

ECOLOGY

First field records of *Pachycrepoideus vindemiae* as a parasitoid of *Drosophila suzukii* in European and Oregon small fruit production areas

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Abstract

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a destructive crop pest native to Southeast Asia that recently invaded countries in Europe and North America, severely impacting commercial fruit production in its new host range. Here we report the results

of a survey aimed at determining the presence of indigenous *D. suzukii* parasitoid populations carried out from May to October 2012 in two areas negatively affected by this fruit pest: Trento Province, Northern Italy, and Oregon in the Pacific Northwest of the USA. We conducted field and laboratory studies in order to determine the status of biological control agents utilizing *D. suzukii* as a host. Our study sites included a range of commercial soft fruits and natural non-commercial habitats. In each site, sentinel traps were baited with either *D. suzukii* or *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) larvae in different food substrates. The generalist parasitoid, *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae), was collected from both *D. suzukii* and *D. melanogaster* pupae in traps deployed in a selection of these sites. This report of *P. vindemiae* in 2012 represents the first identification of *D. suzukii* parasitoids in Europe. A successive parasitism efficacy test was set up under controlled laboratory conditions confirming the ability of *P. vindemiae* to attack *D. suzukii* pupae. In addition, an historical digression with analysis of the original documents in the Italian archives has been provided in order to unravel the correct species name. We finally discuss the possible practical implications of this finding for the biological control of *D. suzukii*.

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Key words: *Drosophila suzukii*, *Pachycrepoideus vindemiae*, *Drosophila melanogaster*, Pteromalidae, pupal parasitoid, biological control.

Acknowledgements: this work was partly funded in Italy by DROSKII project in behalf of Euphresco II. Work in the USA was made possible with funding from USDA-NIFA Specialty Crops Research Initiative (Award number: 2010-51181-21167, Project title: Biology and Management of Spotted Wing *Drosophila* in Small and Stone Fruits). We thank Dr. Luca Bartolozzi, Museum "La Specola", University of Florence, for assistance with lectotype check. We are grateful to Cooperativa Piccoli Frutti Santorsola for the fruit supply.

Contributions: MVRS, DD, BM, CI, VW, GA, conceived and designed the experiments; MVRS, AG, DD, BM, MO, AL, VW, GA, performed the experiments; MVRS, AG, DD, BM, MO, AL, CI, VW, GA, analyzed the data; AG, AL, CI, VW, GA, contributed materials; MVRS, AG, DD, BM, MO, AL, CI, VW, GA, wrote the paper.

Received for publication: 16 April 2013.

Revision received: 9 July 2013.

Accepted for publication: 10 July 2013.

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Entomologia 2013; 1:e3

doi:10.4081/entomologia.2013.e3

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a fly native to Asia that has been recently introduced to Europe (Calabria *et al.*, 2012) and Oregon (Dalton *et al.*, 2011). This species is included in the European Plant Protection Organization (EPPO) A2 list as a quarantine pest and is known to infest commercial small and stone fruits as opposed to most species of drosophilids which only infest overripe, fallen and rotting fruits. In marked contrast, female *D. suzukii* possess a serrated ovipositor, which enables them to lay eggs in healthy, ripening fruits. *Drosophila suzukii* poses a significant risk to marketable soft and thin-skinned fruits (Lee *et al.*, 2011b; Walsh *et al.*, 2011; Cini *et al.*, 2012). Larval feeding may result in fruit to become soft, eventually collapse, and unmarketable (Rota-Stabelli *et al.*, 2013). A preliminary study estimated that in the Trento Province, Italy, the 400-ha soft fruit production areas faced losses of around 500,000 EUR in 2010 and 3 million EUR in 2011 (De Ros *et al.*, 2012). Pacific USA production areas may suffer losses up to \$500 million annually if no pest control

measures are taken (Goodhue *et al.*, 2011). The enormous impact of *D. suzukii* on fruit production is exacerbated by high population build-up, which is facilitated by a high rate of fecundity and a rapid developmental period (Walsh *et al.*, 2011).

After the initial findings in North America in 2008, *D. suzukii* is now recognized as a key pest in all major small and stone fruit production areas of the USA and Canada (Lee *et al.*, 2011a). In Europe, the pest was first recorded in Spain (Calabria *et al.*, 2012) and Italy in 2008 (Cini *et al.*, 2012). The first known damage to commercial small fruit in Italy was found in Trento during 2009 (Grassi *et al.*, 2009). Additional local outbreaks were found in Italy, Spain, France, Slovenia and Croatia during 2010, and the following year in Austria, Switzerland, Germany and Belgium. *Drosophila suzukii* was reported in Great Britain, the Netherlands and Portugal in 2012. A recent genomic survey linked the invasive success of *D. suzukii* in North America and Europe to an ecological pre-adaptation to temperate climates. Its further spread to additional temperate climate areas therefore seems likely (Ometto *et al.*, 2013).

Current control of *D. suzukii* is challenging because of a low number of effective chemical- and semiochemical-based control strategies. Furthermore, the ability of invertebrates and microbial organisms to interfere with populations of the introduced pest is only now beginning to be investigated (Brown *et al.*, 2011; Hamby *et al.*, 2012; Siozios *et al.*, 2013). Natural enemies in newly invaded areas may gradually adapt and establish new associations with *D. suzukii*, thus contributing to its future suppression. In Europe, for example, this has been observed specifically for the leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) (Urbaneja *et al.*, 2000).

A number of hymenopteran parasitoids have been reported in association with *D. suzukii* in the areas of pest origin. In particular, species of the genera *Ganaspis* and *Leptopilina* (Hymenoptera: Figitidae), and *Trichopria* (Hymenoptera: Diapriidae), are reported as parasitoids of *D. suzukii* in Japan (Cini *et al.*, 2012). *Ganaspis* species showed the highest rates of *D. suzukii* parasitism. These figitids lay eggs in larvae that are feeding in fruits and exhibit a high level of specificity for *D. suzukii*. By contrast, *Leptopilina japonica* Novkovic et Kimura and *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae) were able to attack *D. suzukii* larvae and pupae only in fallen decaying fruits and also attacked a wide range of drosophilid hosts (Mitsui *et al.*, 2007; Ideo *et al.*, 2008; Mitsui & Kimura, 2010; Novkovi *et al.*, 2011; Kasuya *et al.*, 2013).

On the other hand, Chabert *et al.* (2012) have already demonstrated under laboratory conditions that several naturally occurring parasitoids of drosophilids in France were able to successfully parasitize *D. suzukii*: two larval parasitoids, *Leptopilina heterotoma* (Thomson) and *Leptopilina bouvardi* Barb. *et al.*, and two pupal parasitoids *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae). Both *Leptopilina* parasitoids showed the highest levels of parasitism. *Leptopilina bouvardi* was able to parasitize 67% of exposed hosts, while one population of *L. heterotoma* had a peak parasitism rate of 95%. The parasitism rate of the local pupal parasitoid *P. vindemiae* averaged 57%. This species has also been found in association with *D. suzukii* in fruit-producing regions of Oregon, USA (Brown *et al.*, 2011). Kacsoh & Schlenke (2012) found in laboratory experiments several promising parasitoids of *D. suzukii*, including *A. japonica*, which are able to overcome the host's defense mechanism of encapsulation.

The present study focused to determine the current presence of indigenous parasitoid biological control agents in two severely affected areas of Trento Province, Italy, and the Willamette Valley, Oregon, USA, using sentinel baits placed in proximity to fruiting host species. Here we report the methodology used to determine the field occurrence of parasitoids of *D. suzukii* in these regions. We additionally describe the procedures conducted to validate parasitism of *D. suzukii* under laboratory conditions.

Materials and Methods

Parasitoids of *D. suzukii* were surveyed in Trento Province, Italy, and the Willamette Valley, Oregon, USA. The field surveys were carried out from July to October in Trento and from April to October in Oregon during 2012. In Trento the field study sites included a commercial blueberry orchard, a commercial vineyard and an alpine forest environment. In Oregon five field sites were established in areas of unsprayed non-commercial hosts in close proximity to commercial host crops.

Preparation of traps

Petri dishes (diameter 9 cm) containing either standard yeast-based medium diet for drosophilids, or fresh banana sliced 1-2 cm thick, were exposed either to *D. suzukii* or to *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) adults in plastic oviposition cages (30×30×30 cm). Dishes were kept in oviposition cages containing ad libitum numbers of *D. suzukii*, for three to seven days depending on fly development time observed in the laboratory. Once removed from the oviposition cages, infested Petri dishes were filled with water in order to stimulate the larvae to come out from the food substrate. Subsequently, the surface of the Petri dishes was carefully rinsed and the larvae were collected on a fine mesh net. New Petri dishes containing about 70-90 g of either standard *Drosophila* food medium, or fruit (sliced bananas, pierced blueberries or sweet cherries, or crushed raspberries) were prepared. Each Petri dish was infested with either 1 g of *D. melanogaster* or 4 g of *D. suzukii* larvae (corresponding to approximately 800-1000 larvae for both species respectively). In Oregon, Petri dishes were substituted with 163 mL plastic soufflé cups (Solo[®], Urbana, IL, USA) (Dalton *et al.*, 2011) in order to minimize desiccation during the hot summer months.

In Italy, two trap types were prepared: one baited with three Petri dishes containing food medium, banana slices and blueberries respectively, each infested by *D. suzukii* larvae (treatment 1); and one with the same food substrates infested by *D. melanogaster* larvae (treatment 2). Both trap types also included a Petri dish filled with medium and left uninfested as blank control (Figure 1).

In Oregon, three trap types were prepared: one baited with two soufflé cups containing food medium and seasonal fruit respectively, each infested by *D. suzukii* larvae (treatment 1); one with the same food substrates infested by *D. melanogaster* larvae (treatment 2), and one with the same food substrates left uninfested as a blank control (Dalton *et al.*, 2011) (Figure 1).

Petri dishes and soufflé containers containing larvae (or no larvae for the controls) in each of the media were placed inside red delta traps to constitute each sentinel trap. Sentinel traps were hung 1-2 m from the ground on either the crop plant or from surrounding vegetation in a relatively protected environment. In Oregon the desiccation levels found in sentinel baits were low enough to allow weekly service of the traps as opposed to many of the areas in Italy where desiccation of the bait materials necessitated more frequent service of traps (twice a week).

Placement of traps

In Italy. Three representative experimental sites in Trento Province were selected, taking into account altitude, presence of *D. suzukii* host plants and high *D. suzukii* population density in both natural and agroecosystems. Two hilly sites were selected, one in Cembra Valley, a commercial organic blueberry orchard in Gaggio (46°20'60"N, 11°28'89"E, 900 m a.s.l.), and the other in Sugana Valley representing a forested environment (Vigolo Vattaro, 45°99'64"N, 11°19'25"E, 600 m a.s.l.). A third was chosen in the bottom of the Adige Valley, in a commercial vineyard (San Michele all'Adige, 46°19'01"N, 11°13'73"E, 200 m a.s.l.). In each site two sentinel traps (treatments 1 and 2) were deployed at least 10 m apart (Figure 1). Traps were collected and replaced twice a

week. The experiment started on 2 July 2012 and continued until the end of October 2012.

In Oregon. Five trap locations were selected based on high diversity of landscape vegetation surrounding a susceptible crop, as well as on preliminary data of percent parasitism from the previous growing season. Traps were first placed in the field during April 2012. The sites in Oregon included an organically managed raspberry field at Oregon State University North Willamette Research and Extension Center (45°21'53"N, 122°44'59"W, 60 m a.s.l.), an unmanaged wild riparian site containing a mix of Himalayan blackberries and seedling cherries (Salem, 44°54'19"N, 123°07'04"W, 43 m a.s.l.), a commercial blueberry and raspberry farm (Riverbend Farm, 44°12'58"N, 123°41'54"W, 89 m a.s.l.), a small-scale, mixed-production-commercial rural homestead with unsprayed soft and stone fruit species (Fraser Creek Farm, 44°37'42"N, 123°15'45"W, 100m), an organically managed mixed-production farm (Gathering Together Farm, 44°31'52"N, 123°22'15"W, 80 m a.s.l.). In each site, three traps (treatment 1, treatment 2 and control) were deployed at least 25 m apart (Figure 1). The first set of traps was placed on April 2, 2012. Traps were collected and replaced once a week until the end of October 2012.

Rearing and identification of parasitoids

Once collected from the field, all Petri dishes or soufflé cups were sealed and transported to the laboratory for further rearing and investigation. In the laboratory, Petri dishes and soufflé cups were placed into 250-720 mL plastic containers with lids equipped with fine mesh netting for ventilation and a moistened dental cotton ball to prevent desiccation of pupae. All containers were kept in controlled conditions (22°C, 55% RH, 14:10 L: D photoperiod) and checked for emerging parasitoids twice a week. Once emerged, flies and parasitoids were counted to determine the percent parasitism occurring in the sentinel traps. After every count, the parasitoids were taken out from the containers and kept in plastic vials with a cotton ball soaked in sugary water (5%), at the same controlled conditions. To determine if a parasitoid had emerged from a puparium, we looked at the exit hole. In contrast to holes made by *Drosophila* species, parasitoids chew a round hole in the puparium from which to exit (Figure 2A and B, respectively). In Oregon, the parasitoid species *P. vindemiae* was initially identified by Howard Thistlewood of Pacific Agri-Food Research Centre in

Summerland, British Columbia, Canada. In Italy it was identified by Marco Valerio Rossi Stacconi of Fondazione Edmund Mach.

Laboratory test of parasitism validation and efficacy

In order to test the ability of *P. vindemiae* to parasitize *D. suzukii*, an additional laboratory experiment was established at Fondazione Edmund Mach. Field collected *P. vindemiae* individuals were maintained in the laboratory on *D. melanogaster* (22°C, 55% RH, 14:10 L:D photoperiod) in order to obtain a first mass rearing. From this stock newly emerged pairs were reared for five days, fed with honey and allowed to mate. Subsequently, six mated *P. vindemiae* females were placed into a plastic cup (720 mL) including a Petri dish filled with standard *Drosophila* food medium infested with 100 *D. suzukii* larvae (3rd-4th instar) and a water soaked cotton ball (N=5). After 5 days *P. vindemiae* females were removed from the cups. Fly and parasitoid emergence was checked daily and the outcomes were divided into three categories: emerged parasitoids, emerged *D. suzukii* individuals and *D. suzukii* individuals that incurred natural mortality. Percent parasitism was calculated considering only emerged fly and parasitoid adults. In order to exclude other possible causes of death, undeveloped *D. suzukii* pupae were examined under the light microscope, checking for any possible indication of parasitism. Emerged *D. suzukii* adults were checked for signs of resistance to the parasitoid, including presence of encapsulated eggs (Poyet *et al.*, 2013).

Results and Discussion

The idiobiont pupal ectoparasitoid *P. vindemiae* was collected from both *D. suzukii* and *D. melanogaster* pupae in traps deployed in a selection of sites in Italy and Oregon.

In Italy, no parasitoids were obtained from any location during the first two months of the experiment. During 24-28 September, 33 specimens of *P. vindemiae* (Figure 3 A-C), were however recovered from Petri dishes containing *Drosophila*-infested blueberries from traps deployed in the vineyard at San Michele all'Adige. Of these, 27 individuals were from *D. melanogaster*- and six individuals from *D. suzukii*-

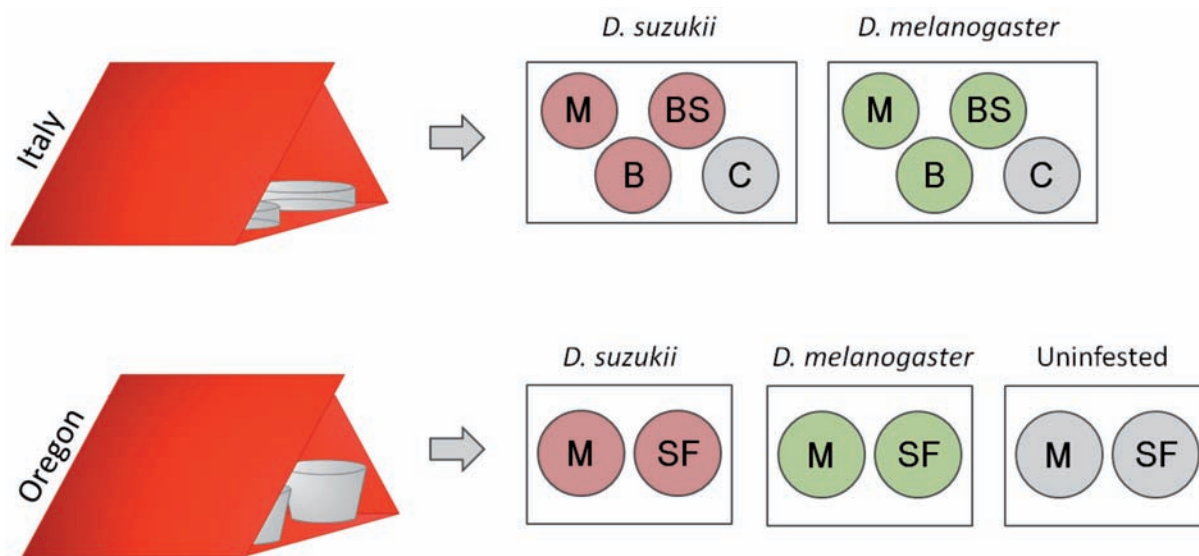


Figure 1. Schematic drawing of the sentinel traps: in Italy two trap types were prepared, while in Oregon three trap types were placed in each site. M: food medium; BS: banana slices; B: blueberries; C: control (uninfested); SF: seasonal fruit.

infested Petri dishes. Adult parasitoid emergence started in the laboratory on 4 October and continued for the following five days. As far as we know this is the first evidence in Europe of parasitization of *D. suzukii* by a local parasitoid. This report supports findings of the same parasitoid species in cherry orchards in the Columbia Gorge of Oregon in the USA (Brown *et al.*, 2011). Interestingly, in our experimental vineyard in Italy, sex pheromone-mediated mating disruption against grapevine moths had been applied for several years allowing a marked reduction in insecticide use (Ioriatti *et al.*, 2011).

In Oregon, *P. vindemiae* were retrieved from all field locations between 29 May to 30 October. *Pachycrepoideus vindemiae* were reared from sentinel baits seeded with both *D. suzukii* and *D. melanogaster*. A total of 1932 *P. vindemiae* emerged from sentinel baits during this period constituting approximately 94% of all parasitoids reared from sentinel baits. Approximately equal numbers of parasitoids emerged from the two host species. This finding supports earlier studies that *P. vindemiae* is a generalist parasitoid found on many dipteran species. In addition we reared 133 (6%) individuals of an unidentified cynipid species coming from sentinel baits seeded with *D. melanogaster*. Parasitism estimations revealed approximately 1% seasonal parasitism rates from our sites in Oregon (Miller B., unpublished data).

It was possible to determine that all developmental larval and pupal stages of host materials were present at equally abundant levels based on the similar protocol used in the preparation of the traps during the exposure period. Standardization of exposure period will not necessarily result in more uniform results between the two continents. The results found in this study probably may be due to differences in the current populations of resident parasitoids in the regions where the trials were conducted.

Pachycrepoideus vindemiae is a major natural enemy of *D. melanogaster* (Martelli, 1910), having a cosmopolitan world distribution and a wide host range comprising over 60 known species belonging to cyclorrhaphous Diptera (including families Anthomyiidae, Calliphoridae, Muscidae, Sarcophagidae, Tachinidae, Tephritidae and Drosophilidae).

In international literature the species is mentioned indifferently as *P. vindemiae* or *P. vindemmiæ*. Actually it was originally described by Rondani (1875) as *Pteromalus vindemiae* (Figure 4). However, within this first report a figure caption reported also the version *P. vindemmiæ* (Figure 4), probably fled by mistake from the Italian language. However, according to the International Code of Zoological Nomenclature, the first citation *vindemiae* would take priority.

It has been demonstrated that *P. vindemiae* can act also as a hyperparasitoid of beneficial Hymenoptera (Goubault *et al.*, 2003; Wang &

Messing, 2004). The broad polyphagy of *P. vindemiae* is explained by its marked physiological flexibility and its ability to adapt to different host sizes.

Our validation test to determine parasitism efficacy confirmed that *P. vindemiae* is able to parasitize *D. suzukii* pupae under controlled conditions. Our data, however, display highly variable rates of parasitism, ranging from 25% to 68%. The overall parasitism rate in this experiment was 53.19 ± 18.6 (SD) (Figure 5), which is compatible with results presented by Chabert *et al.* (2012) using similar methodologies. We observed that *P. vindemiae* females laid their eggs between the pupal body and puparium of the host, in the late pupal stage. The emergence hole was mostly localized toward the anterior end of the puparium, just below the breathing spiracles (Figure 2A). Among undeveloped pupae, no sign of parasitism was found, nor was there evidence of parasitoid resistance in emerged *D. suzukii* adults. The mean percentage of individuals that incurred natural mortality, not due to parasitoid activity, was 20.0 ± 11.1 .

Parasitism of *P. vindemiae* on *D. suzukii* in our experimental field conditions appears to be negligible so far. The preliminary evidence, together with the highly generalist host-choice behaviour including beneficial species, would not make *P. vindemiae* a feasible candidate for development of classic or augmentative biological programs against *D. suzukii* in the production regions where the current experiment was conducted. Sufficient pest suppression in fruit cropping systems would require the preventive and repeated augmentation of effective control agents. Although *P. vindemiae* has been mass-released over a long period for the control of several tephritid pests in the Hawaiian Islands and South America, other parasitoid species of fruit flies have been considered more effective biological control agents (Guillén *et al.*, 2002). Nevertheless, the first finding of a local natural enemy able to attack *D. suzukii* in major European and American fruit production areas is a promising indication toward a gradual adaptation of the local fauna to the newly introduced pest.



Figure 2. Comparative pictures of *Drosophila suzukii* puparia A) The adult *Pachycrepoideus vindemiae* emerges by chewing its way out of the puparium. Circles indicate the exit holes. B) The adult *D. suzukii* emerges from a hinged flap in the puparium. Circle indicates the exit point.



Figure 3. A) *P. vindemiae* adult female on *D. suzukii* puparia. B) *P. vindemiae* developing inside the fly puparium. C) *P. vindemiae* emerged from a *D. suzukii* puparium.

l'istinto della propria specie, seguiranno a collocare la loro progenie nelle larve delle vittime predestinate.

Ho constatato che i due minuti *Vesparii* di cui si parla, appartengono a generi già stabiliti, ma a specie non ancora descritte nè conosciute, e per conseguenza dovrò anche a queste imporre un nome proprio, col quale dovranno passare alla posterità.

Uno lo chiamo Pteromalo della Vendemmia — *Pteromalus vindemiae*.

L'altro: Xisto del mosto — *Xystus musti*.

Ho disegnato a forte ingrandimento le parti principali delle due Drosophile, e quelle dei loro due nemici, come nella unita tavola, affinché si possano meglio distinguere le due specie che abitano più comunemente nelle nostre case; e per conoscere i caratteri più importanti degli uccisori, che han per mandato di opporsi alla sconfinata invasione dei due piccoli Muscarii.

Parma, 10 Ottobre 1875.

PROF. CAMILLO RONDANI.

*Spiegazione delle figure della Tavola
a forte ingrandimento.*

1. Ala della *Drosophila uvarum*. Rndn.
2. » *Drosophila aenopota*. Scop.
3. Tarso anteriore del Maschio nella *Drosophila uvarum*.
4. Capo e Antenna del *Pteromalus vindemiae*.
5. Ala » » »
6. Gamba » » »
7. Antenna del *Xystus musti* (maschio).
8. » » » (femmina).
9. Ala » » »
10. Gamba » » »

Figure 4. Copy of page 148 from the first Italian report describing *P. vindemiae* as a new species. Rondani C., 1875. Notizie sul moscherino dell'uva. Bollettino del Comizio Agrario Parmense VIII (10): 145-148. First mentions of the species name are shown underlined in red.

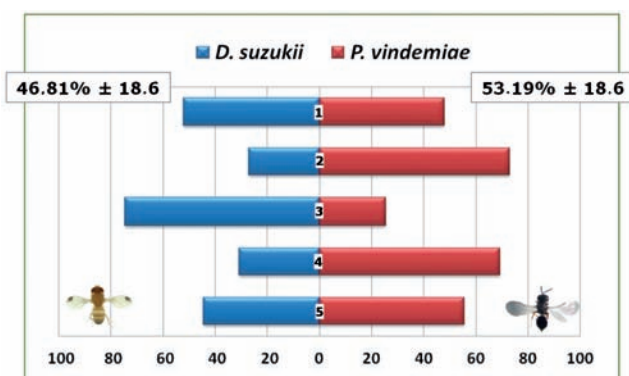


Figure 5. Results of the parasitism validation and efficacy test. Five replicates each with six *P. vindemiae* mated females and 100 3rd instar *D. sukuzii* larvae were performed. The results ranged from 25.35% (3) to 68.13% (2) of parasitized pupae. Mean percentage ± SD of emerged *D. sukuzii* and *P. vindemiae* respectively is shown.

Accordingly, it is noteworthy that a relevant number of more specialist larval parasitoids, belonging to the genera *Asobara* and *Leptopilina*, were by-caught in bottle traps baited with vinegar and wine placed in the Italian experimental sites along the duration of the described survey with the purpose to monitor the *D. sukuzii* population density (personal observation by the authors). This may open the possibility that some parasitoids may undergo a host shift. Species belonging to these two genera were shown to successfully parasitize *D. sukuzii* in their native areas. In particular, *L. japonica* and *Asobara tabida* (Nees) (Hymenoptera: Braconidae) have been reported amongst emerging parasitoids from field sampling of *D. sukuzii* in Japan, even though the latter species was found at a very low rate (Mitsui *et al.*, 2007). Despite its wide European distribution (Carton *et al.*, 1986), *A. tabida* was only recently reported in Italy from an area in Trento Province highly infested with *D. sukuzii* (Anfora *et al.*, 2013). However, European populations of *A. tabida* were not yet able to attack *D. sukuzii* pupae in laboratory experiments (Chabert *et al.*, 2012).

Accumulating evidence reveals that, to take a biological control approach to deal with *D. sukuzii* infestations, the apparently high resistance of this insect pest to larval parasitoids should be taken into account. Indeed, Kacsoh & Schlenke (2012) showed that the constitutively high haemocyte load in *D. sukuzii* enhances the encapsulation process of eggs in response to parasitism by at least 15 parasitoid species. Furthermore, European strains of the pest present a higher level of haemocytes involved in the immune response than Japanese strains (Poyet *et al.*, 2013), making the European *D. sukuzii* populations even more resistant to potential indigenous larval parasitoids. A similar response was seen in North American USA *D. sukuzii* populations (Kacsoh & Schlenke 2012). In addition, in European populations of *L. heteroma*, this effect is not sufficient to prevent the encapsulation reaction (Poyet *et al.*, 2013), even though the immunosuppressive factors in the venom act on some haemocytes of *D. sukuzii* larvae, since they are not sufficiently adapted to this newly introduced host insect. Considering only this aspect, a pupal parasitoid, such as *P. vindemiae*, should be more likely to overcome the resistance of the *D. sukuzii* populations.

In conclusion, the potential for control of *D. sukuzii* by natural enemies present in newly invaded regions may depend on the efficacy with which natural enemies feed in each particular native drosophilid community, as well as on gradual adaptation of indigenous parasitoids to the new host drosophilid. Therefore, continuing investigations on diversity and impact of the natural enemy complex in agro-ecosystems must be performed. A more extensive sampling regime is warranted for 2013, using improved sentinel traps, including more locations and taking into consideration quantitative data on parasitism on both *D. sukuzii* and *D. melanogaster*. Additional foreign exploration for eventual release of more suitable natural enemies is of importance to all industries affected by this pest.

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