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Drosophila parasitoids in northern Italy and their potential to attack the exotic pest Drosophila suzukii

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Abstract *Drosophila suzukii* is an invasive alien pest recently introduced into Europe and North and South America. Several control methods have been tested, and the ability of natural enemies to control this pest has been investigated. This study aimed to identify the main parasitoids of drosophilids in North Italy via field surveys, and to evaluate the ability of some of those species emerged to parasitize *D. suzukii* compared to indigenous *D. melanogaster*. A nine-site survey from July to October 2014 that exposed fruit (banana and blueberry) for 7 and 14 days obtained six parasitoid species, ranked from highest abundance: *Leptopilina boulardi*, *L. heterotoma* (Hymenoptera: Figitidae), *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae), *Trichopria* cf. *drosophilae* (Hymenoptera: Diapriidae), *Asobara tabida* (Hymenoptera: Braconidae), and *Spalangia erythromera* (Hymenoptera: Pteromalidae). The presence and abundance of these species varied greatly among the sites and across the season. The field survey results showed a relationship between parasitoids and indigenous *Drosophila* communities and a high host competition. The ability of larval parasitoids *L. boulardi* and *L. heterotoma* and pupal parasitoid *T.* cf. *drosophilae* to parasitize the exotic and indigenous hosts was laboratory tested. Both larval parasitoids failed to develop on *D. suzukii*, but high mortality was recorded in larvae exposed to *L. heterotoma*. On the contrary, *T.* cf. *drosophilae* developed successfully on *D. suzukii*, with no significant differences between the exotic and indigenous hosts. These results beg further investigations of indigenous enemies, particularly *T.* cf. *drosophilae*, for effective biological control of *D. suzukii*.

Keywords Spotted-wing drosophila · Fruit dish traps · No-choice and choice tests · *Leptopilina boulardi* · *Leptopilina heterotoma* · *Trichopria* cf. *drosophilae*

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Key message

- Knowledge of the distribution and abundance of parasitoid species related to frugivorous Drosophilidae is key to the implementation of a biological control against *Drosophila suzukii*.
- Six parasitoid species (three larval and three pupal parasitoids) related to frugivorous Drosophilidae were detected in North Italy.
- Laboratory experiments demonstrated the capability of *Trichopria* cf. *drosophilae* to parasitize successfully *D. suzukii*.
- Rearing and release of parasitoids, such as *T.* cf. *drosophilae*, could be implemented for effective control of the exotic fly.

Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae), commonly known as the spotted-wing drosophila, is a pest native to Southeast Asia that is now widespread in other parts of Asia, as well as in North America, South America, and Europe (Kanzawa 1939; Hauser 2011; Calabria et al. 2012; Deprà et al. 2014). Unlike many drosophilids, *D. suzukii* females can lay eggs in ripening fruits pre-harvest and cause heavy economic losses, especially to cherries and soft fruits as reported in the United States and Europe (Goodhue et al. 2011; Escudero Colomar et al. 2012; Grassi and Pallaoro 2012; Weydert et al. 2012; De Ros et al. 2013). Besides fruit crops, *D. suzukii* can infest ornamental and wild, alien or native, fruiting plants (including mulberry and fig trees), which can serve as fly reservoirs (Mitsui et al. 2010; Yu et al. 2013; Poyet et al. 2014).

Due to its negative impact on several cropping systems, different control methods have been tested and implemented. Many studies have been directed at finding specific and selective attractants or chemical lures of *D. suzukii* (Landolt et al. 2012a, b; Cha et al. 2012, 2013, 2014, 2015; Iglesias et al. 2014). Although different substances have shown an attractive effect to *D. suzukii*, they have not yet represented a complete solution for most cropping systems (Rossi Stacconi et al. 2015). While spinosyn, pyrethroid, and organophosphate insecticides effectively control *D. suzukii* in both laboratory and field trials (Bruck et al. 2011), the rapid growth of fly populations and damage occurrences near harvest time require several chemical interventions on fruit at the ripening stage that impact human and environmental health negatively. In fact, these treatments raise several risks: residue on fruit, insect resistance, and detrimental effects on pollinators and other beneficial species (Cini et al. 2012; Rota Stabelli et al. 2013). Moreover, research has focused on natural enemies, predators and parasitoids, to identify the most promising candidates for an effective biological control, which is an important component of *D. suzukii* areawide management.

Field surveys and laboratory experiments on potential enemies of *D. suzukii* have been conducted in Japan, North America, and Europe. In central Japan, *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae) and *Ganaspis xanthopoda* (Ashmead) (Hymenoptera: Figitidae) are two common larval parasitoids of frugivorous drosophilids. However, in laboratory trials only *A. japonica* successfully parasitized *D. suzukii* larvae, whereas *G. xanthopoda* rarely, if ever, oviposited in these larvae (Mitsui and Kimura 2010). Similarly, in laboratory experiments conducted in France *A. japonica* caused the highest level of parasitism (more than 95 %) in *D. suzukii* larvae (Chabert

et al. 2012). Indeed, *A. japonica* and other species or populations of the genus *Ganaspis*, native to Southeast Asia as *D. suzukii*, are considered the most effective larval parasitoids to control the pest (Kacsoh and Schlenke 2012; Kasuya et al. 2013). By contrast, *Asobara tabida* Nees, which is the most common parasitoid of frugivorous drosophilids in Europe, and *A. rufescens* Foerster have shown no ability to parasitize *D. suzukii* (Chabert et al. 2012; Cini et al. 2012).

Two larval parasitoids *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault) and *L. heterotoma* (Thomson) (Hymenoptera: Figitidae), spread across Europe (Fleury et al. 2004), have been studied in both Europe and the US (Chabert et al. 2012; Kacsoh and Schlenke 2012; Rossi Stacconi et al. 2015). In French and US studies, these parasitoids successfully oviposited in, and killed *D. suzukii* larvae, yet no adults emerged from the attacked larvae. This finding suggests that they could not develop on this fly, probably because of a strong immune response (Chabert et al. 2012; Kacsoh and Schlenke 2012). However, recently an Italian population of *L. heterotoma* proved to overcome the immunological response of *D. suzukii* (Rossi Stacconi et al. 2015), promoting further investigation into the capability of these larval parasitoids to parasitize successfully the exotic fly.

Under laboratory conditions, pupal parasitoids, such as *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria* cf. *drosophilae* (Perkins) (Hymenoptera: Diapriidae), proved to be more effective than larval parasitoids (Chabert et al. 2012; Rossi Stacconi et al. 2013). The term "cf." used in the taxonomy of this species is the abbreviation of the latin word "confer" (=compare) indicating the uncertain identification of the species. *Pachycrepoideus vindemiae*, which is widespread in Europe, the US, and Japan (Mitsui et al. 2007; Rossi Stacconi et al. 2013), is known to parasitize a wide range of hosts, especially cyclorrhaphous Diptera. It can also act as a hyperparasitoid by attacking beneficial Hymenoptera, including *A. tabida* and *L. heterotoma* (Van Alphen and Thunissenn 1993; Wang and Messing 2004). *Trichopria* cf. *drosophilae* is yet another parasitoid with the capability to develop on various drosophilids, albeit to a lesser degree than *P. vindemiae*, including many frugivorous *Drosophila* species (Mitsui et al. 2007; Chabert et al. 2012; Rossi Stacconi et al. 2015).

Field surveys on parasitoids of frugivorous Drosophilidae have been conducted across the globe (Janssen et al. 1987; Kraaijeveld and Godfray 1999; Allemand et al. 2002; Mitsui et al. 2007). Most of the studies focused on the evaluation of parasitoid resistance and virulence toward indigenous Drosophilidae (Kraaijeveld and Godfray 1999; Fleury et al. 2009). In Italy, the drosophilid–parasitoid

community has been studied far less until the recent flurry of work to identify potential parasitoids of the exotic *D. suzukii* in Northeast Italy after its introduction (Rossi Stacconi et al. 2013, 2015). Therefore, this study aimed first to assess the presence and abundance of indigenous parasitoids of *Drosophila* spp. in three regions of North Italy (Piedmont, Lombardy, and Emilia-Romagna), where *D. suzukii* has become a noxious crop pest (Gargani et al. 2013; Mazzetto et al. 2015). Second, we selected and tested some parasitoid species emerged from the field-collected samples on exotic host *D. suzukii* and indigenous host *D. melanogaster* Meigen in the laboratory, to evaluate their potential as biological control agents of the exotic fly.

Materials and methods

Field monitoring of parasitoids of *Drosophila* spp.

Surveyed sites

Field surveys were carried out in nine total sites (Site) during 2014; three sites were located in each of the three North Italy regions (Locality): Piedmont, Lombardy, and Emilia-Romagna. Following are the fruiting plants (and sites) selected within each region: two blueberry (Boves and Paesana) and one raspberry plantation (Peveragno) in Piedmont; two raspberry (Arcagna and Minoprio) and one blueberry plantation (Minoprio) in Lombardy; one cherry orchard (Cadriano), one uncultivated area with mulberries (*Morus* sp.) (Dodici Morelli), and one experimental garden with blueberry bushes and fruit trees (cherry, fig, and peach) on the University campus (Bologna) in Emilia-Romagna. Locations and characteristics of the surveyed sites are given in Table 1.

The population levels of *D. suzukii* at each site were evaluated by placing a trap baited with 250 mL of apple cider vinegar (ACV) (5 % acidity). The traps were replaced weekly throughout the trial period (July–October). Removed traps were taken to the laboratory, where the ACV was filtered with a funnel (diameter 26 cm) lined with a fine mesh net to retain all insects. Using a brush, all of the Drosophilidae were counted, collected, and preserved in glass tubes (8 × 60 mm) filled with 70 % (v/v) ethanol. Finally, with a stereomicroscope and identification key (Vlach 2010), all *D. suzukii* flies were separated from the other Drosophilidae, and the numbers of male and female *D. suzukii* were recorded.

Field collection and laboratory observation of parasitoids

The presence and abundance of the parasitoids of *Drosophila* spp. were assessed following a modified protocol used by Fleury et al. (2004). Two open traps (Block) were placed in three different positions along the diagonal of each site from July to October 2014. The open traps consisted of a delta trap, on the bottom of which were placed two plastic dishes (diameter 90 mm), one with split banana and one with healthy blueberries (fruit). Split banana was chosen because it was largely used in similar studies (Novković et al. 2012; Fleury et al. 2004; Chabert et al. 2012), while blueberries were chosen both as a favorite host of *D. suzukii* (Kinjo et al. 2013; Mazzetto et al. 2015) and because they were already used to capture parasitoids (Rossi Stacconi et al. 2013). Each dish represented a statistical unit. To allow oviposition of both larval and pupal parasitoids, the dishes were exposed to natural field colonization for 7 or 14 days. The fruit in six of the dishes was changed every 7 days and the fruit in the other six dishes was changed every 14 days. Overall, four treatments, each with three repetitions, were compared in each site: (1) dishes with banana exposed for 7 days; (2) dishes with blueberries exposed for 7 days; (3) dishes with banana exposed for 14 days; (4) dishes with blueberries exposed for 14 days.

Table 1 Locations and characteristics of the sites where field surveys were carried out in 2014

Region	Site	Position	Crop/vegetation
Piedmont	Boves	44°20′54″N 7°32′04″E 548 m a.l.s.	Blueberry
	Paesana	44°40′47″N 7°17′21″E 572 m a.l.s.	Blueberry
	Peveragno	44°19′23″N 7°37′45″E 590 m a.l.s	Raspberry
Lombardy	Arcagna	45°20′17″N 9°27′07″E 79 m a.l.s.	Raspberry
	Minoprio 1	45°43′37″N 9°05′09″E 334 m a.l.s.	Raspberry
	Minoprio 2	45°43′39″N 9°05′12″E 334 m a.l.s.	Blueberry
Emilia-Romagna	Bologna	44°30′53″N 11°24′21″E 54 m a.l.s.	Fruit trees and bushes
	Cadriano	44°32′57″N 11°23′15″E 28 m a.l.s	Cherries
	Dodici Morelli	44°48′24″N 11°17′07″E 15 m a.l.s.	Mulberries in uncultivated area

After field exposure, the dishes were transferred to the laboratory, where they were arranged to allow the adult emergence of *Drosophila* spp. and parasitoids. The fruits from each dish were placed in disposable, net-covered cups (height 76 mm, diameter 60 mm, volume 100 mL). The disposable cups were kept at room temperature (about 25 °C) for 40 days (i.e., the period necessary to obtain parasitoid emergence) and checked every 48 h to observe adult emergence. All *Drosophila* spp. adults were removed, but only the adults emerged in the first 10 days were counted and stored in 70 % (v/v) ethanol inside micro tubes (length 44 mm, diameter 10.8 mm, volume 2 mL) to avoid overlapping generations. Next, they were examined to separate individuals of *D. suzukii* from those of other *Drosophila* species. All parasitoid adults were removed, counted, and stored in 70 % (v/v) ethanol inside micro tubes (length 44 mm, diameter 10.8 mm, volume 2 mL) throughout the 40-day period. The adults were then examined, separated, and identified using specific keys (Bouček 1963; Graham 1969; Forshage and Nordlander 2008; Vlach 2010). Some individuals of each species of the different areas were sent to the respective specialist to assure a correct specific identification.

Laboratory evaluation of parasitoid ability to parasitize *D. suzukii*

Insect rearing

Laboratory trials were carried out to evaluate the ability of local populations of *L. boulardi*, *L. heterotoma,* and *T.* cf. *drosophilae* to parasitize *D. suzukii* compared to that of *D. melanogaster*; these trials necessitated mass rearing of the five species in our laboratories. Instead, *P. vindemiae* was not tested because its capability to parasitize *D. suzukii* has been widely documented (Chabert et al. 2012; Rossi Stacconi et al. 2013, 2015).

The colony of *D. suzukii* was started from individuals emerged from blueberries, raspberries, and blackberries collected in Cuneo and Torino provinces in Piedmont (NW Italy) during the summers of 2013 and 2014, and the colony of *D. melanogaster* was started from individuals obtained in 2013 from the laboratory of G. Gargiulo (University of Bologna, Dipartimento di Farmacia e Biotecnologie). Both colonies were reared on a maize flour-based artificial diet in plastic cylinders (height 6 cm, diameter 3 cm) with mesh-covered lids. The larvae were reared on an artificial diet containing 15 g L^{-1} sucrose, 10 g L^{-1} soy flour, 17 g L^{-1} dead yeast, 71 g L^{-1} maize flour, and 5.6 g L^{-1} agar. The diet was supplemented with propionic acid (4.7 ‰) and a vitamin mixture (2.5 ‰). Adult flies were kept in Plexiglas® cages (20 × 20 × 20 cm) and fed via cotton balls soaked in a honey and water solution (20 % honey). Twice a week, adults were inserted into three cylinders containing 15 mL of the diet, and maintained for 2–3 days to obtain oviposition. Upon removal, the cylinders were closed with lids and transferred to a tray.

The colonies of *L. boulardi* and *L. heterotoma* were started from individuals emerged from additional dishes with banana and blueberries, placed in the Paesana and Peveragno sites during the late summer 2014. The parasitoids were maintained on fruits infested by *Drosophila* spp. larvae inside Plexiglas® cages (20 × 20 × 30 cm) closed at the top by a fine mesh net (<1 mm² mesh size), and fed with honey drops placed on small pieces of paper.

The colony of *T.* cf. *drosophilae* was started from individuals emerged from samples collected at multiple locations in Lombardy in 2014. Newly emerged parasitoid adults were kept in plastic cylinders (height 6 cm, diameter 3 cm) with mesh-covered lids, and fed with honey drops placed on small pieces of paper. Twice a week, about 50 1-day-old *D. melanogaster* pupae were placed in a cylinder. Eight to ten couples of 2–3-day-old *T.* cf. *drosophilae* adults were transferred into the cylinder and removed after 48 h (Romani et al. 2002).

All insect rearing was maintained at 25 °C ± 1 °C, 50–60 % RH, and 16 L:8 D photoperiod.

Laboratory tests with L. boulardi *and* L. heterotoma

To evaluate the parasitism ability of these two larval parasitoids, no-choice tests were carried out using *D. suzukii* or *D. melanogaster* 2nd–3rd-instar larvae, as reported in Fleury et al. (2000). In the tests, 10 larvae of *D. suzukii* or *D. melanogaster* were inserted into a plastic dish (height 2 cm, diameter 5.5 cm) filled with a thin layer of the artificial diet described above. Similar to Campan et al. (2002), 5–7-day-old *L. boulardi* or *L. heterotoma* females were used. A female, previously kept with a male for 48 h to allow mating, was introduced into each plastic dish. After 48 h, the female was removed, and the dishes where the female was kept were checked every 48 h to detect parasitoid or drosophilid adult emergence. For each parasitoid species and each *Drosophila* species, at least eight replicates were performed, each consisting of 10 larvae (=10 *D. suzukii* or 10 *D. melanogaster* exposed to parasitoid). Moreover, 10 replicates only with *D. suzukii* (each with 10 larvae) and only with *D. melanogaster* (each with 10 larvae) were performed as control without parasitoid. The results were evaluated by a number of successfully parasitized larvae (i.e., larvae from which a parasitoid adult emerged), and number of dead larvae (i.e., larvae from which neither a parasitoid nor a drosophilid adult emerged). The tests were conducted at 25 °C ± 1 °C, 50–60 % RH, and 16 L:8 D photoperiod.

Laboratory tests with T. *cf.* drosophilae

To evaluate the effectiveness of *T.* cf. *drosophilae*, no-choice and choice tests were carried out. In both tests, 1-day-old drosophilid pupae were exposed to 2–3-day-old parasitoid females for 48 h, as reported in Romani et al. (2002). The tests were performed in cylinders like those described above, although in this case the bottoms had been previously filled with 15 mL agar-water suspension (6 % agar) to avoid pupa dehydration. Prior to the experiment, the females were kept with males for at least 48 h to allow mating. When the females were removed, the cylinders where the females were alive were checked daily to detect parasitoid or drosophilid adult emergence. The pupal weights of the two *Drosophila* species were compared, and the newly formed pupae (<24 h) were weighed. Six replicates, each consisting of 10 pupae per species, were tested at 25 °C ± 1 °C, 50–60 % RH, and 16 L:8 D photoperiod.

For no-choice tests, 10 pupae of *D. suzukii* or *D. melanogaster* were exposed to one *T.* cf. *drosophilae* female. For each host species, 10 pupae were not exposed to parasitoid females to be maintained as controls. Six replicates were performed, each consisting of 40 pupae (=10 *D. suzukii* or 10 *D. melanogaster* exposed to parasitoids, 10 *D. suzukii* or 10 *D. melanogaster* maintained as controls). The results were evaluated with three measures: number of successfully parasitized pupae (i.e., pupae from which a parasitoid adult emerged); number of dead pupae (i.e., pupae from which neither a parasitoid nor a drosophilid adult emerged); development time (days), calculated as the period from pupa exposure to parasitoid females until parasitoid adult emergence.

For choice tests, five pupae of *D. suzukii* and five pupae of *D. melanogaster* were exposed simultaneously to one *T.* cf. *drosophilae* female in a cylinder. Each replicate consisted of one cylinder, each containing 10 total pupae (i.e., five pupae per species). After exposure, the pupae of the two species were separated into different cylinders. Twelve replicates were performed and the results were evaluated by the number of successfully parasitized pupae, and number of dead pupae, calculated as previously described.

Statistical analyses

Field data were separated into two different datasets. The first set included data obtained from fruit exposed for both 7 and 14 days that was removed on the same date (i.e., every 14 days), where Exposure equaled 7–14 days (Dataset 7–14 days). The second set included all data obtained from the fruit exposed for 7 days and was removed weekly, where Exposure was equal to 7 days (Dataset 7 days).

The emerged adults of each parasitoid species were pooled over the season for each dish and analyzed through a Generalized linear mixed effect model procedure with a Poisson distribution and log link. Locality, Fruit, Exposure, and their two-way interactions were considered as fixed effects; Site (Locality) [Site as a nested variable in Locality], Block (Site), and the interactions fruit×site (locality) and exposure×site (locality) were considered as random effects. When fixed effects were found to be significant, means were separated through the sequential Bonferroni post hoc test.

In laboratory tests, the number of emerged parasitoids and number of dead host individuals were analyzed for each parasitoid species through a Generalized linear model with a binomial distribution and a logit link. Host and eventually presence/absence of parasitoid and their interaction were considered as fixed effects. When effects were shown to be significant, means were separated through the Bonferroni post hoc test.

The pupal weight of *D. suzukii* and *D. melanogaster* and the development time of *T.* cf. *drosophilae* in the two host species were analyzed by the Student's *t* test for independent samples. Data were transformed by square root to achieve homogeneity of variance (Levene test) and normality (Shapiro–Wilk test).

Statistical analyses were performed through SPSS Statistics 22 (IBM Corp. Released 2013, Armonk, NY).

Results

Field monitoring of parasitoids of *Drosophila* spp.

Field monitoring by traps baited with ACV confirmed the presence and abundance of *D. suzukii* in all surveyed sites. Despite the capture of large numbers of the exotic fly in ACV traps, relatively few (below 5.0 %) *D. suzukii*, as compared to other *Drosophila* spp., emerged from the field-exposed fruit dishes (Table 2). Six parasitoid species were obtained from the field-exposed fruit dishes: *L. boulardi*, *L. heterotoma*, *A. tabida*, *P. vindemiae*, *T.* cf. *drosophilae*, and *Spalangia erythromera* Forster (Hymenoptera: Pteromalidae) (Table 2). The presence and abundance of these species varied greatly by site; only *L. boulardi* and *P. vindemiae* emerged from fruit dishes exposed in all sites, while all six species were found only in Minoprio in Lombardy (Table 2). Overall, *L. boulardi*, *L. heterotoma*, *P. vindemiae*, and *T.* cf. *drosophilae* were more common and generally found during field monitoring. On the contrary, *A. tabida* and *S. erythromera* were found occasionally and in fewer numbers in Piedmont and Lombardy (Table 2).

Table 2 Total numbers of *Drosophila* spp. and % of *Drosophila suzukii* collected by apple cider vinegar (ACV) traps and emerged from fruit dishes, and total numbers of parasitoids emerged from fruit dishes exposed in the field in 2014

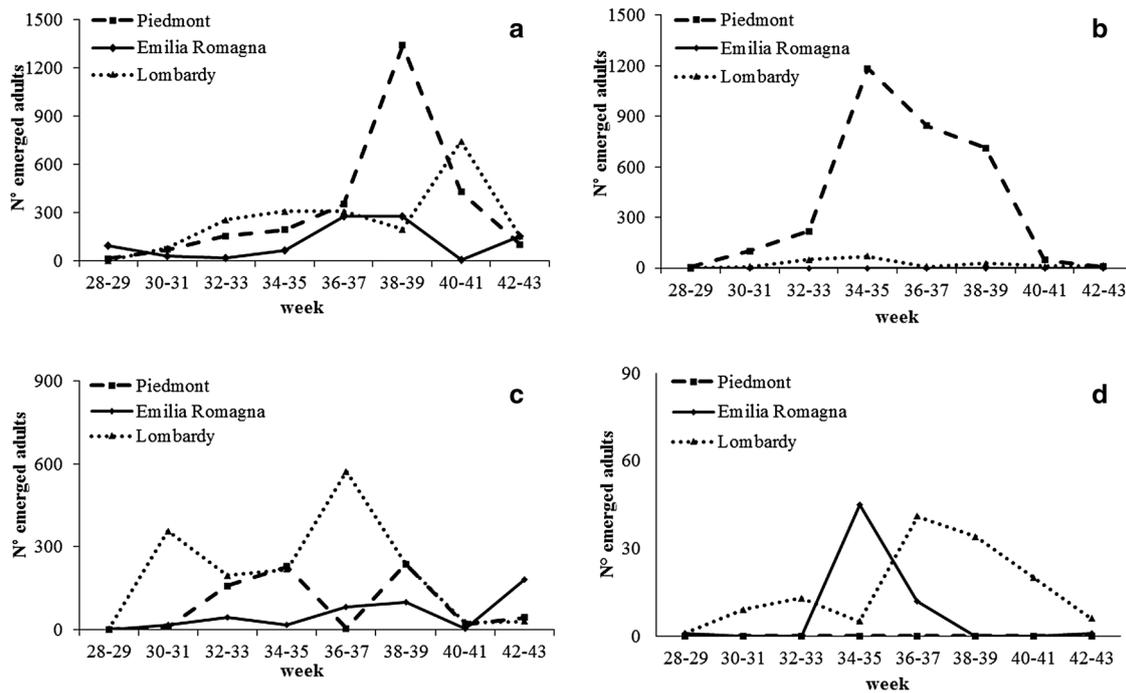| Sites	Time exposure	ACV traps		Fruit dish		Parasitoids (no.)					
		Drosophilidae (no.)	D. suzukii (%)	Drosophilidae (no.)	D. suzukii (%)	L. boulardi	L. heterotoma	P. vindemiae	T. cf. drosophilae	A. tabida	S. erythromera
Boves	7	1619	55.3	12,790	0.70	251	10	4	0	0	0
	14			3203	1.25	99	50	142	0	0	0
Paesana	7	5679	34.9	15,753	1.94	495	482	19	0	1	0
	14			3832	2.61	401	196	453	0	0	21
Peveragno	7	1132	71.9	15,328	2.23	1048	2001	41	0	4	0
	14			3898	1.23	359	372	35	0	0	2
Arcagna	7	709	76.9	9808	0.10	725	7	107	33	22	0
	14			462	4.11	211	8	1133	25	40	0
Minoprio 1	7	5907	86.9	6684	1.35	438	72	119	43	3	1
	14			1744	0.52	163	87	228	10	11	3
Minoprio 2	7	4459	81.2	3365	2.08	393	9	20	2	0	3
	14			920	0.33	110	2	27	16	0	0
Bologna	7	3780	22.3	2158	0.00	343	0	29	14	0	0
	14			180	0.56	12	0	113	4	0	0
Cadriano	7	1415	55.2	1627	1.17	130	0	8	13	0	0
	14			185	0.00	5	0	31	0	0	0
Dodici Morelli	7	3672	56.6	1848	0.49	368	0	73	26	0	0
	14			132	0.76	54	0	185	2	0	0

Fig. 1 Total numbers of *Leptopilina boulardi* (**a**), *Leptopilina heterotoma* (**b**), *Pachycrepoideus vindemiae* (**c**), and *Trichopria* cf. *drosophilae* (**d**) emerged from banana and blueberries exposed in the field in Piedmont, Lombardy, and Emilia-Romagna during 2014

Among the six species, the larval parasitoid *L. boulardi* was the most abundant (Table 2). Adults emerged 15–25 days after fruit dish removal, and in all surveyed sites the presence of *L. boulardi* was recorded from July to October (Fig. 1a). Aside from abundance, differences were found among the regions and across the season. The highest level of parasitism was recorded in Piedmont in late September (weeks 38–39: 1341 wasps). A similar trend was observed in Lombardy during the summer, although the highest level of parasitism occurred 2 weeks later than in Piedmont (weeks 40–41: 742 wasps). In Emilia-Romagna, fewer *L. boulardi* were collected overall, and the peak of parasitism occurred earlier (weeks 36–37 and weeks 38–39: 274 wasps) (Fig. 1a). Statistical analysis of dataset 7–14 days showed that the presence and abundance of *L. boulardi* were significantly affected by fruit used in the dishes, interval of exposure, locality×fruit, locality×exposure, and exposure×fruit (Tables 3, 4). In general, significantly more adults emerged from dishes with banana exposed for 7 days, while no significant differences were found among the localities. Similarly, analysis of dataset 7 days confirmed the significant effect of fruit and locality×fruit on the number of *L. boulardi*, principally based on Emilia-Romagna data (Tables 5, 6). Aside from blocks, random effects did not introduce variability into the results for either *L. boulardi* or other species.

Based on the total number of emerged adults, the second most common parasitoid was *L. heterotoma* (Table 2).

Even though this larval parasitoid was particularly abundant in Piedmont, especially in Peveragno, it was absent in Emilia-Romagna (Table 2). Like *L. boulardi*, adults emerged 15–25 days after fruit dish removal following field exposure during July to October. In Piedmont, the highest level of parasitism was observed in mid-August (weeks 34–35: 1184 wasps) and remained high throughout September, followed by a drastic decrease in October (<100 wasps) (Fig. 1b). *Leptopilina heterotoma* was also recorded in Lombardy throughout the survey period except during the first two weeks (28–29), although specimen numbers were considerably below those in Piedmont. Indeed, fewer than 100 adults in total emerged from fruits with the highest number obtained during August (weeks 34–35: 70 wasps) (Fig. 1b). Despite the high variability in distribution and relative abundance of *L. heterotoma* among the localities, statistical analysis detected no significant differences except for the exposure×fruit interaction, indicating a significant preference for dishes with banana exposed for 7 days where the parasitoid was found (Tables 3, 4).

The species that ranked third in abundance was pupal parasitoid *P. vindemiae* (Table 2). In this instance, adults emerged, albeit in variable amounts, 20–30 days after fruit dish removal following field exposure in all sites from mid-July to October. This pupal parasitoid was most abundant in Lombardy, especially in Arcagna (Table 2). In this region, two main peaks of emergence were observed from

Table 3 Mean number of emerged parasitoid adults pooled over the season for locality, fruit, and exposure and their interactions

Parasitoid	Locality	7 day exposure		14 day exposure		Fruits		Interval of exposure		Total
		Banana	Blueberry	Banana	Blueberry	Banana	Blueberry	7 days	14 days	
L. boulardi	Emilia-Romagna	6.89 ± 11.28	0.15 ± 1.18	0.89 ± 3.23	0.10 ± 0.38	3.89[a]	0.13[b]	3.52[a]	0.50[b]	2.01
	Lombardy	5.15 ± 12.94	3.36 ± 9.44	3.32 ± 7.75	3.40 ± 5.82	4.24	3.38	4.26	3.36	3.81
	Piedmont	9.17 ± 23.22	8.82 ± 17.14	2.96 ± 7.45	8.97 ± 22.27	6.07	8.90	9.00	5.97	7.48
	Total	7.07[a]	4.11[b]	2.39	4.16	4.73[a]	4.14[b]	5.59[a]	3.28[b]	4.43
L. heterotoma	Emilia-Romagna	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
	Lombardy	0.54 ± 1.93	0.04 ± 0.20	0.88 ± 4.30	0.47 ± 1.74	0.71	0.26	0.29	0.68	0.48
	Piedmont	14.75 ± 40.38	3.65 ± 9.62	5.01 ± 13.98	3.57 ± 10.60	9.88	3.61	9.20	4.29	6.75
	Total	5.10[a]	1.23[b]	1.96	1.35	3.53	1.29	3.16	1.66	2.41
P. vindemiae	Emilia-Romagna	0.81 ± 2.49	0.00 ± 0.00	3.93 ± 9.56	0.64 ± 3.48	2.37	0.32	0.41[b]	2.29[a]	1.35
	Lombardy	2.07 ± 8.57	0.29 ± 0.97	18.38 ± 40.75	0.90 ± 2.66	10.23	0.60	1.18[b]	9.64[a]	5.41
	Piedmont	0.18 ± 1.19	0.03 ± 0.17	8.67 ± 34.57	0.08 ± 0.44	4.43	0.06	0.11[b]	4.38[a]	2.24
	Total	1.02[a]	0.11[b]	10.33[a]	0.54[b]	5.67[a]	0.32[b]	0.56[b]	5.43[a]	3.00
T. cf. *drosophilae*	Emilia-Romagna	0.11 ± 0.72	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.37	0.06	0.04	0.06	0.04	0.05
	Lombardy	0.06 ± 0.37	0.53 ± 1.63	0.21 ± 0.67	0.50 ± 2.02	0.14	0.52	0.30	0.36	0.33
	Piedmont	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
	Total	0.06	0.18	0.07	0.19	0.06	0.19	0.12	0.13	0.12

SD was added only to three-way interaction. Data are referred to dataset 7–14 days. When significant, mean values were separated through sequential Bonferroni post hoc test (different letters indicate significant difference between the compared treatments within row). In locality×fruit and exposure×fruit interactions, means referred to fruits were separated in each level of locality or exposure, respectively. In locality×exposure interaction, means referred to exposure were separated in each level of locality

Table 4 Statistical significance of the different fixed and random effects referred to dataset 7–14 days obtained applying a generalized linear mixed effect model procedure with a Poisson distribution and log link

Factors	Significance			
	L. boulardi	*L. heterotoma*	*P. vindemiae*	*T.* cf. *drosophilae*
Fixed effects				
Locality	ns	ns	ns	ns
Fruit	0.005	ns	<0.001	ns
Exposure	0.002	ns	<0.001	ns
Locality×fruit	<0.001	ns	ns	ns
Locality×exposure	0.007	ns	0.007	ns
Exposure×fruit	<0.001	<0.001	0.047	ns
Random effects				
Site (locality)	ns	ns	ns	ns
Block (site)	0.038	ns	0.043	ns
Fruit×site (locality)	ns	ns	ns	ns
Exposure×site (locality)	ns	ns	ns	<0.001

Mean effects are reported in Table 3

fruits exposed during July (weeks 30–31: 357 wasps) and late summer (weeks 36–37: 572 wasps). Thereafter, the number of parasitoids decreased to fewer than 30 wasps in October (Fig. 1c). In Piedmont, larger numbers of *P. vindemiae* were found in the latter halves of both August (weeks 34–35: 228 wasps) and September (weeks 38–39: 236 wasps) (Fig. 1c). On the contrary, the highest number of *P. vindemiae* was observed in Emilia-Romagna in late October (weeks 42–43: 180 wasps), while emergence

during the summer was lower than that of the other regions (Fig. 1c). Statistical analysis of dataset 7–14 days demonstrated that the presence and abundance of *P. vindemiae* were significantly affected by fruit, interval of exposure, locality×exposure, exposure×fruit (Tables 3, 4). Overall, significantly higher numbers of adults emerged from dishes with banana exposed for 14 days. By contrast, no significant effects were found during analysis of dataset 7 days (Tables 5, 6).

Table 5 Mean number of emerged parasitoid adults pooled over the season for locality, fruit, and their interaction

Parasitoid	Locality	Fruits		Total
		Banana	Blueberry	
L. boulardi	Emilia-Romagna	5.72 ± 10.91[a]	0.12 ± 0.93[b]	2.92
	Lombardy	5.95 ± 15.77	4.84 ± 14.76	5.40
	Piedmont	6.22 ± 17.45	6.24 ± 13.78	6.23
	Total	5.96[a]	3.73[b]	4.85
L. heterotoma	Emilia-Romagna	0.00 ± 0.00	0.00 ± 0.00	0.00
	Lombardy	0.50 ± 1.94	0.10 ± 0.55	0.30
	Piedmont	12.35 ± 37.39	4.97 ± 15.69	8.66
	Total	4.28	1.69	2.99
P. vindemiae	Emilia-Romagna	0.76 ± 3.15	0.00 ± 0.00	0.38
	Lombardy	1.47 ± 6.41	0.21 ± 0.75	0.84
	Piedmont	0.37 ± 2.92	0.08 ± 0.68	0.23
	Total	0.87	0.10	0.49
T. cf. *drosophilae*	Emilia-Romagna	0.25 ± 1.19[a]	0.12 ± 0.57[b]	0.19
	Lombardy	0.19 ± 1.18	0.35 ± 1.22	0.27
	Piedmont	0.00 ± 0.00	0.00 ± 0.00	0.00
	Total	0.15	0.16	0.16

SD was added only to three-way interaction. Data are referred to dataset 7 days. When significant, mean values were separated through sequential Bonferroni post hoc test (different letters indicate significant difference between the compared treatments within row). In locality×fruit interaction, means referred to fruits were separated in each level of locality

Table 6 Statistical significance of the different fixed and random effects referred to dataset 7 days obtained applying a generalized linear mixed effect model procedure with a Poisson distribution and log link

Factors	Significance			
	L. boulardi	*L. heterotoma*	*P. vindemiae*	*T.* cf. *drosophilae*
Fixed effects				
Locality	ns	ns	ns	ns
Fruits	0.027	ns	ns	ns
Locality×fruits	0.003	ns	ns	0.044
Random effects				
Site (locality)	ns	ns	ns	ns
Block (site)	0.038	ns	ns	ns
Fruits×site (locality)	ns	ns	ns	ns

Mean effects are reported in Table 5

The pupal parasitoid *T.* cf. *drosophilae* was recorded in Lombardy and in Emilia-Romagna, but it was never found in Piedmont (Table 2). When observed, its adults generally emerged 18–22 days after fruit dish removal. In Lombardy, where its numbers were fewer than *P. vindemiae*, this pupal parasitoid was consistently collected from July to October, reaching the highest level in September (weeks 36–37: 41 wasps) (Fig. 1d). In Emilia-Romagna, *T.* cf. *drosophilae* emerged only from fruits exposed from mid-August to mid-September (weeks 34–35: 45 wasps) except for two specimens that emerged one each at the beginning and end of the survey (Fig. 1d). Statistical analysis of both datasets resulted in no significant effects of locality, fruit, or interval of exposure on the numbers of *T.* cf. *drosophilae* except for locality×fruit during the analysis of dataset 7 days (Tables 3, 4, 5, 6).

The remaining two species, *A. tabida* and *S. erythromera*, were only occasionally found in Piedmont and Lombardy. In Piedmont in particular, five specimens of *A. tabida* emerged during the season as opposed to the 76 specimens that emerged in Lombardy, especially from fruits exposed during September and October (Table 2). Twenty-three and seven specimens of pupal parasitoid *S. erythromera* were detected throughout the season in Piedmont and Lombardy, respectively.

Laboratory evaluation of parasitoid ability to parasitize *D. suzukii*

In the laboratory, all the tested species proved to parasitize successfully *D. melanogaster*, whereas only *T.* cf. *drosophilae* was able to parasitize successfully *D. suzukii* and no significant differences in parasitism rates were found between the two hosts (Table 7). *Trichopria* cf. *drosophilae* took significantly longer to develop in *D. suzukii* (19.3 ± 0.5 days) than in *D. melanogaster* (17.8 ± 0.9 days) (t-test for independent samples, $t = 13.282$, $df = 1, 98$, $P < 0.001$). Conversely, *L. boulardi* and *L. heterotoma* failed to parasitize *D. suzukii*, which produced significantly different parasitism rates between the native and exotic hosts (GLM, *L. boulardi*: $\chi^2 = 171.429$, $df = 1$, $P < 0.001$; *L. heterotoma*: $\chi^2 = 126.452$, $df = 1$, $P < 0.001$) (Table 7). These findings caused two-choice tests to be performed with *T.* cf. *drosophilae* alone.

Dead fly comparison (i.e., larvae or pupae from which no adults of either flies or parasitoids emerged) yielded quite varied results by parasitoid species. In tests with *L. boulardi*, the mean number of dead flies was significantly higher in *D. melanogaster* than in *D. suzukii* (GLM, $\chi^2 = 15.375$, $df = 1$, $P < 0.001$). Nevertheless, no significances were found in the interaction between host and presence/absence of parasitoid or in the mean number of dead flies in the presence or absence of parasitoid (Table 7). By contrast, in tests with *L. heterotoma*, significant differences resulted between tests in the presence or absence of parasitoid; specifically, a higher mortality was detected in the presence of the parasitoid (GLM,

$\chi^2 = 5.815$, $df = 1$, $P = 0.016$). In particular, the mean number of dead *D. suzukii* was significantly higher in tests with *L. heterotoma* than in the control, which showed that this species affected exotic fly mortality (GLM, $\chi^2 = 10.392$, $df = 1$, $P = 0.001$) (Table 7). Finally, in tests with *T.* cf. *drosophilae*, the mean number of dead flies was significantly higher in both *D. melanogaster* versus *D. suzukii* (GLM, $\chi^2 = 4.531$, $df = 1$, $P = 0.033$) and in the presence of parasitoid versus the control (GLM, $\chi^2 = 4.531$, $df = 1$, $P = 0.033$). The interaction between host and presence/absence of parasitoid was not significant (Table 7).

In two-choice tests, *T.* cf. *drosophilae* showed it could attack and parasitize successfully both *D. suzukii* and *D. melanogaster*, with no significant differences noted between the native and exotic hosts. Instead, higher pupal mortality occurred in *D. melanogaster* versus *D. suzukii* (GLM, $\chi^2 = 5.687$, $df = 1$, $P = 0.017$) (Table 7). Additionally, the mean weight of the newly formed *D. suzukii* pupae was significantly above that of *D. melanogaster* pupae (2.39 ± 0.15 vs. 1.04 ± 0.07 mg) (t-test for independent samples, $t = 268.70$, $df = 1, 118$, $P < 0.001$).

Discussion

Our research demonstrated the presence of six parasitoid species related to frugivorous Drosophilidae in North Italy, and identification among these species of a potential candidate to control exotic fly *D. suzukii*. Overall, the survey revealed the high variability in the abundance and population trend of each parasitoid species not only among the

Table 7 Mean number of emerged parasitoids and of dead host individuals in the laboratory tests

Test	Species	Treatment (no. of replicates)	Emerged parasitoids		Dead individuals		Mean dead flies
			D. m.	*D. s.*	*D. m.*	*D. s.*	
No choice	*L. boulardi*	With parasitoid (15)	5.33 ± 2.82^a	0.00 ± 0.00^b	3.33 ± 2.79	1.73 ± 1.49	2.53
		Control larvae (10)			2.90 ± 1.45	1.40 ± 1.26	2.15
		Mean dead			3.12^a	1.57^b	
	L. heterotoma	With parasitoid (8)	6.13 ± 4.12^a	0.00 ± 0.00^b	2.50 ± 2.39^{ab}	4.00 ± 2.07^a	3.25^a
		Control larvae (10)			2.90 ± 1.45^{ab}	1.40 ± 1.26^b	2.15^b
		Mean dead			2.70	2.70	
	T. cf. *drosophilae*	With parasitoid (6)	7.67 ± 1.03	9.00 ± 1.26	2.33 ± 1.03	0.83 ± 0.98	1.58^a
		Control pupae (6)			0.83 ± 0.75	0.33 ± 0.52	0.58^b
		Mean dead			1.58^a	0.58^b	
Choice	*T.* cf. *drosophilae*	(12)	3.50 ± 1.00	4.50 ± 1.00	1.33 ± 0.89^a	0.42 ± 0.90^b	

SD was added to emerged parasitoids or in the case of dead individuals only to two-way interaction. Data were analyzed through generalized linear model with a binomial distribution and a logit link. When significant, mean values were separated through Bonferroni post hoc test (different letters indicate significant differences between the compared treatments). In host×presence/absence of parasitoid interaction, all levels were compared

three localities, but also across sites within a locality. This variability likely related to survey site characteristic differences, and consequently, to the trend in Drosophilidae population levels. Different types of trees, bushes, and shrubs, such as mulberries, blueberries, cherry, peach, and fig trees, most of which are favorite hosts of *D. suzukii*, in the sites in Emilia-Romagna may have provided supplementary or alternative food for *Drosophila* spp. throughout the season, which would account for the lower fly numbers that emerged from fruits exposed in this locality. As would be expected, these lower Drosophilidae numbers mirrored lower amounts of parasitoids emerged from fruits exposed there. The influence of surrounding vegetation on Drosophilidae attraction was also evident from the opposite perspective, that is, when high numbers of other drosophilids were captured in ACV traps. In Piedmont and Lombardy, where monitoring was conducted in blueberry and raspberry plantations with limited presence of other host plants (especially after the harvest), it made the fruits exposed in the field more attractive to drosophilids. The influence of the site on *Drosophila* spp. abundance and composition has been already demonstrated; for example, Ferreira and Tidon (2005) showed that Drosophilidae populations varied according to the level of urbanization.

In all surveyed sites, *D. suzukii* was always present as determined by ACV traps. However, the highly variable rate of trap capture of the exotic flies in comparison with other Drosophilidae highlighted the scarce selectivity of ACV as others have previously observed (Landolt et al. 2012b; Cha et al. 2014; Iglesias et al. 2014; Burrack et al. 2015). Despite its high presence, very few adults of the exotic fly emerged from the field-exposed fruits, probably due to competition for food between *D. suzukii* and native drosophilids. Although the competition between *D. suzukii* and other drosophilids has not yet been investigated, studies of food competition have been conducted in a number of congeneric species (Montchamp-Moreau 1983; Fleury et al. 2004). Consequent to the low number of *D. suzukii*, most parasitoids found in our survey emerged from native drosophilids (more than 80,000), which were not identified because our study was aimed at detecting the parasitoids and evaluating their potential as biological control agents of *D. suzukii*. However, these data could provide useful information on communities of frugivorous drosophilids and their interactions with natural enemies, not yet investigated in our regions and worthy of further studies. Nonetheless, our results have broadened the knowledge on the distribution and abundance of parasitoid species in North Italy, and may represent important achievements in the implementation of biological control strategies against *D. suzukii*.

Among the six parasitoid species obtained, *A. tabida* and *S. erythromera* were only occasionally recorded in North Italy. The relationships of *Asobara* spp. with their hosts have been studied in other countries, especially in Japan (Mitsui et al. 2007; Mitsui and Kimura 2010; Novković et al. 2012; Kohyama and Kimura 2015; Nomano et al. 2015). High levels of competition with other parasitoids, host unsuitability, and egg encapsulation in *A. tabida* may be the principal reason for its scarcity in our study localities. The four other species were more abundant in almost all surveyed sites, which showed that they likely play a role in drosophilid population regulation.

The presence of larval parasitoids *L. boulardi* and *L. heterotoma*, generally observed in Europe (Kraaijeveld and Van Alphen 1994; Fleury et al. 2004, 2009; Moiroux et al. 2013), was confirmed in North Italy from our findings. Our survey also revealed their highly variable distribution by locality; in fact, only *L. boulardi* was found in all sites while *L. heterotoma* was never collected in Emilia-Romagna. As noted above, lower numbers of larval parasitoids reflect lower numbers of Drosophilidae emerged from fruits, but this explanation fails to fully account for the complete absence of *L. heterotoma* in Emilia-Romagna. The role of competition might provide some hints as it has been studied thoroughly in drosophilid parasitoids (Vet and van Opzeeland 1985; Fleury et al. 2000, 2009). Similarly, different resources and environmental factors have been shown to affect the coexistence of *L. boulardi* and *L. heterotoma*. Of these two species, the second has been considered more generalist—and therefore, a better competitor—than the first because it can exploit alternative host species (Fleury et al. 2004). However, in a survey conducted in Tunisia *L. boulardi* proved to be a better competitor than *L. heterotoma* (Carton et al. 1991). A similar competition could explain the variability in the presence and abundance observed in our survey.

Here, both species emerged mainly from fruit exposed for 7 days, in which higher numbers of Drosophilidae larvae suitable for their parasitism were expected. Moreover, they showed a preference for banana over blueberries, which might derive from the fact that the highest number of Drosophilidae emerged from banana (data not shown). Banana traps to capture *Leptopilina* spp. have already been largely adopted (Chabert et al. 2012; Moiroux et al. 2013; Marchiori et al. 2015) because of the strong preference of *Drosophila* spp. to oviposit on fermented banana (Markow and O'Grady 2006).

In laboratory tests, our populations of *L. boulardi* and *L. heterotoma* proved that they were unable to develop on the exotic fly, which aligns with the observations in other studies in Europe and North America (Chabert et al. 2012; Poyet et al. 2013). Therefore, the population of *L. heterotoma* collected in Northeast Italy may represent the only known case to date that demonstrates the ability of this parasitoid to overcome the immunological response and

emerge from *D. suzukii* (Rossi Stacconi et al. 2015). The mechanism regulating the immune response to wasp parasitoid has been widely studied in *D. melanogaster*. Our results found that parasitoid adults emerged from only 50–60 % of the exposed larvae of *D. melanogaster*, a finding that is consistently knowing that parasitism success varies considerably according to geographical population strains and genetics (Kraaijeveld and Godfray 1999). Indeed, recent work has demonstrated that *D. suzukii* has a higher hemocyte load than *D. melanogaster*, which makes it more resistant to several larval parasitoids (Kacsoh and Schlenke 2012; Poyet et al. 2013). Although no adults of *L. heterotoma* emerged in our laboratory tests, a higher mortality of *D. suzukii* larvae exposed to this parasitoid relative to control larvae was recorded, suggesting this species, even if unable to reach adulthood, may possess the capability to begin development and cause death of its host larvae, as reported by other authors (Chabert et al. 2012). In *L. boulardi*, no mortality difference between exposed and non-exposed larvae was recorded.

Pachycrepoideus vindemiae was the main pupal parasitoid found during our field monitoring, as observed in France (Fleury et al. 2009). This parasitoid is reported to be one of the three most abundant frugivorous *Drosophila* parasitoids in South France (Chabert et al. 2012), and its presence has already been assessed in other Italian areas (Nøstvik, 1954). *Pachycrepoideus vindemiae* was collected in all surveyed sites, but showed different population dynamics. Population trend differences between the localities might be influenced by the presence of alternative hosts. In fact, *P. vindemiae* is known to parasitize over 60 fly species, and was shown to dominate interspecific competition (Wang and Messing 2004; Rossi Stacconi et al. 2013). Higher numbers of this pupal parasitoid emerged from the fruit exposed for 14 days, in which higher numbers of pupae promoted the attraction of *P. vindemiae*. This species was also more attracted to banana traps as explained above for *Leptopilina* spp. (Chabert et al. 2012; Rossi Stacconi et al. 2013).

We chose not to test *P. vindemiae* in the laboratory because its capability to parasitize *D. suzukii* at a rate of about 60 % (Chabert et al. 2012; Rossi Stacconi et al. 2015), similar to that recorded on *D. melanogaster* (Delpuech et al. 1994), is well established. Currently, *P. vindemiae* is one of the most widely studied potential biological control agents of *D. suzukii*. Nonetheless, its successful activity on *D. suzukii* is counter balanced by a high number of host species and its role as a hyperparasitoid (Baeza-Larios et al. 2002; Guillén et al. 2002).

Although *T.* cf. *drosophilae* is considered the other main widespread and global pupal parasitoid of drosophilids (Fleury et al. 2009; Asplen et al. 2015), fewer individuals of this species were recorded in our field survey and in

another area of North Italy (Rossi Stacconi et al. 2015). Indeed, in Piedmont this pupal parasitoid was absent, while in Emilia-Romagna and Lombardy, in spite of the few specimens recorded, it displayed similar population dynamics. Little information existed on this parasitoid (Romani et al. 2002, 2008; Small et al. 2012) until introduction of the exotic fly sparked interest. Currently, *T.* cf. *drosophilae* has proved it can successfully parasitize *D. suzukii* in previous laboratory research (Chabert et al. 2012; Gabarra et al. 2015). In our laboratory tests, the population of *T.* cf. *drosophilae* collected in Lombardy parasitized both *D. suzukii* and *D. melanogaster* with the same effectiveness. The longer development time of *T.* cf. *drosophilae* in *D. suzukii* as opposed to *D. melanogaster*, recorded in the no-choice experiment, may have been due to the larger *D. suzukii* pupae (resulting in more food available to parasitoid larvae), as shown in other host–parasitoid systems (Dindo and Grenier 2014). Furthermore, in choice experiments performed for the first time on this species, the parasitoid showed the same preference for pupae of both hosts. These results, together with its limited host range and the feasibility of its mass rearing, make *T.* cf. *drosophilae* a good candidate for augmentative biological control of *D. suzukii*.

Our results suggest the possibility of natural enemy-mediated apparent competition in communities of *Drosophila* spp. and their parasitoids. As a matter of fact, high numbers of indigenous Drosophilidae emerged in spite of the abundance of parasitoids. Consequently, the possibility that native parasitoids can effectively adapt to and control *D. suzukii*, also due to its higher resistance to some parasitoids, still remains limited. Moreover, although more efficient parasitoids were reported in the area of origin, a specific enemy of *D. suzukii* to release in a classical biological control program is yet to be identified. Therefore, further investigations on indigenous enemies, in particular on *T.* cf. *drosophilae*, that was revealed to be a promising biological control agent, should be carried out to achieve effective control of *D. suzukii* through their rearing and release in the field in augmentative biological control programs.

Author contribution statement

LT, MLD, and DL conceived and designed research. FM, EM, NA, SF, and CJ conducted field surveys. FM and EM conducted laboratory tests. DS analyzed data. FM and LT wrote the manuscript. All authors read and approved the manuscript.

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