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Can exotic drosophilids share the same niche of the invasive *Drosophila suzukii*?

N. Amiresmaeili,[1] C. Jucker,[2] S. Savoldelli,[2] D. Lupi[2]

[1]Agroscope, Biosafety Group, Zurich, Switzerland; [2]Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Italy

Abstract

The presence of the four exotic drosophilids *Chymomyza amoena* (Loew), *Drosophila suzukii* (Matsumura), *Zaprionus indianus* (Gupta) and *Zaprionus tuberculatus* Malloch has been investigated in different orchards in Northern Italy for two consecutive years. The presence and the abundance of the population of the drosophilid flies were surveyed with apple cider vinegar traps, fruit baited traps, and fruit collection. *Chymomyza amoena*, *Z. tuberculatus* and *D. suzukii* have been identified in the Apple Cider Vinegar traps in both years. Only *D. suzukii* and *Z. tuberculatus* emerged from fruit baited traps. Except for *D. suzukii,* no other exotic drosofilid was captured from the fruit collection. *Z. indianus* was never observed. Analyses of the presence of the different species, seasonal occurrence and sex ratio are provided.

Correspondence: Daniela Lupi, Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy.
Tel.: +39.02.50316746 - Fax: +39.02.50316748.
E-mail: daniela.lupi@unimi.it

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Dedication: In memory of Dr. Giuseppe Granelli, beloved colleague and friend.

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Introduction

In recent years, four exotic drosophilids, *Chymomyza amoena* (Loew), *Drosophila suzukii* (Matsumura), *Zaprionus indianus* (Gupta) and *Zaprionus tuberculatus* Malloch were detected in Italy. To date, the most dangerous among them is the polyphagous *D. suzukii,* as it is able to lay eggs into the pulp of healthy fruits due to a serrated ovipositor which lacks in the other three species (Asplen *et al*., 2015). This destructive pest of small and stone fruits, native to Eastern and Southeastern Asia, was detected for the first time in 2008 in Tuscany, Italy (San Giuliano Terme, Pisa, Central Italy) simultaneously with Spain, near Barcelona (Calabria *et al*., 2012; Cini *et al.,* 2012). *Drosophila suzukii* rapidly adapted to the climatic conditions of the Mediterranean countries, and nowadays it is widespread in Europe, including several Italian regions where it causes serious economic losses (Cini *et al*., 2014). In Lombardy, where this research was conducted, the pest was detected in 2011 (Griffo *et al*., 2012).

The Nearctic *C. amoena* (Wheeler, 1965) was first found in Europe in Czechoslovakia in 1975 (Màca, 1985; Màca & Bächli 1994), probably introduced through imported fruits, and it is now present in mainland Europe (Bächli *et al*., 2004). In Italy this species was collected for the first time in Italy in Veneto (Northeastern Italy) in 1999 (Bächli *et al*., 1999) and later in Lombardy (Northern Italy) in 2000 in the Alps (Band *et al*., 2003). *Chymomyza amoena* is considered a broad-niched species able to develop and overwinter in a great variety of fruits and nuts (Band *et al*., 2005).

Zaprionus tuberculatus, native to the Afrotropical region and the islands of the Indian Ocean, was detected for the first time for the European continent in Italy in Trentino (Northern Italy) and in Apulia (Southern Italy) in 2013 (Raspi *et al*., 2014; Antonacci *et al*., 2017). No information is available about its presence in Lombardy. Larvae usually develop on decaying fruits, but the insect was also observed laying eggs in flowers. Eggs hatch in a few days, and the feeding larvae make the flower wither and rot (Buruga, 1976).

The species *Z. indianus*, also known as the African fig fly, is native to Africa, but it has widespread into several areas included Asia, North and South America (Vilela, 1999; van der Linde *et al*., 2006). Its presence in the Mediterranean Basin has been reported in several countries (EPPO, 2016). In Italy, the species has been detected for the first time in 1988 (TaxoDros, 2003), and reported again in 2010 by Yassin & David. Recently, the pest was observed in Southern France (Kremmer *et al*., 2017). The polyphagous *Z. indianus* is considered a secondary pest able to infests overripe, fallen, or rotting fruit. However, in Brazil it was reported as a primary pest of figs causing serious economic damages (Vilela *et al*., 1999).

The objective of this two-year study was to evaluate the contemporary presence of the four exotic drosophilids in the same habitat and the possibility for them to share the same host plants. Information on biology and seasonal presence of the drosophilids were also acquired.

Materials and Methods

Sampling areas

Field surveys were carried out in four localities in Lombardy region, Northern Italy during 2014 and 2015 in crops infested by *D. suzukii* in the previous years (Table 1). Investigations were made in Montanaso Lombardo (Lodi), Camnago Volta (Como) and Minoprio (Como) in 2014, and in Camnago Volta, Minoprio, and Guanzate (Como) in 2015. Guanzate was added as a research site in 2015 due to the decision of the farmer in Montanaso Lombardo to renovate the raspberry and blackberry plantation.

Sampling methods and instruments

The population levels and the presence of the four drosophilids were evaluated in each site by placing Apple Cider Vinegar traps (ACV), Fruit Baited traps (FB) or collecting fruits during the season. Details of samplings are given in Table 1.

ACV traps consisted of a plastic bottle (1.5 L) with a screw-top modified with holes of 0.5-0.8 mm diameter in 10 rows and 10 columns, positioned on one side of the top half of the bottle. Each trap was hanged on a plant at 1.5-2.0 m high. The number of ACV traps is indicated in Table 1; one trap every 1,000 sqm was positioned in each sampling site. Traps were baited with 250 mL of apple cider vinegar with 5% acidity. Trap content was replaced weekly during the trial period. The entrapped specimens were rinsed, collected and stored in 70% (v/v) solution of ethanol inside plastic vials (44 ×10.8 mm, 2 mL volume) for subsequent identification.

FB traps were positioned in the crops at about 1.5 m height and consisted of a delta trap where two plastic Petri dishes (diameter 90 mm), one with slices of banana and one with healthy blueberry fruits, were placed. Banana was chosen because it is highly attractive for drosophilids in general (Cha *et al.,* 2012; Schubert *et al.*, 2014), while blueberries were chosen as favorite hosts for *D. suzukii* (Mazzetto *et al.,* 2016). Two delta traps were positioned for each crop. To evaluate if differently overriped fruits can allow the oviposition and development of different drosophilid species (associated to healthy or decaying fruits), as verified by Lasa *et al.* 2016, the Petri dishes were removed every 7 days in one delta trap and every 14 days in the other.

After removal, Petri dishes were positioned inside plastic transparent boxes (750 mL) with a net on the lid for ventilation and prevention of insect escape and transferred to the laboratory of DeFENS, University of Milan. Boxes were maintained at room temperature and checked three times per week. Newly emerged drosophilids were removed during the first ten days in order to avoid overlapping generations; further observations were done in the following ten days to check for *C. amoena* and *Z. tuberculatus* presence, as these species are known to have a longer developing period (Band, 1988a; Buruga, 1976). After removal, specimens were stored in 70% (v/v) solution of ethanol inside plastic vials (2 mL) for subsequent identification.

In 2015, the climate in summer significantly differed from the ten-year period 2004-2014 and was characterized by lower humidity and higher temperatures (ARPA Lombardia 2017) causing the desiccation of the fruits inside FB traps. Consequently, FB traps were discarded and blueberry, raspberry, and blackberry fruits were collected directly from plants. 50 fruits per week per crop were gathered in Guanzate and Minoprio during the fruit-produc-

Table 1. Description of the monitored sites, GPS coordinates and altitude, monitored crops, and surrounded crops or vegetation, monitoring period, and the number of Apple Cider Vinegar (ACV) and Fruit Baited traps (FB).

Sites	GPS coordinates	Altitude	Monitored crops	Surrounding crops/ vegetation	Monitoring period	Traps positioned
Montanaso Lombardo	45° 20' 23" N; 09° 27' 03" E	83 m a.s.l.	Raspberry Blackberry	Fruit trees (mostly apple, plum, pear, kiwifruits) and walnut trees	ACV traps: January – end December 2014 FB trap: June-November 2014	7 ACV 4 FB
Camnago Volta	45° 48' 05" N; 09° 07' 45" E	350 m a.s.l.	Blackberry Apple	Hazelnut and walnut trees	ACV traps June – end December 2014; January – end October 2015	2 ACV
Minoprio	45° 43' 39" N; 09° 05' 09" E	333 m a.s.l.	Raspberry Blueberry	Fruit trees (mostly apple, plum, pear, kiwifruits, loquat, fig) and walnut trees	ACV traps: January – end December 2014 May-October 2015 FB traps: June-November 2014 Fruit: July- October 2015	2 ACV 4 FB
Guanzate	45° 43' 41" N; 09° 00' 49" E	342 m a.s.l.	Raspberry Blackberry	Fruit trees (mostly apple, plum, pear, kiwifruits) and hazelnut and walnut trees	ACV traps: May – end October 2015 Fruit: July-October 2015	2 ACV

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ing season. Fruits were put in plastic aerated transparent boxes (750 mL) and checked with the same methods detailed before.

Identification of drosophilids in laboratory

All specimens were observed under the stereomicroscope (Wild Heerbrugg M5A, Leica Geosystems Gmbh, Heerbrugg, Switzerland) for identification. The following taxonomic keys were used for species classification: Bächli *et al*. (2004) to identify *C. amoena*, Vlach (2010) for *D. suzukii*, Markow & O'Grady (2006) for *Z. tuberculatus,* and Yassin & David (2010) for *Z. indianus.* Among the collected drosophilids, adult males and females of *C. amoena*, *D. suzukii* and *Z. tuberculatus* were identified and counted under the stereomicroscope. When a specimen was not included in the previous four, it was considered and counted as native.

Statistical analysis

SPSS® Statistic (Version 24 for Windows, SPSS Inc. Chicago, IL, USA) was used for all statistical analyses. Prior to analyses, all data were tested for normality and homogeneity of variance with the Shapiro-Wilk W- test and Levene's test respectively. One-way ANOVA was used to compare the mean number of *D. suzukii* emerged weekly from blackberry, raspberry and blueberry fruits (weekly emergences and sites were considered as a replicates). A two way-ANOVA was used to compare *D. suzukii* ACV captures in different localities and months. Where significant differences occurred, Tukey-Kramer's Honestly Significant Difference multiple comparisons test was applied for mean separation ($P<0.05$).

Results

A total of 66,042 and 25,669 drosophilids were captured in 2014 and 2015, respectively (Table 2). This study evidenced the presence of three of the four exotic species in Lombardy region. In detail, *D. suzukii* was collected from all of the sites, traps, fruits and in both years; *C. amoena* was collected in both years only by ACV traps in all localities, exception for Montanaso Lombardo. *Zaprionus tuberculatus* was collected only in 2014 by ACV traps from all study sites and from FB traps in Montanaso Lombardo and Minoprio. No *Z. tuberculatus* was captured in 2015 even in the sites where it was present in the previous year. *Zaprionus indianus* was never collected in the present research. Only *D. suzukii* emerged from the fruit collected in 2015.

In 2014, the population of drosophilids captured by all ACV traps, consisted of 65.95% of *D. suzukii*, 30.34% of native drosophilids, 3.69% of *Z. tuberculatus,* and 0.02% *C. amoena*. Among the adults emerged from FB traps, 1.31% were *Z. tuberculatus,* 0.87% were *D. suzukii,* and the remaining part consisted of native drosophilids.

In 2015, most of the captures with all ACV traps were also represented by *D. suzukii* (84.61%), followed by native drosophilids (14.80%) and *C. amoena* (0.59%). From the fruits collected, *D. suzukii* represented 90.64% of the specimens, while, in the remaining 9.36% only native drosophilids were captured. The absolute abundance of the species collected is shown in Table 2.

The comparison of the infestation of *D. suzukii* in the sampled fruits in 2015 showed that there was a significant difference in the colonization of blackberry, raspberry and blueberry fruits ($F_{2,81}$= 8.90; P=0.01). In particular, raspberry and blackberry were more attractive to *D. suzukii* compared to blueberry [mean values (±ES) of adult emergence per week per 50 fruits: 39.60±4.99 for raspberry; 30.98±4.08 for blackberry; 19.62±4.72 for blueberry].

Seasonal occurrence of *D. suzukii*

The mean captures of *D. suzukii* per ACV traps were significantly influenced by the monitoring period ($F_{11,40}$=3.587; P=0.001), but not by the localities ($F_{3,40}$=0.101; P=0.959) or by an interaction between these main effect ($F_{33,40}$=0.292; P=1.000). Consequently, data of all localities were pulled together to evaluate the population dynamic of *D. suzukii* in the area monitored over the two years of observation (Figure 1). Both years showed the same trend of captures with a peak of the population in October 2014 and in September 2015. The trend of monthly mean temperatures over the two years was nearly the same regarding the minimum temperatures. The maximum temperatures were similar in both years until May, while from June to August they were lower in 2014 (always less than 30°C) than in 2015 (always between 30 and 35°C). As verified by other Authors in Italy (Mazzetto *et al*., 2015), the captures were low in both years until June, then in the summer they augmented showing a slightly different trend. In 2014, captures of *D. suzukii* begun to rise from June and reached the mean value (±SEM) of 101.93±21.80 and 210.10±38.29 captures per trap per week in July and August respectively. The highest temperatures occurred in 2015 have led to a slowdown in the growth of *D. suzukii* population. In fact, in 2015 the captures have always been under 100 individuals per trap until September, with a mean value (±SEM) of 69.10±12.67 in July and of 77.14±18.04 in August.

Seasonal occurrence of *Z. tuberculatus*

During 2014, totally 1,891 *Z. tuberculatus* adults were captured from September to December (Figures 2 and 3). In detail, 89% of the specimens was trapped in Montanaso Lombardo. Most

Table 2. Absolute abundance of drosophilids in the monitored sites using different collection methods (ACV traps in 2014 and 2015, FB traps in 2014, and sampled fruits from crops in 2015).

Year	Sites	ACV traps: No. traps	ACV traps: Total drosophilids	ACV traps: *C. amoena*	ACV traps: *D. suzukii*	ACV traps: *Z. tuberculatus*	FB traps in 2014 and sampled fruits in 2015: No. traps	Total drosophilids	*C. amoena*	*D. suzukii*	*Z. tuberculatus*
2014	Montanaso Lombardo	7	23,482	-	13,223	1,506	6	10,279	-	29	177
2014	Camnago volta	2	11,853	3	9,170	17	-	-	-	-	-
2014	Minoprio	2	7,715	6	5,998	66	6	12,713	-	172	125
2015	Camnago volta	2	10,000	34	8,374	-	-	-	-	-	-
2015	Minoprio	1	1,790	6	1,323	-	-	145	-	132	-
2015	Guanzate	2	8,578	80	7,537	-	-	5,156	-	4,678	-

specimens were present in ACV traps (84%), while only 15.9% emerged from FB traps. In 2015, no *Z. tuberculatus* was captured (Table 2).

ACV traps showed a peak of captures in October in Montanaso Lombardo with a mean value per trap of 136.67±59.65 specimens (Figure 2). FB traps showed a peak in September in Montanaso Lombardo with a mean value (±SEM) of 7.75±5.12 adults per trap and in November in Minoprio, with a mean value of (±SEM) 10.2±8.71 adults per trap (Figure 3).

Seasonal occurrence of *C. amoena*

During 2014, only few *C. amoena* specimens were captured by ACV traps, consequently it was not possible to evaluate the trend of capture throughout the year, whereas in 2015, 120 adults were trapped as shown in Table 2. Figure 4 shows the mean captures of *C. amoena* per week in 2015: the specimens were trapped in Guanzate from May until September, and in the other two localities from July until the end of October. The peak of captures was

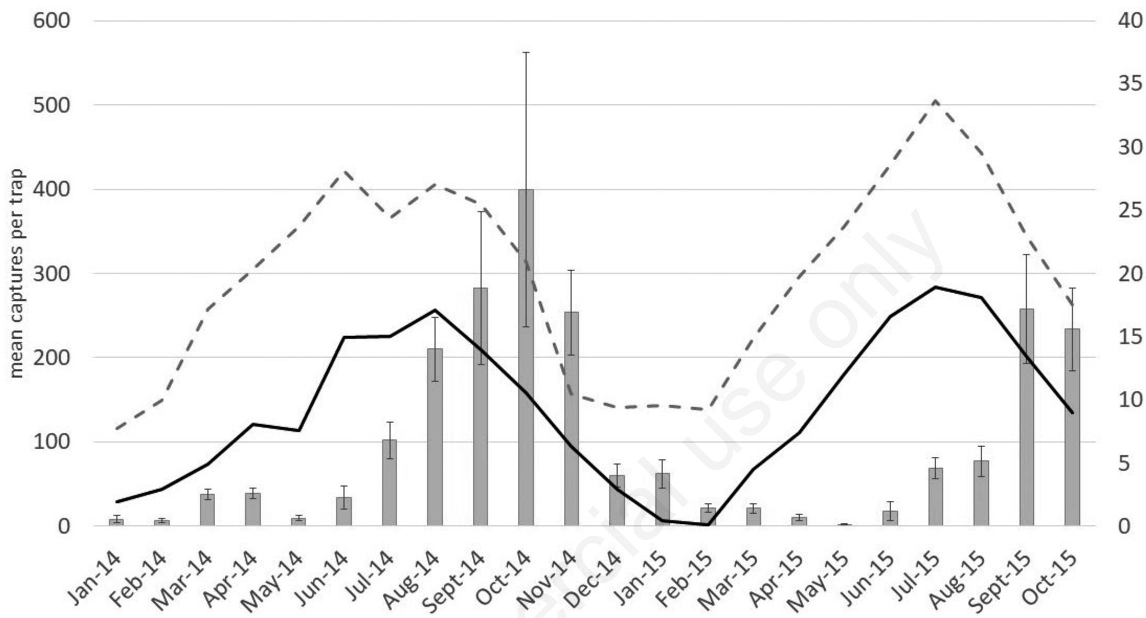

Figure 1. Population dynamic of *D. suzukii* in the monitored area over the two years (the population trend refers to the mean value±SEM obtained from the captures per ACV trap in all the monitored sites from January 2014 to October 2015) and mean monthly minimum (continuous line) and maximum (dotted line) temperatures (°C).

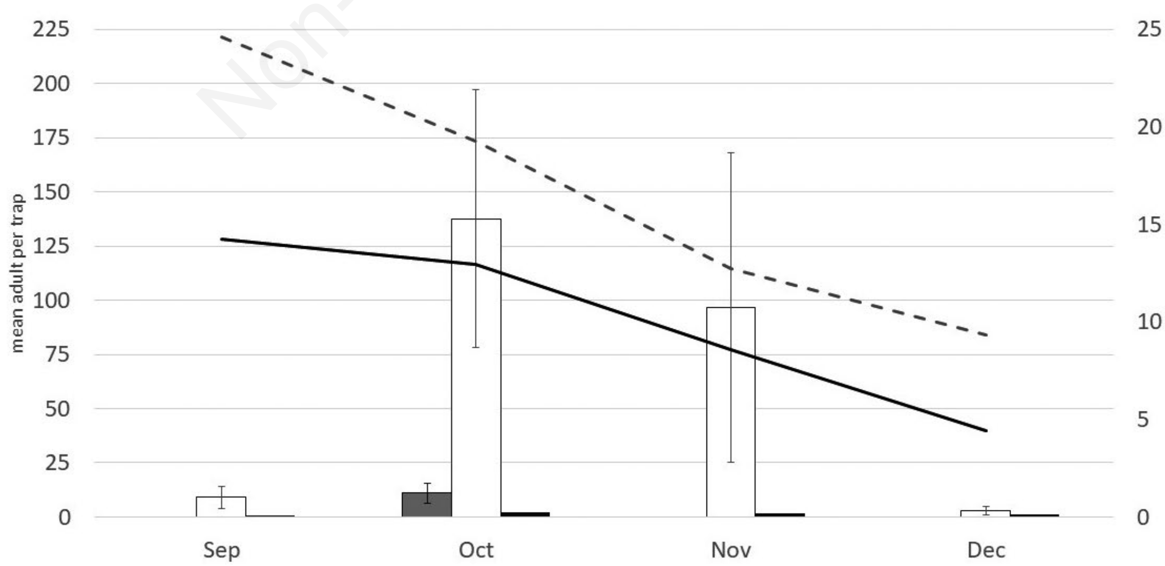

Figure 2. *Z. tuberculatus* adult captures (mean±SEM) per trap per week in ACV traps during 2014 in Minoprio (grey bars), Montanaso Lombardo (white bars), and Camnago Volta (black bars) and mean monthly minimum (continuous line) and maximum (dotted line) temperatures (°C).

observed in July in Guanzate and in Minoprio, while in Camnago Volta the maximum presence of the pest occurred in October.

FB trap attraction

The period of the FB traps exposition in field influenced the emergence of *D. suzukii* and *Z. tuberculatus*. The 77.42% of *D. suzukii* specimens and 81.82% of *Z. tuberculatus* ones emerged from the traps exposed in the field for 7 days. *Drosophila suzukii* and *Z. tuberculatus* emerged from both banana and blueberry baited traps. Comparing the period in which both species were present, a mean value (±SEM) of 5.43±1.40 and 2.4±3.4 *Z. tuberculatus* specimens emerged respectively from banana and blueberry fruits, and 1.87±2.1 and 7.3±1.65 *D. suzukii* emerged from banana and blueberry fruits. Both fruits showed the same attractiveness towards the two exotic drosophilids ($F_{1,158}$=0.479; P=0.491).

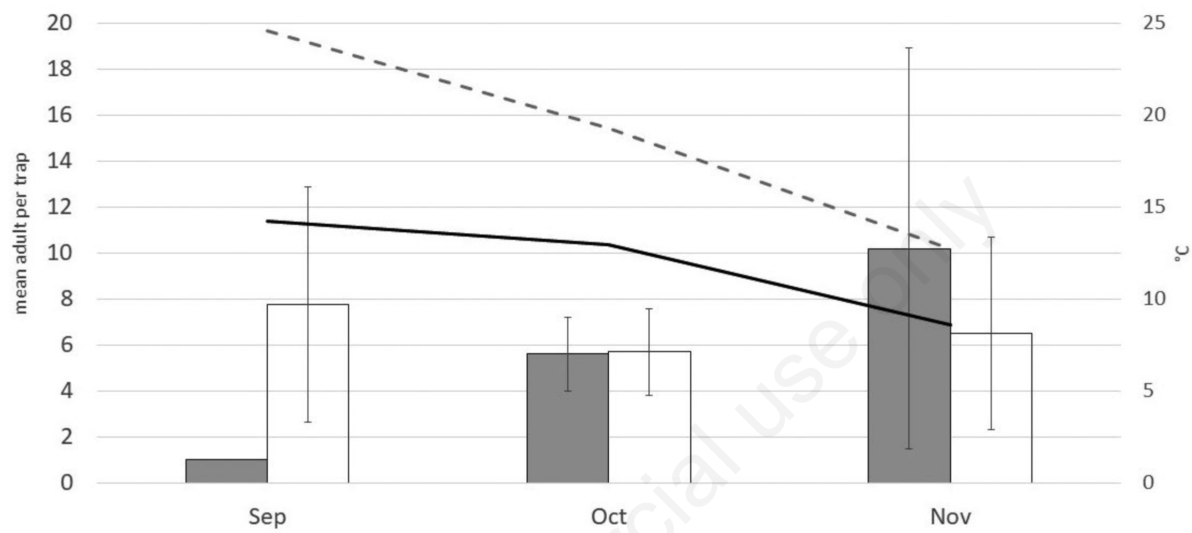

Figure 3: *Zaprionus tuberculatus* adult captures (mean±SEM) per trap per week in FB traps during 2014 in Minoprio (grey bars) and Montanaso Lombardo (white bars), and mean monthly minimum (continuous line) and maximum (dotted line) temperatures (°C).

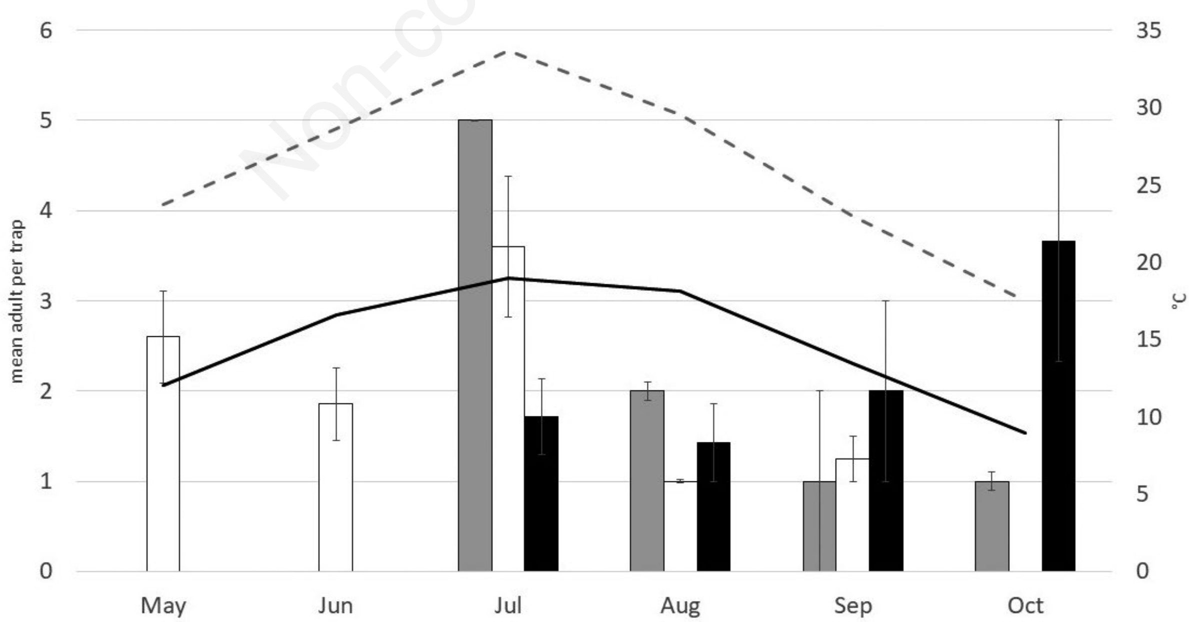

Figure 4. *C. amoena* adult captures (mean±SEM) per trap per week (mean±SEM) during 2015 by ACV traps in Minoprio (grey bars), Camnago Volta (black bars), and Guanzate (white bars) and mean monthly minimum (°C Min) (continuous line) and maximum (°C Max) (dotted line) temperatures.

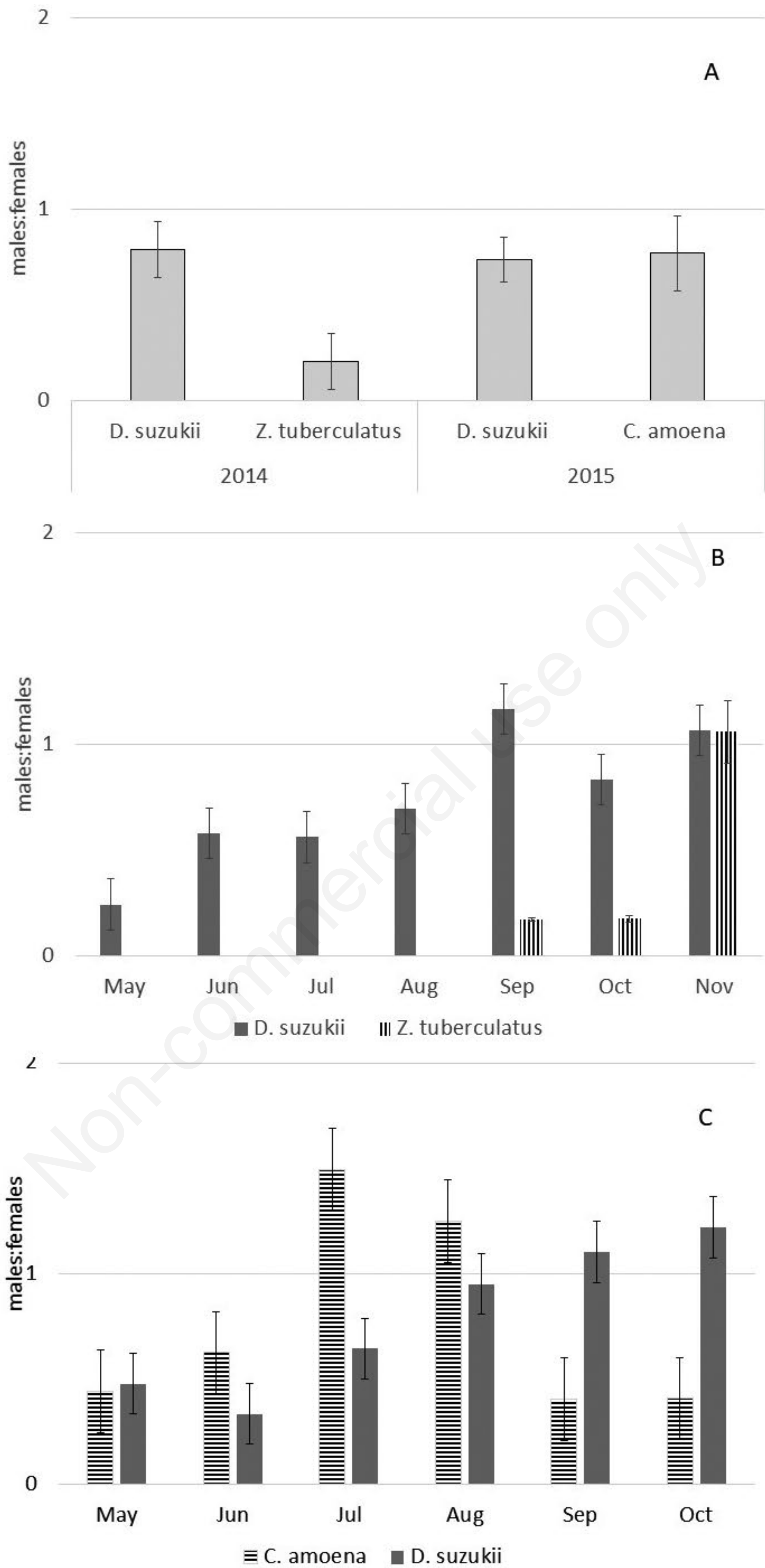

Figure 5. Mean sex ratio (±SEM) over the period of study (A); monthly trend of sex ratio obtained from ACV traps in 2014 (B) and in 2015 (C).

Sex ratio

The observed sex ratio (male:female) of the flies captured by ACV traps was calculated pulling together the captures of all monitored sites (Figure 5). In detail, the mean sex ratio of the three drosophilid species was always female biased in the traps. Fluctuation in monthly sex ratio occurred. *Drosophila suzukii* evidenced a similar trend in both years with a female biased population until August and the increase in the male presence from September. *Zaprionus tuberculatus* was strongly female biased in September and October, while in November we registered similar captures from both sexes. Regarding *C. amoena,* the population was mostly represented by females, exception for July and August when a major number of males was attracted by ACV traps.

Conclusions

The study evidenced the presence of both Nearctic *C. amoena* and Afrotropical *Z. tuberculatus* in Lombardy in the same areas where *D. suzukii* was already detected, while *Z. indianus* was never found. It is known that *D. suzukii* is widespread in Italy (Cini *et al*., 2014) while less information is available about the diffusion and distribution of *C. amoena* and *Z. tubercultatus* in this country. Bächli *et al.* (1999) and Band *et al.* (2003; 2005) evidenced the presence of *C. amoena,* in association with forests or apple fruits, in different sites at altitude major than 450 m a.s.l. in Northern Italy (in Veneto, Lombardy, and Piedmont regions), but this is the first detection at lower altitudes. Raspi *et al.* (2014) and Antonacci *et al.* (2017) reported the presence of *Z. tuberculatus* in Trentino and in Apulia regions from 700 m a.s.l. to 190 m a.s.l in association to different environments.

This is also the first report of the comparison of the phenology of these three exotic drosophilids in the same areas in Italy. ACV traps proved to be a good method to detect the presence of all drosophilids examined. On the contrary, FB traps revealed to be a good method to monitor the presence of *D. suzukii* and *Z. tuberculatus* but not of *C. amoena.* This can be due to the lack of attractiveness by the fruits used as baited traps *versus C. amoena* or to the low density of this species in the fields examined.

The emergence of *Z. tuberculatus* and *D. suzukii* from the same FB traps suggests that these species can share the same niche and develop on the same fruits. As *Z. tuberculatus* is not able to oviposit in undamaged fruit, the insect is probably a secondary colonizer of fruits previously damaged by *D. suzukii*. Both species could emerge majorly in baited traps left only for 7 days as fresh fruits afford adequate moisture, giving drosophilids a suitable site for oviposition and development. Traps left for a longer period probably dehydrated and could not offer a suitable environment for the development and emergence of these species.

The oscillations in the frequencies of the different species reflected their plasticity in tolerance to different environmental climatic conditions and their potential ability to survive in the temperate regions of Northern Italy. *Drosophila suzukii* demonstrated to be very adaptable to different temperatures as it was captured all over the year, and evidenced a trend similar to the one in Asplen *et al*. (2015). The lower population in summer 2015 is due to the fact that *D. suzukii* is less active at temperatures above 30°C as showed by Kinjo *et al.* (2014).

Zaprionus tuberculatus, which has afrotropical origin, also demonstrated to adapt to temperate climate very well. Similarly to other drosophilid species, *Z. tuberculatus* is multivoltine and the emergence in our studies took from 6 to 19 days after FB traps removal. The captures at the end of the season (from September to November) are confirmed also in Antonacci *et al.* (2017). However, the lack of detection of *Z. tuberculatus* in 2015, a year characterized by a particularly hot and dry summer, poses the necessity to deepen the researches on the capability of this species to adapt to different climatic conditions.

Chymomyza amoena also showed a good adaptability to the climate of Northern Italy. It is multivoltine species and the first captures in May confirms also for Italy the beginning of the breeding season in this month (Band, 1988b). From October to May the insect probably overwinters as a third instar larvae inside fruits as described by Band & Band (1980). The lacking of emergence from FB traps and collected fruits cannot confirm the sharing of the same niche of *D. suzukii*, at least in the monitored fruits (banana, blueberry and blackberry). Major captures of *C. amoena* by ACV traps occurred in habitats surrounded by many fruit trees that, according to literature, are preferred hosts of this exotic insect like apple and walnut (Band *et al*., 2005), so it is probable that it emerged from these hosts and was attracted by nearby ACV traps.

If we exclude *D. suzukii*, both *C. amoena* and *Z. tuberculatus* are secondary pest of already damaged fruits. We hypothesize that these species can facilitate decaying processes of fruits already damaged by other factors (*e.g.* pest insects, hailstorm, mechanic damages), but that there is actually no need to consider them harmful for the fruit production. However, as *Z. indianus*, not found in this research, is indicated as a fig fruit phytophagus able to penetrate the natural openings of the mature syconium and lay eggs into the infrutescense (Joshi *et al.*, 2014), a similar behavior in the congeneric species *Z. tuberculatus* is not to be excluded. Therefore, it would be opportune to focus researches on the attack of this species to figs in areas where the two exotic drosophilids are present.

Also, the environmental risk associated to these exotic drosophilids should be taken into account, considering if the novel combination of species (exotic *vs*. native ones) function in the same way as the native assemblages. However, as few and not updated is the knowledge about the interaction among native drosophilids in Italy (Nigro, 1979) it is difficult to assess hypothesis. Finally, the presence of different exotic species that share the same niches can also interfere with their natural enemies by subtracting resources that could be focused only to one enemy. (Amiresmaeili *et al*., 2018).

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