Improved insecticidal control of spotted wing drosophila (*Drosophila suzukii*) using yeast and fermented strawberry juice baits

Ralph Noble [a],[*], Andreja Dobrovin-Pennington [a], Amy Phillips [b], Madeleine F.L. Cannon [c], Bethan Shaw [c], Michelle T. Fountain [c]

[a] Microbiotech Ltd, Pershore Centre, Warwickshire College, Pershore, Worcestershire, UK
[b] Warwickshire College Group, Pershore College, Pershore, Worcestershire, UK
[c] NIAB EMR, East Malling, Kent, UK

ARTICLE INFO

ABSTRACT

The phagostimulant effect of baits on the *Drosophila suzukii* control efficacy of different insecticides applied at sub-lethal concentrations was tested in bioassays. The baits were fermented strawberry juice (FSJ), a suspension of the yeast *Hanseniaspora uvarum*, a combination of the two and Combi-protec, a proprietary mixture of plant extract, proteins and sugars. Without insecticides, the baits did not affect *D. suzukii* mortality. With spinosad, cyantraniliprole or lambda-cyhalothrin, the baits caused higher mortality of *D. suzukii* summer morphs, under summer conditions, compared with using the insecticides in water. The mortality and reduced reproduction efficacy of insecticides was greater with *H. uvarum*, FSJ + *H. uvarum* or Combi-protec treatments compared with FSJ only bait. *H. uvarum* and FSJ baits also increased the mortality of *D. suzukii* winter morphs, held under winter conditions, when used with spinosad or cyantraniliprole but not with lambda-cyhalothrin. Cyantraniliprole + *H. uvarum* also reduced oviposition in winter morphs that were transferred to summer conditions after three days of exposure to treatments under winter conditions. Leaf type did not have an impact on combined bait and insecticide treatments; all were effective when applied to blackberry, blueberry, cherry, raspberry or strawberry leaves. Phagostimulant baits improved the insecticidal control of *D. suzukii* summer and winter morphs by increasing mortality and reducing oviposition and the lethal dose of insecticides. The relative phagostimulant effect of the baits did not fully correspond with their olfactory attraction to *D. suzukii*.

1. Introduction

Spotted wing drosophila (*Drosophila suzukii* Matsumura) is a major invasive pest of soft fruit in North and South America and Europe (Asplen et al., 2015; Schlesener et al., 2017; Cai et al., 2018). Although repeated sprays with insecticides have provided control of *D. suzukii*, some effective active ingredients such as malathion have been or are being withdrawn from use in EU countries (Smirle et al., 2017; Schlesener et al., 2017; Lainsbury, 2018). Spinosad has also provided effective control (Bruck et al., 2011; Rosensteel and Sial, 2017) but recently reported resistance of *D. suzukii* to spinosad in North America (Gress and Zalom, 2018) has increased the urgency for improving the control efficacy of alternative insecticides.

Baits such as sucrose have been used to improve efficacy of insecticides such as spinosad and acetamiprid in the field (Cowles et al., 2015). Corn steep liquor or corn syrup did not improve the *D. suzukii* control efficacy of spinosad or cyantraniliprole in field cherries (*Prunus avium* L.) or raspberries (*Rubus idaeus* L.) (Knight et al., 2016; Fanning et al., 2018). Certain fermentation and refining products, such as vinegar, wine, molasses, yeasts and proteins are known to be attractive to *D. suzukii* (Rouzes et al., 2011; Landolt et al., 2012; Cai et al., 2018) and have therefore been used as baits and/or in monitoring traps for this pest. A mixture of sugar and bakers' or brewers' yeast (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen) improved the control of *D. suzukii* with spinosad in the field, although in laboratory tests, the addition of *S. cerevisiae* to sugar did not significantly reduce egg densities in fruit

compared with sugar alone (Knight et al., 2016). Roubos et al. (2019) did not find an improvement in *D. suzukii* control using spinosad in blueberries (*Vaccinium corymbosum* F.) from using sugar and *S. cerevisiae* as phagostimulant baits. *D. suzukii* is more attracted to the yeast *Hanseniaspora uvarum* (Niehaus), commonly found in its digestive tract and on infested fruit, than to *S. cerevisiae* (Hamby et al., 2012; Scheidler et al., 2015). In laboratory tests, application of both *H. uvarum* and spinosad to leaves increased mortality and reduced oviposition of *D. suzukii* compared with using only spinosad (Mori et al., 2017). A proprietary feeding attractant composed of organic acids and peptides (Suzukii Trap, Bioiberica, Barcelona, Spain), improved the insecticidal activity of dimethoate, lambda-cyhalothrin and spinosad applied to fruits in laboratory assays (Andreazza et al., 2017) and 50% Suzukii Trap improved *D. suzukii* control with spinosad in the field (van Steenwyk et al., 2016). A mixture of 40% corn steep liquor, 30% apple cider vinegar and 30% wine also reduced *D. suzukii* infestation but the acidic vinegar caused foliage damage (van Steenwyk et al., 2016). Combi-protec (Dedetec, Freiburg, Germany), a proprietary mixture of plant extract, proteins and sugars, improved *D. suzukii* control with spinosad and acetamiprid (Dederichs, 2015). The attractiveness of a protein bait made from brewery yeast waste to *D. suzukii* declined when the pyrethroids cypermethrin and cyhalothrin, but not spinosad, were incorporated at 0.05 %v/v (Cai et al., 2018). A proprietary attractant bait combined with spinosad (HOOK SWD, ISCA Technologies Inc., Tiverside, CA) reduced raspberry and blueberry crop fruit infestations with *D. suzukii* by 2–8 times fewer compared with untreated or conventionally insecticide sprayed plots (Klick et al., 2019).

D. suzukii undergoes reproductive diapause in response to moderately cool temperatures of 10–15 °C during larval development. The resulting winter forms of the adults or 'morphs' are larger and darker, and more resistant to cold and starvation than summer morphs (Wallingford and Loeb, 2016). The efficacy of insecticides on *D. suzukii* mortality, such as that referred to above, has primarily been tested on summer morphs which are found throughout the cropping season in the warmer months. The efficacy of insecticides, and the influence of baits to control winter morphs has not been previously investigated although Wong et al. (2018) did not find a clear difference in attraction to fermentation odours between summer and winter morphs. While winter morph *D. suzukii* are not a direct threat to commercial fruit, the lack of feeding resource during the colder months provides an opportunity to reduce over-wintering populations by the use of phagostimulants with insecticides. The fewer flies that survive the winter, the fewer there are to invade commercial crops and result in higher populations building up during the cropping season (Wiman et al., 2014; Rossi-Stacconi et al., 2016).

The above work indicates that the most promising phagostimulant baits for insecticidal control of *D. suzukii* summer morphs are those based on yeasts (particularly *H. uvarum*), fermentation products, plant extracts and sugars. The aims of this work were to (1) compare the olfactory attractiveness of baits and their phagostimulant effect when combined with insecticides at sub-lethal concentrations in a laboratory bioassay on *D. suzukii* summer and winter morph adult flies and (2) to assess whether the baits and/or insecticides changed in efficacy when applied to different fruit plant leaves. Insecticides commonly used to target *D. suzukii* were used with and without Combi-protec and a suspension of *H. uvarum*. Fermented strawberry (*Fragaria vesca* L.) juice (FSJ) was also evaluated as a new bait. Strawberry production results in around five tonnes of waste fruit per hectare which is often disposed of in sealed bins to prevent spread of *D. suzukii* from infested fruit. The resulting fermented liquor contains natural yeasts and is attractive to *D. suzukii* (Noble et al., 2017), potentially providing a free phagostimulant bait for growers to use in conjunction with insecticides.

2. Materials and methods

2.1. Flies

Summer morph *D. suzukii* were reared in Drosophila Quick Mix Medium (Blades Biological Ltd, Edenbridge, Kent, UK) in mesh Bug-Dorm cages ($475 \times 475 \times 475$ mm, Watkins & Doncaster, Leominster, UK) in a room at 21 ± 1 °C with a photoperiod of 16:8 (L:D) h to simulate summer conditions; this was done for all experiments except where stated. Winter morph *D. suzukii* were produced by transferring 10-day old larvae reared under summer conditions, as above, to 13 °C with a photoperiod of 8:16 (L:D) h to simulate winter conditions (Wiman et al., 2014; Shearer et al., 2016). For both morphs, the dishes of food (Drosophila Quick Mix Medium) were removed from cages 12 h before the start of the tests which were conducted with 3–10 day-old *D. suzukii* adults.

2.2. Baits

Baits were prepared with tap water. Yeast cell populations in *S. cerevisiae, H. uvarum* and/or FSJ treatments were determined by counting cell numbers in serial dilutions at $\times 40$ magnification with a haemocytometer (type MNK-400-H, Weber Scientific International Ltd, Teddington, Greater London). Active dry bakers' yeast (*S. cerevisiae*) (Easy Bake Yeast, Allinson Flour, Peterborough, UK) was added at 3.6 g l^{-1} to a solution of sucrose sugar (16 g l^{-1}) to produce a suspension containing 1.4 (SD $\pm$ 0.2) cells $\times 10^9$ ml^{-1}. A suspension of *H. uvarum* (strain 11-382 from the Phaff Yeast Culture Collection, Food Science, UC Davis, CA) was prepared by washing each of ten 90 mm Petri dishes of *H. uvarum* cultures grown on potato dextrose agar (Oxoid Ltd, Basingstoke, Hants., UK) with 7 ml of a sugar solution (16 g l^{-1}). The volume of the retrieved suspension was approximately 60 ml and it contained 1.2 (SD $\pm$ 0.5) cells $\times 10^9$ ml^{-1}. Strawberry juice (SJ) was prepared by crushing fresh, ripe, strawberries with a mortar and pestle followed by filtering through a 1 mm mesh screen. FSJ was prepared by placing crushed strawberries in sealed 2 L containers for 10 days at 20 °C (Noble et al., 2017). The resulting liquid fraction was then separated from the surface solid material and filtered with a 1 mm mesh screen. A combined *H. uvarum* suspension + FSJ bait was prepared by washing the above *H. uvarum* culture plates with FSJ instead of water. Sugar (16 g l^{-1}) was added to the resulting FSJ and FSJ + *H. uvarum* suspensions, which contained 1.0 (SD $\pm$ 0.8) $\times 10^9$ and 1.1 (SD $\pm$ 0.9) $\times 10^9$ yeast cells ml^{-1}. Gasser liquid attractant (Riga AG, Ellikon a.d. Thur, Switzerland), a proprietary vinegar-wine-based attractant, was used at 100%. Combi-protec bait was prepared as a 5% v/v solution (Dederichs, 2015). Molasses and sucrose sugar were prepared as 50 and 160 g l^{-1} solutions respectively. Baits were prepared on the day of setting up experiments. To test for *D. suzukii* phagostimulant effect and lack of toxicity of the above baits, red food colouring containing anthocyanins (Sainsbury's Supermarkets Ltd, London) was added at 5.5 µl ml^{-1} to samples of the prepared *H. uvarum*, Combi-protec and FSJ baits and pipetted as two 10 µl droplets on two blackberry leaves. The leaves were placed in clear plastic containers ($n = 4$) with 10 male and 10 female *D. suzukii*, and maintained in summer conditions. After 24 h, $\geq$90% of males and females in all containers were alive and with red colouring in the digestive tract.

2.3. Olfactory attraction

The relative olfactory attraction of baits to *D. suzukii* was monitored with a 32 channel Locomotor Activity Monitoring rig (type LAM25H, TriKinetics Inc., Waltham, MA), the same as in the photograph in Anon. (2018). The rig consisted of an 8×4 matrix of horizontal plastic tubes (65 mm length $\times$ 25 mm internal diameter), centres spaced 38 mm apart. The tubes were open at one end so that *D. suzukii* could fly in and out. Each tube had a planar array of nine infra-red beams at its

mid-point; when a fly crossed and interrupted the beams, by entering or re-entering a tube, this was registered as a count. The counts were uploaded to a host computer to determine the activity in each tube over a three-day period. A 0.2 ml droplet of the following baits was placed in the closed ends of the tubes: (a) *S. cerevisiae* suspension (b) *H. uvarum* suspension (c) SJ (d) FSJ (e) Gasser (f) Combi-protec (g) molasses (h) sugar. The rig was placed inside a BugDorm cage (475 × 475 × 475 mm) into which 160 (±12) adult summer morph *D. suzukii,* with males and females in approximately equal proportions, were released. The base of the cage was lined with moist tissue paper that was periodically rewetted to maintain a humid environment. The tests were conducted in a laboratory between 15 March and 7 July 2017 in natural, shaded daylight at 20 °C.

2.4. Insecticide bioassays set up

A jar bioassay similar to that described in Mori et al. (2017) was modified to test the effect of baits and different insecticides at sub-lethal concentrations on *D. suzukii* mortality and oviposition. Concentrations within this assay (Table 1) were determined from preliminary tests in which range finding assays were performed, similar to those described below (results not shown). The 750 ml clear plastic jars (103 mm diameter, 95 mm height) (Involvement Packaging Ltd, Banbury, Oxon., UK) had a 10 mm diameter ventilation hole covered with fine mesh in the opaque screw-on lid and were lined with moist filter paper in the base. The jars contained three unsprayed wild blackberry (*Rubus fruticosus* L. agg.) leaves (about 25 × 20 mm), or other leaf types of similar size where stated. Insecticide and/or bait were pipetted as six 10 μl droplets on two of the leaves and six 10 μl droplets of sugar solution (160 g l^{-1}) on the third leaf. Insecticides and bait combinations varied between experiments (see 2.4.1 to 2.4.3 for combinations). The droplets were allowed to dry for 1 h.

An open 30 mm Petri dish with grape juice agar was placed as an oviposition medium in the base of the jars. Grape juice agar was prepared from agar (34.7 g, Agar No. 3, Oxoid Ltd), red grape juice (333 ml, Sainsbury's Supermarkets Ltd), dextrose (33.3 g, Oxoid Ltd) and Nipagin (2.0 g, Sigma-Aldrich, Gillingham, Dorset, UK) per litre distilled water. Preliminary tests with seven mated females and five males of 3–10 day old *D. suzukii* adults and two fruits in the jars showed no significant difference in mean *D. suzukii* oviposition after three days compared with using the grape juice agar dishes (cherries 19 (SD ± 7) eggs; loganberries 18 (SD ± 5) eggs; grape juice agar 15 (SD ± 6) eggs; $n = 4$). However, the

latter medium allowed for easier counting of eggs, and was more uniform with less risk of pesticide residues. *D. suzukii* adults were cold sedated by transferring them into sealed plastic tubes (93 × 23 mm) which were placed in a freezer compartment at −17 °C for 45 s. Seven mated females and five males of cold sedated *D. suzukii* adults were placed in each jar with the filter paper rewetted with 1 ml water after one day, to maintain a relative humidity of 97.3 ± 1.7%. Jars were positioned in the summer or winter environmental conditions depending on the experiment. Mortality and the number of eggs in the grape juice agar were assessed after 3 days, and again after a further 11 days in jar bioassay Experiment 3. In all the experiments, treatments were replicated in time, with replicate sets of jars set up on separate days using different cohorts of flies. This set up was used in the following jar bioassay experiments.

2.4.1. Jar bioassay experiment 1: comparison of baits and insecticides

The bait treatments, applied as droplets to leaves, were: (i) *H. uvarum* suspension (ii) FSJ (iii) FSJ + *H. uvarum* suspension (iv) Combi-protec or (v) water control. These baits were applied with or without the following insecticides (concentrations in Table 1): (i) spinosad (ii) cyantraniliprole (iii) thiacloprid (iv) acetamiprid (v) lambda-cyhalothrin (vi) pyrethrum or (vii) water control. The experiment comprised of 5 baits × 7 insecticides × 4 replicates, hence 140 bioassay jars.

2.4.2. Jar bioassay experiment 2: use of different fruit plant leaves

Leaves were picked by the stalk, which was subsequently trimmed to 5 mm length, and used unwashed within 4 h. The unsprayed leaf types used were: (i) wild blackberry and cultivated (ii) blueberry (iii) cherry (iv) raspberry or (v) strawberry. The bait treatments, used with insecticides or water, were: (i) *H. uvarum* suspension (ii) FSJ or (iii) water control. The insecticides used were: (i) spinosad (ii) cyantraniliprole or (iii) water control (see Table 1 for concentrations). The experiment comprised of 5 leaf types × 3 baits × 3 insecticides × 4 replicates, hence 180 jars.

2.4.3. Jar bioassay experiment 3: comparison of summer and winter morph D. suzukii control

The bait treatments used with insecticides or water were: (i) *H. uvarum* suspension (ii) FSJ or (iii) water control. The insecticides and concentrations (Table 1) used were: (i) spinosad (ii) cyantraniliprole (iii) thiacloprid (iv) lambda-cyhalothrin or (v) water control.

Summer morph *D. suzukii* adults were prepared as previously

Table 1

Concentrations of active ingredients in the insecticide products, UK maximum permitted field doses for cherries and protected strawberries and six 10 μl droplets per leaf in jar bioassays.

Active ingredient	Insecticide product and manufacturer		Concentration of active ingredient in:		
		product (g l^{-1})	permitted field dose[b] (mg l^{-1})		jar bioassays (mg l^{-1})
			strawberries	cherries	
spinosad	Tracer, Dow AgroSciences, Zionsville, IA, USA	480	72	120	2.4[e] or 3.6[f]
cyantraniliprole	Exirel, DuPont, Wilmington, DE, USA	100	75[c]	90	18.9[f] or 37.5[e]
thiacloprid	Calypso, Bayer Crop Science, Monheim, Germany	480	120	150	60.0
acetamiprid	Gazelle, Certis, Cambridge, UK	200[a]	-[d]	75	37.5
lambda-cyhalothrin	Hallmark Zeon, Syngenta, Basel, Switzerland	100	7.5	9.0	3.8
pyrethrum	Pyrethrum 5 EC, Agropharm Ltd. High Wycombe, UK	50	120	120	60.0

[a] g kg^{-1}
[b] If applied in 1000 L water ha^{-1}
[c] Strawberries dose for cyantraniliprole is for the product Benevia, DuPont; in the UK.
[d] At the time of writing, acetamiprid is not permitted for use on strawberries.
[e] Experiment 1.
[f] Experiments 2 and 3.

described and exposed to the treatments for three days under summer conditions. Mortality and oviposition were then recorded. For winter morph flies, jars were placed in winter conditions for the three days of exposure to the bait and/or insecticide combinations, after which mortality was recorded. The treated leaves were then removed and substituted with two leaves with sugar solution droplets; a Petri dish with grape juice agar was also inserted in each jar. The jars were then transferred to summer conditions for a further 11 days. After this point, mortality and oviposition were recorded. The experiment comprised of 2 *D. suzukii* morphs × 3 baits × 5 insecticides × 4 replicates, hence 120 jars.

2.5. Statistical design and analysis

Olfactory attractiveness tests were conducted with a sample of each bait treatment randomly positioned in each of four horizontal rows of eight tubes. Four replicate runs of the experiment were conducted, with the positioning of the baits in the tubes re-randomised between replicate runs. Jar bioassay experiments were designed as randomised blocks with the four replicates of each factorial set of morph, bait, insecticide and/or leaf type treatments set up consecutively. Experiment 3 had a split-plot design, with four paired replicates, each of 15 jars of summer and winter morphs in main plots. The orders in which paired summer or winter morphs were used, treatments applied to leaves and flies introduced in jars, and the positioning of jars, were randomised between each replicate run of the experiments. A square root transformation was used to homogenise the variances in the treatment means in the oviposition data. In each experiment, an ANOVA was conducted to determine if there were significant main effects or interacting effects of the treatments (insecticides, baits, leaves and/or morphs) on mortality or oviposition. The statistical significance of the difference between treatments was determined by conducting Tukey's HSD test on means obtained from the ANOVAs. Results were analysed by GenStat Version 13.1 Throughout, $p \leq 0.05$ was used to determine statistical significance.

3. Results

3.1. Olfactory attractiveness

More *D. suzukii* counts in tubes containing SJ or *H. uvarum* were recorded than in tubes containing *S. cerevisiae*, FSJ, Gasser, Combi-protec, molasses or sugar ($t_{21} > 2.22$; $p < 0.019$) (Fig. 1). There were no significant differences in the numbers of *D. suzukii* counts between tubes containing SJ or *H. uvarum*, nor between tubes containing any of the latter baits.
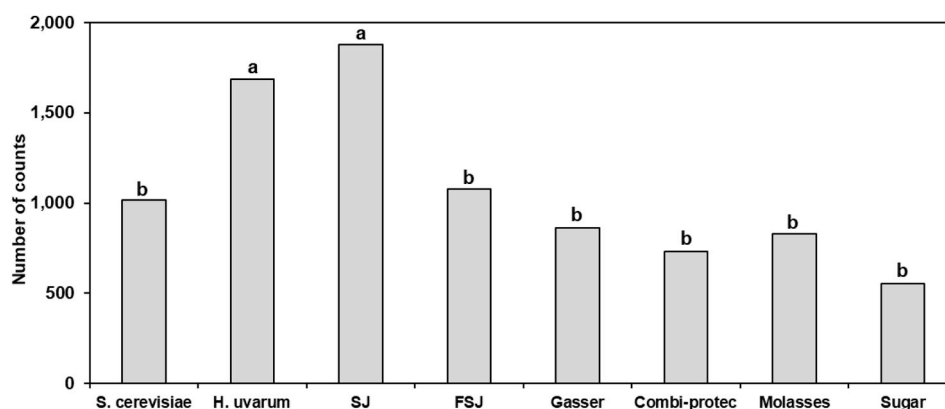
3.2. Jar bioassay experiment 1: comparison of baits and insecticides

There were significant effects of bait ($F_{4,102} = 45.36$; $p < 0.001$) and insecticide ($F_{6,102} = 19.28$; $p < 0.001$) treatments (including water controls), and interacting effects of bait × insecticide ($F_{24,102} = 4.11$; $p < 0.001$) treatments on *D. suzukii* mortality. There were significant effects of bait ($F_{4,102} = 4.43$; $p = 0.002$) and insecticide ($F_{6,102} = 5.99$; $p < 0.001$) treatments, and interacting effects of bait × insecticide treatments ($F_{24,102} = 1.76$; $p = 0.027$) on oviposition.

Without insecticides, none of the baits had a significant effect on *D. suzukii* mortality compared with the water control (Fig. 2a). Without insecticide, FSJ + *H. uvarum* increased oviposition compared with the water control ($t_{102} = 5.38$; $p < 0.001$) (Fig. 2b). Without baits, none of the diluted doses of insecticides affected oviposition compared with the water control (Table 1, Fig. 2b) although mortality was increased by the diluted doses of cyantraniliprole, acetamiprid, pyrethrum ($t_{102} \geq 3.37$; $p \leq 0.001$) and thiacloprid ($t_{102} = 2.12$; $p = 0.036$) but not significantly by spinosad or lambda-cyhalothrin (Fig. 2a). For spinosad, cyantraniliprole and lambda-cyhalothrin, the baits resulted in higher mortality compared with the insecticides in water alone ($t_{102} > 2.91$; $p < 0.005$). However, the baits did not improve the efficacy of acetamiprid, thiacloprid or pyrethrum. Averaged across the six insecticides, the *H. uvarum*, FSJ + *H. uvarum* and Combi-protec treatments resulted in significantly higher mortality than FSJ (average results not shown; $t_{102} = 4.25$; $p < 0.001$). However, mortality was also higher when insecticides were combined with FSJ than using insecticides in water ($t_{102} = 4.92$; $p < 0.001$). Averaged across the six insecticides, oviposition was lower with the *H. uvarum* and Combi-protec baits than with FSJ and FSJ + *H. uvarum* baits (average results not shown; $t_{102} = 2.39$; $p = 0.019$). However, the difference in oviposition between bait and water treatments within individual insecticides was only significantly lower for *H. uvarum* and lambda-cyhalothrin ($t_{102} = 2.53$; $p = 0.013$) (Fig. 2b).

3.3. Jar bioassay experiment 2: use of different fruit plant leaves

There were significant effects of bait ($F_{2,135} = 19.20$; $p < 0.001$), insecticide ($F_{2,135} = 87.44$; $p < 0.001$) and leaf type ($F_{4,135} = 3.23$; $p = 0.014$) treatments, and interacting effects of bait × insecticide ($F_{4,135} = 2.76$; $p = 0.008$) treatments on *D. suzukii* mortality. There were no significant interacting effects of leaf type with bait or insecticide treatments on mortality. There were significant effects of bait ($F_{2,135} = 3.22$; $p = 0.043$) and insecticide ($F_{4,135} = 10.96$; $p < 0.001$) treatments, and interacting effects of bait × insecticide treatments ($F_{4,135} = 2.55$; $p = 0.042$) but no significant effects of leaf type on oviposition.

Fig. 1. Numbers of *D. suzukii* counts over three days in tubes with different baits (undiluted Gasser liquid, SJ strawberry juice, FSJ fermented strawberry juice, aqueous solutions and suspensions of *Saccharomyces cerevisiae* and *Hanseniaspora uvarum*). Values are means of four replicate tests and eight tubes per test. Bars with the same letter are not significantly different ($p = 0.05$).
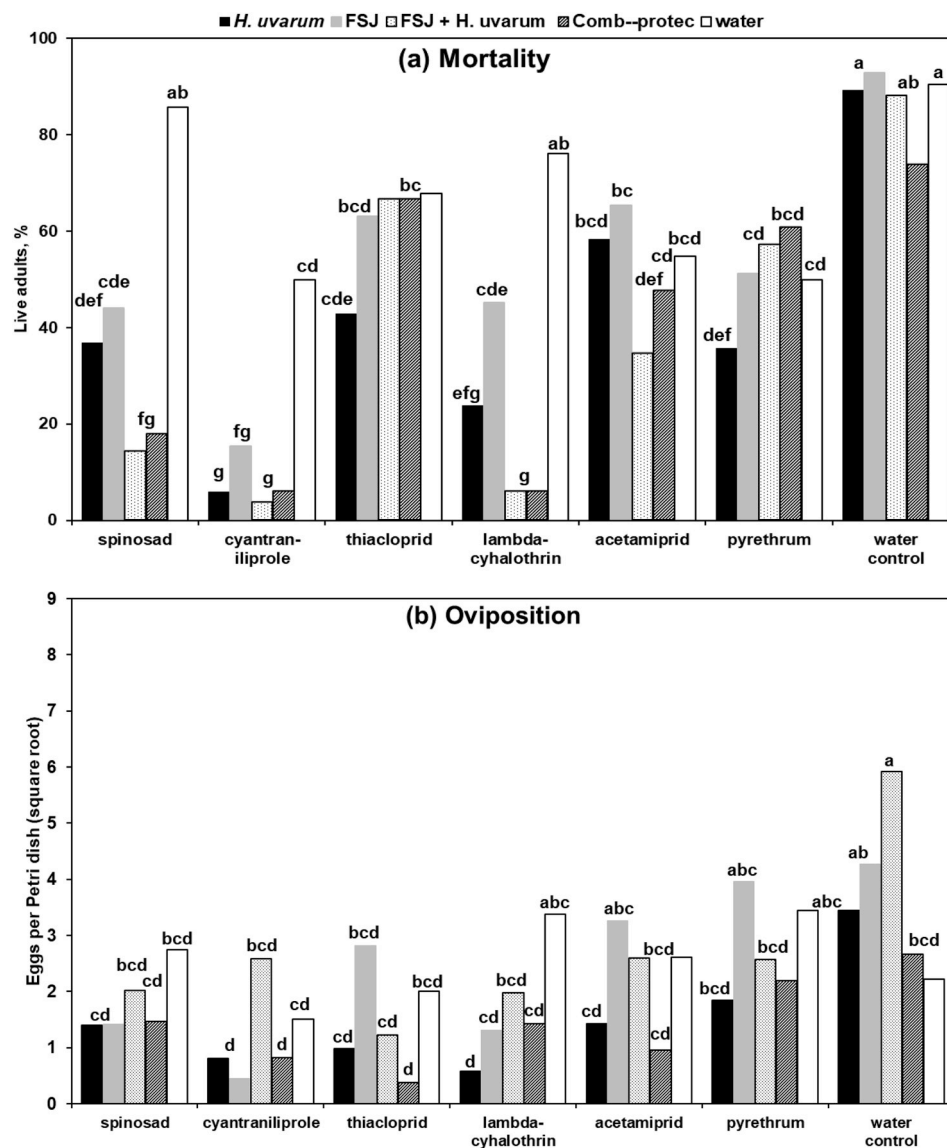
Fig. 2. Effect of bait treatments (*Hanseniaspora uvarum* and/or FSJ fermented strawberry juice, Combi-protec) and insecticides on (a) mortality and (b) oviposition of *Drosophila suzukii*; mean values, $n = 4$. Bars with the same letter are not significantly different ($p = 0.05$).

Irrespective of the presence of insecticide or bait treatments, leaf type significantly affected *D. suzukii* mortality ($F_{4,132} = 3.23$; $p = 0.014$) but not oviposition. Averaged across all insecticide and bait treatments, including water controls, *D. suzukii* mortality was lower using raspberry leaves than using blueberry or strawberry leaves (average results not shown; $t_{132} > 2.49$; $p < 0.014$). However, the effect of leaf type on mortality was small (means for different leaves 46.8–60.2%) when compared with the effect of bait × insecticide treatments (7.1–96.7%). Results averaged across all five leaf types are therefore presented in Fig. 3. When used in water, without baits, cyantraniliprole at 18.9 mg l^{-1} resulted in greater *D. suzukii* mortality than spinosad at 3.6 mg l^{-1} ($t_{132} = 4.63$; $p < 0.001$); both insecticides applied at these diluted doses resulted in greater mortality than the water control ($t_{132} = 7.98$ or 3.37; $p < 0.001$) but did not significantly affect oviposition. Baits in water, without insecticide, did not significantly affect mortality or oviposition compared with the water control. Averaged across both insecticides and all leaf types, *H. uvarum* increased mortality (95.6%) compared with FSJ (86.7%) ($t_{132} = 2.43$; $p = 0.016$). This effect was significant for all five individual leaf types (data not shown; $t_{132} > 2.14$; $p < 0.034$). Mortality was higher following the use of insecticides with FSJ than in water (44.2%) ($t_{132} = 11.52$; $p < 0.001$)

(Fig. 3a). This effect was significant for all five individual leaf types (data not shown; $t_{132} > 3.75$; $p < 0.001$) except for cyantraniliprole with FSJ on blueberry or strawberry leaves. Averaged across all leaf types and both insecticides, both FSJ and *H. uvarum* resulted in similar reductions in oviposition compared with using insecticides in water ($t_{132} \geq 5.82$; $p < 0.001$) (Fig. 3b). However, this negative oviposition impact on *D. suzukii* was only significant for some combinations of leaf type, bait and insecticide treatments: FSJ with spinosad on blueberry, strawberry, cherry and raspberry leaves, and with cyantraniliprole on cherry leaves ($t_{132} > 2.29$; $p < 0.024$), *H. uvarum* with spinosad on raspberry leaves and with cyantraniliprole on blueberry and raspberry leaves ($t_{132} > 2.31$; $p < 0.023$)(data not shown).

3.4. Jar bioassay experiment 3: comparison of summer and winter morph D. suzukii control

There were significant effects of morph ($F_{1,3} = 81.93$; $p = 0.003$), bait ($F_{3,84} = 15.05$; $p < 0.001$) and insecticide ($F_{4,84} = 22.45$; $p < 0.001$) treatments, and interacting effects of bait × insecticide ($F_{8,84} = 2.76$; $p = 0.008$) and morph × insecticide treatments ($F_{4,84} = 3.89$; $p = 0.006$) on *D. suzukii* mortality. There were significant effects of morph
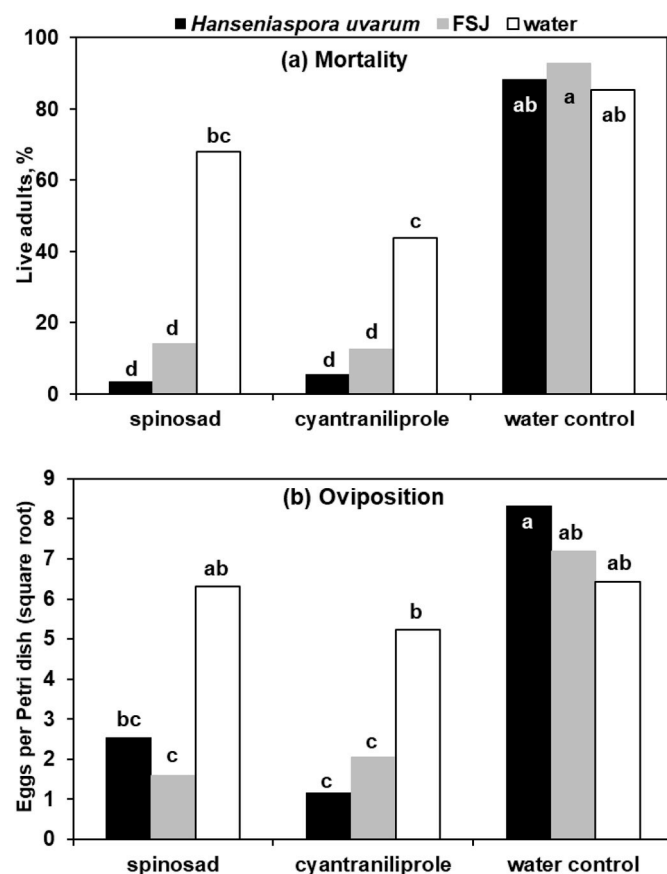
Fig. 3. Effect of bait treatments (*Hanseniaspora uvarum* or FSJ fermented strawberry juice) and insecticides on (a) mortality and (b) oviposition of *Drosophila suzukii*; mean values of five leaf types, $n = 4$. Bars with the same letter are not significantly different ($p = 0.05$).

$(F_{1,3} = 11.91; p = 0.041)$, bait $(F_{3,84} = 9.43; p < 0.001)$ and insecticide $(F_{4,84} = 9.05; p < 0.001)$ treatments, and interacting effects of bait × insecticide treatments $(F_{8,84} = 2.76; p = 0.009)$ on oviposition. After three days, average mortality across all insecticide and bait treatments, including water controls, for winter morph *D. suzukii* (35.1%) was lower than for summer morphs (68.1%) $(t_{84} = 9.05; p < 0.001)$ (Figs. 4a and b and 5a and b). After transfer of the three-day treated winter morphs to 11 days of summer conditions without insecticide and/or bait droplet leaves in the jars, the final mortality was not significantly different to that of the summer morphs after three days (Fig. 4a and c) although oviposition was lower $(t_{84} = 3.42; p < 0.001)$ (Fig. 5a and b). Without insecticides, *H. uvarum* and FSJ had no significant effect on summer or winter morph *D. suzukii* mortality or oviposition compared with the water control (Figs. 4b and c and 5b and c).

3.4.1. Summer morphs

In water without baits, diluted doses of cyantraniliprole and thiacloprid increased mortality $(t_{84} = 3.62 \text{ or } 2.58; p < 0.001 \text{ or } p = 0.012)$. Averaged across all four insecticides, *H. uvarum* resulted in greater mortality than FSJ (average results not shown; $t_{84} = 2.67; p = 0.009$) and there was no significant difference in oviposition between baits. Mortality was higher and oviposition lower following the use of insecticides with FSJ than in water $(t_{84} = 5.08 \text{ and } 2.71; p < 0.001 \text{ or } p = 0.008)$ (Figs. 4a and 5a). *H. uvarum* increased *D. suzukii* mortality with all four insecticides $(t_{84} > 2.93; p < 0.005)$ and reduced oviposition $(t_{84} > 2.12; p < 0.037)$ with all except thiacloprid (Figs. 4a and 5a), compared with using the insecticides in water. FSJ increased mortality with spinosad and cyantraniliprole $(t_{84} = 4.82 \text{ or } 2.93; p < 0.00 \text{ or } p = 0.004)$ and reduced oviposition with cyantraniliprole and lambda-
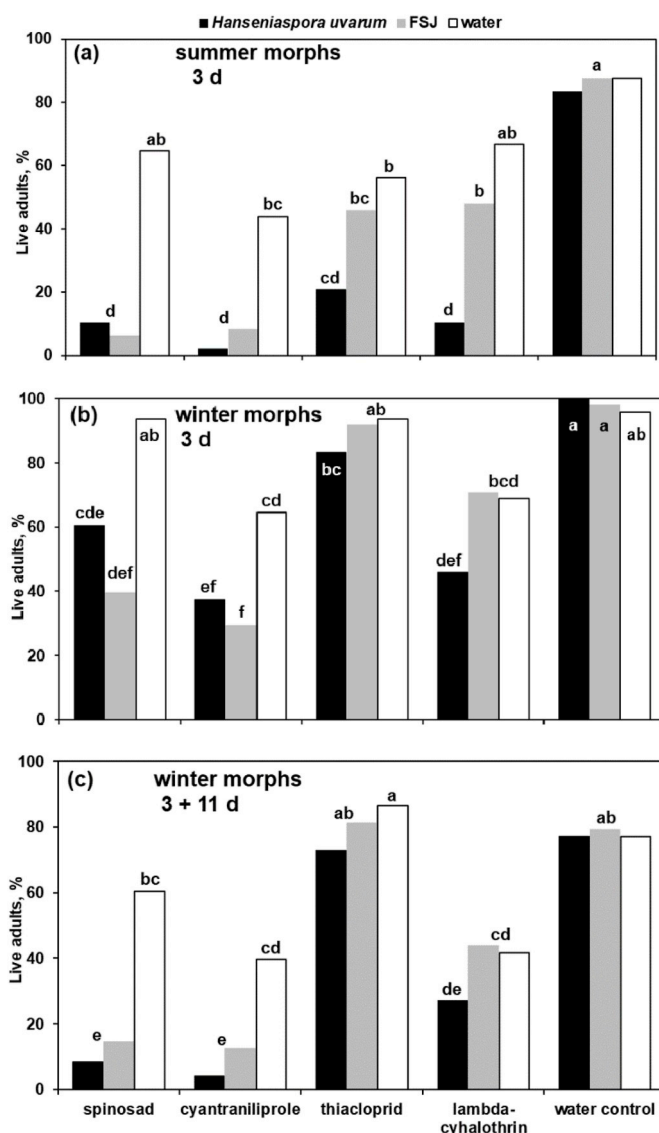

Fig. 4. Effect of bait treatments (*Hanseniaspora uvarum* or FSJ fermented strawberry juice) and insecticides on *Drosophila suzukii* mortality of (a) summer and (b) winter morphs after 3 days and (c) winter morphs after 3 days followed by 11 days of summer conditions; mean values, $n = 4$.
Within the same graphs, bars with the same letter are not significantly different ($p = 0.05$).

cyhalothrin $(t_{84} = 2.21 \text{ or } 2.41; p = 0.030 \text{ or } 0.018)$, compared with using the insecticides in water.

3.4.2. Winter morphs

Without baits, mortality after three days was increased by diluted doses of cyantraniliprole and lambda-cyhalothrin $(t_{84} = 2.54 \text{ or } 2.20; p = 0.013 \text{ or } 0.031)$ but not by spinosad or thiacloprid, when applied at the diluted doses (Table 1). Averaged across bait treatments, the mortality of winter morphs increased during the 11 days of summer conditions in the water controls $(t_{84} = 2.84; p = 0.006)$ and in the spinosad, cyantraniliprole and lambda-cyhalothrin treatments $(t_{84} > 3.44; p < 0.001)$ but not thiacloprid (average results not shown). Averaged across insecticide treatments there was no significant difference between baits in mortality or oviposition of winter morphs, either 3 days after exposure or after a further 11 days without the treated leaves present (Fig. 4b and c). Both baits increased winter morph mortality $(t_{84} > 2.24; p < 0.029)$ when used with spinosad or cyantraniliprole, but not with thiacloprid or lambda-cyhalothrin (Figs. 4b and c and 5b).
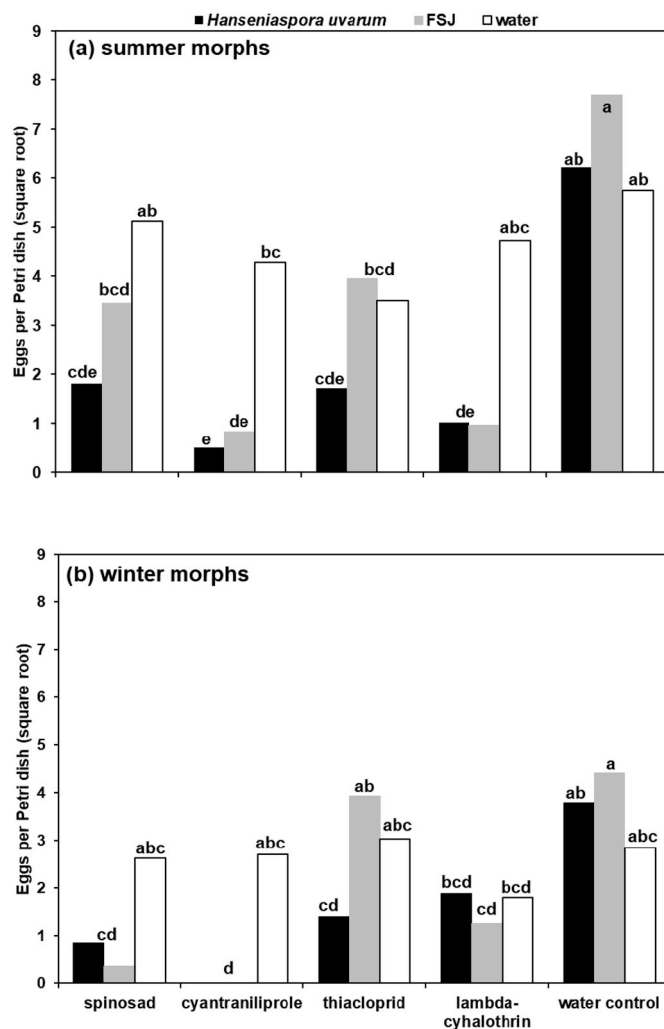
Fig. 5. Effect of bait treatments (*Hanseniaspora uvarum* or FSJ fermented strawberry juice) and insecticides on *Drosophila suzukii* oviposition of (a) summer morphs after 3 days and (b) winter morphs after 3 days followed by 11 days of summer conditions; mean values, $n = 4$.
Within the same graphs, bars with the same letter are not significantly different ($p = 0.05$).

H. uvarum only reduced winter morph oviposition when used with cyantraniliprole ($t_{84} = 2.19$; $p < 0.032$), unlike for summer morphs where oviposition was also reduced when *H. uvarum* was used with spinosad and lambda-cyhalothrin. FSJ did not significantly affect subsequent oviposition of winter morphs, unlike for summer morphs where FSJ reduced oviposition with cyantraniliprole and lambda-cyhalothrin, compared with using the insecticides in water (see 3.4.1).

4. Discussion and conclusions

These tests demonstrate that combining a *D. suzukii* phagostimulant, non-toxic bait (*H. uvarum*, Combi-protec or FSJ) with a dilute dose of insecticide increases *D. suzukii* mortality compared with the same insecticide dose applied in water. Together with using red colouring in the baits (see 2.2), this indicates that *D. suzukii* is feeding on droplets with baits and potentially increasing exposure and hence dose and insect toxicity levels of insecticide. Jar bioassays using insecticides, as 10 μl droplets of leaves, at their full permitted field dose (Table 1) without baits resulted in the following average percentages of mortality of summer morph *D. suzukii* after three days: spinosad or cyantraniliprole 100%, thiacloprid 54% and lambda-cyhalothrin 38% ($n = 4$). This

compares with 63–97%, 94–98%, 57–74% and 76–90% mortality obtained with dilute doses of the same insecticides with *H. uvarum* bait in Experiments 1 to 3. Spinosad and cyantraniliprole were therefore less effective, whereas thiacloprid and lambda-cyhalothrin were at least as effective when used in dilute doses with baits than at their full permitted field dose without baits. *D. suzukii* insecticidal mortality increased with *H. uvarum* and Combi-protec compared with FSJ. However, the relative efficacy of these phagostimulant baits did not fully correspond with their olfactory attraction to baits. In these tests, *H. uvarum* was significantly more attractive than Combi-protec and FSJ which recorded similar numbers of fly activity counts to and from bait samples. None of the diluted doses of insecticides in water significantly affected oviposition, although some of the combined bait and insecticide treatments reduced oviposition compared with water. However, the results for oviposition were more variable than for mortality due to the large oviposition potential for small numbers of surviving female *D. suzukii* (Asplen et al., 2015).

The increased mortality and reduced oviposition resulting from dilute doses of spinosad by incorporation of *H. uvarum* as a phagostimulant bait agrees with the results Mori et al. (2017). Roubos et al. (2019) found no benefit of adding *S. cerevisiae* to spinosad for control of *D. suzukii* in the field and although we confirmed Scheidler et al. (2015) results that *D. suzukii* was more attracted to *H. uvarum* than to *S. cerevisiae,* this may not correspond with their phagostimulant effects. Also, the relative attraction of these yeasts to *Drosophila melanogaster* (Meigen) has been shown to be isolate dependent (Palanca et al., 2013). The greater efficacy in the jar bioassay of the combined use of Combi-protec and diluted doses of spinosad or cyantraniliprole, compared with using these insecticides and doses in water, confirms improved *D. suzukii* control recorded in the field (Dederichs, 2015; Helsen and van der Sluis, 2018). When applied on to leaves with insecticides as six 10 μl droplets, *H. uvarum* and Combi-protec were more effective in *D. suzukii* control than FSJ. The combined use of *H. uvarum* + FSJ did not increase insecticidal mortality greater than when these baits were used individually with insecticides. Baits enable the effective rate of insecticides, including spinosad, to be reduced, leading to fewer non-target effects and lower fruit residues (Mangan et al., 2006; Haviland and Beers, 2012) although the effectiveness of *H. uvarum*, Combi-protec, and other manufactured baits may be limited by their economically viable application rate in the field. For example, the field application rates of a yeast-based bait (Fruition Natlav, Agnova Technologies Pty Ltd, Victoria, Australia), Combi-protec, Suzukii-Trap, and apple cider vinegar and wine were 18.7–46.8 L ha^{-1} and 3.7–16.1 L ha^{-1} for HOOK SWD (Dederichs, 2015; Klick et al., 2019; van Steenwyk et al., 2016) whereas the availability of FSJ, an abundant on-farm by-product (Noble et al., 2017), means that it could be applied at 1000 L ha^{-1}, thereby increasing crop coverage and/or insecticide rate. Although a significant loss in attractiveness to *D. suzukii* from SJ to FSJ was recorded in the olfactory attraction tests, the phagostimulant effect of SJ was not tested, and it would be difficult to store large quantities of by-product juice without subsequent fermentation occurring.

The relative toxicity of insecticides to *D. suzukii* adults found here confirms the decreasing laboratory LC$_{50}$ and LC$_{95}$ values in order of pyrethrin > lambda-cyhalothrin > cyantraniliprole > spinosad (Smirle et al., 2017), and the greater toxicity of spinosad over acetamiprid (Bruck et al., 2011). Spinosad was more effective in controlling *D. suzukii* than acetamiprid in semi-field trials on blueberries, while pyrethrum was ineffective (van Timmeren and Isaacs, 2013). The increased *D. suzukii* control efficacy of lambda-cyhalothrin by adding *H. uvarum*, FSJ and Combi-protec baits confirms the statement of Beers et al. (2011) that the use of adjuvant baits enhances less effective insecticides for *D. suzukii* control. However, lack of consistent improvement in the *D. suzukii* control efficacy of thiacloprid and pyrethrum using baits may be due to a phagorepellent effect of these insecticides at low concentrations on *D. suzukii* (Cai et al., 2018).

In terms of increased insecticidal mortality, *H. uvarum* and FSJ baits

had a phagostimulant effect on both summer- and winter-morph *D. suzukii*. Baits and traps prepared from whole wheat bread dough with an apple cider vinegar (Wallingford and Loeb, 2016) or apple cider vinegar, wine and sugar (Rossi-Staconni et al., 2016) were attractive to both summer- and winter-morph *D. suzukii*. The prevalence of *Hanseniaspora* yeasts in the digestive tracts of winter morph *D. suzukii* indicates that they may be using these and other products of decomposition when fruit and other summer food sources are scarce (Fountain et al., 2018). After three days, the mortality of winter morphs at 13 °C was less than of summer morphs at 21 °C, using the same proportions of males and females. This confirms the results of Shearer et al. (2016) who showed that the time needed to reach 50% mortality decreased with temperature and was shorter in summer morph males than in winter morph males, although the difference in longevity of winter- and summer-morph females kept at the same temperature was not significant. Our results show that the effect of the same insecticide application (with or without baits) on *D. suzukii* mortality was lower for winter morphs than for summer morphs. It was not established whether this was due to differences in insecticide uptake or fly toxicity or activity between summer- and winter-morphs. There were also differences in the relative efficacies of baits between summer and winter morphs. For summer morphs, *H. uvarum* resulted in greater mortality and with more insecticides than FSJ, whereas for winter morphs, *H. uvarum* was only effective with two insecticides and not significantly different to FSJ. This may be due to mated females of summer morphs being particularly attracted to yeasts as a protein source for oviposition (Hamby et al., 2012; Mori et al., 2017). However, the effective bait and insecticide treatments against winter morphs found here could be tested in crops in late winter and early spring to reduce fruit damage and delay the development of summer morph populations. Overwintering females are considered to be the main source of *D. suzukii* infestations in early fruit crops, which are less susceptible to *D. suzukii* infestations than later crops (Wallingford and Loeb, 2016; Panel et al., 2018). For winter applications, *H. uvarum* is more practical due to the requirement for fruit waste for FSJ, although the latter could be frozen or cold stored before use.

From our winter morph assays there were no eggs in the oviposition medium if flies remained in winter conditions for up to four weeks. Oviposition commenced five to seven days after winter morph *D. suzukii* were transferred into summer conditions and then continued to increase after a further three days, which was also reported by Toxopeus et al. (2016). Our work has shown that the effect of bait and insecticide treatments, present for three days of winter conditions, on winter morph mortality and oviposition persisted in a subsequent 11 days of summer conditions. However, further work is needed to determine whether the efficacy of bait and insecticide treatments received during winter conditions persists beyond 11 days of subsequent summer conditions, and with a more gradual and realistic transition from winter to summer conditions.

The attractiveness of phagostimulant baits, and resulting improved insecticidal control, is specific to different groups of dipterans. The product GF-120 Naturalyte (Dow) based on sugars and plant proteins and extracts and containing spinosad was effective in reducing infestations of certain Tephritid fruit fly pests (Yee and Chapman, 2005; Vayssieres et al., 2009) but results with *D. suzukii* control were variable (Walsh et al., 2011). However, Combi-protec has been used successfully to reduce infestation of a Tephritid pest, the walnut husk fly (*Rhagoletis completa* Cresson)(Dederichs, 2013). The impact of phagostimulant baits on non-target dipterans such as syrphids, non-dipteran natural enemies and pollinators in fruit crops requires further investigation (Cowles et al., 2015). Spraying GF-120 on to coffee crops increased the mortality of the Tephritid fruit fly parasitoid *Fopius arisanus* (Sonan)(Wang et al., 2005). Sprays of sugar solutions have not affected the incidence of fruit rots (Cowles et al., 2015; Knight et al., 2016) and fermented liquids or compost teas are capable of plant disease suppression (St Martin, 2014) which may provide an additional benefit of spraying FSJ on to fruit crops.

In *D. suzukii* laboratory bioassays, insecticide treatments were applied on to glass and acrylic surfaces (Smirle et al., 2017; Andreazza et al., 2017), blueberry, cherry and strawberry leaves and/or fruits (Beers et al., 2011; Wiman et al., 2014; Mori et al., 2017). We found that combined bait and insecticide treatments increased *D. suzukii* mortality on a range of fruit plant leaf types, although mortality was slightly lower using raspberry leaves than blueberry or strawberry leaves. The deep ridges in raspberry leaves may have reduced access of flies to treatment droplets, and/or provided a more suitable micro-environment (e.g. humidity) for fly survival in the jars. Blackberry leaves are preferable for the jar bioassay since they are small and abundant in the wild, year-round without pesticide applications. However, the potential impact of leaf morphology on fly survival should be considered in conducting field tests with bait and insecticide treatments. In field tests, as part of these studies, no phytotoxicity was observed after *H. uvarum*, FSJ or Combi-protec baits were sprayed on to blackberry, blueberry, cherry, raspberry or strawberry leaves (data not shown).

In conclusion, these studies support the use of adjuvant phagostimulant baits in mixtures with sub-lethal concentrations of insecticides to control winter and summer morph *D. suzukii* in fruit crops. The results concur with previous studies showing that the most promising phagostimulant baits for insecticidal control of summer morph *D. suzukii* are those based on yeasts (particularly *H. uvarum*), plant extracts, fermentation products and sugars (Combi-protec and FSJ). The ability to apply insecticides at lower doses without compromising efficacy minimises environmental impact by reducing the overall amount of active ingredient applied, and the ability to apply the baits not directly on to fruits, could reduce residues in fresh produce. Application of low doses of insecticides normally increases the risk of pesticide resistance, but in combination with baits, the ingested dose of insecticide may be increased. Future work should focus on how to use these baits in different fruit crops and the optimum application and timing of application for control of summer and winter morph *D. suzukii*.

Declarations of interest

None.

Acknowledgement

References

Andreazza, F., Bernardi, D., Baronio, C.A., Pasinato, J., Nava, D.E., Botton, M., 2017. Toxicities and effects of insecticidal toxic baits to control *Drosophila suzukii* and *Zaprionus indianus* (Diptera: drosophilidae). Pest Manag. Sci. 73, 146–152.

Anonymous, 2018. LAM 10/16/25 Locomotor Activity Monitor. TriKinetics Inc., Waltham, MA, USA. https://www.trikinetics.com/Downloads/LAM25%20Data%20 Sheet.pdf. (Accessed 27 May 2019).

Asplen, M.K., Anfora, G., Biondi, A., Choi, D.S., Chu, D., Daane, K.M., Gibert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchinson, W.D., Isaacs, R., Jiang, Z.-L., Kárpáti, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Vétek, G., Vogt, H., Walton, V.M., Yu, Y., Zappalà, L., Desneux, N., 2015. Invasion biology of spotted wing drosophila (*Drosophila suzukii*): a global perspective and future priorities. J. Pest. Sci. 88, 469–494.

Beers, E.H., van Steenwyk, R.A., Shearer, P.W., Coates, W.W., Grant, J.A., 2011. Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest Manag. Sci. 67, 1386–1395.

Bruck, D.J., Bolda, M., Tanigoshi, L., Klick, J., Kleiber, J., DeFrancesco, J., Gerdemanc, B., Spitlerc, H., 2011. Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Manag. Sci. 67, 1375–1385.

Cai, P., Yi, C., Zhang, Q., Zhang, H., Lin, J., Song, X., Yang, J., Wang, B., Ji, Q., Chen, J., 2018. Evaluation of protein bait manufactured from brewery yeast waste for controlling *Drosophila suzukii* (Diptera: drosophilidae). J. Econ. Entomol. https://doi :10.1093/jee/toy304.

Cowles, R.S., Rodriguez-Saona, C., Holdcraft, R., Loeb, G.M., Elsensohn, J.E., Hesler, S.P., 2015. Sucrose improves insecticide activity against *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 108, 640–653.

Dederichs, U., 2013. Starke Fruchtschäden und Totalausfall, Walnussanbau 2012. Obst Garten 2, 4–6.

Dederichs, U., 2015. Using the bait spray method to control the spotted-wing drosophila. Eur. Fruit Mag. 2015–04, 6–9.

Fanning, P.D., Grieshop, M.J., Isaacs, R., 2018. Efficacy of biopesticides on spotted wing drosophila, *Drosophila suzukii* Matsumura in fall red raspberries. J. Appl. Entomol. 142, 26–32.

Fountain, M.T., Bennett, J., Cobo-Medina, M., Conde Ruiz, R., Deakin, G., Delgado, A., Harrison, R., Harrison, N., 2018. Alimentary microbes of winter-form *Drosophila suzukii*. Insect Mol. Biol. https://doi:10.1111/imb.12377.

Gress, B.E., Zalom, F.G., 2018. Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. Pest Manag. Sci. https://doi:10.1002/ps.5240.

Hamby, K.A., Hernández, A., Boundy-Mills, K., Zaloma, F.G., 2012. Associations of yeasts with spotted-wing drosophila (*Drosophila suzukii*; Diptera: drosophilidae) in cherries and raspberries. Appl. Environ. Microbiol. 78, 4869–4873.

Haviland, D.R., Beers, E.H., 2012. 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.

Helsen, H., van der Sluis, B., 2018. Use of Toxic Baits for the Control of *Drosophila Suzukii*. http://dropsaproject.eu/downloadDocument.cfm?id=323. (Accessed 8 March 2019).

Klick, J., Rodriguez-Saona, C.R., Hernández Cumplido, J., Holdcraft, R.J., Urrutia, W.H., da Silva, R.O., Borges, R., Mafra-Neto, A., Seagraves, M.P., 2019. Testing a novel attract-and-kill strategy for *Drosophila suzukii* (Diptera: drosophilidae) management. J. Insect Sci. 19, 1–6.

Knight, A.L., Basoalto, E., Yee, W., Hilton, R., Kurtzman, C.P., 2016. 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.

Lainsbury, M.A., 2018. The UK Pesticide Guide 2018. CABI, Wallingford, Oxon., UK.

Landolt, P.J., Adams, T., Rogg, H., 2012. Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. J. Appl. Entomol. 136, 148–154.

Mangan, R.L., Moreno, D.S., Thompson, G.D., 2006. Bait dilution, spinosad concentration, and efficacy of GF-120 based fruit fly sprays. Crop Protect. 25, 125–133.

Mori, B.A., Whitener, A.B., Leinweber, Y., Revadi, S., Beers, E.H., Witzgall, P., Becher, P. G., 2017. Enhanced yeast feeding following mating facilitates control of the invasive fruit pest *Drosophila suzukii*. J. Appl. Ecol. 54, 170–177.

Noble, R., Dobrovin-Pennington, A., Shaw, B., Buss, D.S., Cross, J.V., Fountain, M.T., 2017. Fermentation for disinfesting fruit waste from *Drosophila* species (Diptera: drosophilidae). Environ. Entomol. 46, 939–945.

Palanca, L., Gaskett, A.C., Günther, C.S., Newcomb, R.D., Goddard, M.R., 2013. Quantifying variation in the ability of yeasts to attract *Drosophila melanogaster*. PLoS One 8, e75332.

Panel, A.D.C., Zeeman, L., van der Sluis, B.J., van Elk, P., Pannebakker, B.A., Wertheim, B., Helsen, H.H.M., 2018. Overwintered *Drosophila suzukii* are the main source for infestations of the first fruit crops of the season. Insects 9, 145. https://doi:10.3390/insects9040145.

Rosensteel, D.O., Sial, A.A., 2017. Efficacy of insecticides against Drosophila Suzukii in rabbiteye blueberry, 2015, 2017 Arthropod Manag. Tests 1–2. https://doi:10.1093/amt/tsx115.

Rossi-Stacconi, M.V., Kaur, R., Mazzoni, V., Ometto, L., Grassi, A., Gottardello, A., Rota-Stabelli, O., Anfora, G., 2016. Multiple lines of evidence for reproductive winter diapause in the invasive pest *Drosophila suzukii*: useful clues for control strategies. J. Pest. Sci. https://doi:10.1007/s10340-016-0753-8.

Roubos, C.R., Gautam, B.K., Fanning, P.D., van Timmeren, S., Spies, J., Liburd, O.E., Isaacs, R., Curry, S., Little, B.A., Sia, A.A., 2019. Impact of phagostimulants on effectiveness of OMRI-listed insecticides used for control of spotted-wing drosophila (*Drosophila suzukii* Matsumura). J. Appl. Entomol. https://doi:10.1111/jen.12620.

Rouzes, R., Delpac, L., Ravidat, M., Thiery, D., 2011. First occurrence of Drosophila suzukii in the Sauternes vineyards. J. Int. Sci. Vigne Vin 46, 145–147.

Scheidler, M.H., Cheng, L.L., Hamby, K.A., Zalom, F.G., Syde, Z., 2015. Volatile codes: correlation of olfactory signals and reception in Drosophila yeast chemical communication. Sci. Rep. 5, 14059.

Schlesener, D.C.H., Wollmann, J., de Bastos, J., Grützmacher, A.D., Garcia, F.R.M., 2017. Effects of insecticides on adults and eggs of *Drosophila suzukii* (Diptera, Drosophilidae). Rev. Colomb. Entomol. 43, 208–214.

Shearer, P.W., West, J.D., Walton, V.M., Brown, P.H., Svetec, N., Chiu, J.C., 2016. Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. BMC Ecol. 16, 2–18.

Smirle, M.J., Zurowski, C.L., Ayyanath, M., Scott, I.M., MacKenzie, K.E., 2017. Laboratory studies of insecticide efficacy and resistance in *Drosophila suzukii* (Matsumura) (Diptera: drosophilidae) populations from British Columbia, Canada. Pest Manag. Sci. 73, 130–137.

St Martin, C.C.G., 2014. Potential for compost tea for suppressing plant diseases. CAB Rev. 9 (032). https://www.cabi.org/bni/review/20153038604. (Accessed 13 February 2019).

Toxopeus, J., Jakobs, R., Ferguson, L.V., Gariepy, T.D., Sinclair, B.J., 2016. Reproductive arrest and stress resistance in winter-acclimated *Drosophila suzukii*. J. Insect Physiol. 89, 37–51.

van Steenwyk, R.A., Wise, C.R., Caprile, J.L., 2016. Control of spotted wing drosophila, *Drosophila suzukii*, in cherry using a new low volume, reduced-risk technique. Integrated Protection of Fruit Crops Subgroups "Pome fruit arthropods" and "Stone fruits". IOBC-WPRS Bull. 112, 15–20.

van Timmeren, S., Isaacs, R., 2013. Control of spotted wing drosophila (Drosophila suzukii), by specific insecticides and by conventional and organic crop protection programs. Crop Protect. 54, 126–133.

Vayssieres, J.F., Sinzogan, A., Korie, S., Ouagoussounon, I., Thomas-Odjo, A., 2009. Effectiveness of spinosad bait sprays (GF-120) in controlling mango-infesting fruit flies (Diptera: tephritidae) in Benin. J. Econ. Entomol. 102, 515–521.

Wallingford, A., Loeb, G., 2016. Spotted wing drosophila winter biology. N. Y. Fruit Q. 24 (3), 11–13.

Walsh, D.B., Bolda, M.P., Goodhue, R.E., Dreves, A.J., Lee, J., Bruck, D.J., Walton, V.M., O'Neal, S.D., Zalom, F.G., 2011. *Drosophila suzukii* (Diptera: drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integr. Pest Manag. 2, 1–7.

Wang, X., Jarjees, E.A., McGraw, B.K., Bokonon-Ganta, A.H., 2005. Effects of spinosad-based fruit fly bait GF-120 on tephritid fruit fly and aphid parasitoids. Biol. Control 35, 155–162.

Wiman, N.G., Walton, V.M., Dalton, D.T., Anfora, G., Burrack, H.J., Chiu, J.C., et al., 2014. Integrating temperature-dependent life table data into a matrix projection model for Drosophila suzukii population estimation. PLoS ONE 9, e106909. https://doi.org/10.1371/journal.pone.0106909.

Wong, J.S., Wallingford, A.K., Loeb, G.M., Lee, J.C., 2018. Physiological status of *Drosophila suzukii* (Diptera: drosophilidae) affects their response to attractive odours. J. Appl. Entomol. 142, 473–482.

Yee, W.L., Chapman, P.S., 2005. Effects of GF-120 fruit fly bait concentrations on attraction, feeding, mortality, and control of *Rhagoletis indifferens* (Diptera: tephritidae). J. Econ. Entomol. 98, 1654–1663.