

Comparison of attractants for monitoring *Drosophila suzukii* in sweet cherry orchards in Italy

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Abstract

The invasiveness of *Drosophila suzukii* Matsumura is causing sizable damage to soft fruits and cherry. In order to define a targeted strategy for *D. suzukii* management, it is important to have access to highly sensitive trapping tools for detection, monitoring and control that are also selective, practical in use, economic, and with low environmental impact. The aim of this study was to compare different combinations of traps and lures to define a best practice approach for *D. suzukii* monitoring in Italy, extended over a wide elevational gradient and landscape complexity. The high attractiveness was not always combined with high selectivity, as in some cases up to 95% of the *Drosophila* spp. captured were belonging to species different from *D. suzukii*. The commercial Droso-Trap was very efficient providing high capture ability. Overall, the most attractive lure was Droskidrink while Suzukii Trap was the most selective one. Lure attractiveness and selectivity, however, changed during the season in relation to environmental temperatures and phenological developmental stages, suggesting the need for implementing different lures in different periods and for the different purposes (monitoring or mass trapping). In terms of trap management, Droskidrink showed the problem of bacterial-gel formation, while Suzukii Trap and Pherocon SWD were easier to handle as they did not need to be checked at weekly interval. Finally, these results were gathered to convey the most efficient combination of trap and bait able to efficiently perform mass trapping and attract and kill technique.

KEYWORDS

Droskidrink, microclimatic condition, Pherocon SWD, selectivity, Suzukii Trap, trapping system

1 | INTRODUCTION

The spotted-wing *Drosophila*, *Drosophila suzukii* Matsumura (Diptera Drosophilidae), has spread from its native distribution in Asia in recent years and is now a major pest in Europe and Americas (Asplen et al., 2015; Rota-Stabelli, Blaxter, & Anfora, 2012). Due to the capacity to attack and damage ripening fruits close to harvest, rapid developmental cycle and high polyphagy this invasive species is causing severe damage to crop and non-crop species (Cini, Ioriatti, & Anfora, 2012;

Keeseey, Knaden, & Hansson, 2015; Kenis et al., 2016; Lee et al., 2015; Poyet et al., 2015).

The potential of *D. suzukii* to spread worldwide and its recognized detrimental impact have raised the need to specifically monitor its occurrence, promoting the development of effective traps and attractants and their practical application in monitoring and control actions (Cini et al., 2012; eppo.int 2016; Grassi, Palmieri, & Giongo, 2009; Ioriatti et al., 2015; Lee et al., 2011). Monitoring the dynamic of *D. suzukii* populations both at the individual field and at the regional scale

is crucial to understanding the spread into new areas and the potential impact and damage of the population to susceptible crops. Ultimately, effective tools for monitoring and trapping constitute the essential pre-requisite to better predicting pest outbreaks and implementing integrated pest management (IPM) strategies in order to reduce insecticides applications (Cini et al., 2012; Hamby & Becher, 2016; Hamby et al., 2016; Wiman et al., 2014).

However, to develop an effective monitoring method, it is necessary to determine the combination of trap and lure enabling high attractiveness in situations with low population densities, high selectivity, ease of use, low costs and environmental impacts. Selectivity, the ratio between the number of individuals of the target pest and all other *Drosophila* spp. individuals, needs to be accurately assessed and integrated into the framework of an effective monitoring and pest management. In particular, the high selectivity minimizes detrimental effects on the biodiversity of non-target species and, at the same time, facilitates the screening operations during trap sorting (Burrack et al., 2015).

To date, different types of baits and lures for *D. suzukii* have been developed, in respect of the olfactory preference of the species which is sensitive to volatiles associated to both ripe and rotten fruits (Keeseey et al., 2015). Among the different baits, all based on food attractants, the most widely used are fermenting substances (as apple cider vinegar [ACV]) either alone or in a mixture with wine or also in combination with peptides (Basoalto, Hilton, & Knight, 2013; Burrack et al., 2015; Cha et al., 2013, 2014; Cha, Hesler, et al., 2015; Cha, Gill, et al., 2015; De los Santo Ramos, Bello, Gómez, Hernández, & Iribe, 2014; Grassi et al., 2015; Iglesias, Nyoike, & Liburd, 2014; Kleiber et al., 2014; Landolt, Adams, & Rogg, 2012; Lee et al., 2012, 2013). It is worth mentioning that yeast and microorganisms play an important role in determining the volatile profile of attractants (Hamby & Becher, 2016). The Droskidrink bait (75% of ACV, 25% red wine and 20 g/L of cane sugar) has been reported as an effective attractant in the early studies carried out in northern Italy (Grassi et al., 2015). Synthetic lures are also available and are based on chemicals isolated from fermenting mixtures (Cha et al., 2013, 2014) released by specific commercial dispensers.

Comparisons among some baits and lures suitable for trapping *D. suzukii* were carried out in Europe (Cha et al., 2013; Grassi et al., 2015). However, these studies have the limit to analyse the sole attractiveness in narrow elevational gradients and over a short period of time.

Thus, a more accurate evaluation of attractiveness, comparing different baits and synthetic lures together with the continuous monitoring across different years, becomes essential to monitor seasonal dynamic of captures. In addition, such monitoring needs to be performed in different environments to cover a wide elevational gradient and landscape complexity. This extensive approach would provide valuable information for *D. suzukii* management by monitoring the activity density of the pest in different scenarios and, consequently, to timely implement effective IPM strategies. Finally, the accurate evaluation of lures selectivity should implement the analyses of attractiveness to achieve the most targeted monitoring action.

The present work therefore aimed at comparing the attractiveness and selectivity of different baits and lures in time series of catches,

to provide reliable practice for the monitoring and control of *D. suzukii*. In particular, dynamics of captures and selectivity were related to field temperatures and phenological development stages in order to disclose population trends and better address management strategies. All trap comparisons have been carried out in cherry orchards over three years and in different locations in northern Italy. Cherry is one of the most damaged crop due to its ripening period, when few alternative host fruits are available (Kenis et al., 2016), and due to its nutritive value and chemical-physical characteristics, which are optimal for *D. suzukii* development (Lee et al., 2011). This long-term approach performed over a wide geographical range provides large data sets needed to discriminate the variation in lures attractiveness due to unmanageable environmental causes and the effect of competition with the ripening fruits and it allows to determine the most suitable setup to efficiently monitor *D. suzukii* in cherry orchards.

2 | MATERIALS AND METHODS

2.1 | Sites and trap setting

The comparative surveys were conducted in four cherry orchards in the districts of Modena, Trento, and Verona (north-eastern Italy) during 2014, 2015 covering the whole cherry growing season (Table S1). Some trials were further replicated in 2016 in three orchards covering the early phenological stages until the end of blooming (as reported in Table S1). Specific details regarding the single sites, cherry cultivars and orchard management are reported in Table S2. All sites are characterized by standard tree forms and no rain covering protection.

A common protocol was followed at each site sampled. Commercial baits (Apple Cider Vinegar, Acentino, Modena Italy; Droskidrink[®], Prantil, Trento, Italy; Biologische Essigfliegenfalle[®], Riga-Gasser, Ellikon a. d. Thur, Switzerland and Suzukii Trap[®]; Bioiberica, Barcelona, Spain) were selected according to our preliminary results (A. Grassi, L. Tonina, G. Vaccari, unpublished data and supplementary trials showed in Data S1). Droskidrink (specifically provided for our trials without sugar) is a mixture of 75% ACV, 25% red wine (Grassi et al., 2015). It was added with 20 g/L of cane sugar to increase the fermentative activity. Biologische Essigfliegenfalle is a wine vinegar-based attractant (Cahenzli & Daniel, 2016), while Suzukii Trap is a liquid mixture composed of organic acids and protein hydrolysed (7% of protein; De los Santo Ramos et al., 2014). In 2015, Droskidrink was added with a viable culture of selected lactic bacteria (*Oenococcus oeni*) produced by the Fondazione Edmund Mach laboratories and buffered the pH at 4.5 to evaluate their capacity as biocatalysers of the production of biologically active compounds to *D. suzukii* (Anfora, Guzzon, Grassi, Maddalena, & Ioriatti, 2016). The comparison was performed using 250 ml of each bait. The synthetic lures were provided as numbered samples or commercial product (dispenser Pherocon[®] SWD Dual Lure A+B 2014, product code: 5001) by Trécé Inc. (Adair, OK, USA) and were used as preliminary test in combination with 250 ml of ACV (2014) and strictly following the manufacturer recommendation in combination with water drowning solution (2015 and 2016). In 2016, Pherocon SWD was also tested in combination with some of the baits

(Droskidrink and Suzukii Trap) to verify potential synergistic effect. In each trial, the lures were added with one drop of surfactant Triton® X-100 (Sigma-Aldrich Co. LLC.) to break surface tension, except Biologische Essigfliegenfalle and Suzukii Trap.

Four replications of all baits and lures under evaluation were deployed in Droso-Trap® (Biobest, Westerlo, Belgium) and installed within each orchard in a randomized complete block design. In 2014, a homemade trap commonly used in northern Italy for the monitoring of *D. suzukii* until 2014 (Grassi & Pallaoro, 2012) baited with 200 ml of ACV and one drop of Triton was comparatively tested with Droso-Trap baited with 250 ml of the same attractant. From 2015, the Droso-Trap was modified adding a 3 × 3 mm net on the holes to avoid the entrance of bigger insects.

The lures tested, the amount inside a single trap, their replacement in the field and the year are reported in Table S3.

Each batch of traps was set within the orchard and spaced at least two rows apart the border and 20 m the woods, to minimize possible gradients in population density. Each batch was set apart at least 20 m from another and the traps were hung on trees 1.5 m from the ground and 2.5 m apart from each other. The content of each trap (either bait or water drowning solution) was collected weekly and concurrently was renewed or refilled according to the type of attractant. At each sampling, traps were randomly reassigned to a new position. At each trap service the status of liquid and other management aspects such as bacterial gel, sludge and evaporative loss were recorded.

2.2 | Data collection

The content of each trap was sorted under the stereomicroscope, recording the number of females and males of *D. suzukii*, the total number of other *Drosophila* species and the total number of insects other than *Drosophila* species (these last two countings were not performed in Pergine).

For each site in 2015 temperature data loggers were deployed, at 2 m from the ground, to record hourly temperature (HOBO U23 ProV2 with RS1 solar radiation shield; Onset Computer Corporation, Bourne, MA, USA). Hourly temperature data were used to calculate thermal sums and to compute the number of hours in a week when the temperature exceeded the 5°C minimum threshold. This 5°C minimum threshold was selected according to the lower limit of activity of *D. suzukii* (Kanzawa 1939).

2.3 | Data analyses

Data on the weekly capture were used to calculate either the attractiveness as the number of *D. suzukii* caught or the selectivity as the percentage of *D. suzukii* within the total number of *Drosophila* species. Attractiveness and selectivity for each batch were analysed with traps-lure combination as fixed factors and site and data of collection as a random factor, using a linear mixed-effect model (LME). Selectivity data as they are expressed as percentage, were arcsine square root transformed. The means were compared using the Tukey-HSD test following ANOVA (The R Foundation for Statistical Computing <http://www.R-project.org>).

3 | RESULTS

These experimental monitoring trials, performed across different years and extended over a wide elevational gradient and landscape complexity, have provided clear indications for the best combinations of trap and attractant for monitoring *D. suzukii* in cherry orchards in north-eastern Italy.

3.1 | Validation of the traps

The 2014, sampling campaign allowed us to compare the effectiveness of the two different trap types. Employing the same brand of ACV, the commercial Droso-Trap recorded catches of *D. suzukii* four times higher than the homemade trap (averages: 3.13 vs. 0.75 *D. suzukii*/trap/week, LME $F_{1,194} = 22.48, p < .0001$). The average selectivity of both types of traps was similar and corresponding to about 15%; the differences were not statistically significant ($p = .7895$).

3.2 | Validation of the lures

3.2.1 | Attractiveness

Among the four lures tested during the 2014 cherry season, only the pure ACV showed a low capture efficiency. Droskidrink, Biologische Essigfliegenfalle and Pherocon SWD plus ACV showed similar high catching capacity (Figure 1a; LME $F_{3,3411} = 29.59, p < .0001$).

The attractiveness of Droskidrink, either used alone or with *O. oeni* culture, and Suzukii Trap differs from the Pherocon SWD lures, tested in spring 2015, showing five times higher capture rates (Figure 1b; LME $F_{2,2067} = 37.07, p < .0001$).

Synthetic lures over unscented drowning solution as tested in 2016 were significantly less attractive than baits. The use of synthetic lures in combination with baits did not show significant synergistic effect (Figure 1c; LME $F_{4,243} = 8.10, p < .0001$).

3.2.2 | Selectivity

In 2014, ACV showed the worst performance in selectivity among the tested lures and baits, with a value about 14%. Droskidrink, Pherocon SWD plus ACV and Biologische Essigfliegenfalle captured on average 24% of the target species among the captures (Figure 1d; LME $F_{3,411} = 31.22, p < .0001$).

Among the seven lures and baits tested in 2015 (Figure 1e) Suzukii Trap was the most selective, with an average value of 20% *D. suzukii*/all *Drosophila* species, statistically different from the other that are comparable between each other (LME $F_{6,1388} = 19.59, p < .0001$). In two study sites, best selectivity of Suzukii Trap emerged also in relation to all other insects (not *Drosophila* species) captured from the trap (16% vs 5%–11% of other baits; LME $F_{6,864} = 9.55, p < .0001$).

The selectivity of Suzukii Trap in 2016 was comparable with Droskidrink, also when both were combined with Pherocon SWD dispenser, while Pherocon SWD dispenser alone showed a very low selectivity (Figure 1f; LME $F_{4,235} = 5.82, p < .0001$).

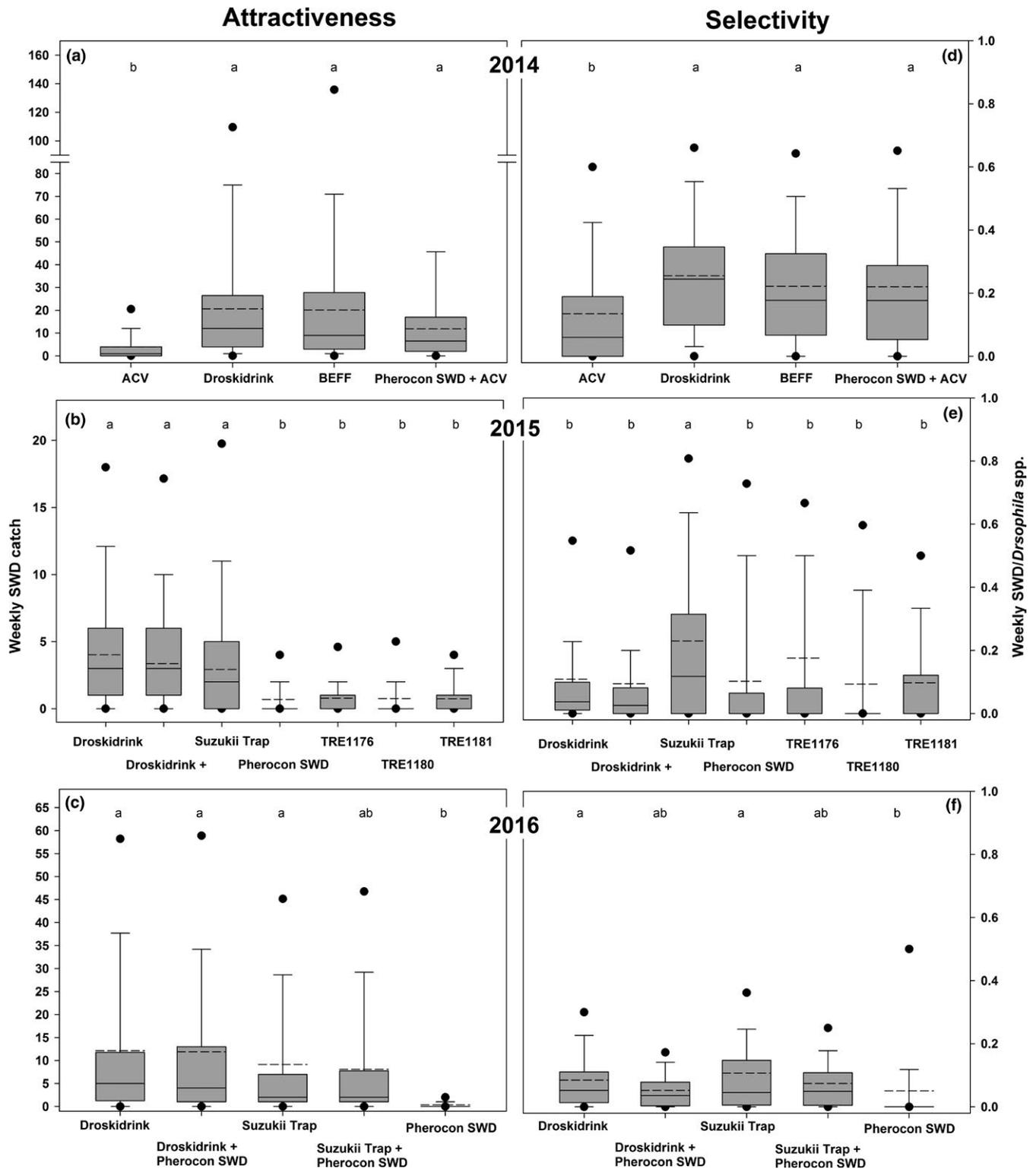


FIGURE 1 Attractiveness and selectivity of the different *Drosophila suzukii* baits and lures in three years of study. The boxes enclose the first and third quartiles; the ends of the whiskers represent the 5th/95th percentiles, the solid lines the median and the dashed lines the mean. Different letters indicate statistically significant differences between treatments (Tukey's test following ANOVA, $N = 52\text{--}308$, $p < .01$). BEFF indicates Biologische Essigfliegenfalle, Droskidrink + indicates Droskidrink added with lactic bacteria, TRE indicates different dispensers of Pherocon SWD Trécé

Overall, Suzuki Trap and Biologische Essigfliegenfalle, consistently among sites, had the highest selectivity (Table S4). ACV, Pherocon SWD and Droskidrink had a lower selectivity, around 14%, but it

is worth noting that the latter showed a highly constant selectivity across all the sites. All remaining lures provided lower selectivity and they are characterized by great variability across sites.

3.3 | Temporal trends

3.3.1 | Attractiveness

The greater efficiency of Droskidrink, Biologische Essigfliegenfalle and Pherocon SWD plus ACV based on average weekly catches in 2014 is supported by data on constancy over time. These attractants showed the best performance for 42% of times for Droskidrink, 37% for Biologische Essigfliegenfalle and 18% for Pherocon SWD; the remaining 3% was for ACV. In 2015, Droskidrink alone or with the addition of *O. oeni* was the most attractive during the period (39% and 36% of time, respectively), followed by Suzukii Trap (21%) and the other lures (4%).

In 2014, Biologische Essigfliegenfalle and Pherocon SWD plus ACV were able to record the first peak of capture before the fruit changing colour phase. Droskidrink alone or with selected bacteria and Suzukii Trap were effective in 2015 during the period from blooming to beginning of ripening. In 2016, Droskidrink and Suzukii Trap were efficient independently of the combination of dispenser Pherocon SWD.

The Figure 2 shows the curves of cumulative catches recorded with the various lures and baits in 2015 according to the number of hours per week with temperature above 5°C. From this analysis the effect of temperature regimes of each site on the early detection ability of different baits is evident. Indeed, while in a warm site (e.g., Vignola) the early detection ability showed a similar trend among lures, in a cold one (e.g., Pergine) Pherocon SWD and Suzukii Trap, with respect to Droskidrink, were able to attract in weeks characterized by lower temperature conditions.

3.3.2 | Selectivity

The selectivity of the best attractants in this study (Droskidrink and Suzukii Trap) is not constant over time and changes greatly among sites (Figure 3). In the three different phenological phases for 2015 the Suzukii Trap was always more selective than Droskidrink, with

value ranging from 5 to 80%. The selectivity over time and sites appears to change with the *D. suzukii* and non-target insect population density. In the three sites where the selectivity was investigated there was a strong increase of this parameter after harvesting, except in Valpolicella.

3.4 | Operational and choosing aspects

In addition to the aspects of attractiveness and selectivity, it appears also important to analyse additional parameters such as easiness to use and duration of attractiveness. Droskidrink presents problems related to the formation of a bacterial gel, especially in periods with temperatures above 20°C. In 2014, in 65% of controls there was the formation of this gel. Biologische Essigfliegenfalle in 50% of controls had produced a black sludge at the bottom of the trap, making the replacing procedures more difficult. The dispensers Pherocon SWD instead are easy to use and their attraction is lasting for more prolonged periods, 4–6 weeks, replacing weekly only the drowning solution. Even Suzukii Trap can be replaced on a monthly basis, but still needs a periodic refill, depending on temperature and relative humidity, to compensate for the evaporation loss. Droskidrink, ACV and Biologische Essigfliegenfalle require weekly replacement. Another very important aspect is related to the costs that must be contained. Also, the ease of purchase must be considered, attractants as ACV and Droskidrink have the advantage that can be easily procured. On this regard, it should be remarked that homemade mixtures may not provide a comparable efficiency to the commercial lures.

4 | DISCUSSION

Cherries are among the first fruits ripening in the year when the population density of *D. suzukii* is still relatively low (Asplen et al., 2015) combining both adults exiting from overwintering diapause and those of the first spring generation. Thus, a highly sensitive monitoring tool

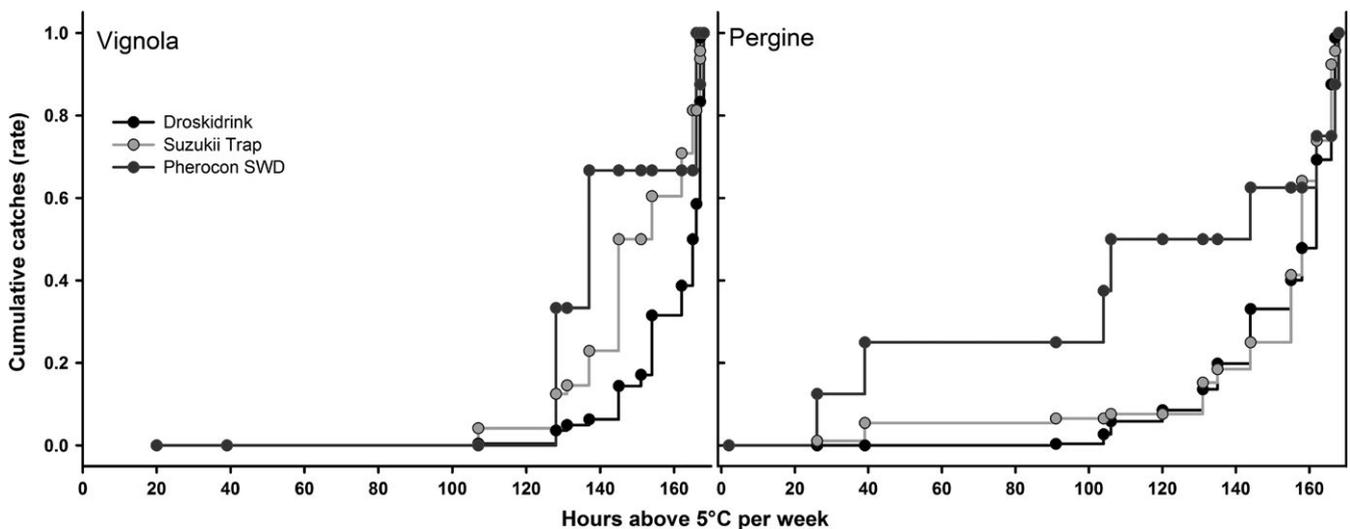


FIGURE 2 Cumulative catches of the three major lures according to the number of hours per week with temperature above 5°C in 2015

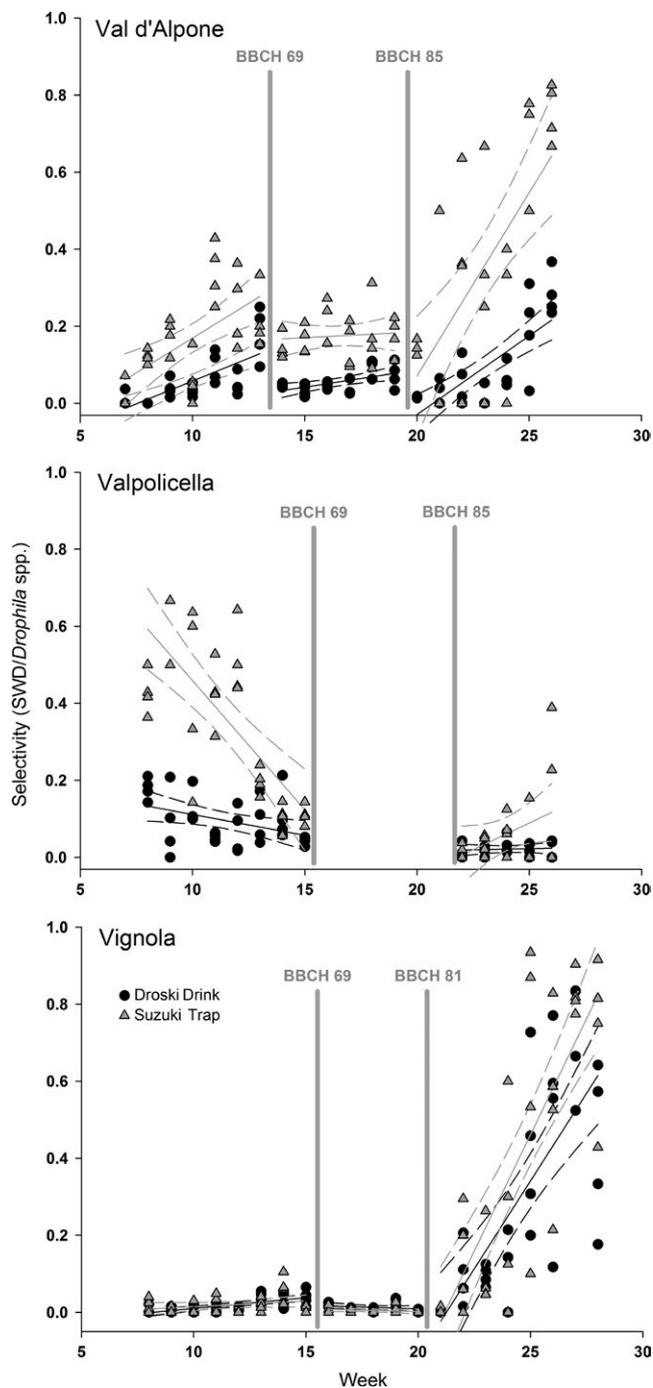


FIGURE 3 Selectivity trends for Droskidrink and Suzukii Trap during the monitoring season 2015. Sampling periods were divided in three blocks, BBCH 69 and 81 (grey vertical lines) point the phenological phases of “End of flowering” and “Beginning of fruit colouring”, respectively. The solid lines indicate the regression lines while the dashed lines indicate the confidence intervals at 95%

becomes a fundamental requirement to inform the timing of insecticides applications and set up selective and effective defences (Hamby & Becher, 2016; Ioriatti et al., 2015; Rossi-Stacconi et al., 2016). In addition, an efficient combination of trap and lure/bait finds immediate application in mass trapping and attract and kill techniques; as well as it provides essential data to compile models able to predict *D. suzukii* population dynamics (Rossi-Stacconi et al., 2016; Wiman et al., 2014).

Overall the highest attractiveness was obtained with Droskidrink, Suzukii Trap and Biologische Essigfliegenfalle. Fermenting substances confirming to be highly effective to attract this species (Cha et al., 2013; Grassi et al., 2015; Keesey et al., 2015).

Droskidrink, Pherocon SWD plus ACV and Biologische Essigfliegenfalle were able to attract more specimens of the target species in respect of ACV. These trials demonstrated that all the trap combinations are characterized by low selectivity ranging, on average, from 6 to 23%, highlighting ratio comparable of those of previous studies (Burrack et al., 2015; Cha et al., 2013). Overall, attractants showing higher attractiveness for *D. suzukii* also capture a higher proportion of other *Drosophila* species, as demonstrated in other research (Burrack et al., 2015).

Compared with the fermenting substances and synthetic lures, Droskidrink has shown a higher selectivity than ACV, confirming that the addition of red wine and cane sugar is effective for *D. suzukii* (Landolt et al., 2012).

Suzukii Trap was the most selective lure in 2015. On this regard, it is worth mentioning that the sampling period in 2016 ended after flowering (week 15–16) missing the collection during the ripening period where the differences in selectivity among Suzukii Trap and Droskidrink maximize. It is reasonable to expect that the selectivity of Suzukii Trap remains consistently higher than other baits if the whole monitoring activity would be performed, as in 2015. The mixture of peptides and organic acid (Suzukii Trap) enhance the attractiveness of *D. suzukii* with respect to other species, and increase the volatile emissions over time, in agreement with previous observations (De los Santos Ramos et al., 2014).

The differences in selectivity between Droskidrink and Suzukii Trap were highly significant in Val d'Alpone and Valpolicella sites and marginal in the Vignola one. The analysis of selectivity points out that the higher abundance of *D. suzukii* is related to the high number of adults emerged from the abandoned fruits on trees and to the lack of competition between lures and ripening fruits during this phase. It is worth noting that Valpolicella site showed a unique trend of higher selectivity at the beginning of the season than after harvesting. This is related to the high activity density of *D. suzukii* in this site due to the mild microclimatic conditions and to the neighbouring woods which constitute a favourable overwintering area.

Altogether it is clear that attractiveness and selectivity are not related each other. Thus, an effective monitoring strategy should take into account the peculiar environmental conditions and balance between the options of a high number of captures and high selectivity, to better approach the population density minimizing at the same time the detrimental effect on insects biodiversity. This aspect would become highly relevant when lures are used to reduce pest population size (e.g., mass trapping technique, attract & kill).

An additional relevant parameter to consider is the early detection ability of an attractant. Indeed, it is important to verify the presence of the pest earlier than the start of damage on fruits especially when the population density is low (Hamby & Becher, 2016). In addition, a reliable early detection requires lures able to attract adults even at temperature below the minimum threshold for larval development, 11°C (Tonina,

Mori, Giomi, & Battisti, 2016). The data collected in 2015 indicate that, below the threshold of 60 hr/week above 5°C, Droskidrink was not able to be attractive, meanwhile Pherocon SWD and Suzukii Trap were able to detect *D. suzukii* early in the season. That because, in addition to the scent of the starting material, the effectiveness of Droskidrink is likely given by fermentation which produces new or higher rates of biologically active volatiles and needs warm temperatures to increase its performance. Attractants which remain effective also at low temperature are needed to implement a trapping strategy across a wide geographical range and different seasons being able to bait overwintering adults and individual in colder areas (Rossi-Stacconi et al., 2016).

In order to improve the lures, it is necessary to consider whether it is better to have lures with a low but constant over time selectivity or with high but not constant selectivity. To improve the efficiency of monitoring programs it could be useful to change the lure types during the period to take advantage of higher selectivity or attractiveness in accordance with practical needs and different *D. suzukii* population structures (Rossi-Stacconi et al., 2016).

In conclusion, Suzukii Trap is the most suitable option in consideration of the high selectivity, the early detection ability, the good attractiveness and the long persistence. While Droskidrink is the best solution in consideration of availability and high attractiveness.

These results suggest that, taking climate and the dynamics of populations into consideration, the use of a combination or a succession of different lures may provide a better estimation of *D. suzukii* population even in critical phases as winter or ripening periods.

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AUTHOR CONTRIBUTION

LT, AGR, NM, SC and CI conceived and designed the research. LT, AGR, SC, NM, AGo, GV conducted the experiments. LT, FG and AGR analysed the data. LT, AGR, NM, FG, GA and CI wrote the manuscript. All authors read and approved the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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