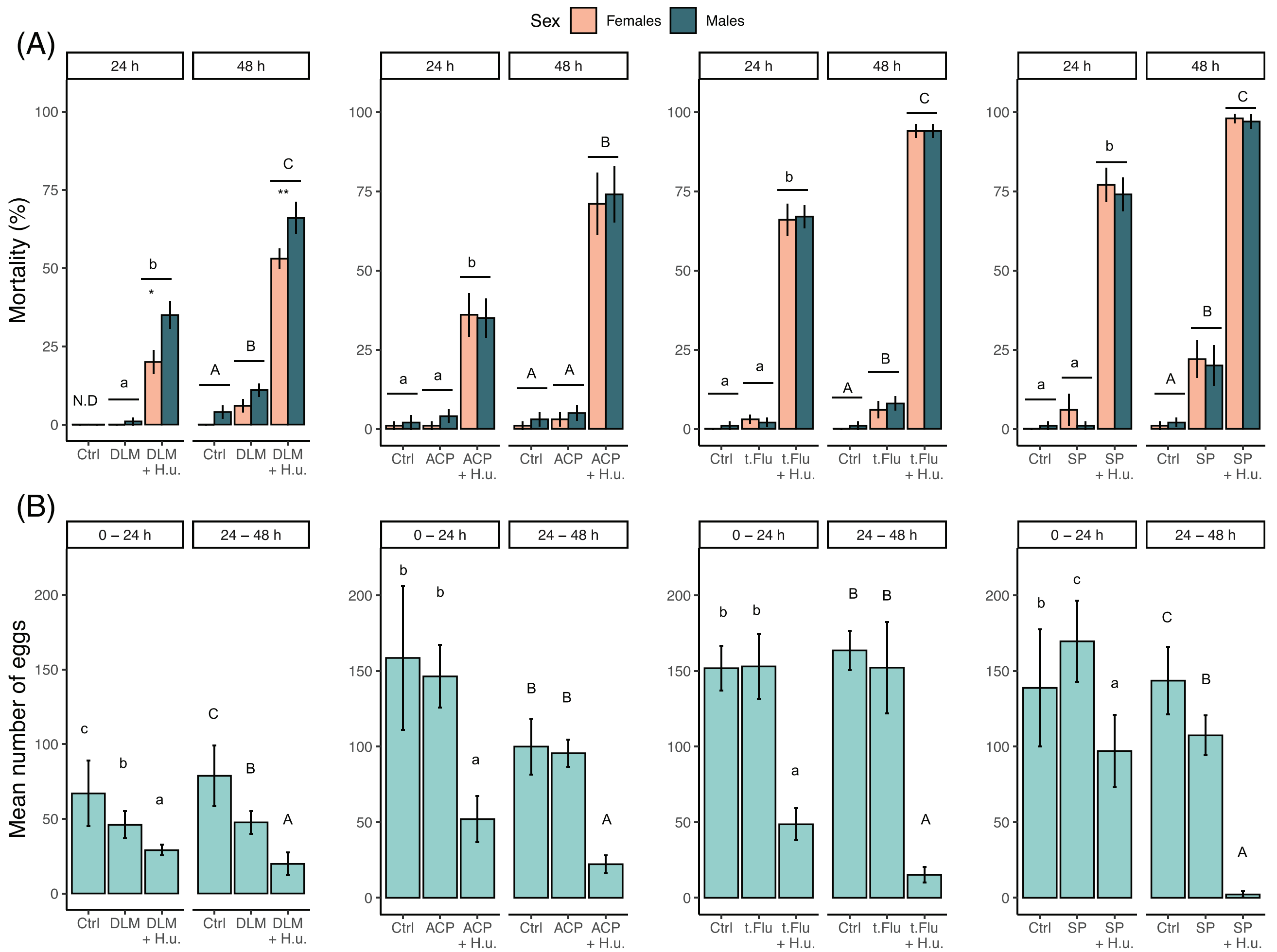

Figure 2. Effect of *Hanseniaspora uvarum*-insecticide formulations on *Drosophila suzukii* mortality (A) and oviposition (B) for 20 females and 20 males per replicate, (n = 5). (A) Cumulative mean percentage of female (light coral) and male (dark cyan) mortality ± standard error of the mean after 24 h and 48 h of continuous exposure to *Vitis vinifera* untreated leaves (Ctrl), insecticide-treated leaves (acetamiprid = ACP, deltamethrin = DLM, tau-Fluvalinate = t.Flu, spinosad = SP) and leaves treated with formulations of insecticide and *H. uvarum* (abbreviated insecticide name + H.u.). Both at 24 h and at 48 h leaves treated with formulations of insecticide and *H. uvarum* caused a significantly higher mortality in both sexes compared to those exposed to insecticide alone or control leaves ($P < 0.01$). (B) A significantly lower number of eggs (Mean ± standard error of the mean) was laid both during the first 24 h as well as from 24–48 h, when flies were exposed to any of the *H. uvarum*-insecticide formulations in comparison to control or the insecticides without yeast. Different letters denote significant differences between treatments ($P < 0.05$, lowercase at 24 h, uppercase at 48 h). Asterisks denote significant differences in mortality between sexes (* $P < 0.05$, ** $P < 0.01$). N.D. means No-Dead flies and data were therefore excluded from data analysis.

$P < 0.0001$). After 120 min, 42.2% (76 individuals in total) of the experimental flies were found on *H. uvarum*-treated leaves compared to only 4.4% (eight in total) on the control leaves (Fig. 1(A)). When given the choice between *V. vinifera* leaves sprayed with *H. uvarum* or the growth medium alone (PDB), flies were again significantly more attracted to *H. uvarum* compared to PDB-treated leaves (GLMM binomial distribution: F = 12.71, df = 27, $P < 0.001$; Fig. S1). Interestingly, when grapes were placed nearby to the treated and untreated leaves (drawing Fig. 1(B)), females still preferred to land and stay significantly more on the *Hanseniaspora*-treated leaves compared to the untreated ones (GLMM binomial distribution: F = 8.52, df = 75; Multiple Comparison of Means (MCM): Z = 4.41, $P < 0.0001$). Yeast-sprayed leaves were as attractive for *D. suzukii* mated females as the grapes placed above (GLMM binomial distribution, MCM: Z = 1.86, $P = 0.22$), but more attractive than the grapes on the untreated side of the experimental cage (GLMM binomial distribution, MCM: Z = 2.96, $P < 0.05$).

3.2 Efficacy of *H. uvarum* formulations with different insecticides

In the first experiment, the addition of *H. uvarum* into insecticide formulations when applied onto green *V. vinifera* leaves significantly increased the mortality of *D. suzukii* individuals in both sexes (Fig. 2(A)) and reduced the total number of eggs laid (Fig. 2(B)), compared to the application of insecticide alone or untreated leaves. This was true for the four tested yeast-insecticide formulations at 24 h and the differences were even clearer at 48 h after exposure (Mortality: GLM binomial distribution, df = 55, $P < 0.001$; Oviposition: GLM Poisson distribution, df = 12, $P < 0.01$; for further details see supporting information, Table S2). Most yeast-insecticide formulations affected female and male mortality similarly ($P > 0.05$), but not deltamethrin-*H. uvarum* (DLM + H.u.) for which mortality in males (percentage mean ± standard error of the mean = 66.0 ± 4.9%) was significantly higher than in females (53.0 ± 3.0%) ($P < 0.05$). At the end of the experiment, on average 59.5 ± 3.0% of flies exposed

Table 1. Comparison of *Hanseniaspora uvarum*-insecticide formulations for their effects on *Drosophila suzukii* mortality and oviposition. Cumulative mean number (± standard error, SE) and percentage of *D. suzukii* adult mortality by *H. uvarum*-insecticide formulations after 24 h and 48 h of continuous exposure to treated *Vitis vinifera* leaves. Total numbers of eggs laid at the end of the experiment are represented by means (±SE). A total of five leaves per treatment was applied with 100 μL of formulation per leaf

	Dead flies after 24 h exposure		Dead flies after 48 h exposure		Total eggs laid
Treatment formulation	Mean (SE)	Percentage	Mean (SE)	Percentage	Mean (SE)
H. uvarum	0.0[†]	0	0.1 (0.1)[a]	0.5	311.0 (45.3)[a]
Acetamiprid + *H. uvarum*	8.4 (1.2)[a]	42	15.2 (0.8)[b]	76	82.2 (13.4)[b]
Deltamethrin + *H.uvarum*	11.5 (1.0)[b]	58	17.0 (0.6)[b]	85	77.6 (14.5)[b]
tau-Fluvalinate + *H. uvarum*	15.2 (0.5)[c]	76	19.0 (0.3)[c]	95	62.2 (16.3)[c]
Spinosad + *H. uvarum*	15.9 (0.5)[d]	80	19.9 (0.1)[c]	100	57.0 (7.5) [c]
	GLM binomial distribution Res. df = 35 $X^2 = 79.6, P < 2.2e\text{-}16$ ***		GLM binomial distribution Res. df = 45 $X^2 = 706.6, P < 2.2e\text{-}16$ ***		GLM Poisson distribution Res. df = 20 $X^2 = 1578.5, P < 2.2e\text{-}16$ ***

Mortality data were analyzed using a generalized linear model (GLM) with binomial distribution and number of eggs laid using a GLM Poisson distribution. Treatment formulation was the fix effect (n = 5) in all models. Column means followed by a different letter denote significant differences between treatments after pairwise comparison ($P < 0.05$). See Table S1 for model details.
† *H. uvarum* treatment was excluded from analyses due to the absence of dead flies in all replicates in the first 24 h.

to DLM + H.u. died compared to 8.5 ± 1.5% on DLM alone, or 3.5 ± 2.5% on untreated leaves (Ctrl). Acetamiprid-*H. uvarum* (ACP + H.u.) caused on average a higher mortality (72.5 ± 6.1%) than ACP (4.0 ± 1.5%) and Ctrl (2.0 ± 1.1%). Finally, tau-Fluvalinate-*H. uvarum* (t-Flu + H.u.) and spinosad-*H. uvarum* (SP + H.u.) formulation treatments showed the highest efficacy, killing on average 97.5 ± 1.1% and 94.0 ± 1.3% of the experimental flies, respectively. In the second experiment, direct comparison of the four insecticide-*H. uvarum* formulations showed a similar pattern as in the first experiment; SP + H.u. and t-Flu + H.u. treatments were the most effective, followed by DLM + H.u. and ACP + H.u. (Table 1). Notably, only 1 out of 200 flies exposed to control H.u. leaves died after 48 h, denoting that no negative impact on flies was caused by *H. uvarum*.

3.3 Flight attraction behavior to *V. vinifera* leaves with *H. uvarum*-insecticide formulations

While *V. vinifera* leaf volatiles induced up-wind flight of 10% (5 out of 50 flies), the application of *H. uvarum* (250 μL) on the surface of the leaves significantly increased attraction resulting in 44% (22 flies) *D. suzukii* females flying up-wind and landing at the odor source within the 5 min test period (GLMM, MCM: Z = 3.55, $P < 0.01$). Notably, the response of flies to odors emitted by *H. uvarum* applied on filter paper (38%, 19 flies) was similar as to

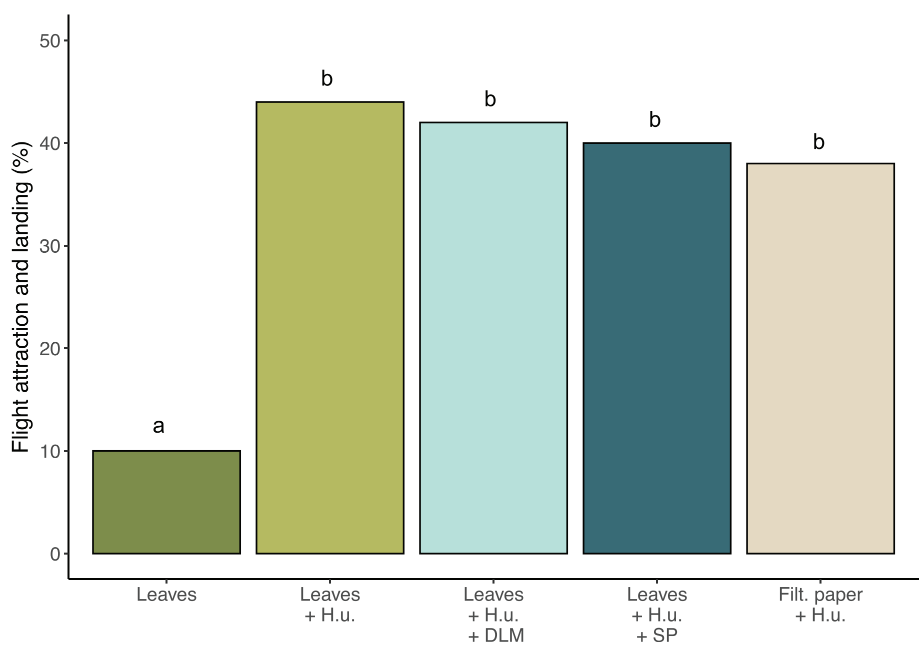

Figure 3. Up-wind flight and landing at the odor source of mated *Drosophila suzukii* females towards headspace volatiles of *Vitis vinifera* leaves (Leaves), leaves sprayed with *Hanseniaspora uvarum* (Leaves + H.u.), leaves sprayed with *H. uvarum* in combination with the insecticides deltamethrin (DLM) or spinosad (SP), and filter paper sprayed with *H. uvarum*. The presence of *H. uvarum*, either alone or in combinations with insecticides increases flight attraction significantly in comparison to untreated green leaves ($P < 0.05$). Different letters illustrate significant differences in response to the treatments. In total, 50 flies for each treatment were tested.

odors emitted by *H. uvarum* applied on leaves (GLMM, MCM: $Z = 0.61$, $P = 0.97$). Furthermore, insecticide formulations of spinosad and *H. uvarum* (40%, 20 flies) or deltamethrin (42%, 21 flies) combined with *H. uvarum* were similar attractive as the *H. uvarum* spray without insecticide (GLMM, MCM: $P > 0.05$).

4 DISCUSSION

The spread of *D. suzukii* causes damage across fruit plantations in many parts of the world.[10,48,49] We studied the impact of yeast in formulation with insecticides on *D. suzukii* attraction in the context of plant leaves to generate a foundation for future field application of a new attract-and-kill strategy.

Our results demonstrate that *H. uvarum* volatiles have a strong capacity to manipulate *D. suzukii* behavior when applied onto plant leaves. In laboratory assays, *D. suzukii* flies were highly attracted to *H. uvarum* sprayed on leaves of *V. vinifera* compared to the yeast growth medium. What is more, mated *D. suzukii* females preferred leaves treated with *H. uvarum* relative to untreated leaves and this preference was competitive to grape berries that were susceptible to *D. suzukii* infestation (Fig. 1). Neither in cage experiments nor wind tunnel tests with mated females we could see a strong attraction to grapevine leaves except when *H. uvarum* was sprayed onto the surface.

Previous research has shown that *H. uvarum* represents a valuable food resource for the flies.[39] In our assay, females were arrested and stayed feeding on *H. uvarum* after landing on the grapevine leaves (Fig. 1(A) and supporting video, SV1). This result is in line with earlier findings showing that shortly after mating, females increased their feeding activity on yeast, possibly to maximize nutrient allocation for egg production.[35] Intriguingly, even in the presence of ripe grape berries, female flies were still attracted in a similar manner to leaves with *H. uvarum* as to fruit (Fig. 1(B)), thus yeast disrupted host infestation at least temporarily. Stronger attraction to yeast than to fruit odors was earlier shown for *D. melanogaster*.[58]

Remarkably, volatiles emitted from *H. uvarum* culture triggered strong up-wind flight in *D. suzukii* even when applied at μL-amounts and dried on the treated leaf surface before testing. In a previous study, we used headspace emitted from 50 mL of fresh culture[35] unaware of the high sensitivity of *D. suzukii* that we now revealed towards a different strain of *H. uvarum*. More than that, in the current study we show that even after addition of insecticide, *H. uvarum* attracted similarly strong as the pure yeast culture. In other words, insecticides blended with *H. uvarum* did not affect the sensitivity and attraction of flies compared to *H. uvarum* alone (Fig. 3). So far experimental evidence about the ability and sensitivity of flies to respond with up-wind flight attraction to yeast-insecticide formulations was lacking. Furthermore, *H. uvarum* applied on filter paper was sufficient to induce *D. suzukii* up-wind flight. Hence, volatiles of *H. uvarum* induce flight attraction even in absence of background odors such as leaf volatiles.

Our study shows that *H. uvarum* acts as an attractant that stimulates the contact with the insecticidal food-bait and enhances insecticide efficacy; when flies were exposed to treated grape leaves, all four tested insecticides combined with *H. uvarum* led to increased mortality and reduced egg-laying as compared to treatments with insecticide alone or exposure to untreated control leaves (Fig. 2).

Noteworthy, manipulative attraction or phagostimulation of *D. suzukii* has been shown in earlier studies even for application of non-nutritive baits as well as traps without insecticides.[26,28,30] Similarly, the attraction to *H. uvarum* offers options for manipulation different to the here studied insecticide-based attract-and-kill.[40–42]

Moreover, in agreement with our work, earlier studies have shown that the addition of yeast to insecticide can lead to increased *D. suzukii* adult and larval mortality, and reduce egg-laying.[31,33,35,43] However, studies on yeast-based attract-and-kill for control of *D. suzukii* need to be compared with caution as test protocols differ substantially, for example with respect to yeast species and strains (*e.g.*: *Saccharomyces cerevisiae*, *Metschnikowia pulcherrima*, *H. uvarum*), different types and concentrations of insecticides, and differing substrates (*e.g.*: acrylic, glass, leaves, fruits) across diverse crops, in both laboratory and field trials. Keeping this in mind, it is understandable that the efficiency of attract-and-kill on the *D. suzukii* target appears variable. For example, the addition of *S. cerevisiae* and sugar to spinosad significantly increased fly mortality and reduced larval density, but not the number of eggs laid in fruit compared with the addition of sugar only.[33] In contrast, Roubos *et al*. (2019)[59] found no improvement when adding *S. cerevisiae* to spinosad formulations for control of *D. suzukii* neither in laboratory nor field experiments. In the case of acetamiprid-formulations contrary to our results, Noble *et al*. (2019)[43] did not find improvement of efficacy. In our work, direct comparison of four insecticide-*H. uvarum* formulations showed that the treatment containing acetamiprid was the least effective (Table 1). With regards to *D. suzukii*, deltamethrin, to our knowledge, was previously only tested alone[17,60] or in combination with the insecticide imidacloprid[16] in laboratory studies on insecticide efficacy and resistance, and no studies were found addressing tau-Fluvalinate effects.

Spinosad is a commonly used and effective insecticide for management of *D. suzukii* also in organic fruit production[14,61] and formulations with *H. uvarum* and spinosad have previously been shown to increase *D. suzukii* mortality and negatively impact fruit infestation or egg-laying.[31,35,43] Spinosad is thus a strong candidate for the development of attract-and-kill strategies. Moreover, spinosad is especially active by ingestion which makes formulation with a phagostimulatory yeast even more compelling.[39] More efficient uptake of spinosad by the flies would furthermore counteract absorption of active compound by the plant material.[62]

Finally, using attractants to lure *D. suzukii* to insecticide reduces the need of comprehensive spray coverage and might allow reduction of spray volumes and drift. Optimized, a reduced spray coverage could result in excluding the fruit from insecticide application and thus minimize pesticide residues on the harvested crop. Developing strategies of targeted insecticide application is timely with respect to current advance of precision technology in viticulture.[63–65]

Overall, our data suggest high efficacy of our novel yeast-insecticide formulations and application methods targeting specifically green-plant leaves rather than the whole plant with consumable fruit. Our data show that *H. uvarum*-based attract-and-kill is compatible with different kinds of insecticides, facilitating resistance management by rotating products with different modes of action.[61] The corroborated concept of yeast-based behavioral manipulation could be applied even in strategies different to attract-and-kill. For example, *H. uvarum* volatiles might be used to attract *D. suzukii* to baits for monitoring or mass trapping.

In summary, the collective findings provide a foundation for the design of a behavioral management strategy targeting *D. suzukii*.

Formulations with the highly attractive *H. uvarum* enhance insecticide efficacy even when applied on leaves, not fruit, and might allow to decrease spray volumes, coverage of the plant and chemical residues on the crop. All-importantly, we think that the suggested approach is complementary to existing management practices and should therefore be evaluated for implementation within IPM programs. Thus, field experiments assessing the efficacy of *H. uvarum*-insecticide formulations as well as insecticide residues on the crop after spray application on canopy exclusively, is the logical next step in the development of the suggested *D. suzukii* management method.

ACKNOWLEDGEMENTS

The authors wish to thank the European Regional Development Fund (ERDF) 2014-2020 for funding DROMYTAL project (FESR1021, CUP H32F16000420009) to conduct the research. GR was in addition funded by Uruguayan National Agency for Research and Innovation (ANII Fellowship POS_EXT_2016_1_134106). PGB was in addition supported by the Swedish Research Council Formas (Grant 2015-1221) and the SLU Centre for Biological Control (CBC). Advice in data analysis by Franca Stábile is acknowledged.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad DigitalRepository at https://datadryad.org/stash.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Ehler LE, Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. *Pest Manag Sci* **62**:787–789 (2006).
2 Foster SP and Harris MO, Behavioral manipulation methods for insect pest-management. *Annu Rev Entomol* **42**:123–146 (1997).
3 Allan SA, Behavior-based control of insect crop pests, in *Insect Behavior: From Mechanisms to Ecological and Evolutionary Consequences*, ed. by Cordoba-Aguilar A, Gonzales-Tokman D and Gonzalez-Santoyo I. Oxford University Press Mexico, New York, pp. 309–331 (2018).
4 Rodriguez-Saona CR and Stelinski LL, in *Behavior-Modifying Strategies in IPM: Theory and Practice. In: Integrated Pest Management: Innovation-Development Process*, ed. by Peshin R and Dhawan AK. Springer, Dordrecht, pp. 263–315 (2009).
5 Witzgall P, Kirsch P and Cork A, Sex pheromones and their impact on pest management. *J Chem Ecol* **36**:80–100 (2010).
6 Davis TS, Crippen TL, Hofstetter RW and Tomberlin JK, Microbial volatile emissions as insect Semiochemicals. *J Chem Ecol* **39**:840–859 (2013).
7 Wong AC-N, Wang Q-P, Morimoto J, Senior AM, Lihoreau M, Neely GG *et al*., Gut microbiota modifies olfactory-guided microbial preferences and foraging decisions in drosophila. *Curr Biol* **27**:2397–2404 (2017).
8 Knight AL and Witzgall P, Combining mutualistic yeast and pathogenic virus — a novel method for codling moth control. *J Chem Ecol* **39**: 1019–1026 (2013).
9 Becher PG, Hagman A, Verschut V, Chakraborty A, Rozpędowska E, Lebreton S *et al*., Chemical signaling and insect attraction is a conserved trait in yeasts. *Ecol Evol* **8**:2962–2974 (2018).
10 Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ *et al*., *Drosophila suzukii* (Diptera: Drosophilidae): invasive Pest of ripening soft fruit expanding its geographic range and damage potential. *J Integr Pest Manag* **2**:1–7 (2011).
11 Cha DH, Adams T, Werle CT, Sampson BJ, Adamczyk JJ, Rogg H *et al*., A four-component synthetic attractant for *Drosophila suzukii* (Diptera: Drosophilidae) isolated from fermented bait headspace. *Pest Manag Sci* **70**:324–331 (2014).
12 Ibouh K, Oreste M, Bubici G, Tarasco E, Rossi Stacconi MV, Ioriatti C *et al*., Biological control of *Drosophila suzukii*: efficacy of parasitoids, entomopathogenic fungi, nematodes and deterrents of oviposition in laboratory assays. *Crop Prot* **125**:104897 (2019). https://doi.org/10.1016/j.cropro.2019.104897.
13 Lee JC, Wang X, Daane KM, Hoelmer KA, Isaacs R, Sial AA *et al*., Biological control of spotted-wing drosophila (Diptera: Drosophilidae)—current and pending tactics. *J Integr Pest Manag* **10**:13 (2019). https://doi.org/10.1093/jipm/pmz012.
14 Bruck DJ, Bolda M, Tanigoshi L, Klick J, Kleiber J, DeFrancesco J *et al*., Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops: Controlling *D. suzukii* in berries. *Pest Manag Sci* **67**:1375–1385 (2011).
15 Beers EH, Van Steenwyk RA, Shearer PW, Coates WW and Grant JA, Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. *Pest Manag Sci* **67**:1386–1395 (2011).
16 Smirle MJ, Zurowski CL, Ayyanath M-M, Scott IM and MacKenzie KE, Laboratory studies of insecticide efficacy and resistance in *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) populations from British Columbia, Canada. *Pest Manag Sci* **73**:130–137 (2017).
17 Shaw B, Hemer S, Cannon MFL, Rogai F and Fountain MT, Insecticide control of *Drosophila suzukii* in commercial sweet cherry crops under cladding. *Insects* **10**:196 (2019). https://doi.org/10.3390/insects10070196.
18 Haviland DR and Beers EH, Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries. *J Integr Pest Manag* **3**:1–6 (2012).
19 Leach H, Moses J, Hanson E, Fanning P and Isaacs R, Rapid harvest schedules and fruit removal as non-chemical approaches for managing spotted wing drosophila. *J Pest Sci* **91**:219–226 (2018).
20 DiGiacomo G, Hadrich J, Hutchison WD, Peterson H and Rogers M, Economic impact of spotted wing drosophila (Diptera: Drosophilidae) yield loss on Minnesota raspberry farms: a grower survey. *J Integr Pest Manag* **10**:1–6 (2019).
21 Knapp L, Mazzi D and Finger R, Management strategies against *Drosophila suzukii*: insights into Swiss grape growers choices. *Pest Manag Sci* **75**:2820–2029 (2019).
22 Van Timmeren S and Isaacs R, Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. *Crop Prot* **54**:126–133 (2013).
23 Van Timmeren S, Mota-Sanchez D, Wise JC and Isaacs R, Baseline susceptibility of spotted wing drosophila (*Drosophila suzukii*) to four key insecticide classes: spotted wing drosophila baseline susceptibility. *Pest Manag Sci* **74**:78–87 (2018).
24 Gress BE and Zalom FG, Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. *Pest Manag Sci* **75**:1270–1276 (2019).
25 Disi JO, Van Timmeren S, Gress B, Zalom F, Isaacs R and Sial A, Insecticide residue longevity for on-site screening of *Drosophila suzukii* (Matsumura) resistance. *Pest Manag Sci* **76**:2918–2924 (2020).
26 Choi M-Y, Tang SB, Ahn S-J, Amarasekare KG, Shearer P and Lee JC, Effect of non-nutritive sugars to decrease the survivorship of spotted wing drosophila, Drosophila suzukii. *J Insect Physiol* **99**:86–94 (2017).
27 Rice KB, Short BD and Leskey TC, Development of an attract-and-kill strategy for *Drosophila suzukii* (Diptera: Drosophilidae): evaluation of Attracticidal spheres under laboratory and field conditions. *J Econ Entomol* **110**:535–542 (2017).
28 Tait G, Kaiser C, Rossi Stacconi MV, Dalton D, Anfora G and Walton VM, A food-grade gum as a management tool for *Drosophila suzukii*. *Bull Insectol* **71**:295–307 (2018).
29 Gullickson MG, Rogers MA, Burkness EC and Hutchison WD, Efficacy of organic and conventional insecticides for *Drosophila suzukii* when combined with erythritol, a non-nutritive feeding stimulant. *Crop Prot* **125**:104878 (2019).
30 Rossi Stacconi MV, Tait G, Rendon D, Grassi A, Boyer G, Nieri R *et al*., Gumming up the works: field tests of a new food-grade gum as

behavioral disruptor for *Drosophila suzukii* (Diptera: Drosophilidae). *J Econ Entomol* **113**:1872–1880 (2020).
31 Bianchi F, Spitaler U, Castellan I, Cossu CS, Brigadoi T, Duménil C *et al.*, Persistence of a yeast-based (*Hanseniaspora uvarum*) attract-and-kill formulation against *Drosophila suzukii* on grape leaves. *Insects* **11**: 810 (2020). https://doi.org/10.3390/insects11110810.
32 Klick J, Rodriguez-Saona CR, Cumplido JH, Holdcraft RJ, Urrutia WH, da Silva RO *et al.*, Testing a novel attract-and-kill strategy for *Drosophila suzukii* (Diptera: Drosophilidae) management. *J Insect Sci* **19**:1–6 (2019).
33 Knight AL, Basoalto E, Yee W, Hilton R and Kurtzman CP, Adding yeasts with sugar to increase the number of effective insecticide classes to manage *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in cherry. *Pest Manag Sci* **72**:1482–1490 (2016).
34 Hamby KA and Becher PG, Current knowledge of interactions between *Drosophila suzukii* and microbes, and their potential utility for pest management. *J Pest Sci* **89**:621–630 (2016).
35 Mori BA, Whitener AB, Leinweber Y, Revadi S, Beers EH, Witzgall P *et al.*, Enhanced yeast feeding following mating facilitates control of the invasive fruit pest *Drosophila suzukii*. *J Appl Ecol* **54**:170–177 (2017).
36 Hamby KA, Hernández A, Boundy-Mills K and Zalom FG, Associations of yeasts with spotted-wing drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in cherries and raspberries. *Appl Environ Microbiol* **78**:4869–4873 (2012).
37 Bellutti N, Gallmetzer A, Innerebner G, Schmidt S, Zelger R and Koschier EH, Dietary yeast affects preference and performance in *Drosophila suzukii*. *J Pest Sci* **91**:651–660 (2018).
38 Scheidler NH, Liu C, Hamby KA, Zalom FG and Syed Z, Volatile codes: correlation of olfactory signals and reception in drosophila-yeast chemical communication. *Sci Rep* **5**:14059 (2015). https://doi.org/10.1038/srep14059.
39 Spitaler U, Bianchi F, Eisenstecken D, Castellan I, Angeli S, Dordevic N *et al.*, Yeast species affects feeding and fitness of *Drosophila suzukii* adults. *J Pest Sci* **93**:1295–1309 (2020).
40 Lasa R, Tadeo E, Toledo-Hérnandez RA, Carmona L, Lima I and Williams T, Improved capture of *Drosophila suzukii* by a trap baited with two attractants in the same device. *PLoS ONE* **12**:e0188350 (2017). https://doi.org/10.1371/journal.pone.0188350.
41 Bueno E, Martin KR, Raguso RA, Mcmullen JG, Hesler SP, Loeb GM *et al.*, Response of wild spotted wing drosophila (*Drosophila suzukii*) to microbial volatiles. *J Chem Ecol* **46**:688–698 (2020).
42 Jones R, Fountain MT, Günther CS, Eady PE and Goddard MR, Separate and combined *Hanseniaspora uvarum* and *Metschnikowia pulcherrima* metabolic volatiles are attractive to *Drosophila suzukii* in the laboratory and field. *Sci Rep* **11**:1201 (2021). https://doi.org/10.1038/s41598-020-79691-3.
43 Noble R, Dobrovin-Pennington A, Phillips A, Cannon MFL, Shaw B and Fountain MT, Improved insecticidal control of spotted wing drosophila (*Drosophila suzukii*) using yeast and fermented strawberry juice baits. *Crop Prot* **125**:104902 (2019). https://doi.org/10.1016/j.cropro.2019.104902.
44 Liu HM, Guo JH, Cheng YJ, Luo L, Liu P, Wang BQ *et al.*, Control of gray mold of grape by *Hanseniaspora uvarum* and its effects on postharvest quality parameters. *Ann Microbiol* **60**:31–35 (2010).
45 Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M & EFSA Panel on Biological Hazards *et al.*, Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 10: Suitability of taxonomic units notified to EFSA until March 2019. Available: https://data.europa.eu/doi/10.2903/j.efsa.2019.5753 (2019). Accessed November 2021.
46 Baser N, Broutou O, Verrastro V, Porcelli F, Ioriatti C, Anforа G *et al.*, Susceptibility of table grape varieties grown in South-Eastern Italy to *Drosophila suzukii*. *J Appl Entomol* **142**:465–472 (2018).
47 Entling W, Anslinger S, Jarausch B, Michl G and Hoffmann C, Berry skin resistance explains oviposition preferences of *Drosophila suzukii* at the level of grape cultivars and single berries. *J Pest Sci* **92**:477–484 (2019). https://doi.org/10.1007/s10340-018-1040-7
48 Shrader ME, Burrack HJ and Pfeiffer DG, *Drosophila suzukii* (Diptera: Drosophilidae) Oviposition and adult emergence in six wine grape varieties grown in Virginia. *J Econ Entomol* **112**:139–148 (2019).
49 Ioriatti C, Walton V, Dalton D, Anfora G, Grassi A, Maistri S *et al.*, *Drosophila suzukii* (Diptera: Drosophilidae) and its potential impact to wine grapes during harvest in two cool climate wine grape production regions. *J Econ Entomol* **108**:1148–1155 (2015).
50 Fleet GH, Lafon-Lafourcade S and Ribéreau-Gayon P, Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. *Appl Environ Microbiol* **48**:1034–1038 (1984).
51 Barata A, Malfeito-Ferreira M and Loureiro V, The microbial ecology of wine grape berries. *Int J Food Microbiol* **153**:243–259 (2012).
52 Bokulich NA, Thorngate JH, Richardson PM and Mills DA, Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *PNAS* **111**:139–148 (2014).
53 Pertot I, Caffi T, Rossi V, Mugnai L, Hoffmann C, Grando MS *et al.*, A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Prot* **97**:70–84 (2017).
54 Aka J, Ugaglia AA and Lescot J-M, Pesticide use and risk aversion in the French wine sector. *J Wine Econ* **13**:451–460 (2018).
55 Revadi S, Lebreton S, Witzgall P, Anfora G, Dekker T and Becher P, Sexual behavior of Drosophila suzukii. *Insects* **6**:183–196 (2015).
56 Becher PG, Bengtsson M, Hansson BS and Witzgall P, Flying the Fly: Long-range flight behavior of *Drosophila melanogaster* to attractive odors. *J Chem Ecol* **36**:599–607 (2010).
57 R Core Team, *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing*. Vienna, Austria, (2020). https://intro2r.com/citing-r.html
58 Becher PG, Flick G, Rozpędowska E, Schmidt A, Hagman A, Lebreton S *et al.*, Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Funct Ecol* **26**:822–828 (2012).
59 Roubos CR, Gautam BK, Fanning PD, Van Timmeren S, Spies J, Liburd OE *et al.*, Impact of phagostimulants on effectiveness of OMRI-listed insecticides used for control of spotted-wing drosophila (*Drosophila suzukii* Matsumura). *J Appl Entomol* **143**: 609–625 (2019).
60 Hoffmann Schlesener DC, Wollmann J, De Bastos PJ, Grützmacher AD and Mello Garcia FR, Effects of insecticides on adults and eggs of *Drosophila suzukii* (Diptera, Drosophilidae). *Rev Colomb Entomol* **43**: 208 (2017). https://doi.org/10.25100/socolen.v43i2.5945.
61 Sial AA, Roubos CR, Gautam BK, Fanning PD, Van Timmeren S, Spies J *et al.*, Evaluation of organic insecticides for management of spotted-wing drosophila (*Drosophila suzukii*) in berry crops. *J Appl Entomol* **143**:593–608 (2019).
62 Thompson GD, Dutton R and Sparks TC, Spinosad – a case study: an example from a natural products discovery programme. *Pest Manag Sci* **56**:696–702 (2000).
63 Campos J, Llop J, Gallart M, García-Ruiz F, Gras A, Salcedo R *et al.*, Development of canopy vigour maps using UAV for site-specific management during vineyard spraying process. *Precis Agric* **20**:1136–1156 (2019).
64 Di Gennaro SF and Matese A, Evaluation of novel precision viticulture tool for canopy biomass estimation and missing plant detection based on 2.5D and 3D approaches using RGB images acquired by UAV platform. *Plant Methods* **16**:91 (2020). https://doi.org/10.1186/s13007-020-00632-2.
65 Mermer S, Pfab F, Hoheisel GA, Bahlol HY, Khot L, Dalton DT *et al.*, Canopy spray deposition and related mortality impacts of commonly used insecticides on *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) populations in blueberry. *Pest Manag Sci* **76**:1531–1540 (2020).

