Can *Saccharomycopsis vini* and *Z*4-11Al be used in a push-pull system against the invasive pest *Drosophila suzukii*?

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Faculty of Landscape Architecture, Horticulture and Crop Production Science
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Kan Saccharomycopsis vini och Z4-11Al användas i ett push-pull system mot den invasiva flugan Drosophila suzukii?

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Sammanfattning

Drosophila suzukii är en invasiv skadegörare på bär som hittades i Sverige första gången 2014. Till skillnad från andra arter av *Drosophila* har *D. suzukii* ett sågtandat äggläggningsrör vilket möjliggör äggläggning på omogna och mognande bär. Som värdväxt för sina larver använder *D. suzukii* en stor mängd kultiverade och vilda växter.

Det är känt att arter av *Drosophila* har samutvecklats med vissa jästsorter vilka återfinns både i deras matspjälkningssystem och i flugornas miljö. Hos *D. suzukii* har det visat sig att parade honor söker sig till jästrika substrat och jästarten S*accharomycopsis vini* har visat sig vara mycket attraktiv.

*D. suzukii*s larver klarar inte konkurrens med larver av *Drosophila melanogaster* och därför undviker *D. suzukii* att lägga ägg på substrat som exponerats för *D. melanogaster*. Det för *D. melanogaster* honor artspecifika feromonet *Z*4-11Al tros minska äggläggningsviljan hos *D. suzukii*. Denna kunskap skulle kunna användas för att utveckla bekämpningsmetoder mer specifika för *D. suzukii*.

Syftet med den här uppsatsen var att testa om jästen *S. vini* och feromonet *Z*4-11Al kan användas i ett push-pull bekämpningssystem mot *D. suzukii*. Experimenten utfördes i laboratoriemiljö. Tio honor släpptes ut i en arena på vilken fällor var placerade. Som äggläggningssubstrat placerades ett blåbär i mitten. *Z*4-11Al introducerades bredvid blåbäret.

Resultaten bekräftar att *D. suzukii* attraheras av *S. vini* och att jästarten har potential att användas som lockbete i fällor. Om *Z*4-11Al kan användas som avskräckande mot äggläggning kan inte definitivt avgöras. Möjligtvis gjorde närvaron av *Z*4-11Al jästen mindre attraktiv för *D. suzukii*.

Nyckelord: *feromon, jäst, bekämpning, invasiv*

Abstract

Drosophila suzukii is a pest with global spread that lays its eggs in soft fruits. It has been deemed potentially invasive to Sweden. Contrary to other *Drosophila* species, *D. suzukii* possess a serrated ovipositor which makes it possible for it to lay its eggs in ripening fruits. It thereby avoids competition with *D. melanogaster*, whose larvae outcompetes those of *D. suzukii*. Moreover, it has an extensive host range including both cultivated and wild plants.

Mated *D. suzukii* females are drawn to yeast for feeding in the period after mating. The yeast S*accharomycopsis vini* has been found attractive. When presented with a substrate *D. suzukii* actively avoids oviposition in substrate inoculated by *D. melanogaster.* The species-specific female pheromone of *D. melanogaster* *Z*4-11Al is thought to induce oviposition aversion in *D. suzukii*.

The objective of this study was to test if *S. vini* and *Z*4-11Al have the potential of being used in a push-pull system against *D. suzukii*. Testing took place in a laboratory setting. Ten female *D. suzukii* flies were released into arenas containing traps and a blueberry providing oviposition substrate. *Z*4-11Al was dispensed from a vial placed near the blueberry.

Results confirms previous findings that *D. suzukii* finds the yeast *S. vini* attractive. *S. vini* has potential for being used as a trap lure. Regarding the potential of *Z*4-11Al as an oviposition deterrent, results are inconclusive. There are some indications that *Z*4-11Al might work to deterred *D. suzukii* from yeast.

Keywords: *(Z)-4-undecenal, pheromone, yeast, volatiles, pest management, spotted wing drosophila*

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Abbreviations

IPM	Integrated pest management
PDA	Potato dextrose agar
PDB	Potato dextrose broth
SLU	Swedish University of Agricultural Sciences
SWD	Spotted wing drosophila

1. Introduction

Drosophila suzukii (Matsumura) is a pest of soft skinned fruit that is widely distributed, present nearly all over the globe. Reaching Europe in 2008 and first reported in Sweden in 2014, where it has been classified to have a high potential of becoming invasive (Calabria et al. 2012; Manduric 2017; Strand et al. 2018; Kwadha et al. 2021).

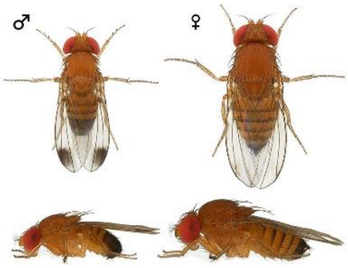


Figure 1 Male and female Drosophila suzukii (Agroscope 2013) (CC BY-ND 2.0)

The adult males of *D. suzukii* (Figure 1) are distinctive with their dark spots on the leading edge of the wings that has given rise to the species common name Spotted Wing Drosophila (SWD). The spots emerge during cuticular sclerotization but may in rare cases be absent or pale. In addition, the males can be distinguished from the females by the presence of black sexcombs on the first and second tarsi of the front legs. Female *D. suzukii* possess a serrated ovipositor with which the skin of ripening and ripe intact fruits is perforated allowing for ovipositing inside the fruit. The eggs are translucent white with two breathing filaments that stick out on the outside of the fruit (Figure 2) (Hauser 2011; Atallah et al. 2014).


Figure 2 Filaments of Drosophila suzukii eggs laid on a blueberry. (Andersson 2021)

D. suzukii is known for having an extensive host range spanning from important cultivated crops such as raspberries, strawberries, blueberries and cherries, to wild plants with soft skin (Bellamy et al. 2013; Kenis et al. 2016). Attacks on fruit with harder skins have been reported but soft skinned cultivars are preferred over fruit with skin that requires a higher penetrative force to pierce (Entling et al. 2019).

When ovipositing in ripening fruit *D. suzukii* creates an opening in the fruits' surface thus making the crop more susceptible to fungi, bacteria and insects that attack damaged fruit. *D. suzukii* has been shown capable of introducing sour rot in grapes and making way for *Drosophila melanogaster* to be able to lay eggs in an otherwise inaccessible host niche (Rombaut et al. 2017; Ioriatti et al. 2018). When the eggs of *D. suzukii* hatch, after 24-48 hours, the larvae feed on the flesh making the fruit become soft, collapse and rapidly rendering it unmarketable (Walsh et al.

2011). Controlling the adult stage of *D. suzukii* is key since extensive damage is done when the females perforate the fruit skin, and the eggs deposited inside are not easily accessible by control agents.

Devising traps for this polyphagous and highly adaptive pest has proven to be a challenge (Lee et al. 2012; Atallah et al. 2014). Like other members of the *Drosophila* group, *D. suzukii* is known to be associated with an array of yeast species (Hamby et al. 2012; Stefanini 2018). Current trapping methods make use of this, using yeast, vinegars, wine or synthetically produced volatile components of the former three as lures. Some traps also use volatiles from *D. suzukiis'* host plants (Landolt et al. 2012; Cha et al. 2013; Lasa et al. 2017; Little et al. 2021). When used in integrated pest management (IPM), traps are important elements used for both monitoring and/or mass trapping. Problems with current trapping methods are that they are non-specific, trapping other species of *Drosophila* and other insects (Renkema et al. 2014; Little et al. 2021). Trap efficiency also varies with season and is not correlated with fruit infestation, making monitoring *D. suzukii* in IPM hard (Hamby et al. 2014; Burrack et al. 2015; Jaffe et al. 2018). Ineffective traps are costly, require extra work and when faced with potentially losing the harvest there is a risk of growers abandoning IPM strategies in favour of regular insecticide spraying (Beers et al. 2011; Lee et al. 2012). This increases the risk of rendering *D. suzukii* resistant towards insecticides (Gress & Zalom 2019). Furthermore, insecticide use is costly (Diepenbrock et al. 2016) and residue levels can become a problem when growers want to export their crop (Haviland & Beers 2012). Finding a sustainable management and trapping method for *D. suzukii* thus is imperative.

A push-pull strategy has been put forward as a possible part in battling *D. suzukii* infestation. Combining an effective trap that attracts flies, pulling them away from the host plant, with a deterrence compound that – pushes – *D. suzukii* away from the host (Wallingford et al. 2018; Alkema et al. 2019).

It has been hypothesised that *D. suzukii* is drawn to yeast volatiles for finding food and to fruit volatiles to locate oviposition sites. *Drosophila* flies have a mutualistic relationship with yeast, it providing nutrients needed for development and the flies contributing to dispersal of yeast (Christiaens et al. 2014; Karageorgi et al. 2017). Mated female *D. suzukii* flies were more attracted to fruit and yeast odours than their unmated counterparts and mated flies overall consumed more yeast. When given a choice between feeding on the yeast *Hanseniaspora uvarum* and laying eggs on a blueberry, females choose to feed more and oviposit less during a 24 h period following mating (Mori et al. 2017). The possible benefit of this increased yeast feeding is supported by findings that female *D. suzukii* that had been feeding on yeast laid more eggs. In 2012 Bellutti et al. (2018) isolated *Saccharomycopsis vini* from *D. suzukii*-infested grapes. Rehermann (n.d.) showed that *S. vini* elicited as strong a response as the for *D. suzukii* most common yeast species, *H. uvarum*. *S. vini* trapped the most flies over a 24 h period and was preferred over *H. uvarum* in a choice assay.

The fact that *D. suzukii* uses volatiles associated with ripening fruit to locate oviposition sites can be the result of interspecies competition (Keesey et al. 2015). *D. suzukii* are more tuned to volatiles emitted by ripening fruit than their close relatives (Revadi et al. 2015b; Karageorgi et al. 2017). Being able to lay eggs in ripening berries gives *D. suzukii* larvae a head-start over competitors. *D. suzukii* larvae do not fare well in competition with *D. melanogaster* larvae and *D. suzukii* prefers to avoid or lay less eggs in substrate previously inoculated by *D. melanogaster* (Shaw et al. 2018; Kidera & Takahashi 2020).

(*Z*)-4-undecenal (*Z*4-11Al) is an aldehyde pheromone produced during oxidation of (*Z*,*Z*)-7,11-heptacosadiene (7,11-HD) and specific to female *D. melanogaster* (Figure 3). *Z*4-11Al is used for long-range communication and attracts both male and female flies. Attraction however varies between *D. melanogaster* strains. In addition, *Z*4-11Als precursor 7,11-HD inhibits the close relative *Drosophila simulans* to mate with *D. melanogaster* (Lebreton et al. 2017). In ongoing experiments, *D. suzukii* exhibited oviposition aversion in the presence of *Z*4-11Al. Based on the attractive properties of *S. vini* and the repelling potential of *Z*4-11Al this project aimed to test if they might be used in a push-pull system for managing *D. suzukii*.

To test the push-pull system, the project investigated four questions: 1) could *S. vini* be used as a trap lure? 2) how does the presence of *Z*4-11Al affect trapping of *D. suzukii*? 3) does *Z*4-11Al affect oviposition in *D. suzukii*? And 4) does the use of *S. vini* as an attractant and *Z*4-11Al as deterrent have potential of working in a push-pull system?

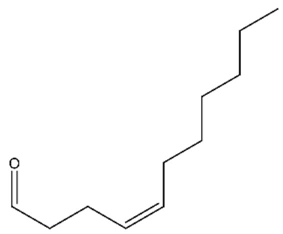


Figure 3 Z4-11Al molecule

2. Materials and methods

2.1. *Drosophila suzukii* colony

The flies used were from a laboratory colony maintained at SLU Alnarp originally established with *D. suzukii* collected from northern Italy. They were kept on standard *Drosophila* cornmeal diet (Revadi et al. 2015a) under a 12:12 h photoperiod. Newly emerged (during a 24 h period) females and males were transferred together onto new medium in plastic *Drosophila* rearing vials, in which they were kept for 5-7 days. At the day of experiment, female flies were picked out with the blunt end of a Pasteur-pipette and placed in empty vials (10 flies/vial) topped with a damp cotton ball. If there were any doubts regarding sex, it was double checked with a 20x magnification loupe.

2.2. Blueberries

To test for a correlation between oviposition and attraction, blueberries (*Vaccinium corymbosum*) sourced from a local supermarket were used as substrate. To minimize the effect of variation, the differences in weight of blueberries between replicates was kept at 0.10 g. Similarly, to minimize variation due to ripening stage, berries used were of the same ripening stage with blue pulp. Ripeness was ascertained by opening a small flap by the calyx using a pair of extra fine tipped stainless tweezers (Dumont® Swiss Made, Montignez, Switzerland). They were thereafter washed individually under running deionized water and dried with a paper towel.

2.3. Yeast culture

The yeast, *Saccharomycopsis vini*, was isolated from *D. suzukii*-infested grapes in South Tyrol, Italy (Bellutti et al. 2018) and maintained on potato dextrose agar (PDA) in a Petri dish. For every *S. vini* culture used for trapping, two colonies from a culture on PDA plate were inoculated in potato dextrose broth (PDB, Difco™ Becton-Dickinson, France) and grown for 24 h at room temperature. Before used in traps, the optical density of the yeast culture was determined in a spectrophotometer (UV-18800 UV, Shimadzu) at λ 595 nm.

2.4. Push and pull assay

To test if *S. vini* and *Z*4-11Al could be applied as pull and push components respectively and combined in a push-pull system against *D. suzukii*, BugDorm cages (30x30x30cm, Megaview, Taiwan) were used as arenas, see Appendix 1.

Each cage contained two traps. The traps were assembled as follows: a 200 µL pipette tip was cut as to attain a 2.5 mm opening and was inserted into a 1000 µL pipette tip that had been cut to approximately 1.5 cm in length (Figure 4). The tips were then inserted into a 4.5 mL glass vial (Genetec Inc., Gothenburg, Sweden) containing 2 mL of either PDB or *S. vini* in PDB (here after referred to as only *S. vini*). In each arena, the traps were placed in diagonally opposite corners, one in the right bottom corner near the cage opening and one in the upper left corner, see Appendix 2. The position of the PDB and *S. vini* traps was switched between replicates to minimise positional effect. To enhance humidity, a plastic cup with a netted lid, containing a wet cotton ball was placed in the right upper corner of each arena, see Appendix 2. A blueberry was placed with the calyx facing downward on a white lid in the middle of the arena. The flies were introduced to the cage by placing a vial containing 10 mated female flies (5-7 days old), in the left bottom corner and removing the cotton ball.

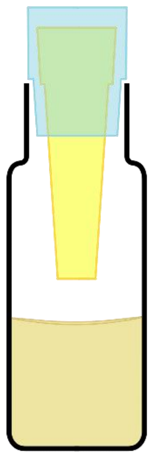


Figure 4 Illustration of a trap (Andersson 2021)

For the experiments involving *Z*4-11Al, 500 µL of *Z*4-11Al (2 ng/µL in ethanol) was added in to a 1.5 mL glass vial (Genetec Inc., Gothenburg, Sweden) with a dental roll (5-6 mm and 18 mm in diameter respectively in length) as a dispenser. About 2.5-3 mm of the dental roll was left sticking out of the vial. The *Z*4-11Al vial was placed leaning against the lid (on which the blueberry was placed), secured on the underside with a piece of double-sided tape (Scotch®, 3M) (Figure 5). The *Z*4-11Al dispenser was placed in the cages 30 minutes before the flies were introduced. After 24 h, flies were removed from the cages and the number of flies trapped and the eggs laid were recorded. Eggs were counted using a microscope.

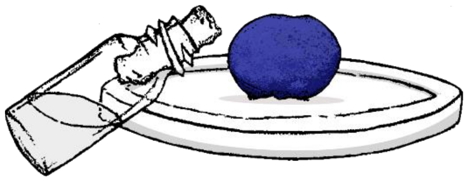


Figure 5 Illustration of a Z4-11Al vial leaning against lid with blueberry on it. (Andersson & Paulusson 2021)

Cage arenas were set up with either two PDB traps (PDB/PDB) or one PDB trap and one *S. vini* trap (*S. vini*/PDB). Experiments with *Z*4-11Al, will be denoted as PDB/PDB *Z*4-11Al and *S. vini*/PDB *Z*4-11Al respectively. One experiment where *Z*4-11Al was omitted, ran concurrent with the PDB/PDB *Z*4-11Al, here after referred to as PDB/PDB control. All experiments were conducted between the end of February to the end of March 2021 at SLU, Alnarp. The order and days in which the experiments were conducted is described in Figure 6.

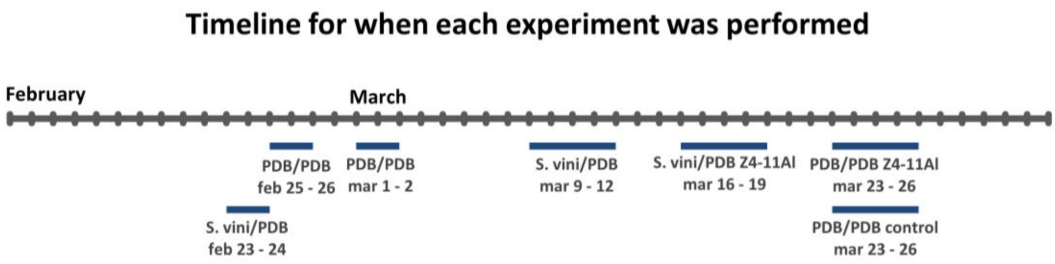


Figure 6 Blue lines represents days during which the different experiments were performed, with exact dates underneath each. The distance between each point on the timeline represents one day.

2.5. Statistics

Trapping and trap position data from *S. vini*/PDB arenas was tested using Wilcoxon signed rank tests. For comparing trapping data between cage setups with and without *Z*4-11Al a Mann-Whitney test was preformed following Kruskal-Wallis test. Results on oviposition between setups were tested using Kruskal-Wallis H test followed by Dunn's multiple comparison. Pearson correlation was computed to assess if there was a connection between the number of flies trapped and eggs laid and between the number of eggs laid and the fruit weight. All statistic tests were performed using Minitab® version 19.2020.

3. Results

3.1. Results for trapping

To test for attractiveness of *S. vini*, one trap with either PDB or *S. vini* was provided in each cage. There were more female *D. suzukii* trapped in *S. vini* (3.64 ± 2.52 flies, n = 22) than in PBD traps (0.41 ± 0.20 flies, n = 22) (Figure 7, Wilcoxon signed rank $p < 0.0005$). No effect of position of the traps was observed (Wilcoxon signed rank test, $p = 0.404$).

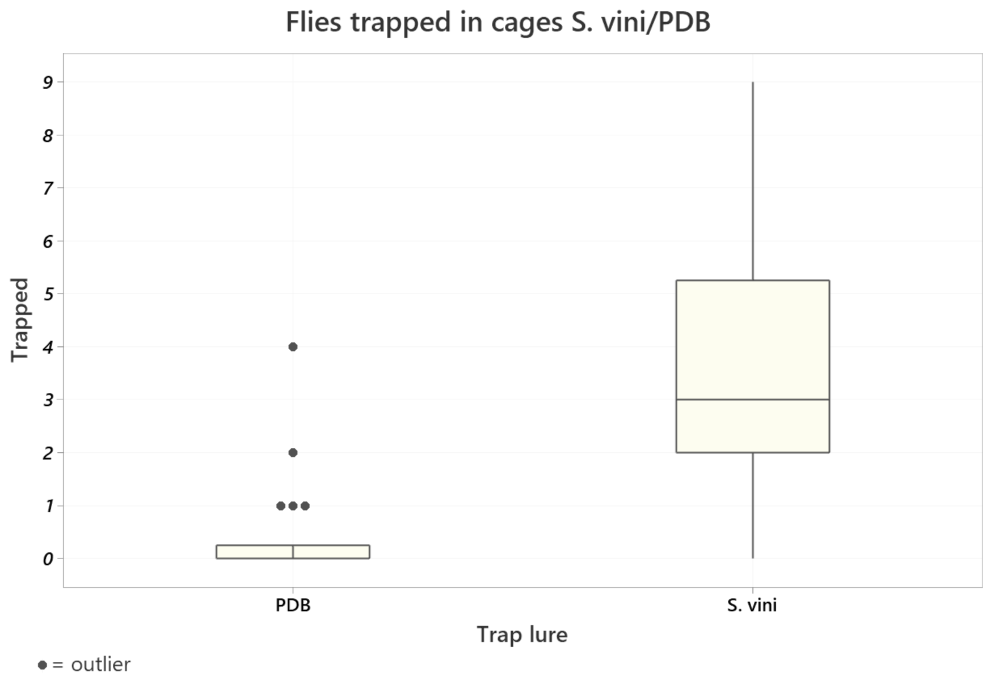


Figure 7 Number of female Drosophila suzukii in traps baited with either PDB or Saccharomycopsis vini (S. vini). Significantly more flies were trapped in S. vini than in PDB traps (Wilcoxon signed rank; n = 22 cages, p < 0.0005).

When reviewing the results, there is a difference in the number of flies trapped in *S. vini*/PDB (n = 22) and *S. vini*/PDB *Z*4-11Al (n = 16) setups (Kruskal Wallis p = 0.0002). When *Z*4-11Al was present in *S. vini*/PDB arenas, fewer *D. suzukii* females were trapped in *S. vini* (Mann-Whitney, p = 0.005) than in arenas without *Z*4-11Al (Figure 8). Presence of *Z*4-11Al did not seem to affect the number of flies trapped in PDB (Mann-Whitney, p = 0.564). Similarly, there was no significant effect of *Z*4-11Al in arenas with only PDB traps - the two setups that ran concurrently (Figure 9, Mann-Whitney, p = 0.209). The potential effect of *S. vini* on the number of flies trapped in PDB should be noted (Figure 8).

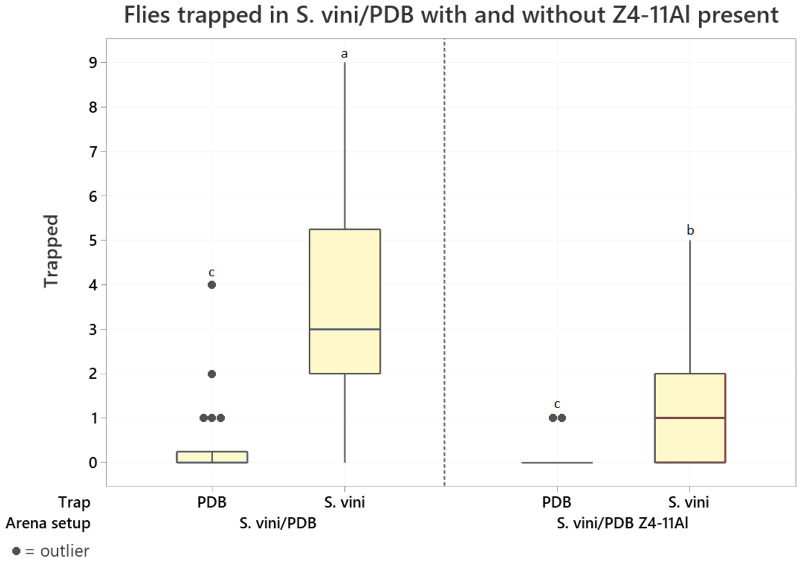


Figure 8 Number of female Drosophila suzukii trapped in cages with and without Z4-11Al present. Treatments that share letters do not differ significantly from one another. Separation by dotted line means the experiments were not run concurrently.

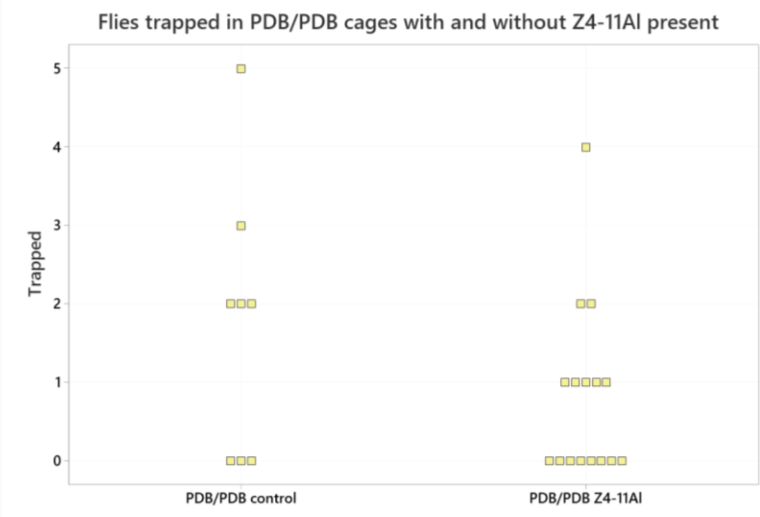


Figure 9 Number of female Drosophila suzukii trapped in PDB when Z4-11Al was present and not. (PDB/PDB control, n = 8 and PDB/PDB Z4-11Al, n = 16, Mann-Whitney, p = 0.209).

3.2. Results for oviposition

Highest number of eggs laid occurred in PDB/PDB arenas (50.13 ± 9.20, n = 16). This was more than in all other arena setups except *S. vini*/PDB (39.14 ± 11.97, n = 22) (Table 1, Kruskal-Wallis H test followed by Dunn's multiple comparison; H = 49.17, p < 0.00001).

Table 1 Compilation of egg laying per fruit and fly, and mean fruit weight (g).

Cage setup	N cages	Mean fruit weight ± SD	Mean eggs per fruit ± SD	Mean eggs per released fly ± SD	Mean eggs per fly not trapped ± SD	Mean eggs per trapped fly ± SD
PDB/PDB	16	2.55 ± 0.18	50.1 ± 9.2[a]	5.0 ± 0.9	6.5 ± 1.6	2.5 ± 1.9
PDB/PDB control	8	1.83 ± 0.10	15.0 ± 6.8[b]	1.5 ± 0.6	1.9 ± 0.8	0.4 ± 0.4
PDB/PDB *Z*4-11Al	16	1.88 ± 0.09	15.7 ± 12.0[b]	1.6 ± 1.2	1.7 ± 1.2	0.5 ± 0.7
S. vini/PDB	22	1.72 ± 0.27	39.1 ± 12.0[a]	3.9 ± 1.0	10.2 ± 11.7	1.5 ± 1.1
S. vini/PDB *Z*4-11Al	16	1.86 ± 0.14	25.9 ± 9.8[b]	2.6 ± 1.0	3.3 ± 1.7	0.7 ± 0.8

D. suzukii females laid a larger number of eggs per blueberry in *S. vini*/PDB (n = 22) arenas than in *S. vini*/PDB *Z*4-11Al arenas (n = 16) (Kruskal-Wallis H test followed by Dunn's multiple comparison, H = 49.17, p = 0.0087). The number of eggs laid by females on blueberries in PDB/PDB control (n = 8), PDB/PDB *Z*4-11Al (n = 16) and *S. vini*/PDB *Z*4-11Al (n = 16) did not differ (Figure 10).

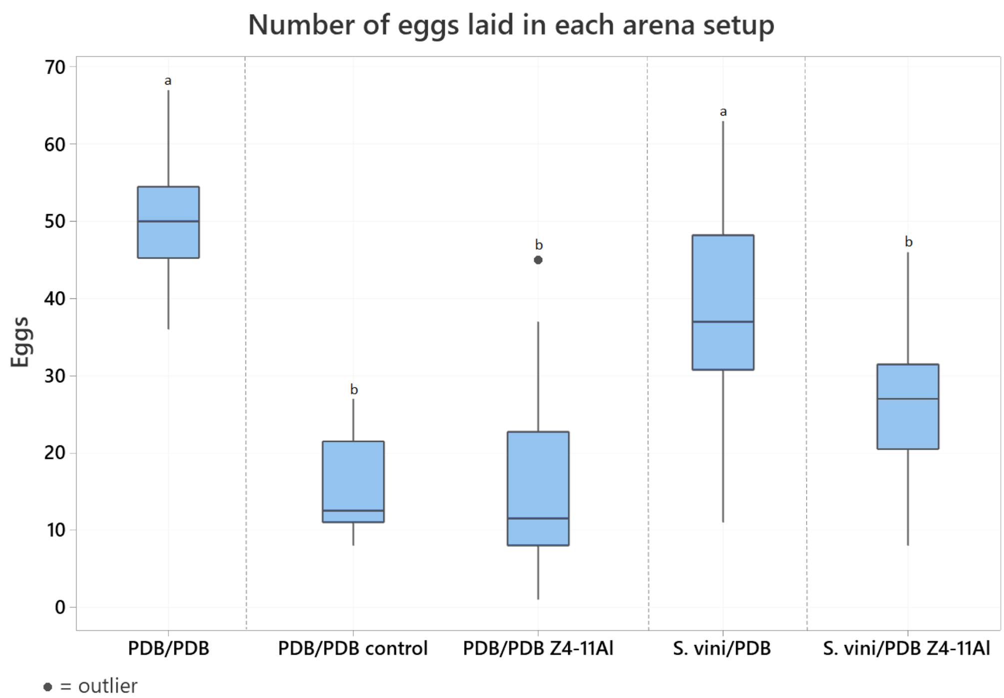
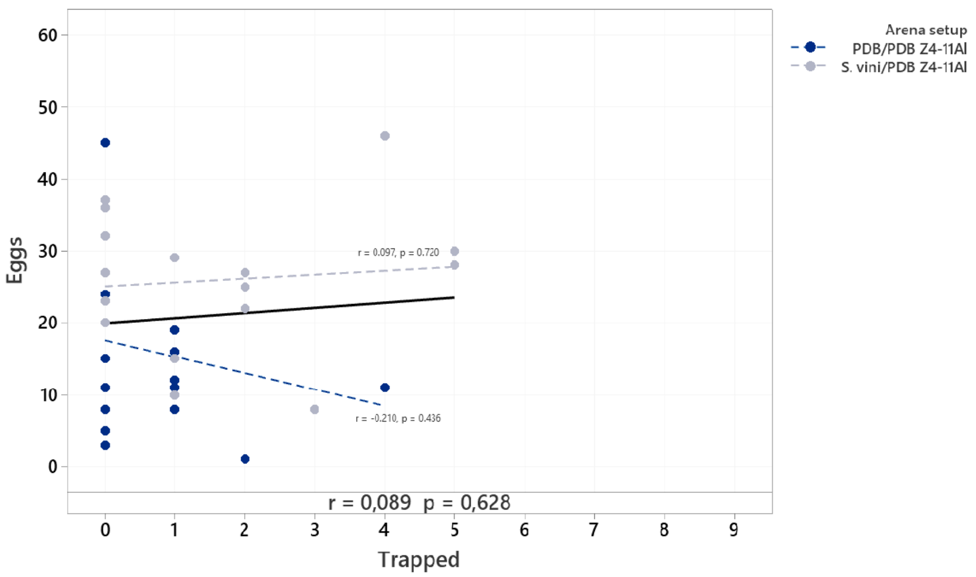


Figure 10 Number of eggs laid by Drosophila suzukii on blueberries with and without Z4-11Al present in cages. Separation by a dotted line means the experiments were performed on different days. Groups that differ significant are denoted with different letters.

There is no clear correlation between number of eggs laid and flies trapped. In general, the amount of oviposition did not decrease when the number of trapped flies increased. This was true for both within arena setups, see Appendix 3, and when looking at pooled data from arena setups with and without *Z*4-11Al respectively (Pearson correlation, α = 0.05, Figure 11a; r = 0.089, p = 0.628, Figure 11b; r = 0.110, p = 0.467).

a) Eggs laid in relation to number of trapped flies in setups with Z4-11Al



b) Eggs laid in relation to number of trapped flies in setups without Z4-11Al

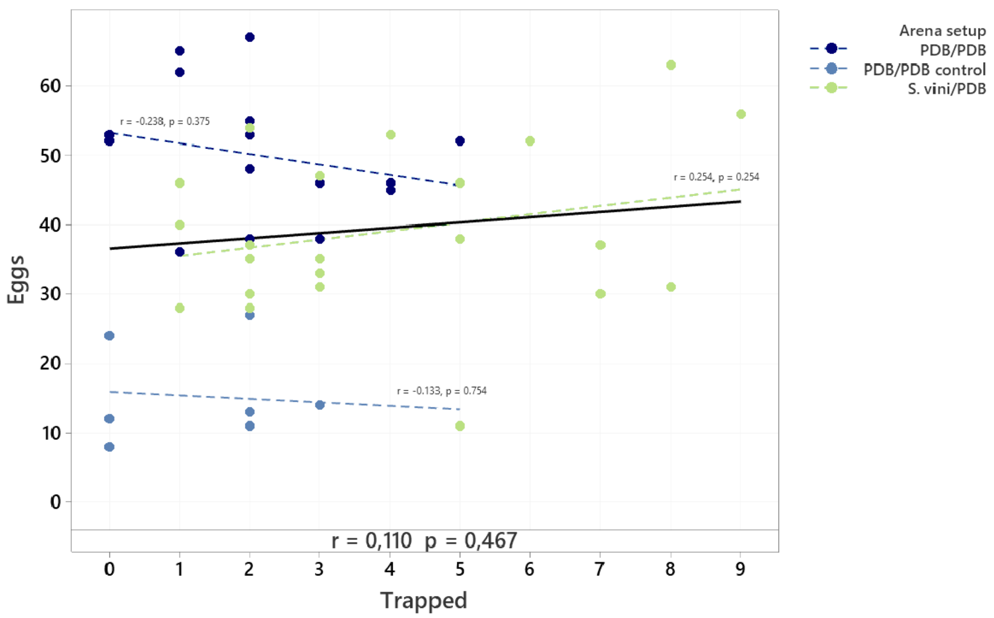


Figure 11 Number of eggs laid in relation to the number of trapped flies in arenas with (a) and without (b) Z4-11Al (Pearson correlation, α = 0.05). Solid line is correlation for pooled data.

3.3. Results in connection to fruit weight

Due to variation in the origin and cultivar of blueberries in the supermarket over time the goal of 0.10 g variation was hard to uphold between days (Figure 12). The fruit weight of blueberries used in PDB/PDB differs from the weight of blueberries in all other setups (Table 1), well above the set goal of 0.10 g difference. Mean berry weight also differs more than 0.10 g between the setups *S. vini*/PDB and *S. vini*/PDB *Z*4-11Al, with 0.75 g for the most extreme difference between individual berries.

No clear connection between fruit weight and the amount of oviposition could be seen, see Appendix 4. In arenas containing *Z*4-11Al no association between oviposition and fruit weight could be observed (Pearson correlation, $\alpha = 0.05$, $r = 0.001$, $p = 0.996$). A weak correlation can be seen when looking at egg lying with respect to fruit weight in arenas without *Z*4-11Al (Pearson correlation, $r = 0.424$, $p = 0.003$). It should however be kept in mind that this test contains data from setups done with weeks between them, see timeline in Figure 6, and with different blueberry cultivars.

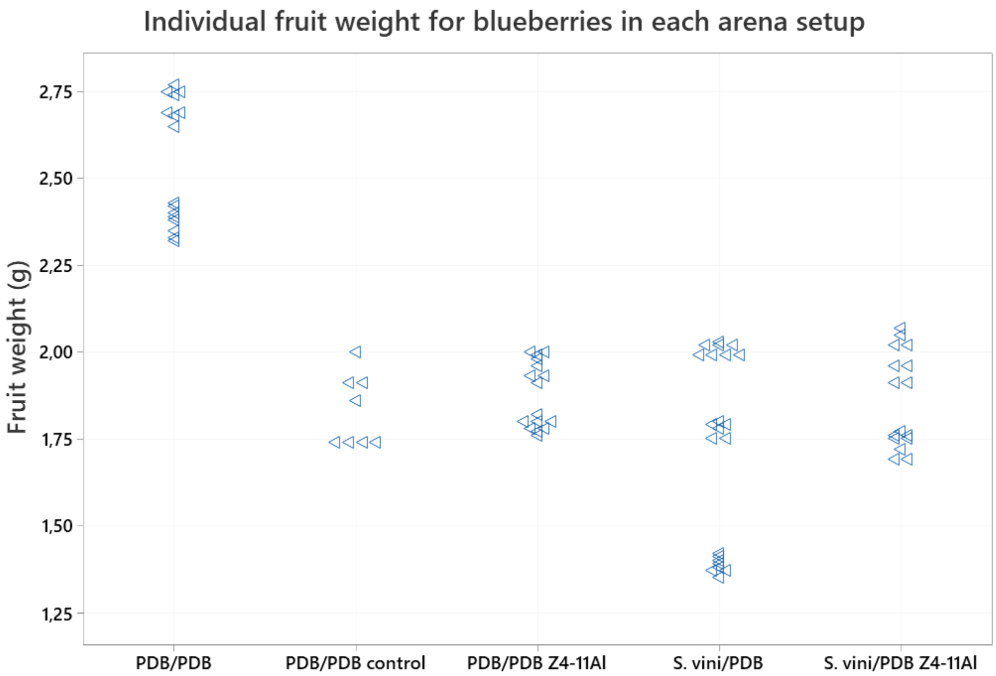


Figure 12 Weight of individual blueberries in the different arena setups.

4. Discussion

Finding a sustainable management strategy for the invasive pest *D. suzukii* is urgent and a push-pull system could contribute to pest control. This study set out with the aim to test if the yeast *S. vini* and the female *D. melanogaster* pheromone *Z*4-11Al have the potential to be used in a push-pull system against *D. suzukii*.

The trapping experiments demonstrated that *D. suzukii* clearly is attracted to *S. vini* and almost no flies were trapped in PDB in *S. vini*/PDB arenas indicating that *S. vini* as a bait was the main triggering factor for attraction. The results show that *S. vini* has potential for being used as a trap lure for *D. suzukii* and reasserts previous studies finding *S. vini* to be attractive (Bellutti et al. 2018; Rehermann n.d.).

New methods for controlling *D. suzukii* are desired because of its great damage potential. For these methods to be sustainable they should reduce the amount of insecticide needed and limit off-target effects. A push-pull system involves two components - ideally working synergistically by deterring the pest from the crop and luring it away with an attractant. In the context of this study this means *Z*4-11Al should work to deter *D. suzukii* from laying eggs, making the host plant less attractive and counteract any spillover-effect from traps. Insects can become desensitized to the push component and the pull component should work to mitigate this by offering an attractive alternative resource, in this case *S. vini* (Cook et al. 2007; Alkema et al. 2019). Under the tested conditions, *Z*4-11Al seems to have a supressing effect on trapping potential of *S. vini*, reducing the number of trapped *D. suzukii*. It might be that *Z*4-11Al made *D. suzukii* avoid the yeast. Whether this was the case would need further studying. When Kidera & Takahashi (2020) tested *D. suzukii* oviposition behaviour in a choice assay they found that *D. suzukii* preferred a yeast and *D. melanogaster* free substrate over a substrate with yeast but inoculated by *D. melanogaster*. The *D. suzukii* relative *D. simulans* lost attraction to yeast volatiles in presence of *Z*4-11Al (Lebreton et al. 2017). Presence of *D. melanogaster* is not favourable for *D. suzukii* larvae and having ways of avoiding competition means increasing offspring survival. *D. suzukiis'* lowered attraction to yeast might be similar to how the moth *Spodoptera littoralis* responded less to an attractive pheromone when combined with a volatile cue indicating the presence of a competitor on the host plant (Hatano et al. 2015).

The results on whether *Z*4-11Al had the desired suppression effect on oviposition by *D. suzukii* are not conclusive. Oviposition on blueberries in the setups that ran concurrently, PDB/PDB control and PDB/PDB *Z*4-11Al did not differ. There was significantly less egg laying in *S. vini*/PDB cages with *Z*4-11Al than in *S. vini*/PDB cages without *Z*4-11Al. These experiments were however performed on different days making it impossible to tell if the lower egg laying was caused by the *Z*4-11Al and *S. vini* presence or something else making egg laying differ over time. To rule out if ethanol was affecting *D. suzukii* behaviour a control with ethanol without pheromone could have been done. Egg laying was overall higher in experiments conducted between 23rd of February to 12th of March than in those conducted between 16th of March to 26th of March, see timeline for exact setups (Figure 6). Suggesting that egg laying declined over the duration of the study. Besides, the PDB/PDB control experiments were performed in a different lab than PDB/PDB *Z*4-11Al, though under similar laboratory conditions. This was done to avoid *Z*4-11Al interaction with flies in PDB/PDB control cages. Whether *Z*4-11Al could work as a push component needs further studying.

It can be argued that this lower egg laying in *S. vini*/PDB *Z*4-11Al setups was due to flies getting trapped before they had time to oviposit. But contrary to what might have been expected, ovipositing did not decrease with increased trapping. Since mated females increase yeast intake in the 24 h after mating (Mori et al. 2017) females would be expected to be attracted to the yeast – and get trapped – before choosing to oviposit. Female flies used in the experiments had been kept together with males for 5-7 days, allowing for mating. Caveat to this is that we do not know exactly when mating occurred, and it may have been longer than 24 h before they were introduced into the experiment arenas. Thus, the period for enhanced yeast feeding might have passed and flies came into cages ready for egg laying. To avoid this uncertainty, newly eclosed males and females could have been kept separated until the day they were to be used in testing, then controlled mating could have been done.

The number of eggs in blueberries could also be dependent on how long the flies were alive and not trapped. It is not known for how long the flies lived and if it was trapped or non-trapped fly/flies that laid eggs. Based on the mean number of eggs calculated to have been laid by non-trapped flies in *S. vini*/PDB arenas (Table 1) female flies probably lay eggs before getting trapped. *D. suzukii* females in this study could access the fruit multiple times during 24 h. When between 5-7 days old, *D. suzukii* females have been reported to be able to lay around 10 eggs per fly (Cai et al. 2019). It might not be very likely that the single surviving non-trapped fly laid all 56 eggs in the *S. vini*/PDB replicate. Though upwards of 40 eggs have been recorded being laid by a single fly, when presented with multiple blueberries (Kienzle et al. 2020). To know how flies behaved before getting trapped in yeast and for how long non-trapped flies that were dead after 24 h, had been active they would have to be observed in the cages.

It is known that susceptibility of fruits to *D. suzukii* differs depending on fruit cultivar and firmness, which determines the penetrative force needed to perforate the fruit skin thus influencing oviposition behaviour (Kinjo et al. 2013). Together with the variation in blueberry cultivar and weight, these factors might have influenced results between experiments conducted on different days. Weight and the diameter of the host fruit can affect the amount of oviposition (Poyet et al. 2015). In the current study, the same ripeness was prioritised over maintaining the same fruit weight between experiments running on different days. Ripening stage affects egg laying behaviour in *D. suzukii*, *D. suzukii* is less interested in immature and overripe berries (Lee et al. 2011) and lay more eggs on ripe blueberries than on rotten fruit (Cai et al. 2019). Blueberries soften when ripening and green immature fruits are substantially firmer than later development stages (Chea et al. 2019). By ensuring that all berries used were ripe, but not over ripe, they should all have been in the for *D. suzukii* preferred range and more uniform in firmness than if not controlled. If variation in fruit weight was the dominant fruit characteristic affecting egg laying behaviour of *D. suzukii* in this study is not clear. The weak correlation between the number of eggs laid and fruit weight in arenas without *Z*4-11Al should be interpreted with caution, considering that egg laying in general seems to have declined over the course of the study. PDB/PDB control experiments were done in the end of Mach and PDB/PDB and *S. vini*/PDB experiments were the first to be performed. This also means that the cultivar used was not the same in these setups. In multiple cases an identical number of eggs were laid on blueberries differing in weight, see Appendix 4. Either way, to minimize the effect on the results by a factor not planned to be studied, future experiments should use berries of the same weight. Firmness and penetration force needed to pierce the skin might also be measured. In addition, a major improvement to this study would be having a control run concurrent with all setups. Further improvements could be observing the behaviour of *D. suzukii*, specifically, if they oviposit before being trapped and at which point they get trapped.

S. vini, being highly attractive for *D. suzukii*, has great potential for working as a trap lure. Yeast can work as a phagostimulant reducing the insecticide dose needed for killing. This could be used as an attract and kill management component in the push-pull system. Besides the reduced dosage needed, there is the advantage of insecticide being kept inside the trap hopefully reducing the risk of non-target effects and resistance developing (Sarkar et al. 2020). It also might protect against the weather; rain reduces the effect of some insecticides (Van Timmeren & Isaacs 2013).

Improving trapping results in the field could potentially be done by employing complementary management tactics, such as removing old fruit from the field. This practise improved the number of females with mature eggs being trapped (Swoboda-Bhattarai et al. 2017). Removing fermenting and rotting fruits ties in with the preference for yeast in mated *D. suzukii* females (Mori et al. 2017). This highlights the importance of understanding the complexity of *D. suzukii* behaviour in devising sustainable management practises.

To definitively answer the question if *S. vini* and *Z*4-11Al could be used together in a push-pull system for *D. suzukii* further studies are needed. Tests to investigate the mechanisms of the system could be done by separately testing the push and pull components for their specific effect on egg laying in two choice assays. It would also be interesting to look into whether egg laying and trapping happens before or after the content of the *Z*4-11Al dispenser has completely evaporated. Having longer distance between the traps and the *Z*4-11Al dispenser would make for a more realistic setting. Maybe with push on one side and pull on the other with an oviposition substrate running in between. *Z*4-11Al lowering trapping might not be a problem if efficient enough in deterring from oviposition. In the end it would be down to how the tested components work in a field setting. Finding a sustainable way of deterring *D. suzukii* from laying eggs on crop plants would be a big step in pest management of this invasive species.

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Hedda Andersson
12th of August 2021

Appendix 1 – Cages and trap positions

BugDorme cages placed four by four on tables, surrounded by white curtains. (Andersson 2021)

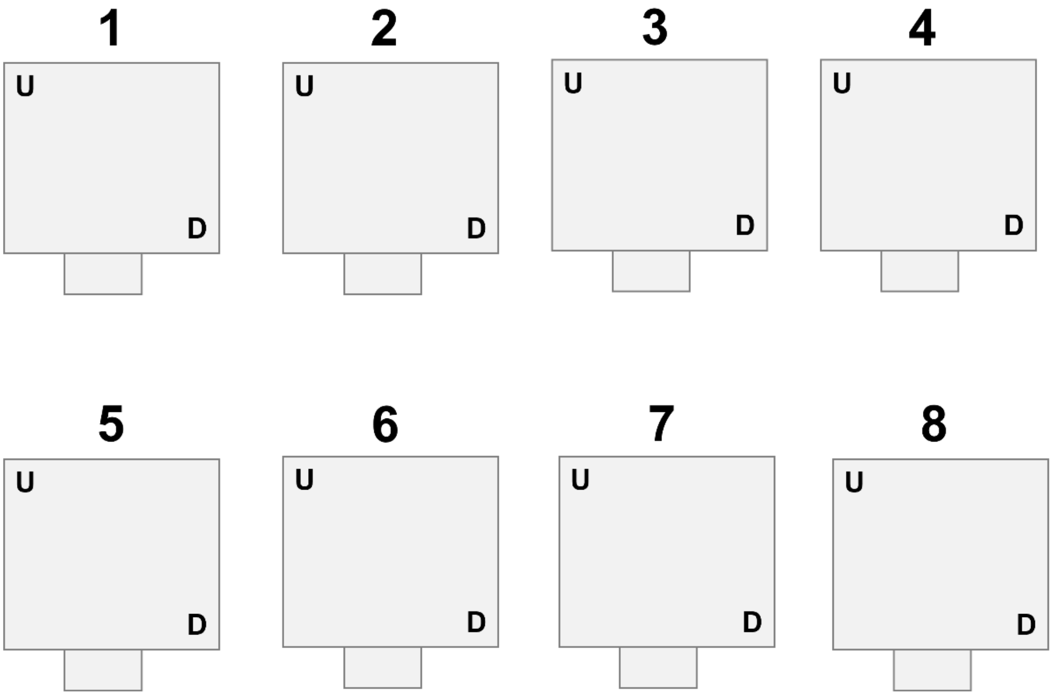


Illustration of the cage placement, U (up) and D (down) denotes placement of traps. (Andersson 2021)

Appendix 2 – Arena setup

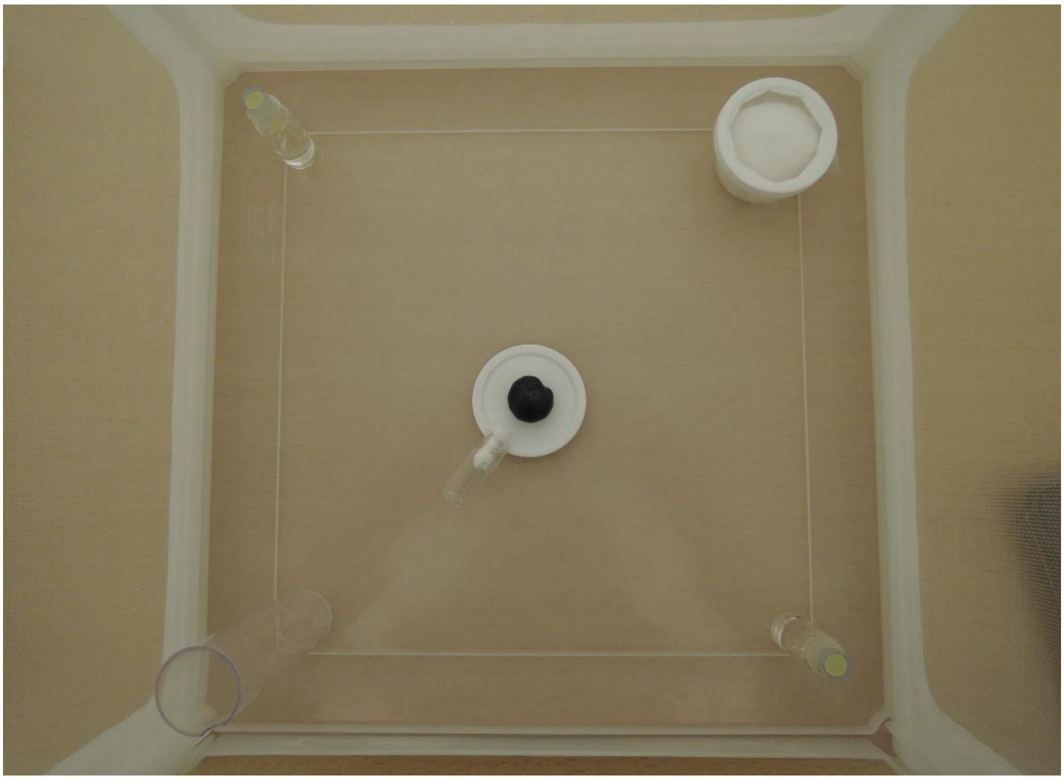


Photo of arena setup with Z4-11Al vial and fly release vial in left bottom corner. (Andersson 2021)

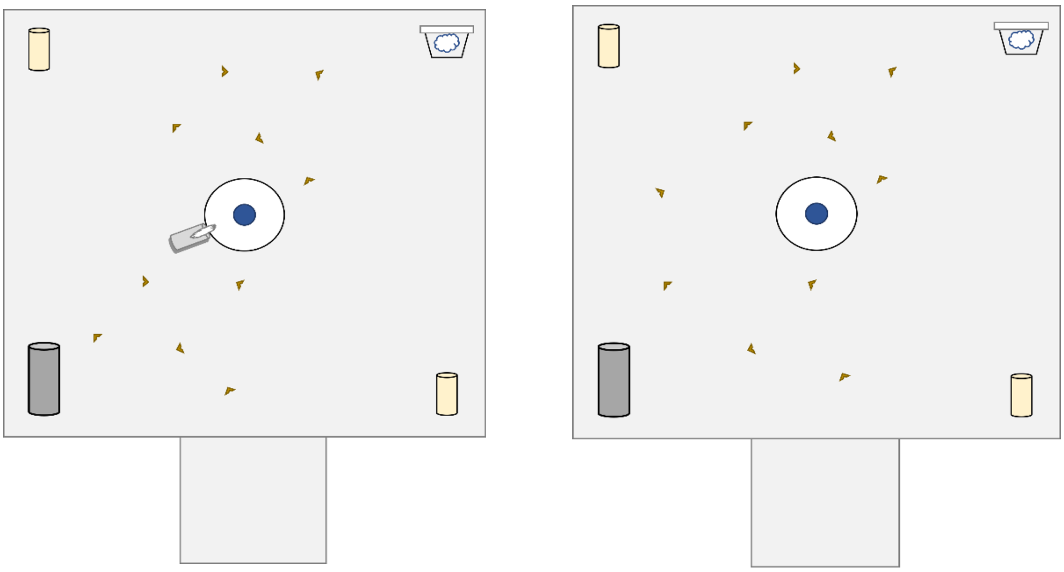
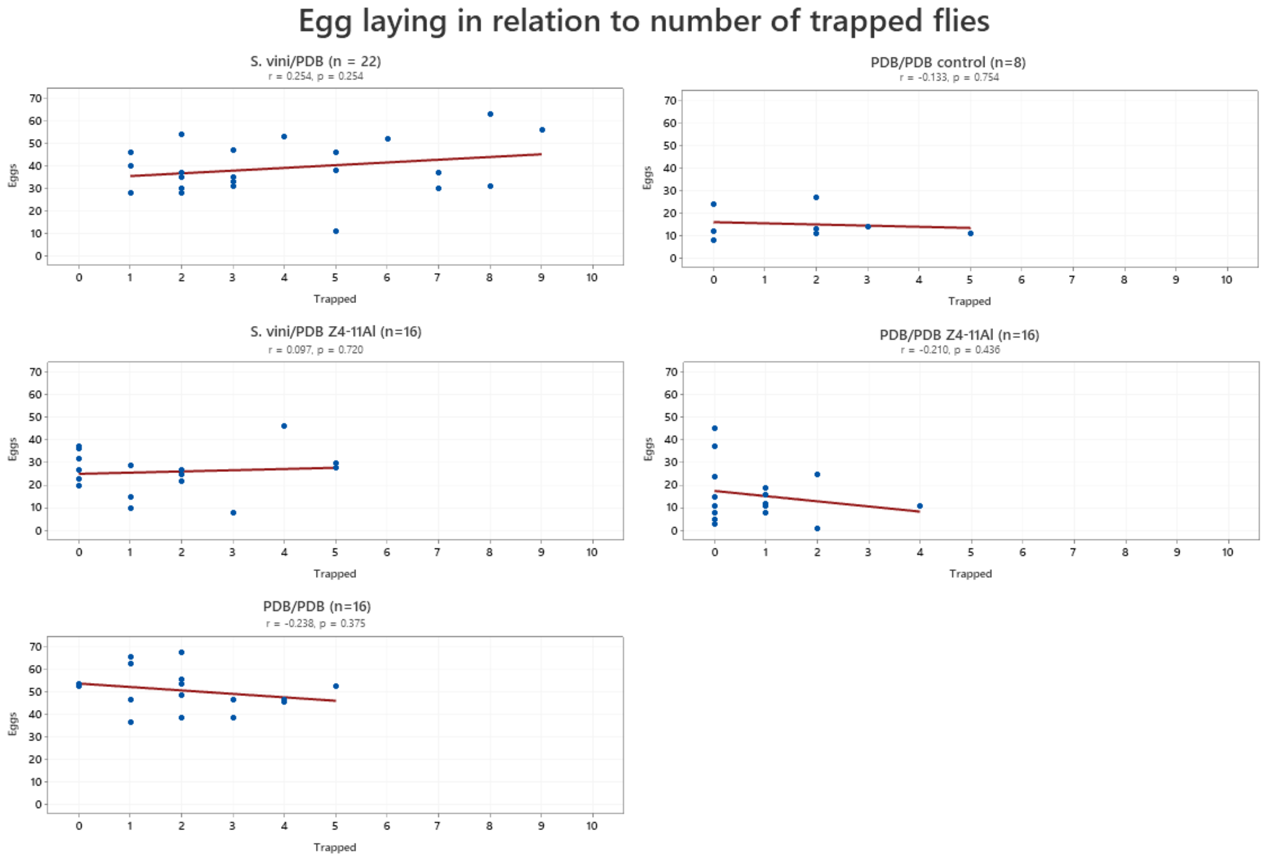


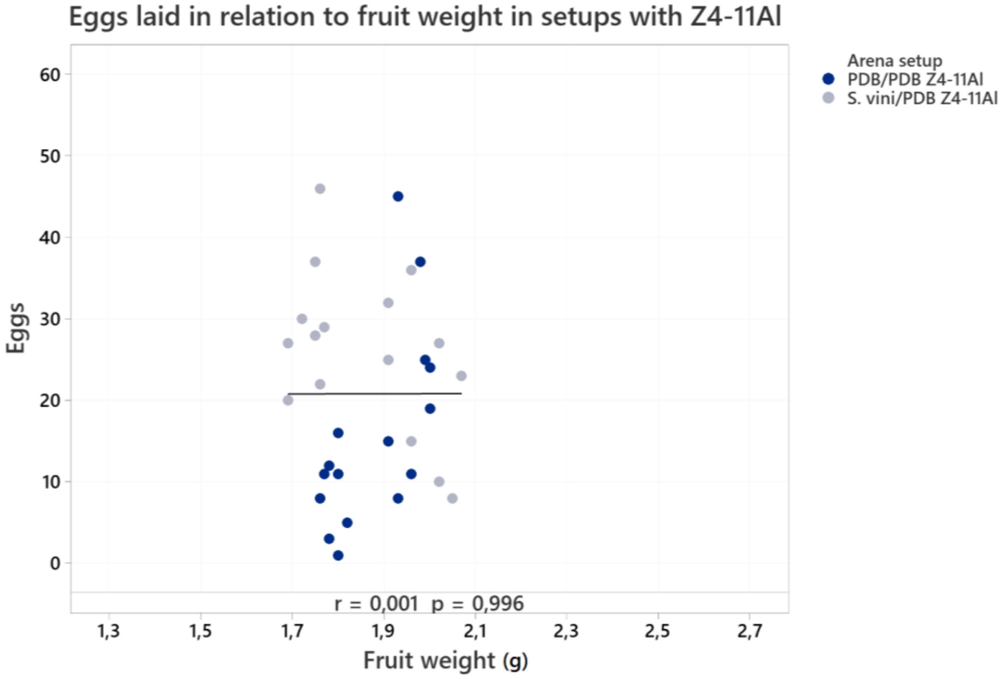
Illustration of arena setups with (left) and without Z4-11Al vial (right). (Andersson 2021)

Appendix 3 – Oviposition and trapped flies

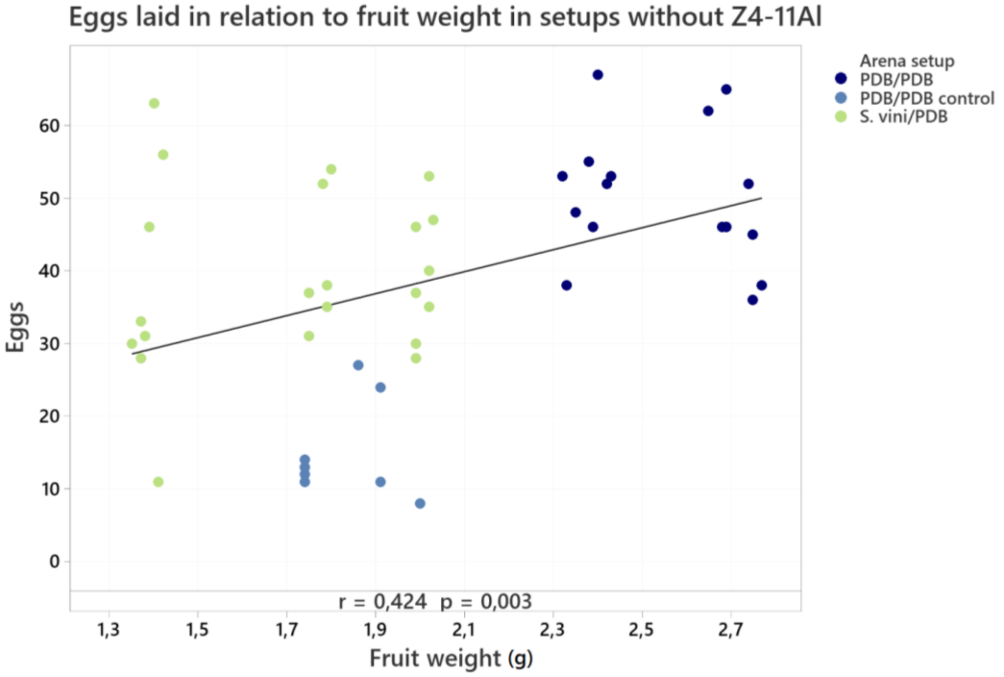


Number of eggs laid on individual blueberries in relation to total number of trapped flies in the cage in which it was placed (Pearson correlation, α = 0.05).

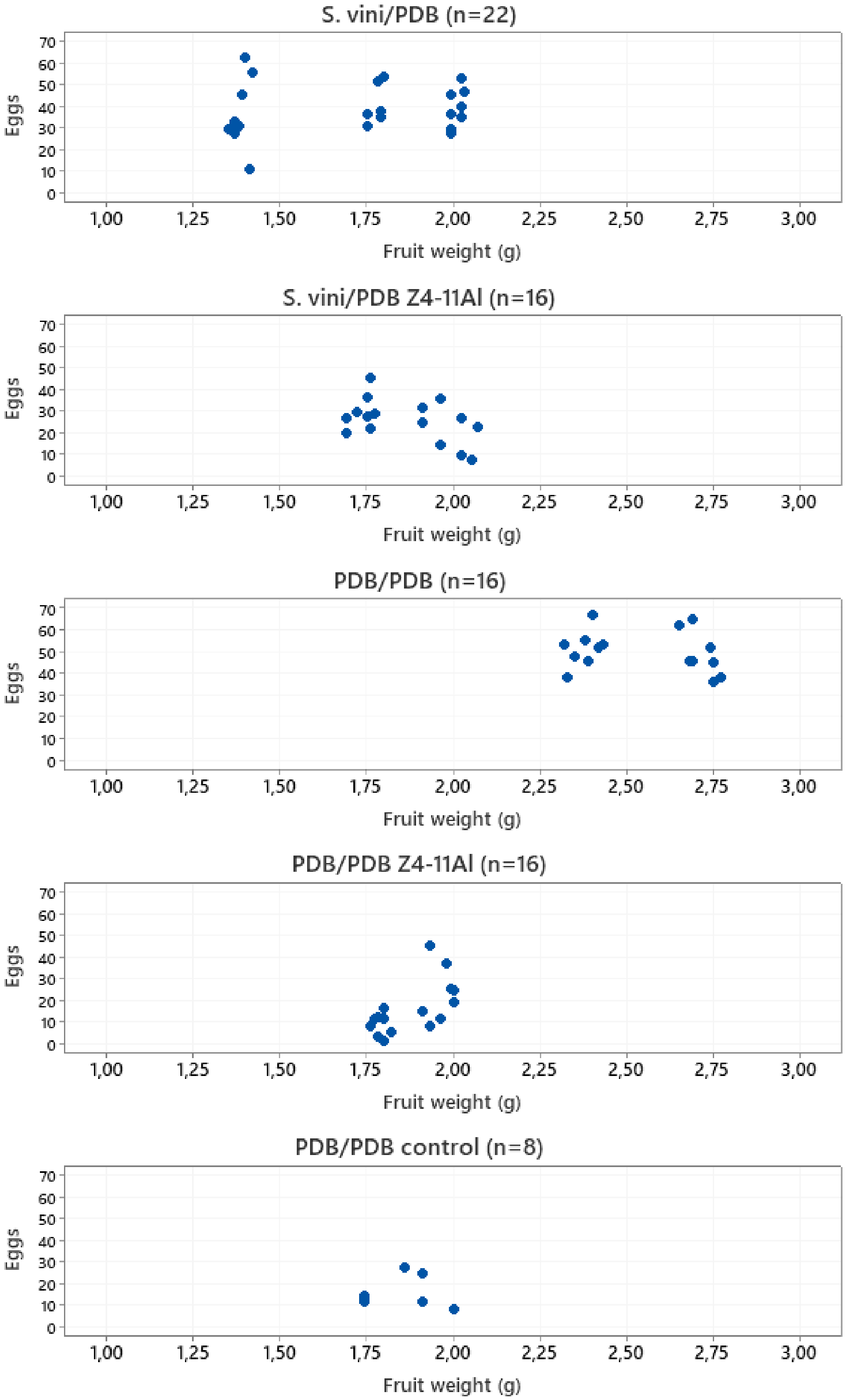
Appendix 4 – Oviposition and fruit weight



Eggs laid in setups with Z4-11Al in relation to fruit weight (Pearson correlation, α = 0.05).



Eggs laid in setups without Z4-11Al in relation to fruit weight (Pearson correlation, α = 0.05).



Number of eggs laid on individual berries in relation to the fruit weight.