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Seasonal occurrence of resident parasitoids associated with *Drosophila suzukii* in two small fruit production regions of Italy and the USA

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Abstract

For the first time we report the results of a survey to determine the presence, seasonal phenology and biological control status of indigenous parasitoid populations utilizing *Drosophila suzukii* (Matsumura) and *Drosophila melanogaster* Meigen (Diptera Drosophilidae) as hosts in Trento Province, Northern Italy, and the Willamette Valley, Oregon, USA. Larval and pupal parasitoids were sampled using sentinel traps baited with larvae of *D. suzukii* or *D. melanogaster*, or traps baited with fruit or yeast-based host substrates. Two generalist parasitoids, *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera Pteromalidae) and *Leptopilina heterotoma* (Thomson) (Hymenoptera Figitidae) emerged from the sentinel traps in both regions, and a third generalist parasitoid, *Trichopria drosophilae* Perkins (Hymenoptera Diapriidae), was found in Italy. *L. heterotoma* was present during the early portion of the season in Italy while *P. vindemiae* was found throughout the growing season in both production regions. Low numbers of parasitoids relative to initial larval load in baits suggest a limited effect of indigenous parasitoids on *D. suzukii* in these two important fruit production regions. These findings highlight the need for improved biological control of *D. suzukii* through introduction or augmentation of specialist parasitoids from the native range of *D. suzukii*. This report provides baseline data on the current status of biological control of *D. suzukii* in Italy and Oregon.

Key words: *Pachycrepoideus vindemiae*, *Trichopria drosophilae*, *Leptopilina heterotoma*, parasitism, biological control, invasive pest.

Introduction

Drosophila suzukii (Matsumura) (Diptera Drosophilidae) has a vast native range in Southeast Asia (Kanzawa, 1939; Oku, 2003; Lin *et al.*, 2013). Since its first detections in 2008 in California (USA), Spain, and Italy, this significant pest of soft- and thin-skinned fruit has rapidly expanded its invaded range to include most of the major fruit producing regions in North America, Europe, and recently South America (Walsh *et al.*, 2011; Burrack *et al.*, 2012; Cini *et al.*, 2012; CABI, 2014; Cini *et al.*, 2014; Deprá *et al.*, 2014; NAPIS, 2014). Successful establishment of the fly may be enhanced by its climatic adaptability, short generation time and high reproductive rate, or by limited natural enemies (Emiljanowicz *et al.*, 2014; Tochen *et al.*, 2014; Wiman *et al.*; 2014; Kimura, 2004; Blumenthal, 2006; Chabert *et al.*, 2012). Unlike most other fruit-feeding drosophilids, female *D. suzukii* oviposit into ripening fruits using a serrated ovipositor (Lee *et al.*, 2011; EPPO/OEPP 2013; Rota-Stabelli *et al.*, 2013). Economic impacts occur due to ovipositional wounds and larval feeding that facilitate spoilage of attacked fruit, as well as increased inputs for monitoring and chemical application, increased harvest frequency and storage costs, reduction of fruit shelf life, and decreased access to foreign markets (Goodhue *et al.*, 2011).

Current control efforts in North America rely heavily on the use of insecticides targeting adult *D. suzukii* prior to oviposition into susceptible host fruits (Beers *et al.*, 2011; Bruck *et al.*, 2011). The effectiveness of pesticide-based programs may be limited by reservoirs of *D. suzukii* in wild fruits in unmanaged habitats surrounding commercial fields (Lee *et al.*, 2015). Organic production is particularly threatened because few effective organically approved insecticides are available for control of *D. suzukii* (Walsh *et al.*, 2011; Van Timmeren and Isaacs, 2013; Woltz *et al.*, 2015). Chemical control is further limited by pre-harvest intervals of insecticide applications and maximum residue limits strictly enforced by countries importing fruit (Walsh *et al.*, 2011; Haviland and Beers, 2012). From this perspective, biological control may offer a landscape-level management strategy for this highly polyphagous and mobile pest.

Worldwide, a diverse group of at least 50 hymenopteran parasitoids attacks various species of *Drosophila* (Carton *et al.*, 1986). The majority of these are larval parasitoids in the genera *Asobara* (Braconidae), *Leptopilina* and *Ganaspis* (Figitidae). Common pupal parasitoids of *Drosophila* include *Trichopria drosophilae* Perkins (Diapriidae), and *Pachycrepoideus vindemiae* (Rondani) (Pteromalidae). Most studies focus on three common larval parasitoids of *Drosophila* which attack larvae living within fermenting substrates: *Leptopilina heterotoma* (Thomson) and *Leptopilina boulardi* Barbotin and *Asobara tabida* Nees (Prévost, 2009). Under optimal conditions, these parasitoids can provide a high average rate of parasitism, for example, reaching 90% in southern France (Fleury *et al.*, 2004). Information on parasitoids attacking *D. suzukii* in the pest's native range is so far limited to a few studies in Japan. Generalist larval parasitoids that utilize *D. suzukii* as a host in this region include *Asobara japonica* Belokobylskij, *A. tabida*, *Ganaspis xanthopoda* (Ashmead), *Leptopilina japonica* Novković et Kimura (Mitsui *et al.*, 2007; Ideo *et al.*, 2008; Mitsui and Kimura, 2010; Novković *et al.*, 2011; Kasuya *et al.*, 2013). *A. japonica* has shown a high rate of successful development from *D. suzukii* (79%) (Kacsoh and Schlenke, 2012). Generalist pupal parasitoids observed to attack *D. suzukii* in Japan include *Trichopria* sp. and *Pachycrepoideus* sp. (Mitsui *et al.*, 2007). Known specialist parasitoids of *D. suzukii* in Japan include one undescribed *Asobara* species and one strain of *G. xanthopoda* that exhibits a high level of specificity for *D. suzukii*, suggesting the existence of different host races or host-specific races of this species (Nomano *et al.*, 2014; Mitsui and Kimura, 2010; Kasuya *et al.*, 2013).

Following pest invasion in the USA and Europe, several studies have examined the suitability of *D. suzukii* as a host for common indigenous parasitoids (Chabert *et al.*, 2012; Kacsoh and Schlenke, 2012; Poyet *et al.*, 2013). Populations of two undescribed *Ganaspis* sp. collected in Florida and Hawaii have been shown to develop successfully on *D. suzukii* (Kacsoh and Schlenke, 2012). Kacsoh and Schlenke (2012) tested 15 species from wide geographic origins, and found that only 7 of 24 parasitoid populations were able to complete development on *D. suzukii*. The failure of the other parasitoid populations to develop was presumably due to strong host immune response against the parasitoids, whereas these same parasitoids could successfully develop on the common vinegar fly *Drosophila melanogaster* Meigen (Kacsoh and Schlenke, 2012; Poyet *et al.*, 2013). The only other larval parasitoid able to develop from *D. suzukii* was *Asobara citri* Fisher from Ivory Coast. Similarly, Chabert *et al.* (2012) tested five European parasitoids and found that only the two pupal parasitoids *Trichopria* cf. *drosophilae* and *P. vindemiae* successfully developed on *D. suzukii*, while the three larval parasitoids *L. heterotoma*, *L. boulardi*, and *A. tabida* were unsuccessful. The two pupal parasitoids were also found to readily attack *D. suzukii* in Italy, Spain, and California (Rossi Stacconi *et al.*, 2013; Gabarra *et al.*, 2014; Xingeng Wang, unpublished data).

The specialized ecological niche of the exotic *D. suzukii* likely limits access by most indigenous larval parasitoids because *D. suzukii* larvae are protected inside host fruits. Classical biological control is a potentially useful management strategy for an exotic pest species whenever effective resident natural enemies are lacking in the new distribution range. It is prudent, however, to first understand the composition, distribution, and host range of resident natural enemies and their possible impacts on the exotic pest before considering the introduction of exotic natural enemies for the control of the pest. To date, there are relatively few published field studies on the importance of indigenous parasitoids or natural enemies in the control of *D. suzukii* despite the widespread establishment of this pest and significant levels of damage caused by *D. suzukii* (Rossi Stacconi *et al.*, 2013; Gabarra *et al.*, 2014; Woltz *et al.*, 2015). Information is lacking regarding the parasitoid species composition and potential role of indigenous parasitoids on *D. suzukii* in Oregon, Italy, and most other regions invaded by this pest (Rossi Stacconi *et al.*, 2013; 2015). Therefore, the objective of the present study is to provide baseline information on the current status and comparative importance of resident parasitoids against commonly occurring *Drosophila* species in important small fruit production regions in northern Italy and western Oregon, USA.

Materials and methods

Parasitoids of *D. suzukii* and *D. melanogaster* were field-surveyed in Trento Province, Italy, and the Willamette Valley, Oregon, USA. The surveys were conducted at four sites in Trento Province from 5 May to 18 October 2013, and at five sites in Oregon from 16 May to 30 October 2012 and 12 May to 30 October 2013. The sites were established in either unsprayed commercial crops or on non-commercial fruit-bearing plants in close proximity to commercial host crops. Presence of *D. suzukii* at all sites was confirmed in 2011.

Insect colonies

Colonies of *D. suzukii* were established using adults collected from various field sites in the respective areas where surveys were conducted. Collected adults were released into plastic cages and their offspring reared on a yeast-based standard artificial medium diet for drosophilids, henceforth referred to as 'medium' (Dalton *et al.*, 2011). Wild-collected individuals were released into the rearing cages periodically to maintain genetic diversity. Colonies of *D. melanogaster* originated from a long-established university laboratory colony and were reared in the same method as described above.

Preparation of traps

Petri dishes (9 cm diameter) containing either medium (in Oregon), or fresh banana sliced 1-2 cm thick (in Italy), were exposed either to *D. suzukii* or *D. melanogaster* adults in plastic oviposition cages (30 × 30 × 30 cm). Dishes were kept in oviposition cages containing *ad libitum* numbers of *D. suzukii* or *D. melanogaster* for seven and three days respectively at 21 °C and 65% relative humidity in both regions. This time period allowed for the development of second and third instar larvae of each of these species. Infested Petri dishes were subsequently filled with water in order to stimulate the larvae to emerge from the food substrate and the surface of each Petri dish was rinsed onto a 0.25 mm diam. mesh (Soiltest Inc., Chicago, IL, USA). Second and third instar larvae remaining on the top surface of the sieve were transferred and weighed in order to estimate the weight corresponding to number of larvae for each species. For *D. suzukii* and *D. melanogaster* 4 g and 2 g of larvae corresponded to approximately 800 larvae respectively. These second and third instar larvae were then added to 70-90 g of host substrate in fresh containers (Rossi Stacconi *et al.*, 2013). In Italy, host substrates were placed in a Petri dish and included medium, banana slices, blueberries or cherries (termed 'fruit'). In Oregon, host substrates were placed in a 163-mL plastic soufflé cup (Solo®, Urbana, IL, USA) and included blueberries, cherries or raspberries in 2012, and blueberries, blackberries, strawberries, raspberries, or a mix (blueberries, blackberries and raspberries) in 2013 (Dalton *et al.*, 2011). Because of differences in growing seasons and availability of fruit, the fruits in each region reflected both seasonal and regional availability for that area of study. For each container with *Drosophila* larvae within the substrate, a second container with identical substrate, but without laboratory-reared larvae, was deployed to serve as a control treatment.

Each Petri dish or soufflé cup containing host substrate with or without larvae was placed inside a red delta trap to constitute a sentinel trap. Sentinel traps were hung 1-2 m from the ground from the crop plant or from surrounding vegetation in a protected and predominantly shaded environment. At each site, six traps were randomly deployed, each with a different combination of substrate (fruit or media) and larvae (*D. melanogaster*, *D. suzukii* or none).

Placement of traps

In Northern Italy the four representative survey sites were selected to take into account elevation, presence of *D. suzukii* host plants and high levels of *D. suzukii* in both natural and agro-ecosystems. Three hilly sites were selected: a commercial organic blueberry orchard in Cembra Valley (Gaggio, 46°20'36"N 11°28'53"E, 900 m a.s.l.), a commercial cherry orchard in Mocheni Valley (Loc. Franchini, 46°09'35"N 11°28'43"E, 790 m a.s.l.), and a natural forest environment in Sugana Valley (Vigolo Vattaro, 46°99'38"N 11°19'25"E, 720 m a.s.l.). The fourth location was situated at the bottom of the Adige Valley, in a commercial vineyard (San Michele all'Adige, 46°19'01"N 11°13'43"E, 230 m a.s.l.).

In Oregon, the five sites where sentinel baits were deployed were selected based on high diversity of landscape vegetation surrounding a susceptible crop. The sites included: an organically managed raspberry field (Oregon State University, North Willamette Research and Extension Center, NWREC, 45°16'55"N 122°45'00"W, 60 m a.s.l.); an unmanaged wild riparian site containing a mix of Himalayan blackberries and seedling cherries (Salem, 44°54'11"N 123°07'13"W, 181 m a.s.l.); a commercial blueberry and raspberry farm (Riverbend Farm, RF, 44°40'51"N 122°58'40"W, 89 m a.s.l.); a small-scale, mixed-production-commercial rural homestead with unsprayed soft and stone fruit species (Frasier Creek Farm, FCF, 44°37'40"N 123°15'46"W, 100 m a.s.l.); and an organically managed mixed-production farm (Gathering Together Farm, GTF, 44°31'40"N 123°21'57"W 80 m a.s.l.). All sentinel traps were collected and replaced once per week.

Rearing and identification of parasitoids

Assessment of exposed traps was similar in Oregon and in Italy. At the end of each field exposure, each Petri dish or soufflé cup was placed in an escape-proof 250-720 mL container and taken to the laboratory. Containers were equipped with a fine mesh netted lid for ventilation and a moistened dental cotton ball placed next to the substrate to prevent desiccation of contents. All containers were incubated for four weeks under ambient laboratory conditions of 21 °C and 65% relative humidity. Unpublished preliminary data from the previous season indicated that parasitoid emergence during the four week incubation period was negligible under these rearing conditions. Following the incubation period, containers were held at 22 °C and 55% RH (14:10 L:D photoperiod) and examined weekly for parasitoids emerging from host pupae. Containers were examined for a period of nine weeks to ensure recording of all emerged parasitoids would be observed. All parasitoids were aspirated and counted during observation. Voucher specimens were placed into vials of 95% ethyl alcohol. Containers with highly active flies were placed in a walk-in cold room (4 °C) for a maximum of 120 minutes prior to the collection of parasitoids. This technique temporarily decreased fly activity without increasing mortality, and concurrently allowed for the aspiration of parasitoids. After the nine-week observation period was completed, containers were dismantled.

In order to assess parasitism for cups containing *D. suzukii* within host medium, a parasitism index (P) was estimated using the total number of emerged parasitoids (p_i) for each species from each cup. We assumed that the total number of *D. suzukii* (Th) within the host media to be 800, based on the initial larvae placed within the cups (Rossi Stacconi *et al.*, 2015). The index was calculated on a weekly basis using $P = (p_i / Th) \times 100$ to approximate the percent parasitism. All sites within a region were pooled for this calculation. This index is a conservative value, looking only at the number of emerged parasitoids, and does not account for the number of parasitoids that attacked, but did not successfully emerge from *D. suzukii*.

Statistical analysis

The effects of host species, substrate and location were analyzed using one-way ANOVA with region and year as independent factors. For all effects but that of location, sites for each region were pooled for analysis. Differences of the means were separated using Tukey's HSD. Data were analyzed at seven-day intervals. Analyses were conducted using Statistica (StatSoft 7.1, Tulsa, OK, USA).

Parasitoid species collected in Oregon were identified by M. Buffington with voucher specimens deposited at the Smithsonian Institute in Washington D.C. Italian parasitoids were identified by A. Loni with voucher specimens deposited at Pisa University.

Results

Two parasitoid species, *P. vindemiae* and *L. heterotoma*, emerged from host pupae in field-placed sentinel traps in both Oregon and Italy. Three individual *T. drosophilae* females emerged from a trap placed in Vigolo Vattaro (forest environment), Italy, on 23 August 2013, but since this was the only finding, this species was not included in the calculation of parasitism index.

Datasets for each region were analyzed separately. In Oregon, the species of host drosophilid larvae initially present in the host substrate had no significant effect on numbers of emerging *P. vindemiae* from sentinel bait stations in 2012 ($F_{2,\,439} = 1.0$, $p = 0.37$, table 1), with similar numbers emerging from sentinel bait stations containing *D. melanogaster*, *D. suzukii* and the control sentinel traps. During 2013, significantly more parasitoids emerged from *D. melanogaster*-baited traps, followed by *D. suzukii*-baited traps, and lastly the control traps ($F_{2,\,608} = 21.0$, $p < 0.001$, table 1). Significantly more *L. heterotoma* parasitoids emerged from *D. melanogaster*-baited traps and the control traps, compared to sentinel stations seeded with *D. suzukii* larvae ($F_{2,\,439} = 3.6$, $p = 0.025$, table 1) during 2012. This trend was numerically consistent during 2013, although the differences were not statistically significant ($F_{2,\,608} = 1.3$, $p = 0.260$).

In Italy, the species of host fly present in host substrate had a significant effect on the number of individuals of *P. vindemiae* that emerged ($F_{2,\,789} = 8.2$, $p < 0.001$; table 1), with higher numbers of *P. vindemiae* emerging from sentinel bait stations containing *D. melanogaster*, followed by *D. suzukii*, and then the controls. For *L. heterotoma*, a statistically similar number of parasitoids emerged irrespective of the host species initially present in host substrate ($F_{2,789} = 2.0$, $p = 0.131$, table 1).

In Oregon, the type of fruit substrate used in the sentinel station did not significantly affect the number of emerged parasitoids during 2012 ($F_{4,\,437} = 2.2$, $p = 0.072$; table 2). There was, however, a numeric trend of greater numbers of *P. vindemiae* emerging from sentinel bait stations containing raspberries, and fewer parasitoids emerging from the other stations. During 2013, substrate

Table 1. Effect of sentinel trap host fly on the mean *P. vindemiae* and *L. heterotoma* per trap (± SEM) in the Willamette Valley (Oregon, USA) and Trento Province (Italy) during 2012 and 2013. Means followed by different letters are statistically different as determined by ANOVA followed by Tukey's HSD (N = number of Petri dishes or soufflé cups).

Year	Region	Host fly	Mean number *P. vindemiae*/trap	N	Mean number *L. heterotoma*/trap	N
2012	Oregon	Control	7.65 ± 1.65 a	63	0.06 ± 0.038 a	63
2012	Oregon	*D. melanogaster*	7.9 ± 0.98 a	180	0.03 ± 0.015 a	180
2012	Oregon	*D. suzukii*	6.06 ± 0.93 a	199	0 b	199
2013	Oregon	Control	0.63 ± 0.17 c	204	0.50 ± 0.23 a	204
2013	Oregon	*D. melanogaster*	3.58 ± 0.41 a	204	0.48 ± 0.27 a	204
2013	Oregon	*D. suzukii*	1.93 ± 0.33 b	203	0.06 ± 0.039 a	203
2013	Italy	Control	0.09 ± 0.03 c	264	0.54 ± 0.32 a	264
2013	Italy	*D. melanogaster*	0.84 ± 0.21 a	264	0.19 ± 0.07 a	264
2013	Italy	*D. suzukii*	0.35 ± 0.09 b	264	1.01 ± 0.38 a	264

Table 2. Sentinel trap host substrate and mean *P. vindemiae* and *L. heterotoma* per trap (± SEM) in the Willamette Valley (Oregon, USA) and Trento Province (Italy) during 2012 and 2013. Means of parasitoid followed by different letters are statistically different as determined by ANOVA followed by Tukey's HSD (N = number of Petri dishes or soufflé cups).

Year	Region	Substrate	Mean number *P. vindemiae*/trap	N	Mean number *L. heterotoma*/trap	N
2012	Oregon	Banana	4.1 ± 1.30 a	13	0.23 ± 0.16 a	13
2012	Oregon	Blueberry	6.00 ± 2 a	29	0 c	29
2012	Oregon	Cherry	6.80 ± 0.7 a	103	0.02 ± 0.01 b	103
2012	Oregon	Medium	6.60 ± 0.7 a	263	0.02 ± 0.01 b	263
2012	Oregon	Raspberry	13.20 ± 4 a	34	0 c	34
2013	Oregon	Blackberry	2.90 ± 0.81 a	30	0.06 ± 0.03 b	30
2013	Oregon	Blueberry	1.05 ± 0.77 a	15	0 c	15
2013	Oregon	Mixed fruit	0.01 ± 0.01 a	12	0 c	12
2013	Oregon	Medium	2.02 ± 0.26 a	305	0.03 ± 0.03 b	305
2013	Oregon	Raspberry	2.30 ± 0.37 a	204	0.69 ± 0.30 a	204
2013	Oregon	Strawberry	1.44 ± 0.57 a	45	1.35 ± 0.92 a	45
2013	Italy	Banana	0.68 ± 0.18 a	264	0.82 ± 0.35 a	264
2013	Italy	Medium	0.29 ± 0.12 a	264	0.45 ± 0.22 a	264
2013	Italy	Fruit	0.33 ± 0.09 a	264	0.47 ± 0.27 a	264

Table 3. Location and mean *P. vindemiae* and *L. heterotoma* emergence (± SEM) in the Willamette Valley (Oregon, USA) and Trento Province (Italy) during 2012 and 2013. Mean followed by different letters are statistically different as determined by ANOVA followed by Tukey's HSD (N = number of Petri dishes or soufflé cups).

Year	Region	Location	Mean number *P. vindemiae*/trap	N	Mean number *L. heterotoma*/trap	N
2012	Oregon	FCF	6.50 ± 0.96 b	133	0.37 ± 0.02 a	133
2012	Oregon	GTF	6.97 ± 1.05 b	119	0 c	119
2012	Oregon	NWREC	13.64 ± 3.7 a	50	0 c	50
2012	Oregon	RF	3.50 ± 0.36 b	66	0.03 ± 0.03 a	66
2012	Oregon	Salem	6.70 ± 1.12 b	74	0.04 ± 0.02 a	74
2013	Oregon	FCF	3.11 ± 0.5 a	144	0.52 ± 0.28 a	144
2013	Oregon	GTF	1.24 ± 0.3 c	144	0.62 ± 0.38 a	144
2013	Oregon	NWREC	1.85 ± 0.43 bc	120	0.22 ± 0.20 a	120
2013	Oregon	RF	1.84 ± 0.45 bc	89	0.14 ± 0.12 a	89
2013	Oregon	Salem	2.03 ± 0.39 b	114	0.07 ± 0.06 a	114
2013	Italy	Gaggio	0.10 ± 0.03 a	198	0.33 ± 0.03 a	198
2013	Italy	Loc. Franchini	0.50 ± 0.19 a	198	0.90 ± 0.3 a	198
2013	Italy	San Michele	0.60 ± 0.16 a	198	1.13 ± 0.33 a	198
2013	Italy	Vigolo Vattaro	0.50 ± 0.18 a	198	0.27 ± 0.03 a	198

type had no effect on numbers of emerging parasitoids ($F_{5,605}$ = 1.0, p = 0.4; table 2). During 2012 in Oregon, significantly more *L. heterotoma* emerged from banana compared to the other fruits ($F_{4,\ 436}$ = 5.0, p < 0.001; table 2). During 2013, more *L. heterotoma* were reared from strawberries and raspberries compared to the other fruit ($F_{5,605}$ = 2.3, p = 0.038).

In Italy, the role of substrate was not a significant factor for *P. vindemiae* emergence from sentinel bait stations ($F_{2,789}$ = 2.6, p = 0.07; table 2). Likewise, for *L. heterotoma* no significant differences of substrate on emergence were observed ($F_{2,789}$ = 2.5, p = 0.6; table 2).

In Oregon, the role of location was significant in both years. During 2012, greater numbers of *P. vindemiae* emerged from sentinel bait stations at NWREC compared to the other locations ($F_{4,437}$ = 4.5, p = 0.001; table 3). During 2013, the most parasitoids were reared from FCF, followed by Salem, NWREC, Riverbend Farm and GTF ($F_{4,\ 606}$ = 3.0, p <0.001; table 3). For *L. heterotoma* location played no significant role in the number of parasitoids reared in 2012 ($F_{4,\ 437}$ = 1.15, p = 0.33; table 3) or 2013 ($F_{4,606}$ = 0.81, p = 0.51; table 3). In Italy in 2013, the role of location did not significantly affect emergence of *P. vindemiae* ($F_{3,778}$ = 2.1, p = 0.098; table 3) or *L. heterotoma* ($F_{3,778}$ = 0.1, p = 0.06; table 3) from sentinel bait stations.

No significant trends in the timing of emergence of *P. vindemiae* from sentinel traps were found in Oregon. The parasitism index for *P. vindemiae* increased to a maximum of 3.8 during 17 July and 9 October during 2012, and the seasonal mean was 1.24 (figure 1a). Recorded parasitism was lower in 2013, with a peak parasitism index on 18 June at 1.36 with a seasonal mean of 0.43 (figure 1b).

In Italy, the number of *P. vindemiae* emerging from *D. suzukii*-baited traps displayed a significant temporal trend, with a parasitoid emergence peak of 2.6 and parasitism index of 0.66 on 18 August, after which emergence decreased ($F_{21,242}$ = 2.4, p < 0.001; figure 1c). The seasonal mean parasitism index for *P. vindemiae* was 0.08 in 2013. The number of *L. heterotoma* emerging from *D. suzukii*-baited traps displayed a non-significant trend with peak parasitoid emergence of 1.39 on 26 May (parasitism index of 2.6), after which emergence decreased ($F_{21,\ 242}$ = 1.1, p = 0.32; figure 1d). The seasonal mean parasitism index for *L. heterotoma* was 0.43.

In Italy, the number of *P. vindemiae* emerging from *D. suzukii*-baited traps displayed a significant temporal trend, with a parasitoid emergence peak of 2.6 and parasitism index of 0.66 on 18 August, after which emergence decreased ($F_{21,242}$ = 2.4, p < 0.001; figure 1c). The seasonal mean parasitism index for *P. vindemiae* was 0.08 in 2013. The number of *L. heterotoma* emerging from *D. suzukii*-baited traps displayed a non-significant trend with peak parasitoid emergence of 1.39 on 26 May (parasitism index of 2.6), after which emergence decreased ($F_{21,\ 242}$ = 1.1, p = 0.32; figure 1d). The seasonal mean parasitism index for *L. heterotoma* was 0.43.

Discussion

The current study represents the first surveys for parasitoid species associated with *D. suzukii* and *D. melanogaster* in Northern Italy and Western Oregon using sentinel host traps. In both regions, two parasitoids (*P. vindemiae* and *L. heterotoma*) emerged from puparia in traps baited with larvae of *D. suzukii* or *D. melanogaster*. In addition, a third parasitoid (*T. drosophilae*) was recovered from a single sentinel trap in Italy. All three species are generalist parasitoids of *Drosophila* (Carton *et al.*, 1986). Both *P. vindemiae* and *T. drosophilae* showed a relatively high parasitism rate on *D. suzukii* under laboratory conditions (Chabert *et al.*, 2012; Rossi Stacconi *et al.*, 2015) and have also been found associated with *D. suzukii* in other regions of Europe and the USA (Kacsoh and Schlenke, 2012; Gabarra *et al.*, 2014; Xingeng Wang, unpublished data). Laboratory work has further confirmed that both the Oregon and Italian populations of *P. vindemiae* can readily develop

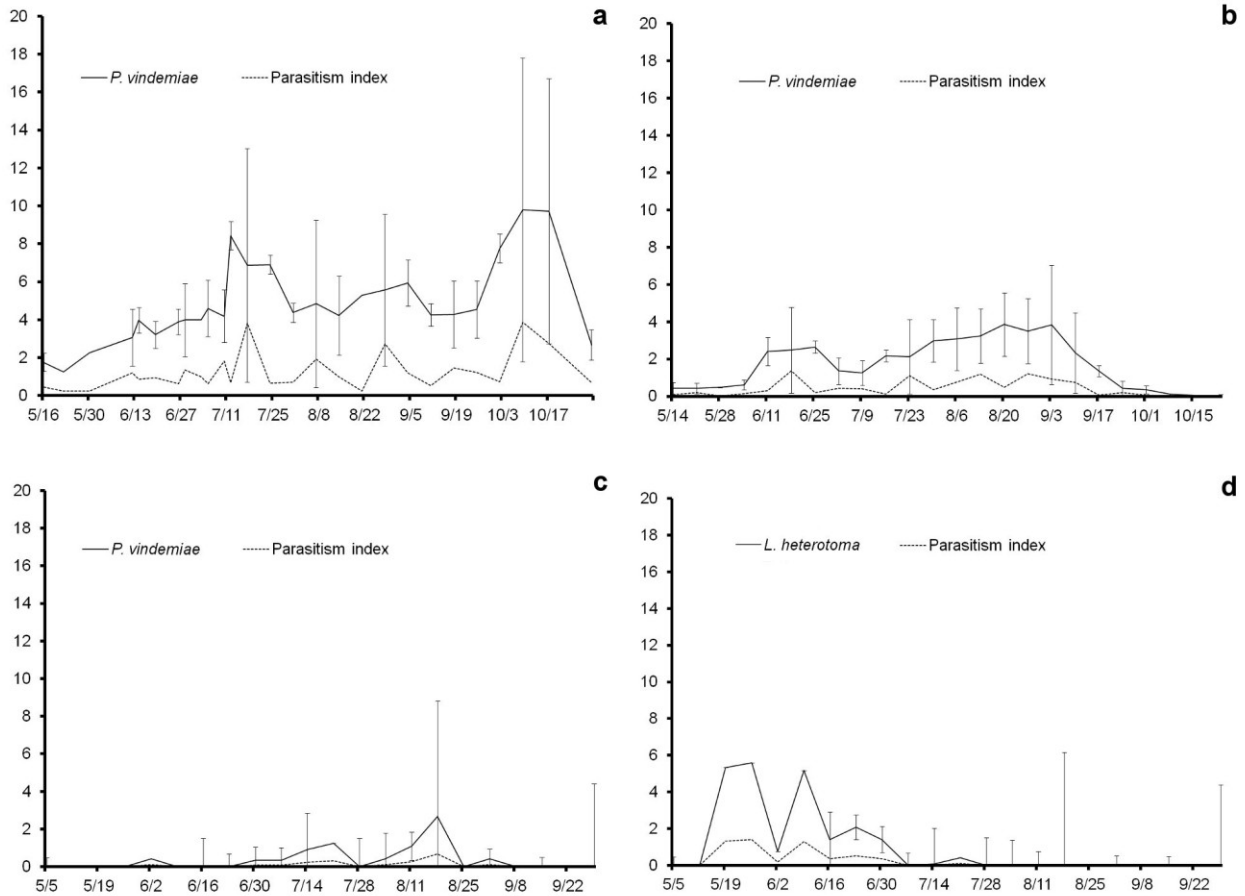

Figure 1. Mean seasonal parasitoids per sentinel trap baited with *D. suzukii* (± SEM) and parasitism index of two key parasitoids of *D. suzukii* in the Willamette Valley, Oregon, USA and Trento Province, Italy during 2012 and 2013. a) *P. vindemiae* in Oregon during 2012; b) *P. vindemiae* in Oregon during 2013; c) *P. vindemiae* in Italy during 2013; d) *L. heterotoma* in Italy during 2013.

on *D. suzukii* and *D. melanogaster*, as can the Italian strain of *L. heterotoma*, whereas the Oregon strain of *L. heterotoma* has not been observed to develop on *D. suzukii* (Rossi Stacconi *et al.*, 2015).

Both *P. vindemiae* and *L. heterotoma* were retrieved from control traps that were not infested with larvae prior to placement in the field. This indicates that field-occurring *D. suzukii*, *D. melanogaster*, or other drosophilid species colonized the media in un-baited controls, as well as larva-baited sentinel traps during the seven-day exposure period. However, the heavily infested substrate and short exposure period limited the introduction of naturally occurring fly hosts within seeded traps. Thus, our results may not necessarily reflect natural rates of field parasitism of *D. suzukii*, but they do provide baseline data on the current field presence and seasonal occurrence of parasitoids of *Drosophila* species in these two regions.

The parasitoid composition and parasitism index varied between seasons and locations or were affected by the use of different bait substrates in both regions. First, in most cases, more *P. vindemiae* emerged from sentinel traps baited with *D. melanogaster* larvae, followed by traps baited with *D. suzukii* larvae, and control sentinel traps baited with neither host. This was observed in both Oregon and Italy during 2013. These data suggest that *P. vindemiae* may prefer to attack *D. melanogaster* over *D. suzukii* or is more efficient in parasitizing *D. melanogaster* in the field. Second, greater numbers of *P. vindemiae* emerged than *L. heterotoma* in Oregon. No *L. heterotoma* emerged from *D. suzukii*-seeded sentinel traps in Oregon during 2012, and numerically more *L. heterotoma* emerged from *D. suzukii*-seeded traps in Italy. Host suitability laboratory work (Rossi Stacconi *et al.*, 2015) suggests that the Italian strain of *L. heterotoma* is able to overcome the defenses of *D. suzukii* and successfully parasitize *D. suzukii*. Third, the raspberry host substrate resulted in the highest emergence of *P. vindemiae* during 2012 in Oregon. Raspberry and blackberry were the substrates that resulted in the highest emergence of *P. vindemiae* in 2013. Banana used as a substrate resulted in numerically higher *P. vindemiae* emergence in Italy during 2013, compared to the Oregon sites where this fruit resulted in numerically lower numbers of *P. vindemiae* emergence. Banana as a host substrate resulted in numerically higher *L. heterotoma* emergence from sentinel traps in both Oregon during 2012 and Italy during 2013. Taken together, there was no clear trend to indicate the most attractive host substrate to optimize attraction of the current assemblage of parasitoids of drosophilids in any of the regions. Of greater importance when choosing a substrate are the seasonal availability of fruit substrates and substrate resistance to desiccation. In addition, the practicality of trap preparation must be considered when using the current experimental methods to recover parasitoids.

In Oregon, sentinel trap emergence of *P. vindemiae* at NWREC and FCF was greatest during 2012 and 2013, respectively, but no statistical difference could be found between locations for *L. heterotoma*. In Italy, location had no impact on the emergence of either species of parasitoid during 2013. Both NWREC and FCF are locations with limited chemical management practices, which may have resulted in higher parasitoid population levels. This aspect needs additional study. The fact that *L. heterotoma* was present during the early portion of the season in Italy, while it was absent during this time period in Oregon needs further study. *P. vindemiae* was present during the majority of the season in both production regions. A trend of increasing populations of *P. vindemiae* was visible in both production regions as the season progressed. A probable explanation for this is that host populations increased during the latter portion of the season. The parasitism index for *P. vindemiae* on *D. suzukii* remained low throughout both observed seasons in Oregon and in 2013 for Italy. This suggests that *P. vindemiae* has a very limited impact on *D. suzukii* pest populations. Sentinel traps were incubated for four weeks after field exposure in order to allow for development of parasitoids. The *Drosophila* host media used in the experiments deteriorated during the initial four-week period. Unpublished preliminary data from the previous seasons of work indicated that parasitoid emergence during this four week period was negligible. Low numbers of parasitoids were collected and removed on a weekly basis during the subsequent nine-week parasitoid collection period. The media most probably did not support additional development of drosophilids, or a second generation of parasitoid wasps, during the nine-week parasitoid observation period, as indicated by the low estimations of parasitism levels. The currently surveyed parasitoids play a limited role in biocontrol against *D. suzukii*.

The current study has several limitations for accurately estimating parasitoid impact on *D. suzukii* populations. This index does not take into consideration additional suitable larval hosts that were deposited in the sentinel traps by resident drosophilids during the seven-day exposure period. In addition, *D. suzukii* mortality caused by parasitoids that attack, but fail to develop to the adult stage, was not accounted for because only adult parasitoids that emerged from the samples were counted. Observing collected traps for a nine-week period introduced the potential for proliferation of hosts and parasitoids in the laboratory, thus skewing the estimation of field parasitism. However, the vast majority of parasitoids emerged during the first 5 weeks of observation, with little to no emergence in weeks 6 through 9 of the observation period, suggesting that this had little to no impact on the estimation of field parasitism. We therefore suggest that the protocol for future surveys can be amended to reduce the length of the observation period.

Taking these factors into consideration, the exact impact of parasitism on the total *D. suzukii* population is unclear. In Oregon the parasitoid currently believed to have the highest impact on *D. suzukii* is *P. vindemiae.* In Italy, the *D. suzukii* parasitism index was greater with *L. heterotoma*, and significantly more of this species were collected in *D. suzukii*-baited traps, suggesting that *L. heterotoma* is the parasitoid with the highest impact in this region. We believe that the resident parasitoids in these study regions cause limited suppression of *D. suzukii* pest populations given host stage suitability characteristics (Rossi Stacconi *et al*., 2015). The current study therefore supports continued search for other biological agents that can additively diminish fly populations in unmanaged habitats near commercial berry fields (Woltz *et al*., 2015). Specialist parasitoid species will likely have a greater effect to control populations of pestiferous *Drosophila* species.

In conclusion, baseline data on the current status of biological control by parasitism of drosophilids by indigenous parasitoids in Trento Province, Italy, and the Willamette Valley of Oregon, USA, indicate that the suppression of *Drosophila* populations is currently limited. These findings highlight the importance of work to implement biological control methods of *D. suzukii* management. Future study in these regions may indicate potential impacts such as parasitoid adaptation, impact of classical or augmentative biological control programs, and related impacts of non-target control measures such as pesticide use or alternative cultural management.

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