



Host location and dispersal ability of the cosmopolitan parasitoid *Trichopria drosophilae* released to control the invasive spotted wing *Drosophila*

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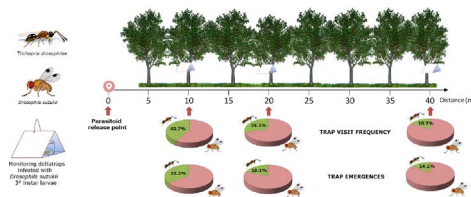
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GRAPHICAL ABSTRACT



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ABSTRACT

Biological control remains unutilized as yet in the framework of *Drosophila suzukii* Matsumura management. Although several parasitoid species attack the pest under laboratory conditions, information is lacking on their host-finding and dispersal capabilities in natural environments. We tested the effect of repeated parasitoid releases on *D. suzukii* populations in infested orchards. The pupal parasitoid *Trichopria drosophilae* (Perkins) was released on different crops at eight sites. Parasitism was monitored using traps placed at various distances from the parasitoid release point (RP). A second experiment was carried out under semi-field conditions to evaluate augmentation of the parasitoid. In both experiments, *D. suzukii* infestation was evaluated through fruit samplings, both from the plant, and from the ground. In the open field trials, *T. drosophilae* attacked *D. suzukii* in traps up to 40 m away from the RP, and pest emergence was significantly reduced within a radius of 10 m at seven out of eight sites. In the semi-field trials, parasitoid releases significantly reduced *D. suzukii* emergence from ground-sampled fruit, and augmentation enhanced parasitism, increasing the numbers of parasitoids

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emerging from host pupae. Although further field studies are required, these results suggest that *T. drosophilae* may be considered a potential biocontrol agent for *D. suzukii*.

1. Introduction

The invasive spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a polyphagous pest that breeds in most wild and cultivated soft-skinned fruits (Asplen et al., 2015; Kenis et al., 2016). It is native to Asia and, since its first detections in Europe and in the United States in 2008 (Calabria et al., 2012; Lee et al., 2011), the control of this pest has been extremely challenging (Asplen et al., 2015). Despite many studies and numerous approaches to controlling this pest (Haye et al., 2016; Wiman et al., 2016; Del Fava et al., 2017), *D. suzukii* still poses a serious economic threat for cherry and small fruit growers (Bolda et al., 2010; Goodhue et al., 2011; Grassi et al., 2012; De Ros et al., 2013).

Biological control plays a key role in the integrated pest management (IPM) of invasive insect pests (Ragsdale et al., 2011; Daane and Johnson, 2010; Biondi et al., 2018). Reducing *D. suzukii* populations through predation and parasitism would help to improve the outcome of other control strategies. For instance, several predator species have been observed feeding on the juvenile stages of *D. suzukii* (Gabarra et al., 2015; Renkema et al., 2015; Woltz et al., 2015) and their contribution to biological control has been recently quantified by Woltz and Lee (2017). However, within-farm augmentation of ground predators (i.e. ants and spiders) is not practical in the short term, although they might be conserved through cultural practices (Altieri, 1999; Zehnder et al., 2007; Lu et al., 2012).

Several parasitoid species have been investigated in the hope that they might be effective against *D. suzukii* in biocontrol programs. Studies have been carried out on both coevolved *D. suzukii* parasitoids (Nomano et al., 2015; Daane et al., 2016) and on native parasitoids that fortuitously attack it in newly invaded areas (Chabert et al., 2012; Rossi Stacconi et al., 2013; Cancino et al., 2015; Gabarra et al., 2015; Miller et al., 2015; Mazzetto et al., 2016; Wang et al., 2016a). The first offer advantages in terms of efficacy and specificity, but their importation and release would be challenging owing to restrictive laws that prevent the introduction of alien species and prioritize the conservation of biological diversity (Mason et al., 2017).

Native parasitoids may represent an alternative that does not suffer legal restrictions and, in certain cases, could be immediately employed at the farm level. However, many local larval parasitoids are strongly limited by the *D. suzukii* immune response (i.e. egg and larva encapsulation) (Chabert et al., 2012; Kacsoh and Schlenke, 2012; Poyet et al., 2013; Wang et al., 2016b), whereas other species are considered generalists with wide host ranges and potential side effects on non-target species if mass released into the field (van Alphen and Thunnissen, 1983; Chen et al., 2015; Knöll et al., 2017).

Currently, the pupal parasitoid *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) appears to be the best candidate for augmentation against *D. suzukii*. In fact, this cosmopolitan parasitoid

coexists with other *D. suzukii* parasitoids (Daane et al., 2016; Wang et al., 2016c), has no preference between *D. suzukii* and *D. melanogaster* Meigen (Mazzetto et al., 2016), and may even preferentially attack *D. suzukii* in dual choice parasitization tests (Wang et al., 2016a). In addition, *T. drosophilae* is able to parasitize *D. suzukii* on a wide temperature condition range, spanning from 15 °C up to 30 °C (Rossi Stacconi et al., 2017). This suggests that it could be used in early spring release strategies to tackle the first *D. suzukii* generations just after the overwinter bottleneck (Dalton et al., 2011; Ometto et al., 2013; Stephens et al., 2015; Enriquez and Colinet, 2016; Rossi-Stacconi et al., 2016; Shearer et al., 2016; Wallingford et al., 2016; Wiman et al., 2016).

A reduction in the pest load before the start of fruit productions coupled with an area-wide parasitoid establishment or increase may have an impact on the *D. suzukii* population dynamic throughout the season (i.e. lowering or delaying population outbreaks). Although many laboratory studies have been conducted to assess the ability of various parasitoids to attack *D. suzukii*, little information is available on their efficacy in field conditions.

Here, we describe two experiment series, performed both in open and semi-field conditions. In the first experiment, we assessed the host location and the dispersal ability of *T. drosophilae* when released on *D. suzukii* infested crops. In order to replicate the trial in different environments and on different crops, a national research network, involving several universities and research institutes (Table 1), was set up. In the second experiment, we assessed the performance of *T. drosophilae* in a confined environment (high tunnel conditions) as well as the effect of the *augmentorium* technique for enhancing the parasitoid's action (Deguine et al., 2011; Jang et al., 2007; Klungness et al., 2005).

2. Material and methods

2.1. Insects

The *D. suzukii* populations used in this study were direct offspring of live adults collected from multiple locations in Italy. Flies were provided with standard cornmeal and yeast-based artificial medium and maintained in plastic cages under laboratory conditions (23 ± 1 °C, 16L:8D, 70 ± 10% RH). The *T. drosophilae* individuals used for the field releases were provided by Bioplanet s.c.a. (Cesena, Italy) in commercial bottles each containing about 500 individuals (TRICHOPRIA®).

The original *T. drosophilae* population derived from individuals collected during the 2014 season in northern Italy (Lodi; 45°33'65.69"N, 9°48'06.14"E). Insect bottles were packed into polystyrene boxes provided with ice packs and shipped to the various laboratories (see Table 1) by express courier one or two days prior to field releases. In one case, i.e., Cesena, insects were picked at the private

Table 1
Details on the *Trichopria drosophilae* release locations and crops.

Site	Working group	Locality (Province)	Latitude	Longitude	Altitude (m a.s.l.)	Crop	First release
1	Edmund Mach Found.	Pergine (Trento)	46°07'73.84"N	11°26'44.59"E	567	Raspberry	10 May
2	Edmund Mach Found.	Samone (Trento)	46°07'62.81"N	11°53'26.87"E	803	Blueberry	15 June
3	Univ. of Padua	Valpolicella (Verona)	45°34'21.40"N	10°55'07.0"E	640	Cherry	20 May
4	Univ. of Padua	Cesena (Forlì-Cesena)	44°4'54.77"N	12°14'23.68"E	210	Cherry	17 May
5	Univ. of Bologna & C.F.P. Modena	Vignola (Bologna)	44°27'49.07"N	10°59'30.54"E	122	Cherry	10 May
6	Univ. of Torino & Agrion	Peperagno (Turin)	44°18'42.98"N	7°38'20.96"E	658	Blueberry	06 July
7	Univ. of Milan	Sondrio (Milan)	46°10'53.57"N	9°39'17.59"E	594	Blueberry	08 June
8	Univ. of Catania	Maletto (Catania)	37°49'26.02"N	14°54'7.42"E	1050	Strawberry	18 May

company on the same day as the release. In two locations, the Edmund Mach Foundation and the University of Catania representing north and south Italy, respectively, quality controls were carried out on the received insect bottles. The amount of live and dead parasitoids, their sex-ratio and the possible presence of unemerged puparia were recorded. A comparative assessment of the parasitoid size, initial eggload and longevity (both on water and 50% honey solution) was also carried out on both commercial *T. drosophilae* (reared on *D. melanogaster*) and laboratory *T. drosophilae* reared on *D. sukuzii* and deriving from wild adults collected by sweep netting on *Rubus* sp. bushes located in a natural area of Biancavilla (Sicily, Italy), during fall 2015.

2.2. Field trials

Field trials were carried out in eight sites, at different altitudes, and on different crops (Table 1). For each site, a control plot and a treated plot were selected. The two plots were selected as they had a very similar width, elevation, orientation, cultivar, crop management and records of *D. sukuzii* presence. The distance between the two plots was sufficiently short to ensure the same climatic conditions, but large enough to reasonably prevent any significant migration of parasitoids between the two areas (range: 0.3–2 km). Each plot was close to a buffer zone hosting wild vegetation.

One week before the first release of parasitoids, two sentinel traps were placed in each plot (one in the buffer zone and one in the orchard), in order to assess the potential presence of *T. drosophilae* natural populations. Each trap consisted of a white Delta trap containing a plastic cup (300 mL) closed by a fine-mesh net (20 mesh, ≈ 0.8 mm) allowing parasitoids to pass through but stopping drosophilids. The effectiveness of the net was tested prior to the beginning of field trials (data not shown). Plastic cups were baited with a fresh substrate (banana slices) infested by a known number of 3rd instar *D. sukuzii* larvae close to pupation (30–60 individuals).

For all sites, the first parasitoid release was scheduled in the week before the full maturation of the fruit in the field. Consequently, for each site, the timing of the trial varied according to the crop, the cultivar and the pedo-climatic characteristics of the plots (Table 1). Parasitoids were released into the treated plot from a single release point (RP) located at the border of the field, within the buffer zone. A corresponding point (CP) was set within the buffer zone of the control plot. For the treated plot, nine sentinel traps (prepared as described above) were placed along three sampling lines rising from the RP. The distances between the RP and the traps along each line were 10, 20 and 40 m. In the control plot, four traps were deployed along a single line rising from the CP, except for Peveragno, Maletto and Vignola where fewer traps were set due to the characteristics of the orchards.

Trichopria drosophilae adults were released weekly at the RP for five consecutive weeks. The number of individuals per release was 1000 adults, corresponding to the content of two commercial bottles (sex-ratio 50:50). Fruit was sampled weekly from the plants, and when available from the ground. Sampling from the plants consisted of 20 ripe berries/fruits, randomly picked from the plants surrounding each trap of the treated and the control orchards. For the traps located within the buffer zone, the sampling was performed on wild berries/fruits, if present at that time. Fruit sampling from the ground was performed by dividing the plots into three zones: the buffer zone, the proximal zone (orchard area within 20 m from the RP/CP), and the distal zone (orchard area beyond 20 m from the RP/CP). For each zone, 50 berries/fruits were sampled weekly. Collected traps and sampled fruit were incubated under controlled conditions ($23^{\circ}\text{C} \pm 2$; 60–70% RH) for 25 days. During this time, *D. sukuzii* hatchings were recorded only for the first 10 days, in order to avoid tally of the 2nd generation individuals, while parasitoid emergences were recorded for the entire incubation period. The number and sex ratio of emerged flies and parasitoids were recorded for both traps and sampled fruit, as well as the percentage of infestation of the sampled fruit.

2.3. Semi-field trials

Semi-field trials were set up in a high tunnel (30×5 m) covered by an aphid-proof net (135 g/m^2), in order to prevent flies and parasitoids from escaping. Two walls made of the same net, were placed internally at 10 and 20 m, in order to divide the structure into three sectors (50 m^2 each). Within the tunnel, two raspberry rows were cropped (cv. Tulameen and Heritage). Each row contained 15 potted plants, so that every sector housed five plants of each cultivar. For Tulameen, the fruiting period covered the entire month of August, whereas for Heritage, fruiting started towards the end of August and continued until the end of September. One week before the beginning of the fruit ripening (week 0), an artificial infestation of *D. sukuzii* was performed in each sector (1 couple/m^2).

The central sector was set as a control, whereas the east and west sectors were set as treatment 1 (T1) and treatment 2 (T2) respectively. In particular, T1 consisted of one release of *T. drosophilae* during week 1 and during week 2 ($0.5 \text{ couple/m}^2/\text{week}$), while T2 consisted of the same parasitoid releases plus the presence of *augmentoria*. The *augmentorium* consists of containers of various sizes, where infested fruit is regularly deposited. The structure is isolated from the external environment by a fine mesh net such that emerging pests remain confined inside the container whereas parasitoids are able to exit. This sequesters part of the pest population from the field, and also increases the number of the natural enemies (Klungness et al., 2005). In our experiment, two cubic *augmentoria* (30 cm side; 0.027 m^3 each) were placed in the T2 sector.

During the entire fruiting period, fruit was sampled weekly from each sector as follows: 1) from the plants, by randomly picking 20 fruits/plant, 2) from the ground by collecting 20 fruits/plant that had dropped from 0 to 7 days beforehand (i.e. newly dropped fruit, NDF), and 3) from the ground by collecting 20 fruits/plant that had been on the ground from 8 to 14 days (i.e. old dropped fruit, ODF). The NDF and ODF on the ground were physically separated: each week, part of the NDF was moved onto plastic plates (30×60 cm) located between the raspberry rows and collected the following week as ODF. The distinction between these two categories was made in order to verify whether the *T. drosophilae* emergence rate was affected by the fruit sampling time. After each collection, the plates were cleaned and loaded with other NDF. In T2, all the NDF that remained on the ground after fruit samplings and being loaded onto plastic plates was collected in the *augmentoria*.

2.4. Statistical analysis

Differences in the quality controls on commercial parasitoids and the comparative evaluation of *T. drosophilae* strains were tested with the Mann-Whitney *U* test (Mann and Whitney, 1947). For the field trials, the distribution of the parasitoid was expressed as the frequency of parasitoid emergence from the traps and tested with the likelihood ratio G-test followed by Ryan multiple comparisons for proportions (McDonald, 2014; Ryan, 1960). The average number of both *D. sukuzii* and *T. drosophilae* adults emerged from traps over the five weeks of releases was tested using the Kruskal-Wallis test followed by Dunn's post hoc for possible differences between sites and distances, whereas the Mann-Whitney *U* test was used to test possible differences between treated and control plots. For the semi-field trial, a Friedman test (nonparametric repeated measures ANOVA) with five replications followed by the Bonferroni post hoc test (Siegel and Castellan, 1988) was performed to test differences in the overall percentage of *T. drosophilae* emergence from the ground-sampled fruit between the control and four treatments (NDF and ODF in T1 and T2).

The weekly *D. sukuzii* emergences from the ground-sampled fruit were tested using the Kruskal-Wallis test followed by Dunn's post hoc for possible differences between control and treatments. In order to evaluate the *augmentorium* effect, the weekly difference in *T. drosophilae*

emergence between T1 and T2 from the ODF ground-sampled fruit was calculated and a turning point (TP) analysis was performed to estimate the beginning of a new trend along the series. A TP corresponded to the point at which the cumulative difference between the average value and each individual value reached the largest absolute value, and was estimated using the cumulative sum statistic method (Pettitt, 1979). To test the null hypothesis that there was no TP, the Mann–Whitney *U* test was used to compare the two data series, before and from the candidate TP (Siegel and Castellan, 1988). All analyses were run using Statistica 64© 12 (StatSoft. Inc., Tulsa, OK).

3. Results

3.1. Parasitoid quality controls

No difference was observed between the commercial parasitoids in the quality controls carried out in northern Italy and in southern Italy, both in terms of living individuals and sex ratio (Table 2). In the bottles shipped to Trento, significantly more dead males were found ($U = 33$; $p < .05$), whereas no difference was observed for the females (Table 2). The *D. melanogaster*-reared parasitoids (commercials) were significantly smaller than the *D. suzukii*-reared ones and carried a lower initial eggload ($U = 0$; $p < .01$ and $U = 108.5$; $p < .01$ respectively; Table 2). Nonetheless, the commercial *T. drosophilae* lived longer than the laboratory parasitoids when provided only with water ($U = 126$; $p < .01$), whereas no difference in longevity was observed when provided with both water and honey (Table 2).

3.2. Field trials

3.2.1. Presence of parasitoid natural populations

No natural population of *T. drosophilae* was recorded from the traps during the week prior to the first parasitoid release. During the release period, *T. drosophilae* adults emerged from traps located in the control plots in only two sites out of eight (Fig. 1). These emergences were scant in amount, representing 5.59% and 0.47%, respectively, of the total trap emergences from Vignola and Sondrio control plots over the five weeks of sampling. These rates were taken into account for the calculation of the treated plot parasitism rate in the two sites. No larval parasitoids emerged from the traps, whereas parasitism activity by *P. vindemiae* was recorded throughout the trials. This activity varied according to the site and the week, ranging between 0% and 36.4% of the overall parasitization observed. However, *P. vindemiae* emergences were never significantly different between the control and the treated plots ($U = 12$; $p > .05$).

3.2.2. *Trichopria drosophilae* operating range and dispersal

In all sites but Maletto, *T. drosophilae* infested the traps located at the maximum distance from the release point (Fig. 1). The average emergence frequency gradually decreased from 10 to 40 m along the sampling line ($G = 23.57$; $df = 1$; $p < .001$). Statistical analysis showed that both the sampling area and the altitude were determinants of the frequency of *T. drosophilae* emergences from the traps, suggesting the parasitoid's specific needs for temperature and humidity. In particular, *T. drosophilae* emerged more often from traps located in the buffer zones than in the orchards ($G = 3.82$; $df = 1$; $p = .0476$) and in those sites located within 700 m a.s.l. ($G = 49.37$; $df = 1$; $p < .001$). No effect on the emergence frequency was observed considering the crop, sampling time (both on a monthly and weekly basis), and number of releases (data not shown).

3.2.3. *Trichopria drosophilae* biocontrol efficacy

Drosophila suzukii emergences were significantly reduced in the traps located at 10 m ($U = 1579$; $p = .0011$), moreover a strong tendency to reduction was observed at 20 m ($U = 1944$; $p = .072$) (Fig. 2a). The increase in emerged flies along the sampling lines

($H = 8.53$, $df = 2$, $p = .014$) was proportional to the reduction in parasitoid emergence ($H = 23.99$; $df = 2$; $p < .001$) (Fig. 2a). The average *T. drosophilae* and *D. suzukii* emergences were different among sites ($H = 11.92$; $df = 6$; $p < .05$ and $H = 98.94$; $df = 7$; $p < .001$ respectively; Fig. 2a). A reduction in *D. suzukii* emergence spanning from 60% up to 93% was observed in those traps from which *T. drosophilae* emerged with respect to those where this parasitoid was absent (Fig. 2b). In Maletto, only one *T. drosophilae* individual emerged from the traps throughout the entire trial period, whereas *D. suzukii* emergence from the traps with no parasitoid was comparable to most of the other sites (Fig. 2b). In both the orchards and the buffer zones, fruit samplings on the plant did not show any significant differences between the treated and the control plots, both in terms of infestation rate and of *D. suzukii* emergence. Valpolicella was the only site in which *T. drosophilae* emergences were observed on the plant sampled fruit (two adults in the second week). Fruit sampling on the ground revealed very scant *T. drosophilae* emergences (Fig. 2c), which were not consistent with the high pest infestation level observed in the same fruit and with the parasitization rate observed within the traps.

3.3. Semi-field trials

No difference was observed in plant sampled fruit between the two treatments and the control, both in terms of fruit infestation rate and pest emergence (Fig. 3a-b). With regard to the ground sampled fruit, no parasitoids emerged from the control, while in both T1 and T2, a significantly higher *T. drosophilae* emergence was observed from the ODF with respect to the NDF ($\chi^2 = 33,904$; $DF = 4$; $p < .001$; Fig. 3c). The *augmentorium* treatment (T2) significantly increased the *T. drosophilae* emergence from ODF starting from week 4. This corresponded to a significant turning point according to the Mann–Whitney *U* test after the Pettitt test (asterisk in Fig. 3d, $U = 89$; $p = .043$). Similarly, in T2 *D. suzukii* emergences from ODF were significantly reduced with respect to the control starting from week 4 ($H = 7.28$; $df = 4$; $p = .0263$), whereas in T1 this reduction was observed only at week 7 ($H = 6.08$; $df = 4$; $p = .0478$; Fig. 3e).

4. Discussion

Our study provides new important information about the potential exploitation of *T. drosophilae* for *D. suzukii* augmentative biocontrol programs. We tested the *D. suzukii* biocontrol and dispersal capacity of

Table 2

Median and interquartile range (IQR, 25th–75th percentile) of the quality control tests carried out on commercial packages of *Trichopria drosophilae* received in northern Italy (Foundation Edmund mach) and southern Italy (University of Catania), and of the comparative biological analysis of laboratory and commercial parasitoids reared on *Drosophila suzukii* and *Drosophila melanogaster*, respectively. Single and double asterisks indicate significant differences ($p < .05$ and $p < .01$, respectively) after the Mann–Whitney *U* test.

Quality checks on commercial <i>T. drosophilae</i>	Living individuals per bottle	% females	Living individuals (%)	
			Males	Females
North Italy	540 (506–581)	53 (49–57)	87 (84–91)	88 (86–93)
South Italy	574 (531–670)	55 (52–58)	97 (90–98)	95 (71–96)
	ns	ns	*	ns
Comparative evaluation of <i>T. drosophilae</i> populations	Hindleg tibia (μ m)	Initial eggload (n° eggs)	Longevity at 23 °C (days)	
			Water	Honey
Commercial	390 (383–401)	47 (45–49)	12 (9–13)	43 (34–50)
Laboratory	520 (490–540)	52 (49–62)	9 (7–11)	37 (29–45)
	**	**	**	ns

Single and double asterisks indicate significant differences ($p < .05$ and $p < .01$ respectively) after Mann–Whitney *U* test. Ns = not significant.

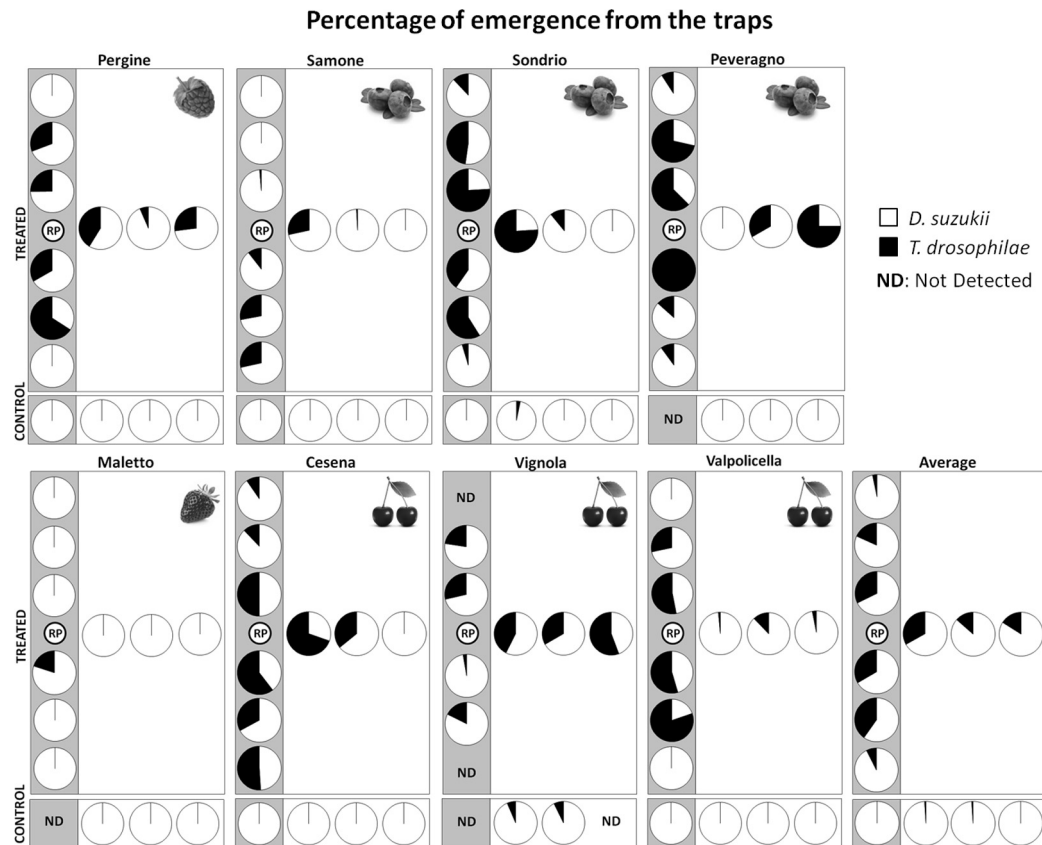


Fig. 1. Field trials. Average percentage of emergences of both hosts and parasitoids from the baited traps during the five weeks of releases. Data are sorted by site and crop. Grey areas represent buffer zones, white areas represent orchards.

this parasitoid in multiple environments, on different crops, at different altitudes and in various geographical areas of Italy. The results were consistent among seven sites out of eight and clearly suggested that *T. drosophilae* has the potential to impact the *D. suzukii* emergence rate in the field.

In the open field trials, *T. drosophilae* infested the sentinel traps up to 40 m from its release point and toward multiple directions, nonetheless the *D. suzukii* emergence was significantly reduced only in those traps located at 10 m along the sampling lines. This was likely due to the low number of released parasitoids (1000 individuals per week, 50% sex ratio) and their consequent dispersion within the environment, which resulted in a parasitism gradient over the distance from the release point.

In biocontrol programs, parasitoids are distributed according to a precise scheme, which should guarantee an even dispersion of the natural enemy over a certain area. In this regard, information on parasitoid dispersal capacity is fundamental for optimizing its release frequencies and numbers (Heimpel and Asplen, 2011; Zappalà et al., 2012).

Our data provide some initial data, which we believe can be useful for future biocontrol strategies using *T. drosophilae*. In our experimental conditions, the overall trap visit frequency per distance (Fig. 1) rapidly decreases over the sampling lines. To maintain the same trap infestation rate observed at 10 m, release points should be set every 30 m, since such distance corresponds to a 50% decrement of the observed trap visit frequency. However, it is clear that this distribution is merely theoretical and needs to be adapted to each case, since in the field several environmental constrains (e.g. vegetation structure, main wind direction, temperature and humidity gradients, etc.) create preferential pathways for the parasitoid movements. We observed a slight but significantly higher frequency of *T. drosophilae* emergences from the traps

located in the buffer zones than in those located in the orchards.

Most of our field trials were carried out under conventional cropping systems. In these conditions many factors, such as the use of chemicals, the prunings or lack of non-crop vegetation, make the orchard less attractive for natural enemies (Ruberson et al., 1998; Pimentel, 2008). In order to improve this aspect, conservative approaches are key to creating the right humidity, temperature and food availability conditions for predators and parasitoids (Gillespie et al., 2016; Jonsson et al., 2008; Barbosa, 1998).

The creation of a suitable environment within the crop may be of particular importance for *T. drosophilae*, since this pupal parasitoid seems to prefer semi-natural rather than agricultural areas (Knöll et al., 2017). Studies on faunistic surveys of *D. suzukii* natural enemies have shown that *T. drosophilae* is not one of the most abundantly caught species and that its occurrence was far from regular among the sites considered (Gabarra et al., 2015; Daane et al., 2016; Mazzetto et al., 2016; Knöll et al., 2017). Our preliminary assessment of the parasitoid presence was consistent with these findings and showed no presence of *T. drosophilae* before the parasitoid releases, whereas few individuals were recorded in the control plots during the trials and from just two sites (Vignola and Sondrio).

In contrast with the trap parasitism level, the number of *T. drosophilae* emerged from the ground-sampled fruit was almost null. This lead us to hypothesize four possible reasons: 1) a real absence of parasitism, 2) a sampling bias due to the operator's tendency to collect freshly fallen intact fruits in instead of rotten or melted ones, that tend to host *D. suzukii* larvae, 3) a loss of parasitized hosts due the larval tendency to come out from the fruit and pupate on the ground (Gabarra et al., 2015; Woltz and Lee, 2017), and 4) a loss of parasitized hosts due to predation by ground predators (Woltz and Lee, 2017). These hypotheses were partially verified through the semi-field trials, in which

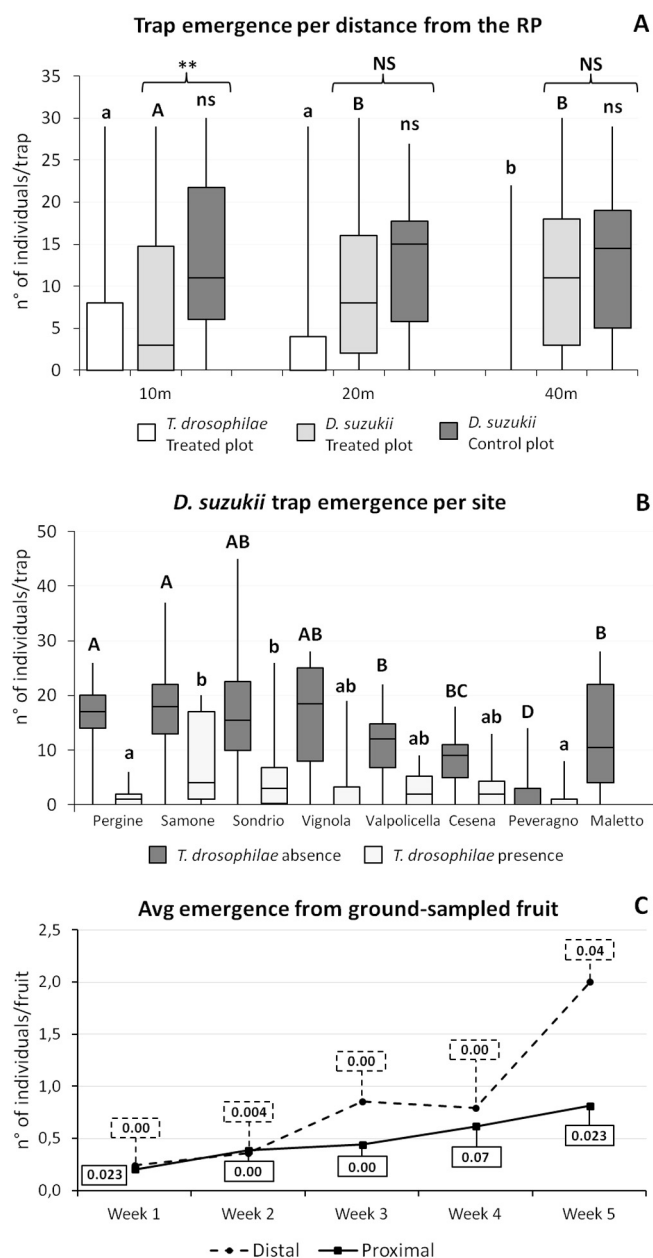


Fig. 2. Field trials. A) Overall emergences (either flies and parasitoids) from the traps placed at various distances from the release point (RP), and B) *D. sukuzii* emergences from the traps of each site in the presence or absence of *T. drosophilae* attack. Different letters indicate significant differences ($p < .05$) after the Kruskal-Wallis test followed by Dunn's post hoc test. Double asterisks indicate significant differences ($p < .01$) after the Mann-Whitney *U* test. C) Average number of individuals emerged per ground-sampled fruit during the five weeks of releases (all sites). Solid and dashed lines indicate the weekly *D. sukuzii* emergence rates from the proximal and the distal zone respectively. The corresponding *T. drosophilae* emergence rates are reported in the squares.

we observed a significant increase in the parasitoid's emergence rate when collecting older fallen fruits (i.e. 8–14 days old) together with the ground-dropped pupae (plastic plates collections).

This suggests either that the ground-sampled fruit collected in the field trial was too fresh, containing mainly larvae rather than pupae, or that most of the *D. sukuzii* larvae pupated onto the ground, dropping from the fruit before they could be collected. In both cases the *T. drosophilae* parasitism rate in the field may have been underestimated. The role played by other natural enemies in removing *D. sukuzii* pupae is still an open issue. Woltz and Lee (2017) observed a 61–91% increase in *D. sukuzii* pupae in the treatments in which ground predators were

excluded. As direct observation, we often found ants and rove beetle species within the sentinel traps, thus part of the parasitized *D. sukuzii* pupae may have been lost due to high predation levels. In the semi-field trials, the parasitoid releases significantly reduced the emergences of *D. sukuzii* from the fruit with respect to the control in both treated sectors. The *augmentorium* technique enhanced parasitoid activity by increasing the number of its emergences. This increment became evident in the third week, corresponding to the emergence of the first *T. drosophilae* generation from the *augmentoria*-collected fruit. Previously, the *augmentorium* technique has been successfully applied against different fruit fly species in Hawaii and Reunion Island (Jang et al., 2007; Deguine et al., 2011).

There are two key factors for the success of this technique: 1) the choice of net, which should be able to sequester the target pest but allow its parasitoids to pass through it, and 2) the application of the *augmentoria* with an area wide approach and over a long time period (Deguine et al., 2011). For the semi-field trials we used a 20 mesh net (0.8×0.8 mm) which excludes more than 95% of *D. sukuzii* individuals but allows *T. drosophilae* to pass through (Rossi Stacconi pers. comm.). As concerns the second point, we performed an artificial *D. sukuzii* infestation in a closed environment (50 m^2) and for a limited time (seven weeks), thus not taking into account the issues dealing with pest population boosts from external sources or parasitoid dispersion.

Another important aspect of the *augmentorium* is the creation of patches in the host distribution pattern. Although several biotic and abiotic factors contribute to define the parasitoid attack rate in a patchy environment (van Lenteren and Bakker, 1978; Abrams, 1982; Fellowes et al., 2005), three main types of density dependent responses (functional response) have been described for parasitoids (Holling, 1959; Fellowes et al., 2005). The high level of trap exploitation observed in our field trials and the parasitoid population increase in the greenhouse *augmentorium* treatment suggest that *T. drosophilae* would benefit from the use of *augmentoria*. In fact, when *T. drosophilae* is offered with different host density patches of *D. sukuzii* pupae (either singly or simultaneously), it reacts with a strong aggregation response towards high host density patches both in terms of residence time, frequency of first encounter, and increase in parasitism (Kaçar et al., 2017).

As a pupal parasitoid, *T. drosophilae* parasitism activity is performed once the damage has already occurred. Therefore, its use for augmentation biocontrol (both through inoculative or inundative strategies) should be implemented on a yearly basis before the ripening of the first *D. sukuzii* host fruits (i.e. cherry). In addition, if an effective *D. sukuzii* larval parasitoid is identified (Daane et al., 2016), a combined use with *T. drosophilae* may lead to an increase in total efficacy, as already demonstrated for the biological control of the fruit fly *Anastrepha fraterculus* Wiedemann (Van Nieuwenhove et al., 2016).

The comparative evaluation of *D. melanogaster*-reared (commercial) and *D. sukuzii*-reared (laboratory) *T. drosophilae* showed significant differences between the two populations. In line with previous findings (Wang et al., 2016c), parasitoids reared from *D. melanogaster* were smaller than those reared from *D. sukuzii*. The laboratory *T. drosophilae* carried more eggs at emergence, further suggesting a fitness advantage. Positive size-fitness relationships have been previously investigated in both egg, larval and pupal parasitoids, often observing a size range above which fitness reaches a plateau or even decreases (Visser, 1994; Kazmer and Luck, 1995; West et al., 1996; Ellers et al., 1998). Further investigations on this topic are required in order to assess the effect of the natal host on the adult parasitoid performances, as evaluated for other indigenous parasitoids adapted to exotic pests (Bodino et al., 2016). In fact, an increased fitness of *D. sukuzii*-reared *T. drosophilae*, could have an impact on *D. sukuzii* biocontrol, not only by increasing the average per capita efficacy of the parasitoid (Kazmer and Luck, 1995), but also by driving its host preference toward the target pest through pre-immaginal learning (Gandolfi et al., 2003; Papaj and Alcinda, 2012). Nonetheless, *D. sukuzii* may not be a good candidate for rearing *T. drosophilae* on a large scale, due to its lower fitness and more

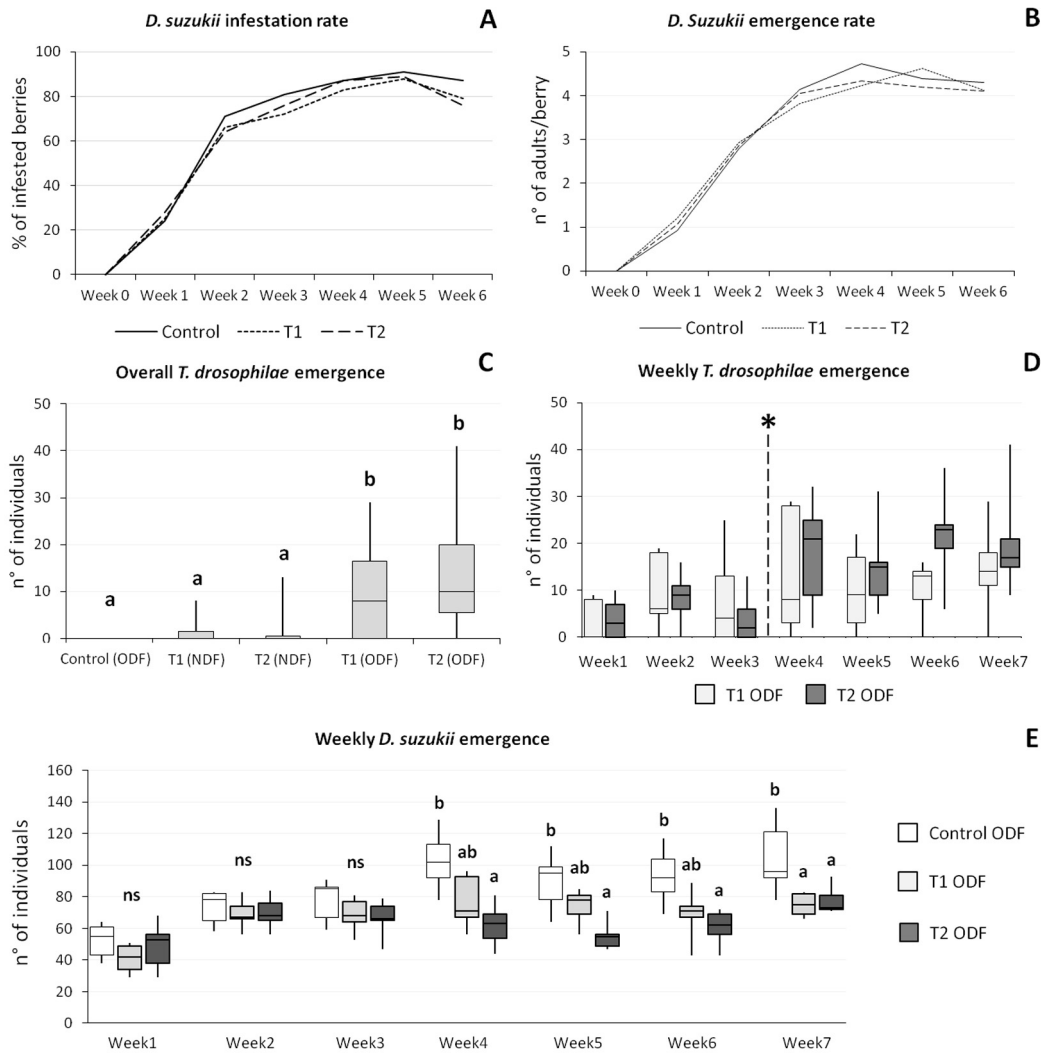


Fig. 3. Semi-field trials. A) Weekly berry infestation rate and B) *D. suzukii* emergence rate from the plant-sampled fruit. C) *T. drosophilae* emergences from the ground-sampled fruit during the whole trial period. Different letters indicate significant differences ($p < .05$) after Friedman's test with replication followed by Bonferroni post hoc test. D) Weekly *T. drosophilae* emergences from the old dropped fruit (ODF). The dashed line indicates the presence of a turning point (TP) in the emergence trends. The asterisks indicate significant differences ($p < .05$) between the two data series, before and after the TP, following Mann-Whitney *U* test. E) Weekly *D. suzukii* emergence from the old dropped fruit. For each week, different letters indicate significant differences ($p < .05$) after the Kruskal-Wallis test followed by Dunn's post hoc test.

advanced nutritional requirements compared to *D. melanogaster* (Hamby et al., 2016), thus an economic evaluation of *T. drosophilae* mass production using *D. suzukii* as host is also necessary.

5. Conclusions

We have demonstrated that artificially released *T. drosophilae* can attack *D. suzukii* in multiple open-field environments and this ability could be exploited to set up a *D. suzukii* biological control strategy. In our opinion, the strategy should focus on reducing the *D. suzukii* population just at the very beginning of the season, when the few *D. suzukii* adults that survived the winter are looking for alternative food sources (wild vegetation flowering and fruiting, compost, etc.). In this scenario, periodic augmentative releases of *T. drosophilae* over a wide area and at *D. suzukii* feeding hotspots could regulate the pest population dynamics prior to fruit ripening in the orchards and then later throughout the entire season.

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