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ORIGINAL CONTRIBUTION

Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol

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Abstract

Recommendations for monitoring spotted wing drosophila (SWD) *Drosophila suzukii* (Matsumura) are to use either vinegar or wine as a bait for traps. Traps baited with vinegar and traps baited with wine, in field tests in northwest Oregon, captured large numbers of male and female SWD flies. Numbers of SWD trapped were significantly greater with a mixture of vinegar and wine compared with vinegar alone or wine alone. Attraction of SWD to vinegar and wine may be due in part to responses to acetic acid and ethanol evaporated from the two baits, respectively. Numbers of SWD captured in traps baited with 2% acetic acid in water were significantly greater than in unbaited traps, indicating a fly response to acetic acid. Very few flies were captured in traps baited with 10% ethanol in water. Traps baited with a combination of acetic acid and ethanol in water captured more SWD flies than traps baited with acetic acid or ethanol solutions alone. These results indicate a synergy of the two materials, and of the two chemicals, as lures for SWD. A comparison of a mixture of acetic acid with ethanol in water versus a mixture of vinegar with wine showed stronger fly attraction to the vinegar/wine mixture, indicating potential attractiveness of vinegar and wine volatiles in addition to acetic acid and ethanol.

Introduction

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), is widely distributed in temperate and subtropical Asia (Delfinado and Hardy 1977) and has recently been found in the eastern United States and western North America (Steck et al. 2009; Walsh 2009; Beers et al. 2010). The fly is of concern to fruit growers because of its ability to attack ripening soft fruits. It is considered to be a threat to the production of berries, cherries, grapes and other fruits.

Presently, programmes are in place to detect and monitor the presence and relative abundance of the fly throughout much of the fruit production regions of California, Oregon, and Washington of the United States, and British Columbia, Canada, to determine the presence and rate of spread of SWD, as well as to assess its threat to the fruit production industries in these areas. As a part of these programmes, traps are maintained following recommended trap and bait designs and trapping protocols. These recommendations include the use of apple cider vinegar or grape wine as the bait in traps (Steck et al. 2009; Beers et al. 2010). The trapping of male and female SWD with baits may have potential for management or control of SWD through attract and kill or mass trapping approaches, as shown by Kanzawa (1934). Although both bait types in use are successful in luring SWD to traps, improvements to the lure might improve detection efforts if those improvements significantly increase trap sensitivity and might improve trap efficacy in 'trap out' or mass trapping attempts for control of fly populations. Identification

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of chemicals that are attractive to SWD would also provide opportunities to develop optimized lures with controlled release dispensers that could be used in dry traps.

The search by insects for mates, food sources and oviposition sites often involves attraction responses to semiochemicals such as pheromones, kairomones and feeding attractants. Sex pheromones of *Drosophila* flies are involved in courtship-type interactions (often referred to as aphrodisiac pheromones) (Antony and Jallon 1982; Nemoto et al. 1994; Oguma et al. 1992), or short-range attraction as demonstrated in olfactometer and flight tunnel assays (often referred to as aggregation pheromones because of the responses of both sexes (Bartelt et al. 1985a,b; Schaner et al. 1987). There are no *Drosophila* pheromones reported to be attractants useful as lures in traps, and no pheromones have been identified for SWD.

Volatiles from food baits can attract *Drosophila* spp. flies to food, mates and oviposition sites, or combinations of these functions. In some *Drosophila* species, fly response to aggregation pheromone may be enhanced by the presence of food materials (Landolt 1997), and flies may feed at the same sites that serve as oviposition opportunities. Some species of *Drosophila* can be baited with human food materials such as vinegar (hence the common name vinegar fly) and fermenting fruits that can be presented as juices and purees in traps. *Drosophila melanogaster* Meigen and other *Drosophila* spp. are readily attracted to and trapped with fermented fruits. For example, Brito da Cunha et al. (1951) used cut up bananas in plastic cups to evaluate the influence of yeasts on bait attractiveness to *Drosophila* spp. Barrows (1907) tested a number of fermenting fruit volatiles as attractants for adult *Drosophila ampelophila* Loew in a laboratory assay and found consistent strong responses to ethanol and to acetic acid, and less so to amyl alcohol, lactic acid and acetic ether. Barrows (1907) also found that acetic ether, isobutyl acetate and methyl acetate increased *D. ampelophila* response to ethanol. Hutner et al. (1937) used an indoor release and trap bioassay system to show responses of *D. melanogaster* to several compounds, but concluded that they are weak in response to a corn meal-yeast control bait. Reed (1938) demonstrated *D. melanogaster* fly attraction to ethanol and to acetic acid in a laboratory olfactometer. West (1961) used olfactometer assays, baited traps tested indoors and traps placed in the field to evaluate materials and chemicals attractive to *D. melanogaster* and *Drosophila virilis* Sturtevant. *Drosophila melanogaster* was attracted in laboratory cage tests to a blend of ethanol, acetic acid and 2-phenylethanol isolated from odours of overripe mango fruits (Zhu et al. 2003).

We are not aware of published experimental findings of *D. suzukii* (SWD) attraction to or trapping with pheromones, food baits or chemicals derived from food baits, although they clearly respond to survey traps baited with vinegar or wine. As indicated by results of studies on *D. melanogaster* and *D. ampelophila*, we hypothesize that acetic acid and ethanol probably play significant roles as attractants resulting in arrival of SWD at traps baited with vinegar or wine.

We evaluated SWD responses in the field to the trap baits vinegar and wine, and to the two chemicals acetic acid and ethanol, which are major volatile components of those baits respectively. Experiments tested the hypotheses that (i) both sexes of the fly are attracted to vinegar, wine, acetic acid and ethanol, (ii) that vinegar and wine are additive or synergistic in attracting SWD, (iii) that acetic acid and ethanol are additive or synergistic in attracting SWD, (iv) that much or all of the fly response to vinegar is because of the presence of acetic acid and (v) that much or all of the fly response to wine is because of the presence of ethanol. Results of these experiments suggest methods to enhance the power of trapping systems for detection of SWD, and additional hypotheses for the pursuit of further improvements to SWD lures.

Materials and Methods

All experiments were conducted near Salem, Marion County, Oregon in areas of abundant roadside blackberry (*Rubus* spp.). Two types of traps, dome and cup, were used in these tests. The Agrisense Dome trap (dome; Agrisense, BCS Ltd., UK) is similar to the McPhail trap in design (Newell 1936). It is opaque yellow on the bottom 1/3, clear on the top 2/3, and with an invaginated 5-cm-diameter opening at the bottom that forms a reservoir in the trap to hold a liquid bait or drowning solution. The cup trap (figured by Beers et al. 2010) is a 946-ml clear plastic drinking cup with a clear plastic lid (Solo, Urbana, IL), with four 1-cm-diameter holes in the sides of the cup near the top. A drowning solution or drowning solution plus bait was placed within the cup. Forty grams of boric acid and 0.5-ml liquid dishwashing soap (Palmolive Pure and Clean Spring Fresh Dishwashing Soap; Colgate-Palmolive Company, New York, NY, USA) were added to a batch of 3.8 l of water or aqueous bait to make a drowning solution used in all traps. Boric acid served to inhibit microbial activity of baits and captured insects, and soap reduced surface tension to promote capture and submersion of trapped insects. For both types of traps and all treatments in all experiments, each trap contained either 300 ml of drowning solution or 300 ml of bait in drowning solution. Traps were placed 10 m apart, in replicated blocks. Blocks of traps were at least 20 m apart. Traps were randomized within treatment blocks when placed in the field.

Synergy of vinegar and wine

This experiment tested the three hypotheses that vinegar is attractive, wine is attractive, and the two materials are additive or synergistic in attracting SWD. Dome traps were used in this experiment. Trap bait treatments were (i) drowning solution, (ii) apple cider vinegar (Safeway Brand, Apple Cider Vinegar, 5% acetic acid) diluted to make 2% acetic acid, (iii) a red grape wine (Carlo Rossi Reserve Merlot) diluted to make 7.2% ethanol and (iv) a 40 : 60 ratio of apple cider vinegar and red grape wine. The ethanol content of the wine was 12%, and the acetic acid content of the vinegar was 5%. The vinegar was diluted as two parts vinegar to three parts water to achieve 2% acetic acid content. The 40 : 60 mixture of the diluted vinegar and the wine provided 2% acetic acid and 7.2% ethanol in a solution as the combination treatment. Twelve replicate blocks were placed in the field and were maintained for only 4 days because of the very high numbers of flies trapped per day.

Synergy of acetic acid and ethanol in drowning solution

This experiment tested the three hypotheses that acetic acid is attractive, ethanol is attractive, and the two compounds are coattractive or synergistic in attracting SWD flies. The dome trap was used for this test. The four trap bait treatments were (i) drowning solution, (ii) 2% acetic acid in the drowning solution, (iii) 10% ethanol in the drowning solution and (iv) 2% acetic acid plus 10% ethanol in the drowning solution as a combination treatment. The 2% acetic acid solution was made by adding 80 ml of glacial acetic acid to 3920 ml of water, for each 4 l batch. The 10% ethanol solution was made by adding 400 ml ethanol to 3600 ml water for each 4 l batch. The same amounts of acetic acid (80 ml) and ethanol (400 ml) were both added to 3520 ml of the drowning solution to make batches of the combination treatment.

Six blocks of the four treatments were set up in the field. Traps were checked and bait solutions were replaced every week. Catch data for each trap were summed for the 7-week period of the test to provide six replicates.

Synergy of acetic acid and ethanol dispensed from vials

This experiment also tested the hypotheses that acetic acid is attractive, ethanol is attractive, and the two compounds released together are coattractive or synergistic in attracting SWD. The dome trap was used, with the drowning solution described above, but the chemicals tested were dispensed from 15-ml polypropylene vials with a 3-mm-diameter hole for the acetic acid and a 6-mm-diameter hole for ethanol and drilled into the vial lid. This vial-controlled release system was effective as a dispenser for acetic acid with 3-methyl-1-butanol as a lure for several noctuid moths (Landolt and Alfaro 2001), and as a lure for acetic acid with isobutanol as a lure of *Vespula* wasps (Landolt et al. 2005). Chemicals were applied to cotton balls at the bottom of the vial. Vial(s) were suspended by wire inside of the top of the dome trap. The four experimental treatments were as follows: (i) no attractant, (ii) 10 ml acetic acid in a vial, (iii) 10 ml ethanol in a vial and (iv) the combination of 10 ml of acetic acid in a vial and 10 ml ethanol in a second vial.

Six block replicates of the four treatments were set up in the field. Traps were checked and drowning solutions were replaced each week, and vials were replaced every 2 weeks. Catch data for each trap were summed for the 4-week test period to provide the six replicates.

Comparison of acetic acid/ethanol mixture versus vinegar/wine mixture

The objective of this experiment was to determine how much that SWD response to acetic acid and ethanol accounts for SWD response to vinegar and wine respectively. Mixtures of the two chemicals and of the two materials were tested as part of the drowning solution of the dome trap. Experimental treatments were as follows: (i) control with no chemical lure or bait, (ii) a 40 : 60 mixture of apple cider vinegar and red grape wine and (iii) 2% acetic acid and 7.2% ethanol. Ten replicate blocks of the three treatments were set up in the field. Traps were checked and maintained for 1 day, because of very high numbers of flies captured.

Comparison of trapping systems

This experiment compared the dome trap and cup trap, using an optimum bait from this study compared to a bait currently recommended for use to detect and monitor SWD. One of the current recommendations is to use diluted vinegar in a trap made of a 946-ml clear plastic disposable drinking cup and lid, with four equally spaced 1-cm-diameter holes in the side of the cup near the top for entry of flies (Beers et al. 2010). The bait is placed in the drinking cup, functioning also as a drowning solution. The four experimental treatments were as follows: (i) the cup trap baited with vinegar, (ii) the cup trap baited with a mixture of vinegar and wine, (iii) the dome trap baited with vinegar and (iv) the dome trap baited with a mixture of vinegar and wine. The vinegar placed in traps for treatments 1 and 3 was diluted to 40% vinegar in water (to constitute 2% acetic acid in the solution). The mixture of vinegar and wine for treatments 2 and 4 was 40 : 60 to make the mixture 2% acetic acid and 7.2% ethanol.

Twenty replicate blocks of the four treatments were set up in the field. Traps were maintained for 5 days. Catch data for each trap were summed for the 5-day period to provide the 20 replicates.

Data were analysed by analysis of variance (ANOVA) for a complete randomized block design. Counts were square root square root transformed before analysis (Steel and Torrie 1960). In those assays in which combinations of compounds or materials were not examined (e.g. comparison of trap types), treatments were compared by Tukey's Honestly Significant Difference (HSD) Test, following a significant F-value from a one-way ANOVA (DataMost 1995). To examine whether combinations of compounds acted synergistically, we analysed the data as a 2 × 2 factorial (compound A [present or absent] × compound B [present or absent]) ANOVA. A significant interaction in the ANOVA would be evidence of synergistic or antagonistic effects. Analyses were carried out using PROC MIXED in SAS (SAS Institute 2002). Some specific comparisons of interest in these analyses were made with LSD tests using the LSMEANS and PDIFF options in PROC MIXED.

Results

Synergy of vinegar and wine

Very few flies were captured in control traps. For both sexes of SWD, numbers of flies were significantly greater in traps baited with vinegar (males: $F_{1,33} = 26.3$, $P < 0.0001$; females: $F_{1,33} = 27.3$, $P < 0.0001$) or wine (males: $F_{1,33} = 9.6$, $P = 0.004$; females: $F_{1,33} = 10.4$, $P = 0.003$) than in traps without these compounds. Large numbers of flies were captured in traps baited with the combination of vinegar and wine. A test of the interaction term with ANOVA indicated that the two products acted additively rather than synergistically (vinegar × wine: $F_{1,33} < 1.0$, $P > 0.40$ for both sexes). For both males and females, numbers trapped with vinegar alone were numerically higher (Table 1), but not significantly greater, compared with traps baited with wine alone (males: $t_{33} = 1.4$, $P = 0.16$; females: $t_{33} = 1.4$, $P = 0.17$).

Synergy of acetic acid and ethanol in drowning solution

There were nearly no SWD flies in unbaited traps, few in ethanol-baited traps, and numerous males and females in traps baited with the other two treatments (Table 2), with the acetic acid and ethanol as part of the trap drowning solution. There was strong statistical evidence that the two compounds acted synergistically in capturing male SWD (acetic acid × ethanol: $F_{1,15} = 6.6$, $P = 0.02$) and weaker evidence for the same effect for females (acetic acid × ethanol: $F_{1,15} = 3.8$, $P = 0.07$). Numbers of flies in traps baited with acetic acid were significantly higher than in unbaited traps (males: $t_{15} = 3.2$, $P = 0.006$; females: $t_{15} = 3.7$, $P = 0.002$), but numbers of flies in traps baited with ethanol were not significantly greater than in unbaited traps ($P > 0.30$ for both sexes).

Synergy of acetic acid and ethanol dispensed from vials

Few SWD flies were captured in unbaited traps or traps baited with ethanol dispensed from vials (Table 3). There was no evidence for synergism between acetic acid and ethanol for either sex (acetic

Table 1 Mean (±SE) numbers of male and female SWD flies captured in dome traps baited with vinegar, wine or a mixture of vinegar and wine, used as the drowning solution for the trap

	Control	Vinegar	Wine	Combination
Male SWD	2.4 ± 1.1	1141.8 ± 598.2	486.4 ± 195.2	2120.3 ± 811.4
Female SWD	2.3 ± 1.1	906.5 ± 498.0	401.8 ± 185.0	1319.3 ± 575.3

Table 2 Mean (±SE) numbers of male and female SWD flies captured in dome traps baited with 2% acetic acid (AA), 10% ethanol (ETH) or a mixture of 2% acetic acid and 10% ethanol as part of the drowning solution of the trap

	Control	AA	ETH	AA + ETH
Male SWD	0.2 ± 0.2	16.2 ± 9.4	0.8 ± 0.4	54.2 ± 11.3
Female SWD	0.2 ± 0.2	13.8 ± 7.5	1.8 ± 0.9	42.2 ± 7.9

Table 3 Mean (±SE) numbers of male and female SWD flies captured in dome traps baited with acetic acid (AA) in a vial, ethanol (ETH) in a vial or the combination of acetic acid in a vial and ethanol in a vial

	Control	AA	ETH	AA + ETH
Male SWD	1.2 ± 0.4	30.2 ± 18.1	1.3 ± 0.8	55.8 ± 26.0
Female SWD	0.7 ± 0.5	24.3 ± 11.3	0.5 ± 0.3	42.5 ± 14.7

acid × ethanol: $F_{1,15} < 1.5$, $P > 0.25$ for both sexes). Ethanol had no effects on capture rates (ethanol main effect: $F_{1,15} < 1.5$, $P > 0.30$ for both sexes). Numbers of flies were significantly higher in traps baited with acetic acid compared with traps fee of acetic acid (acetic acid main effect: $F_{1,15} = 21.1$, $P = 0.0004$ for males; $F_{1,15} = 37.9$, $P < 0.0001$ for females).

Comparison of acetic acid/ethanol mixture and vinegar/wine mixture

Numbers of male and female SWD in traps baited with solutions of acetic acid plus ethanol, or with vinegar plus wine, were significantly greater than in unbaited traps (Table 4). For both sexes, numbers in traps baited with vinegar plus wine were more than 10 times higher than in the traps baited with acetic acid plus ethanol. Nearly no SWD flies were captured in unbaited traps. ANOVA $F = 84.85$, $P = 7.6 \times 10^{-13}$ for males; $F = 104.96$, $P = 5.3 \times 10^{-14}$ for females.

Table 4 Mean (±SE) numbers of male and female SWD flies captured in dome traps baited with a mixture of vinegar and wine, or a mixture of acetic acid and ethanol, used as the drowning solution for the trap[1]

	Control	Vinegar + Wine	Acetic acid + Ethanol
Male SWD	0.5 ± 0.4a	145.8 ± 23.7c	13.2 ± 3.5b
Female SWD	0.5 ± 0.3a	95.6 ± 13.1c	9.0 ± 2.2b

[1]Means in a row followed by the same letter are not significantly different by Tukey's HSD test.

Table 5 Mean (±SE) numbers of male and female SWD flies captured in cup traps or dome traps baited with either vinegar or a mixture of vinegar and wine used as the drowning solution for the trap[1]

	Cup trap vinegar	Cup trap vinegar/wine	Dome trap vinegar	Dome trap vinegar/wine
Male SWD	415.4 ± 90.0a	937.3 ± 241.2b	1249.8 ± 331.4bc	2088.7 ± 424.4d
Female SWD	206.7 ± 41.6a	460.9 ± 122.1b	586.5 ± 167.0bc	1135.7 ± 233.1cd

[1]Means in a row followed by the same letter are not significantly different by Tukey's HSD test.

Comparison of trapping systems

For both cup and dome types of traps, the numbers of male and female SWD captured were greater in traps baited with vinegar plus wine, compared with vinegar alone (Table 5). For both the vinegar and vinegar plus wine types of baits, the numbers of male and female SWD captured were greater in dome traps compared with cup traps (Table 5). ANOVA $F = 6.58$, $P = 0.0005$ for males; $F = 7.61$, $P = 0.0002$ for females.

Discussion

We interpret the capture of flies in traps to be the likely end result of oriented or directed flights by the flies to the odour source which is inside the trap. In this sense, we refer to the capture of flies as indicating an attraction response. Vinegar and wine are recommended as baits for trapping SWD (Beers et al. 2010), and our results experimentally demonstrated and confirmed the attraction of SWD to vinegar and to wine, with the trapping of large numbers of SWD in traps with either of those materials, and in comparison to very few flies in un-baited traps. Numerically more flies were trapped with vinegar than with wine. However, this difference was not statistically supported. Vinegar is an attractant (Becher et al. 2010) and trap bait for *D. melanogaster*, which also can be trapped with wine (Reed 1938). The combination of vinegar and wine attracted greater numbers of SWD into traps compared with vinegar or wine alone. Use of this combination of food materials in place of the recommended baits of apple cider vinegar or grape wine (Beers et al. 2010) would provide a significant increase in the power of a trap-bait system.

These experiments demonstrated attraction of both male and female SWD to acetic acid, but not to ethanol. This finding was obtained when the chemicals were placed in the drowning solution of the trap, or when the chemicals were dispensed from vials as a controlled release device from which the compound evaporated and diffused. We are not aware of prior experimental demonstrations of attraction (including trapping) of SWD to these chemicals, although the recommended uses of vinegar and wine as baits suggest the possibility of SWD attraction to acetic acid and ethanol, which are principal volatile chemical components of vinegar and wine, respectively. However, additional studies should be conducted over a range of concentrations and release rates of both chemicals to clarify the optimum release conditions for both chemicals as SWD attractants. Such a study would permit a direct comparison between an optimum acetic acid concentration (in the trap drowning solution) or release rate (from a dispenser) in comparison with a concentration or release rate of ethanol. *Drosophila melanogaster* is also attracted to acetic acid, and to ethanol, as demonstrated in laboratory assays by Dethier (1947). SWD attraction to the combination of acetic acid and ethanol was considerably greater than their responses to either chemical presented alone. Trapping SWD with the combination of acetic acid and ethanol should be superior to trapping with either chemical alone.

The fly response to the combination of vinegar and wine was greater than the response to acetic acid or the combination of acetic acid and ethanol. This finding indicates that other volatile chemicals emitted by vinegar and wine in addition to acetic acid and ethanol may also be attractive to male and female SWD flies. Further study is needed to determine whether the greater attractiveness of that combination of baits is because of volatile chemicals in the vinegar, to volatile chemicals in the wine, or both. Determination of respective roles of vinegar and wine volatiles in the SWD attraction responses will facilitate the isolation and identification of additional attractants from these materials. Identification of a more complete synthetic chemical attractant from such baits might provide a more powerful lure to potentially replace the use of vinegar or wine as a trap bait. A chemical lure provided from a controlled release dispenser should be more stable in attractiveness over time, compared with a likely large variance in the release of attractive volatiles from solutions of vinegar or wine over time. An additional advantage of a chemical blend as an attractant would be the opportunity to develop and use attractant dispensers in dry traps, which are often easier to service than wet traps, and to develop a more selective lure that attracts fewer non-target insects. However, it appears that the combination of acetic acid and ethanol is weak in comparison with the combination of vinegar and wine and is not recommended as a suitable lure or bait replacement.

Insect feeding attractants are usually not specific, and other species of 'non-target' insects are often captured in traps baited with feeding attractant lures. For example, the combination of acetic acid and 3-methyl-1-butanol, developed as a feeding attractant for pest species of cutworms and armyworms (Landolt 2000), also attracts many other species of moths (Landolt and Hammond 2001), as well as other insects such as wasps (Landolt et al. 2007). In this study of SWD baits, other types of flies were trapped, including other species of Drosophilidae. Spotted wing drosophila was, however, the most abundant fly in our traps, and information on non-target flies was not recorded. In any detection or monitoring programme, these non-target species may at times be abundant enough to increase trap handling time and the cost of the programme and have potential also to alter the attractiveness of a bait or lure if allowed to decompose in traps.

The comparison of two traps and two baits in experiment 5 indicated greater numbers of SWD flies are captured in dome traps compared with cup traps. There are multiple aspects that differ between the two trap designs, such as vertical versus horizontal movement of insects needed to enter the trap, the large difference in entrance diameters, the trap coloration and the drowning solution surface area that should impact the amounts released of attractive volatiles from the solutions. It is not known what trap characteristics impacted SWD catches with these trapping tests, and a far more detailed assessment of trap characteristics and designs is needed. The function or objective of a trapping programme may also impact trap choice, and trap efficacy in capturing attracted flies (power of the trap) may not be the most important factor. Costs of the trap and the handling and maintenance of the trap are also important aspects to a detection or monitoring programme.

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