Field performance of different lures for *Drosophila suzukii*

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Abstract

Drosophila suzukii *is an invasive fruit-pest, which became a considerable problem in soft fruit production in Europe over the last years. The serious yield losses are due to the rapid reproductive output and the ability to oviposit into healthy immature and ripe fruits. Its wide host range of wild and cultivated plants makes it extremely difficult to control the pest.*

Insufficient management practice and lack of knowledge of the biology of Spotted wing drosophila (SWD), and imminence of further dispersion of the pest urges the intense study of D. suzukii *aiming towards development feasible crop protection strategies. Trapping is currently used for monitoring and is investigated for prospective mass trapping. To identify cheap and attractive volatile organic compounds as lures, a field experiment in the area of Stuttgart in South-West Germany has been conducted. In two high-value crops, i.e. raspberry and grapevine, the trapping performance of different bait substances were compared, namely apple cider vinegar (ACV), a mixture of red wine and red wine vinegar (RWV), the commercial lure Dros'Attract (Biobest) (DROS), cherry juice (CHER), and natural aromas of raspberry and wine.*

The presence of D. suzukii *in the study area was confirmed in the growing season of raspberry and grapes in 2015. However, fruit damage by SWD was negligible, most likely due to the prevailing hot and dry weather conditions during the summer, which were unfavorable to the flight activity of the pest. Natural aromas failed entirely as lures. RWV performed better than ACV in raspberry and wine. CHER, initially similar to ACV, revealed good results with increasing temperatures, probably due to the formation of fermentation volatiles. Catch results of the commercial lure DROS was inferior to RWV and CHER. Based on the results for cherry juice, a further research on fermentable substrates is suggested. It may be useful as possible additives to lures to increase the trapping performance.*

Keywords: trapping performance, bait, mass trapping, monitoring, Spotted wing drosophila

Introduction

Today, fruit production in Europe is challenged by the incidence and spread of the invasive fruit pest *Drosophila suzukii* Matsumura (Diptera, Drosophilidae).

Originally native to Southeast Asia (Kanzawa, 1939), *D. suzukii* was introduced by fruit imports to Europe in 2009 and dispersed thereafter first throughout Italy, Germany, Spain, France and nowadays also to Scandinavia, Great Britain, and Eastern Europe (Cini *et al.*, 2012). Unlike other Drosophilid flies, due to its serrated ovipositor the female *D. suzukii* is able to penetrate red, healthy and soft skinned cultivated and wild stone and berry fruits as well as grape varieties for oviposition (Walsh *et al.* 2011). As a result of larval feeding, fruits collapse subsequently which causes quantitative yield losses. However, *D. suzukii* may appear as post-harvest pest after oviposition shortly before or during harvest and during fruit marketing, causing qualitative loss and the risk of passive dispersion. Its wide host range together with the ability of early infestation of still ripening fruit imply the high potential damage of *D. suzukii* towards the fruit producing industry. Insufficient

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management practice and the imminence of further dispersion of this invasive pest urges to an intense study of *D. suzukii* with the aim to develop feasible crop protection systems.

The present study contributes to the development of mass trapping – a possible method for cultural control of *D. suzukii* - with the aim to find suitable baits in two relevant crops in South-West Germany.

Material and Methods

For both crops, raspberry and grapevine, trapping experiments were conducted during summer 2015 in two sites around Stuttgart, Baden-Württemberg in South-West Germany.

Traps were constructed of translucent polyethylene mineral water bottles of 1 l volume. Ten 4 mm entry holes were placed at a height of 15 cm from the bottom equally spaced around the circumference of the bottles. A broad red strip, made of colored tape, was fixed around the holes to attract flies to the entry. A round sheet of stiff, white tarpaulin (21 cm diameter) covered the bottleneck as rain shelter to avoid any dilution of the lure. Traps were filled with 200 ml of the respective liquid lure. Traps were fixed with wire to the stakes (Stickel) in the wine or raspberry rows, with the entry holes at ca. 1.10 m above soil.

Six different lures were tested in both crops. A first group of three lures consisted of substances that were already known to be relatively suitable to catch *D. suzukii*: the commercial product Dros' Attract (Biobest) (DROS), a 1:1 mixture of red wine and red wine vinegar (RWV) and a 1:1 mixture of water and apple cider vinegar (ACV). A second group consisted of nature-identical fruit aromas (fine chemicals) diluted in water. In the test in raspberry two natural flavors from Joh. Vögele KG were tested: raspberry aroma (AR) (2 ml per 100 ml) and muscatella wine aroma (AW) (1 ml per 100 ml). Raspberry aroma was excluded in the second test and replaced by a second apple cider vinegar mixture, stained red by adding 10 ml red barb juice (ACV+R). Thirdly, cherry juice (CHER) was tested to determine the performance of the liquid of one of the major host crops of *D.suzukii* as attractant. To exclude a possible attractive effect of the traps themselves, a control treatment - a trap baited with water alone – was included.

The experiment was laid out as a randomized complete block design with four blocks per experimental site. The traps remained in the once assigned position for the whole experiment and were not randomly reallocated within the blocks, causing a potential correlation of measurements from the same trap, that was later accounted for in statistical analysis.

Traps were installed in raspberry (cv. 'Glen Ample' and 'Tulameen') the 15th and 18th of June, 2015, when first berries started coloring red. In vineyards (cv. 'Trollinger', 'Merlot' and 'Regent'), traps were installed the 26th of August and 1st September, 2015 when grapes were found in BBCH 83.

Trap catches were evaluated in weekly intervals, when the old lures were replaced by fresh ones. The captured *D. suzukii* were identified, sexed, and counted under a microscope.

To determine the infestation level in the treatment, each week ten berries were randomly picked from each block and kept at room temperature in plastic cups. Three days later, the cups were filled with saline water to force *Drosophila*- larvae to leave the berries to be counted.

Results

Raspberry

Traps baited with treatments ACV, CHER, DROS and RWV were suitable to detect *D. suzukii* and caught substantial numbers over the experimental period of five weeks. Taps baited with aromas of raspberry (AR) and wine (AW) were almost completely inefficient because almost no *D. suzukii* individuals were caught. Thus, these two treatments were excluded from the statistical evaluation. The water baited control traps did not attract neither *D. suzukii* nor any other insect species.

Except the last evaluation date, RWV and DROS catches were superior to those of ACV and CHER (Figure 1). However, an increasing trapping performance of CHER treatment at the end of the sampling could be observed. Very few catches were made with ACV over the entire experimental period. Significant differences between the treatments were scarcely found due to the high variability.

The distributions of sexes clearly shows significantly higher number of females over the whole sampling period at both experimental sites. However, sex-ratio differed significantly between treatments (Table 1).

Table 1: Numbers of *D. suzukii* individuals caught per 7 days in raspberry. Median, proportions of females and confidence limits.

Date	Lure treatment							
	ACV		CHER		DROS		RWV	
	Count **a)**	Prop. **b)**	Count	Prop.	Count	Prop.	Count	Prop.
Jun 23-25	3.51 (0.7,10)ab	**0.88**bc	0.14 (-0.6,2.21)b	**0.87** c	30.94 (11.08,83.48)a	**0.94**ab	13.5 (4.48,37.35)ab	**0.96** a
Jul 01-03	15.12 (5.87,36.86)bc	**(0.76,0.94)**	8.49 (3.04,21.29)c	**(0.74,0.99)**	84.60 (35.44,200.03)a	**(0.87,0.97)**	69.8 (29.15,165.26)ab	**(0.91,0.98)**
Jul 08-10	15.1 (5.85,36.82)a	*	37.46 (15.33,89.61)a	*	58.31 (24.13,138.95)a	*	70.9(29.58,167.95)a	*
Jul 15-17	18.78 (7.40,45.61)b		32.67 (13.29,78.34)ab		72.31 (30.11,171.75)ab		86.04 (35.94,204.10)a	
Jul 22-24	10.34 (3.68,26.5)a		46.61 (19.08,111.86)a		30.87 (12.44,74.55)a		32.72 (13.23,79.0)a	

a) Linear mixed model for log $(y_t + 1)$-transformed total counts using model in equation (1) with compound symmetric variance-covariance structure with heterogeneous variances (CSH). Effect $(\beta\tau)_{jm}$: Num DF = 20, Den DF = 86.4, F = 3.49, $p < 0.0001$. F-test was followed by mean comparisons (Tukey). Back transformed means are presented ("Count") and serve as estimators of the medians. Medians of different treatments that share a common letter do not differ significantly at the 5 % level.

b) Linear mixed effects model for weighted elogits (2) using model (1) with compound symmetric variance-covariance structure with heterogeneous variances (CSH). Effect $(\beta\tau)_{jm}$: Num DF = 20, Den DF = 8.99, F = 2.68, p = 0.0657. Effect τ_m: Num DF = 4, Den DF = 25.8, F = 2.89, p = 0.0421. Effect β_j: Num DF = 5, Den DF = 5.04, F = 0.52, p = 0.7543. Followed by mean comparison (Tukey) for factor τ_m. Elogits are presented as back transformed proportions. Proportions that share a small letter do not differ significantly at the 5% level. Proportions followed by an asterisk (*) differ significantly from equalproportions of sexes (1:1).

Berries sampled over the whole observational period used to assess oviposition by *D. suzkii*, were found infested only to a low extent, despite the high population density as assumed from the heavy abundance of *D. suzukii* females detected in traps (Table 2)

Table 2: Mean number of larvae per 10 berries and sampling interval separated by cultures and sites.

Culture	Site	Weeks after experiment start						
		1	2	3	4	5	6	7
Raspberry	Möhringen	0	0	0	0.9	0	-	-
	Fellbach	0.25	2.5	0.25	3.5	-	-	-
Wine	Degerloch		0	0	0	7.25	0	-
	Hohenheim	0	0	0	0	0	0	-

For weeks with missing entry no record was taken as in the case of the first week at Degerloch. Minus signs indicate that the crop was harvested and no berries to sample were left.

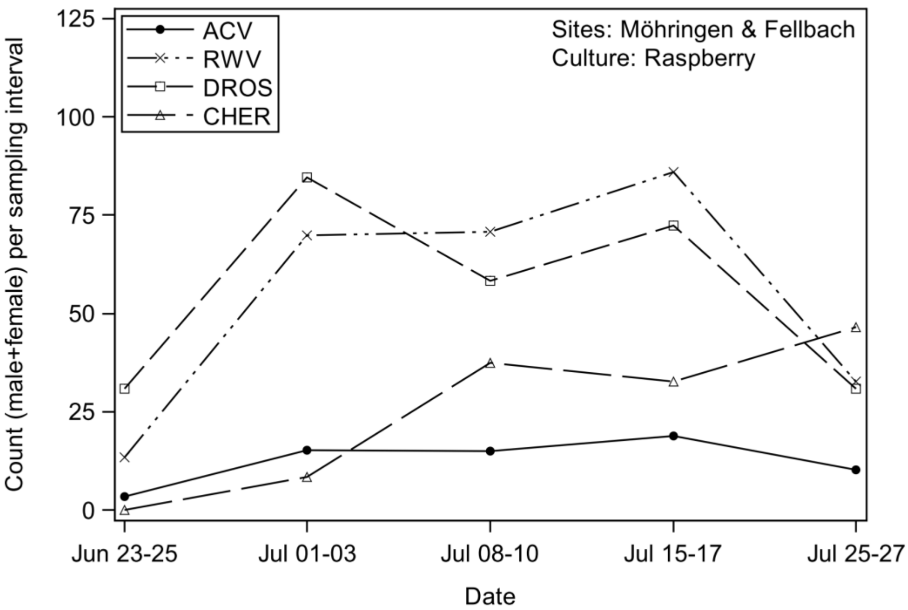

Figure 1: Catches of *D. suzukii* individuals per sampling interval in raspberry.

<u>Wine</u>

Similar to the test in raspberry, the water baited control traps caught no flies at any occasion. The wine aroma treatment (AW) was inefficient as well. Both treatments were excluded from statistical analysis.

Initial numbers of *D. suzukii* caught were relatively low at the first sampling date. At this low initial levels, when ripening process began and attractivity to *D. suzukii* was still relatively low. With further ripening, the number of flies caught increased continuously.

The trapping performance of CHER was slightly better than in raspberry treatments. RMC and DROS were nearly as good as the cherry juice. Apple cider vinegar lures have caught the fewest *D. suzukii* in this treatment. It is noticeable, that treatments ACV and ACV+R do not differ significantly, neither in terms of total counts nor in terms of sex ratios. Hence, the red staining of the yellow apple cider vinegar treatment did not improve or impair the attractivity to *D. suzukii* to a detectable extent (Figure 2).

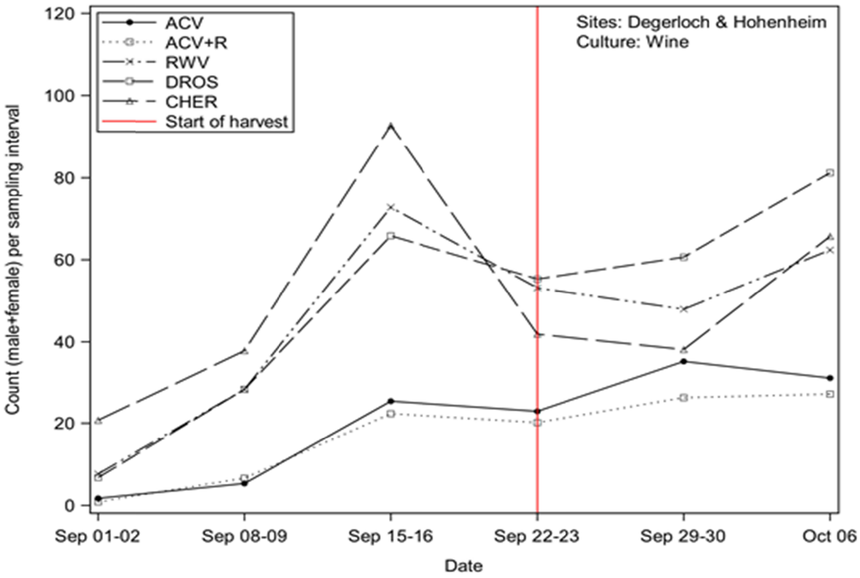

Figure 2: Catches of *D. suzukii* individuals per sampling interval in wine.

The sex ratios were similar to those in the raspberry trials. The fraction of females in the catches were significantly higher than those of males at any sampling date (Table 3).

Table 3: Numbers of *D. suzukii* individuals caught per 7 days in wine. Median, proportions of females and confidence limits.

Date	Lure treatment									
	ACV		ACV+R		CHER		DROS		RWV	
	Count **a)**	Prop. **b)**	Count	Prop.	Count	Prop.	Count	Prop.	Count	Prop.
Sep 01-02	1.70 (0.27,4.74)bc	**0.74a**	0.84 (-0.14,2.91)c	**0.76a**	20.74 (9.22,45.24)a	**0.80a**	6.85 (2.69,15.68)ab	**0.70a**	7.65 (3.1,17.39)ab	**0.68a**
Sep 08-09	5.44 (2.14,12.19)b	**(0.65,0.81)**	6.7 (2.76,14.78)b	**(0.68,0.83)**	37.74 (17.91,78.36)a	**(0.68,0.82)**	28.4 (13.35,59.22)a	**(0.61,0.77)**	28.4 (13.35,59.22)a	**(0.58,0.75)**
Sep 15-16	25.44 (12.34,51.41)b	*	22.51 (10.86,45.59)b	*	92.78 (46.31,184.8)a	*	65.89 (32.75,131.5)a	*	72.81 (36.24,145.3)a	*
Sep 22-23	23.10 (11.30,46.22)bc		20.27 (9.86,40.68)c		41.91 (20.90,83.7)ac		55.29 (27.73,109.3)a		53.12 (26.5,105.5)ab	
Sep 29-30	35.13 (16.62,73.12)a		26.38 (12.18,56.0)a		38.10 (18.10,79.20)a		60.63 (29.04,125.41)a		47.91 (22.6,100.6)a	
Oct 06	31.15 (13.53,70.13)a		27.2 (11.75,61.5)a		65.77 (29.18,146.73)a		81.19 (36.15,180.9)a		62.43 (27.67,139.4)a	

a) Linear mixed model for log (yt + 1)-transformed total counts using model in equation (1) with compound symmetric variance-covariance structure with heterogeneous variances (CSH). E□ect (βτ) jm: Num DF = 20, Den DF = 86.4, F = 3.49, p < 0.0001. F-test was followed by mean comparisons (Tukey). Back transformed means are presented ("Count") and serve as estimators of the medians. Medians of di□erent treatments that share a common letter do not di□er significantly at the 5 % level.

b) Linear mixed e□ects model for weighted elogits (2) using model (1) with compound symmetric variance-covariance structure with heterogeneous variances (CSH). E□ect (βτ) jm: Num DF = 20, Den DF = 8.99, F = 2.68, p = 0.0657. E□ect τm: Num DF = 4, Den DF = 25.8, F = 2.89, p = 0.0421. E□ect βj: Num DF = 5, Den DF = 5.04, F = 0.52, p = 0.7543. Followed by mean comparison (Tukey) for factor τm. Elogits are presented as back transformed proportions. Proportions that share a small letter do not di□er significantly at the 5% level. Proportions followed by an asterisk (*) di□er significantly from equalproportions of sexes (1:1).

Similar to the test in raspberry, earlier in the season, no larvae in fruits were found over most of the observational period. Again, only in the very last observation dates nearly grape harvest, larvae were found in sampled berries. At Hohenheim until harvest no single larva was found in the samples. At Degerloch the first larvae were recorded only late before harvest, but samples showed a very high number of 7.25 larvae per ten berries (Table 2) in one occasion.

Discussion

The good trapping performance of CHER in wine and raspberry treatments may be explained with yeast growth (mother of vinegar) after a few days, producing a characteristic smell. This may also explain of the changes in trapping performance of CHER traps, increasing towards the end of the weekly exposure periods. Many drosophilids are known to be associated with yeasts in a mutualistic relationship, where adult flies feed on yeasts and the latter are distributed through the flies (Starmer & Aberdeen, 1990). Communication between yeasts and drosophilids is mediated by volatile compounds (Scheidler *et al.*, 2015). Due to its high total sugar content and especially the high amount of fructose (Scardina, 2009), cherry juice is an ideal substrate for microbes such as yeasts (Querol & Fleet, 2006; Scardina, 2009). Many yeast species have been identified in cherry juice that have as well been found associated with *D. suzukii* (Hamby *et al.*, 2012). With increasing temperatures yeast proliferation and fermentation is likely to have increased. Indeed, most yeasts grow best between 20 and 30 °C (Querol & Fleet, 2006). The formation of a bouquet of yeast and fermentation related volatiles evolving from cherry juice traps might have increased the attractiveness of that bait to *D. suzukii*.

The performance of traps baited with RWV in comparison to ACV was far higher. It could be presumed, that these differences between ACV and RWV might be due to an additional visual cue exerted by the red color of RWV. In fact, all treatments found more effective, i.e. RWV, DROS and CHER, are of intense red color. However, the comparison of ACV and ACV+R did not reveal any differences at any sampling date. Attraction might be influenced by very specific spectral compositions of the involved colors as Basoalto *et al.* (2013) suggest. After all, it seems that RWV, DROS and CHER had favorable properties apart from color in comparison to ACV. Backed by the results of Cha *et al.* (2014), that identified the four most attractive substances in apple cider vinegar and Merlot wine, it may be hypothesized, that the concentrations of these substances might be higher in RWV, CHER and DROS in comparison to ACV.

The negligible amount of flies caught with natural aromas might also have something to do with the ingredients of the lures. They showed completely unsuited as attractant to *D. suzukii*, at least in the tested concentrations. Their concentrations were arbitrarily chosen as no experience with such substances as lures was available. Whether higher or lower concentrations would have been more successful remains uncertain. Additionally, the translucent color of the aroma mixtures probably provided a weaker visual cue in comparison to the bright red color of other treatments. The low hatchability in the grapes may have different reasons. On the one hand grapes have a considerable ability to close the injures created by oviposition through an increased callus formation. Therefore, deposited eggs may not be oxygenated properly and embryogenesis could not be completed. On the other hand, the low fruit infestation, also in the raspberry treatment might be due to a low population density of *D. suzukii* that could be mainly explained by the adverse climatic conditions during summer 2015 (Tochen *et al.*, 2014). In addition, the fruit skins of grape berries were particularly solid due to the dry conditions and high temperatures and radiation. The increased hardness of the fruit skin could have compromised the acceptance of berries as oviposition sites (Lee *et al.*, 2012). In fact, Ioriatti *et al.* (2015) discovered the thickness of the fruit skin, together with the absence of injuries in the fruits, to be of highest importance for low levels of oviposition by *D. suzukii*.

All treatments showed a higher proportion of females without an obvious higher specificity of any lure. A possible reason could be a lower temperature tolerance compared to the female *D. suzukii* (Dalton *et al.*, 2011). The overwintering mortality of males in 2014/2015 could have been so high that the imbalance has taken over the entire growing season.

Likewise, the hot and dry summer have triggered an increased mortality rate for male *D. suzukii*.

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