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Halyomorpha halys (Hemiptera: Pentatomidae) on kiwifruit in Northern Italy: phenology, infestation and natural enemy assessment

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ABSTRACT

The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Stål), is an Asian invasive plant-feeding insect and an emerging kiwifruit pest. Knowledge about the BMSB dynamics and damages on kiwifruit outside the bug native range is scarce. This two-year study was aimed at describing phenology and infestation dynamics of BMSB in green-fleshed and yellow-fleshed kiwifruits. Natural enemies were investigated as well. Field surveys were performed weekly in two sites in Northern Italy by pheromone-baited traps and visual samplings, from early Spring to mid-Autumn. All BMSB life stages were collected and kept under observation to assess occurrence of parasitoids. A sample of fruit was dissected weekly to detect BMSB feeding injuries. In 2018-2019, BMSBs were found throughout the growing season and completed two generations per year with a large overlapping of all life stages. Pheromone-baited traps and visual samplings gave consistent results and can be both considered effective sampling methods on kiwifruit. Fruit injuries were significantly related to kiwifruit development stage, weekly captures of BMSBs by traps and cultivar. Several BMSB egg masses were found on kiwifruit leaves. *Anastatus bifasciatus* Geoffroy, a native egg parasitoid with Palearctic distribution, emerged from 20.14% of eggs in 2018 but the percent parasitism dropped to 0.47% in 2019. In both years, other natural enemies were found exerting a negligible pressure on BMSB populations. The determination of reliable economic thresholds for BMSB on kiwifruit is urgently needed to develop a robust and sustainable IPM strategy, and this study provides data towards that direction.

Keywords

Brown Marmorated Stink Bug, *Actinidia chinensis*, monitoring, pheromone traps, parasitoids.

INTRODUCTION

In 2018, approximately 4.3 million tons of kiwifruit were produced worldwide, with a revenue of 7.6 billion USD (Global Trade, 2019). In the same year, the global export stood at 1.4 million tons and amounted to 2.8 billion USD (Global Trade, 2019). Italy is the second biggest producer of kiwifruit in the world after China, with an overall production of 555 thousand tons in 2018 (FAOSTAT, 2019). The Italian kiwifruit industry makes up only a small portion of the overall Italian horticulture (CSO, 2019), but it is profitable and still expanding. Italy ranks second as kiwifruit supplier worldwide and, in 2018, 289 thousand tons (approximately 20% of total global exports) were exported (Global Trade, 2019). From 2007 to 2018 the average annual increase rate for the Italian kiwifruit industry, in terms of value, was +2.3% per year (Global Trade, 2019). The two most commercially cultivated kiwifruit species worldwide are *Actinidia chinensis* var. *deliciosa* (A. Chev) A. Chev and *A. chinensis* var. *chinensis* Planch. (Actinidiaceae), respectively the green-fleshed and yellow-fleshed kiwifruit, with the former being prevalent, i.e. the green cv. Hayward (Pinto and Vilela, 2018).

For many years, insects have not been a serious problem for the Italian kiwifruit growers. Many species, that often cause yield losses to other crops, are potentially pests of kiwifruit, but severe damages were sporadic (Testolin and Ferguson, 2009; Testolin, 2015). Damages have been due to the transmission of pathogens, e.g. *Pseudomonas syringae* pv. *actinidiae* (Psa) by *Metcalfa pruinosa* Say (Hemiptera: Flatidae) (Donati et al., 2017). The Brown Marmorated Stink Bug (BMSB) *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), an invasive plant-feeding insect native to East Asia (Hoebeke and Carter, 2003; Leskey and Nielsen 2018; Kriticos et al., 2017), has, however, the potential to become a major pest of kiwifruit in Italy and other countries. BMSB is widespread in Europe and North America and it has also been recorded or intercepted in the Southern hemisphere (MacLellan, 2013; Valentin et al., 2017; Leskey and Nielsen, 2018). This pest is causing millions of USD of damage worldwide (Leskey et al., 2012a; Rice et al., 2014; Cesari et al., 2015). In Italy, the first detection occurred in 2007 near Genova, Liguria region, but the insect has been reported as a pest only since 2014, when it was found in the Modena area, Emilia Romagna region (Northern Italy) (Cianferoni et al., 2018). In Southern Europe, BMSB was found to be bivoltine (Costi et al., 2017) and suitable climate and high density of crops provide excellent conditions for the establishment of large populations. In 2019, in the fruit-growing areas of Northern Italy, BMSB caused approximately 590 million euros (about 690 million USD) of losses (CSO, 2019).

BMSB can feed on more than 300 different plants (Bariselli et al., 2016; Kriticos et al., 2017). This species may switch from one host plant to another according to its life cycle requirements. In fact, it requires plants with ripening fruit to complete its development and it moves among hosts following plant phenology (Acebes-Doria et al., 2016; Zobel et al., 2016; Bergmann et al., 2016; Leskey and Nielsen, 2018). Therefore, plants can be differenced in breeding hosts, on which all life stages (egg to adult) are usually observed, and feeding hosts, where only adults can feed on (Oda et al., 1980). Moreover, BMSB presents life stage-specific changes in host exploitation. Few plants are known to be used for oviposition, while nymphs feed on a wide number of plants and adults have an even wider host range (Nielsen and Hamilton, 2009; Acebes-Doria et al., 2016; Bergmann et al., 2016; Zobel et al., 2016). This behavior is facilitated by the high dispersal ability of BMSB. Adults can fly up to 5 km per day on average, although flights up to 117 km have been reported (Leskey and Nielsen, 2018). Nymphs have also shown great dispersal ability both in field and laboratory experiments (Leskey and Nielsen, 2018). Despite the broad host range, different genotypes or varieties of the same plant species may exhibit completely different suitability (Bergmann et al., 2016). Reliable and long-lasting control strategy for BMSB has not yet been developed. Chemical control, including orchard border sprays and crop perimeter restructuring (Blaauw et al., 2014), has been the first strategy adopted against BMSB in the invaded areas. However, results have been erratic (Leskey and Nielsen, 2018; Leskey et al., 2012a, b) and secondary pest resurgence have often been reported (Leskey et al., 2012a). In its native areas, BMSB is maintained below the economic injury level by several entomophagous insects, among which the most effective are egg parasitoids of the genus *Trissolcus* (Hymenoptera:

Scelionidae), i.e. *Trissolcus japonicus* (Ashmead) (Lee et al., 2013). Adventive populations of this species, and the congeneric *T. mitsukurii* (Ashmead), have been recorded in some countries of introduction of BMSB, including Italy and Switzerland (Talamas et al., 2015; Sabbatini Peverieri et al., 2018; Haye et al., 2020). In June 2020, field releases of *T. japonicus* were performed in about 700 sites in Italy, 300 of which located in Emilia Romagna region (Conti et al., 2020). Moreover, in Europe and in the USA, studies on the potential of native entomophagous insects to control BMSB have been performed (Abram et al., 2017, Stahl et al., 2019). According to Haye et al. (2015) in Europe the egg parasitoid *Anastatus bifasciatus* Geoffroy (Hymenoptera: Eupelmidae) seems to be the only indigenous candidate for augmentative biological control of BMSB.

Although both *A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* have been reported as feeding hosts of BMSB in its native range (Lee et al., 2013; Lara et al., 2018) and in areas of introduction including Italy (Bariselli et al., 2016; Andreadis et al., 2018; Ak et al., 2019), the knowledge on the interactions between BMSB and kiwifruit vines is scarce out of their native areas (Teulon and Xu, 2018). BMSB, in different life stages, was observed in kiwifruit orchards in Greece and Turkey, where feeding injuries were recorded (Andreadis et al., 2018; Ak et al., 2019). The damage may be amplified by the lack of fully effective insecticides (Leskey et al., 2012b; Kuhar and Kamminga, 2017; Morehead and Kuhar, 2017). Laboratory trials have shown that BMSB can feed on both *A. chinensis* var. *deliciosa* cv. Hayward and *A. chinensis* var. *chinensis* cv. Zesy002 (commercially known as 'SunGold'). In no-choice experiments, damage was higher on Hayward variety, while in choice tests between the two varieties, no difference was found (Lara et al., 2018).

Given the remarkable pest status of BMSB, it is worth studying the infestation pattern of this insect on kiwifruit, as a crucial step in refining control strategies on this crop. In this framework, we undertook this study to: 1) describe the phenology and infestation of BMSB, on the two most cultivated *Actinidia* varieties in Northern Italy; 2) compare sampling methods; 3) assess the occurrence of natural enemies of BMSB or other stinkbugs; 4) evaluate the relationships between BMSB density and injuries to fruit.

MATERIALS & METHODS

Sites

Field monitoring of BMSB was performed in 2018 and 2019 in two kiwifruit orchards (sites1 and 2) located near Faenza, Ravenna Province (Emilia-Romagna region). In both sites, two transects (30 m long x 3 rows wide, 36 vines) were established at the border of two orchards of different varieties, *A. chinensis* var. *deliciosa* cv. Hayward and *A. chinensis* var. *chinensis* cv. Zesy002, both trained as Pergola. The orchards characteristics are reported in Table 1. The transects were not treated with insecticides, contrary to the rest of orchard, which was sprayed twice per growing season with etofenprox applied as Trebon® UP Sipcam Italia S.p.A. at the rate of 50 mL/hL) (Table 1). In both sites, 'Hayward' orchard was separated from 'Zesy002' by a 50-m wide uncultivated strip. Inter-rows were grassed and weed control was performed by monthly mowing. Cultural management (e.g. fertigation, pruning, thinning, disease control) was performed according to Emilia Romagna cultivation guidelines.

Monitoring of BMSB and other stinkbugs

In each transect of each orchard, the first two rows were sampled, because BMSB is mainly a border-driven pest (Leskey and Nielsen, 2018; Maistrello et al., 2017).

Sampling was performed weekly from bud break (BBCH 7 [Salinero et al., 2009]) occurring in April till harvest (BBCH 85) in October. Samplings were performed for 31 weeks and 30 weeks in 2018 and 2019, respectively. Two sampling methods were used: i) two commercial pyramid traps per transect baited with aggregation pheromone (RESCUE® Stink Bug Trap) and ii) fixed-time visual monitoring. The two traps were placed on the central row of the transects at 5 m from each edge, and hung at approximately 1.5 m. For the visual monitoring, two operators thoroughly examined the canes, leaves and fruit of each vine of the sampled rows for 60 min.

Natural enemy assessment

All the stink bugs found by both sampling methods (BMSB but also other species) were taken to the laboratory at the Department of Agricultural and Food Sciences. Bugs were counted and maintained under observation up to 50 days to assess occurrence of natural enemies. All life stages were collected including egg masses (unhatched and hatched with first instar nymphs on), small nymphs (second and third instar), large nymphs (fourth and fifth instar) and adults. Once died, the adult stinkbugs were examined and dissected under a stereo-microscope to check the presence of parasitoid eggs on their body or larvae inside.

Visual samplings were also extended to ecological infrastructures (maple trees, persimmon trees, Judas trees) nearby the orchards, in order to collect egg masses of stink bugs for parasitoid detection. The efficacy of egg parasitoids was evaluated in terms of 1) discovery efficiency (= percentage of parasitized egg masses, calculated as a ratio based on the number of egg masses from which at least one adult parasitoid emerged to the total number of collected egg masses); 2) percentage of successful parasitism, calculated as a ratio based on the number of eggs from which an adult parasitoid emerged to the total number of collected eggs and 3) exploitation efficiency (= percentage of successfully parasitized eggs per each parasitized egg mass) (Zapponi et al., 2020). The percentage of BMSB first instar nymphs was also calculated, as a ratio based on the number of nymphs to the total number of collected eggs.

Injury evaluation

In both years, for each transect, 21 randomly-picked fruits were collected weekly until harvest (BBCH 85) to assess damage. Fruit collection started when fruit had reached at least 3-4 cm in length (BBCH

71). Due to the different phenology between the two *Actinidia* varieties (Testolin and Ferguson, 2009), the samplings started a couple of weeks earlier in *A. chinensis* var. *chinensis* (22 May 2018, 11 June 2019), than in *A. chinensis* var. *deliciosa* (5 June 2018, 25 June 2019). Because of difference in the harvesting times, in both years fruit samplings ended up in the first week of October for *A. chinensis* var. *chinensis* and in the last week of October for *A. chinensis* var. *deliciosa*. To detect damage (which cannot be scored from outside) each fruit was cut in 0.5 cm slices, where feeding injury appeared as dark spots in the pulp for recent punctures and suberified areas for the older ones (Lara et al., 2018). Fruit was considered as "injured" regardless of the number of punctures detected.

Data analysis

Relationship between pheromone-baited traps and visual samplings

Bivariate Pearson's linear correlation was used to describe the relationship between BMSBs caught by pheromone-baited traps and BMSBs counted by means of visual sampling. Three separate analyses were carried out for small nymphs (i.e. second and third instars as the first ones remain on egg masses and cannot be sampled by traps), large nymphs (fourth and fifth instars) and adult bugs. The captures by the trap and the insects visually sampled in the transect in the same plot were paired for each sampling week. Overall, 152 points were considered for each correlation analysis. Bias corrected and accelerated bootstrap confidence intervals at 95% (CI95%) of correlation coefficients were calculated on 1000 resamplings.

Regression analysis on percentages of injured fruit

Curvilinear regression ($Y=A/(1+B*\rho^x)$) was used to describe the relationship between the sampling dates and the percentage of injured yellow and green kiwifruits (Snedecor and Cochran, 1980). Multiple linear regression was used to modelling the effect of kiwifruit cultivar, BBCH scale and total number of bugs (mobile nymphs + male and female adults) caught by pheromone traps on the percentage of injured fruit. The assumptions of the linear regression were all met. No outliers were detected by checking residuals analysis or Cook's distances. No relevant correlation among the explanatory variables could be detected. The Durbin-Watson test, which was carried out on dataset ordered by site (first ordination criterion) and by date (second ordination criterion) did not show any relevant level of autocorrelation among data. CI95% of coefficients as well as their standard errors (SE) were calculated by bias corrected and accelerated bootstrap based on 1000 resamplings.

The software package IBM SPSS Statistics ver. 23 (IBM, 2019) was used for linear correlation analysis and multiple regression analyses; for curvilinear regression, the software Statistica version 10 (Statsoft, 2010) was utilized.

RESULTS

BMSB phenology

During the overall monitoring activity 12,702 BMSB individuals were collected (pooling years, sites, sampling methods and considering mobile nymphs and adults). The overall number of BMSBs was higher in site 2 (8,375) than in site 1 (4,327). A total number of 7,527 bugs were collected by pheromone traps whereas 5,175 bugs were sampled in visual surveys. The captures by pheromone-baited traps showed a similar trend in both years, although the total number of individuals was different between 2018 (3,660) and 2019 (9,042). In both years, the first overwintering adults were caught in April (mid-Spring) and their abundance remained generally low until the end of May (Fig. 1). Then three peaks of adult captures were observed. The first one occurred during late flowering (BBCH 68, 69), the second during the final phase of fruit development (BBCH 79) and the third at fruit maturity/senescence (BBCH 85, 93). The nymphs appeared in late Spring and peaked in early Summer, because of the overlapping of the first and second generations. Nymphs decreased steeply in Autumn when the number of overwintering adults increased. Some of them were found in kiwi orchards also after harvest.

In visual samplings, adults were rarely observed before September (Fig. 2). The first nymphs were observed on 5 June 2018 and 18 June 2019, respectively, and their abundance remained high throughout Summer (Fig. 2). In total, 204 egg masses (71 unhatched and 133 hatched with first instar nymphs on) were detected on kiwifruit leaves pooling both years. These egg masses were found from late Spring to late Summer.

Comparison of sampling methods

The two sampling methods gave consistent results as demonstrated by significant and positive correlations. Traps captured approximately eight times more adults than those detected in visual samplings (r=0.56 [CI95%,0.31-0.71] P<0.001) (Fig.3 A). The two methods were very similar in detecting late instar nymphs (r=0.57 [CI95%,0.40-0.71] P<0.001) (Fig.3 B), whereas a larger number of early instar mobile nymphs was recorded by visual sampling than by traps (r=0.36 [CI95%,0.20-0.54] P<0.001) (Fig.3 C).

Other stinkbugs

Although BMSB was the dominant species, also other stinkbugs, found by visual sampling, were collected. The second most abundant species was the Southern Green Stink Bug *Nezara viridula* (L.), that occurred on kiwifruit plants in different life stages (from egg to adult). We found sporadically also *Palomena prasina* (L.), *Rhaphigaster nebulosa* Poda, *Acrosternum heegeri* Fieber, *Graphosoma lineatum* (L.) (Table 2). In 2018, 4 nymphs of the predatory bug *Arma custos* Fabricius were found, one of which was observed feeding on a BMSB nymph.

BMSB natural enemies

The occurrence of potential natural enemies of BMSB was also investigated. European earwigs (*Forficula auricularia* L. [Dermaptera: Forficulidae]) were detected throughout the sampling period inside the traps, often together with eaten remains of BMSB. However, we have never observed this species feeding on BMSB. Moreover, several spiders were seen inside and outside the traps. In one case, in 2018, some *Crematogaster scutellaris* (Olivier) (Hymenoptera: Formicidae) were found on an egg mass of BMSB. The eggs appeared intact, but never hatched.

Parasitoid eggs, laid by unidentified tachinid species, were found on 239 BMSB adults (79 in 2018, 160 in 2019) mainly under wings or scutellum. Stereo- microscope observations revealed that 126 parasitoid eggs hatched, the larvae penetrated the host body and developed until the second instar before the bug died.

In 2018, on 32 unhatched egg masses of BMSB, 13 were successfully parasitized by the egg parasitoid *A. bifasciatus*, with a discovery efficiency of 40.63%. Eight of the parasitized egg masses were detected outside the orchards on a Judas tree (in site 1) and five on the leaves of kiwifruit vines. Instead, in 2019 only one egg mass out of 39 was parasitized by *A. bifasciatus*, resulting in a discovery efficiency of 2.56%. As a consequence, the percentage of successful parasitism was dramatically higher in 2018 (20.14%) than 2019 (0.47%) (Table 3). The exploitation efficiency was calculated only for 2018 with a median of 46.43 (Fig. 4).

Aridelus rufotestaceus Tobias (Hymenoptera: Braconidae), a parasitoid of nymphs (Shaw et al., 2001), emerged from 5 *N. viridula* individuals. The species was determined according to Shaw et al. (2001).

Injury Evaluation

In Figs. 5-6, the temporal trends of damage, described by curvilinear regression, are reported for each *Actinidia* variety. In both years, the first evidence of injuries to fruit was already found at the early stages of fruit sampling (BBCH 71). From the beginning of Summer, the damage dramatically increased in both cultivars. The damage followed a typical logistic trend, which can be useful to optimize the field sampling and in general for scouting purposes.

The highest percentages of damaged fruit were found in late Summer/early Autumn, when the fruit, had reached at least half of the final size (BBCH 75). The three predictors used for the linear model (BBCH scale, *Actinidia* varieties, total BMSB per trap) were responsible for a high variation in damage percentages ($R^2 = 0.50$; $F_{3,\ 148} = 49.56$; $P< 0.001$). BBCH scale and BMSB infestation, as detected by the traps, had a similar and relevant impact on fruit injuries (standardized β value of 0.46 and 0.41, respectively). Although the percentage of damaged fruit was often higher in *A. chinensis* var. *chinensis* orchards, especially in late Summer, the effect of kiwifruit variety was less relevant than those by the former predictors; a 6.57 increase in percentage of injured fruit is predicted for *A. chinensis* var. *deliciosa* in comparison to *A. chinensis* var. *chinensis* (Table 4).

DISCUSSION

In Northern Italy, in 2019 BMSB caused a loss of 55 million euros just in green-fleshed kiwifruit (CSO, 2019). Despite its importance as a pest, little information is available on the phenology of this stink bug on kiwifruit outside China (Andreadis et al., 2018). Our study was thus aimed at filling this gap, as regards kiwifruit orchards in Northern Italy. In both years, all BMSB life stages were detected on kiwifruit plants, regardless of the monitoring technique. Therefore, following the categorization of hosts by Oda et al. (1980), both ‘Hayward’ and ’Zesy002’ could be considered as reproductive hosts for BMSB, serving as food for nymphs and adults and providing oviposition sites for females. The overall BMSB captures were two-folds higher in site 2 than in site 1, although the distance between the two sites was only 2.1 km. The difference may be linked to the surrounding the two sampling areas. In the neighboring of site 1, peach and persimmon trees, both well-known hosts for BMSB (Lee et al., 2013; Nielsen and Hamilton, 2009), were present. Instead, the transects of site 2 were surrounded only by kiwifruit orchards. The diversity of suitable host plants in site 1 may have lured BMSB away from the sampling transects, due to its wide host range. A similar level of infestation was recorded both in ‘Hayward’ and ‘Zesy002’.

BMSB life cycle showed both analogies and differences in the two years of study. Traps caught the first overwintered adults two weeks earlier in 2019 than in 2018. Moreover, the number of these adults was also higher in 2019 than in 2018. The milder winter in 2018-2019 than in 2017-2018 likely allowed the survival to overwintering of a higher proportion of individuals (Supp Figure S1, S2). Considering the BBCH scale of kiwifruit, in both years the first adults were found during bud development (BBCH 07) and the first juveniles were observed between late flowering and early fruit development (BBCH 69-71).

Our field data confirmed that in Emilia-Romagna BMSB successfully carried out two generations per year, being active from April through the end of October, as previously observed in semi-field conditions by Costi et al. (2017). We considered as the beginning of a generation the field occurrence of egg clusters and/or nymphs of the first two instars. In 2018, the first and second generation appeared in early June and around the second half of July, respectively. The overlap of all BMSB life stages was only observed in July, when the first generation ended and the second started. Conversely, in 2019 the first generation appeared later, in the second half of June. It was difficult to precisely detect the beginning of the second generation, due to the overlap of life stages during the whole monitoring activity. In May 2019, the temperatures were 4-5°C lower than the seasonal average and a total rainfall of 225 mm was recorded in comparison to a mean for May of 65 mm (Supp Figure S1, S2). For this reason, the full reproductive development of the overwintered adults may have been delayed compared to 2018. As a result, in 2019 the oviposition also occurred later and lasted longer than in 2018. Costi et al. (2017) reported that, in semi-field conditions, the BMSB oviposition period can last up to 14 weeks for the overwintered adults and up to 8 weeks for the Summer generation adults.

Before the introduction of BMSB, stink bugs were not considered as key pests in kiwifruit cultivations in Italy (Testolin and Ferguson, 2009). Therefore, it is not surprising that a few individuals belonging to other Pentatomidae species were found. The abundance of BMSB confirmed that this species has quickly become the dominant one in kiwifruit orchards where it has become established. A similar trend has been reported for other fruit tree orchards (Leskey and Nielsen, 2018).

As regards the natural enemies, *F. auricularia*, which is considered as a possible BMSB predator (Poley et al., 2018), was frequently sampled in the traps during monitoring, but no feeding on BMSB was ever observed. Although spiders were found in traps and on kiwifruit vines, predation was observed only once, on a fifth instar nymph.

Concerning parasitoids, macrotype eggs of unidentified tachinid species were found on the body of BMSB, but no parasitoid successfully completed development in the accepted hosts. In some areas of introduction of the USA, BMSB was found to be parasitized, though at low level, by the native tachinid species *Trichopoda pennipes* (F.) (Joshi et al., 2019; Anderson et al., 2020). This parasitoid of stink bugs is now naturalized in Italy following its fortuitous introduction in the 1980s (Colazza et al., 1996), with *N. viridula* as a preferred host (Cerretti and Tschorsnig, 2010). Although a few *T. pennipes* emerged from *N. viridula* adults collected in the sampled sites, it is unlikely that the eggs laid on BMSB belonged to this species, based on their location on the host body (Francati et al, 2019). More studies are necessary to confirm the potential of *T. pennipes* or other tachinid species to exploit BMSB in Italy or other countries of introduction, thus contributing to the control of the target species.

Only the egg parasitoid *A. bifasciatus* emerged from BMSB eggs collected in the field in both years. The percentage of successful parasitism for this species in 2018 was one of the highest recorded until now, conversely, the exploitation efficiency was in line with those already reported (Moraglio et al., 2020). The remarkable difference in parasitism between the two years was unexpected and difficult to explain. The different climatic conditions recorded in the two years (Supp Figure S1, S2) may have affected the *A. bifasciatus* lifecycle, as observed by us for BMSB. Neither *T. japonicus* nor *T. mitsukurii*, the two Asian egg parasitoids first recorded in Italy in 2018 (Sabbatini Peverieri et al., 2018) were found. Moreover, *A. rufotestaceus* was recorded as a *N. viridula* parasitoid in our study areas. A future shift of this species to the invasive stink bug may not be excluded, as new host-parasitoid associations have already been observed for other allochthonous insects (Francati et al., 2015; Dindo et al., 2016; Rossi Stacconi et al, 2018).

BMSB was confirmed to be able to inflict severe injuries to kiwifruit. We found green rounded spots and irregular white spots under the fruit skin, which were also reported by Chen et al. (2020). Moreover, we detected extensive irregular light brown spots in the fruit pulp. These different kinds of injuries could be a consequence of different times of attack by BMSB. The green spots seemed a reaction to fresh punctures, as they were frequent at the beginning of the fruit surveys. The white spots could be due to old punctures, because they were commonly observed in mid-season when the fruit had reached nearly their final size. Finally, light brown spots were likely caused by multiple punctures.

Given that only three predictor variables (i.e. BBCH scale, variety and total number of bugs per trap) were considered and that no climatic parameter was included, the linear regression model explained a high rate of variance in kiwifruit damages. Moreover, the significant correlations demonstrated between traps catches and visual samplings for all developmental stages of BMSB allowed the inclusion in the model only of trap data as a measure infestation. This could encourage the implementation of the model, because routine monitoring activities relying on trap catches are easier to check and less time consuming than visual surveys. If validated in subsequent years and in other countries where kiwifruit is grown in conditions like Northern Italy (e.g. France, Portugal, Spain, Turkey, Chile), this model could represent a step toward the development of reliable economic injuries thresholds to guide BMSB management on kiwifruit vines.

The linear model showed a significant effect of variety: *coeteris paribus* a 6.6% higher damage was predicted in *A. chinensis* var. *deliciosa* in comparison with *A. chinensis* var. *chinensis*. This is contrast with Lara et al. (2018) and Chen et al. (2020), who found that both *Actinidia* varieties were equally susceptible to damage by BMSB. Their experiments were, however, carried out using artificial infestations.

This study reinforced the concerns due to BMSB invasions in areas where kiwifruit is grown. In both yellow- and green-fleshed varieties injuries were serious and caused relevant economic losses to growers. Moreover, current management strategies, which mostly rely on broad-spectrum insecticide sprays, did not seem either fully effective or sustainable. Our experiments cast some light on BMSB population dynamics in kiwifruit orchards and provide indication to refine monitoring activity. Further research aimed at defining reliable economic thresholds are urgently needed in kiwifruit, as well as in other fruit crops, to develop effective and sustainable management strategies for BMSB.

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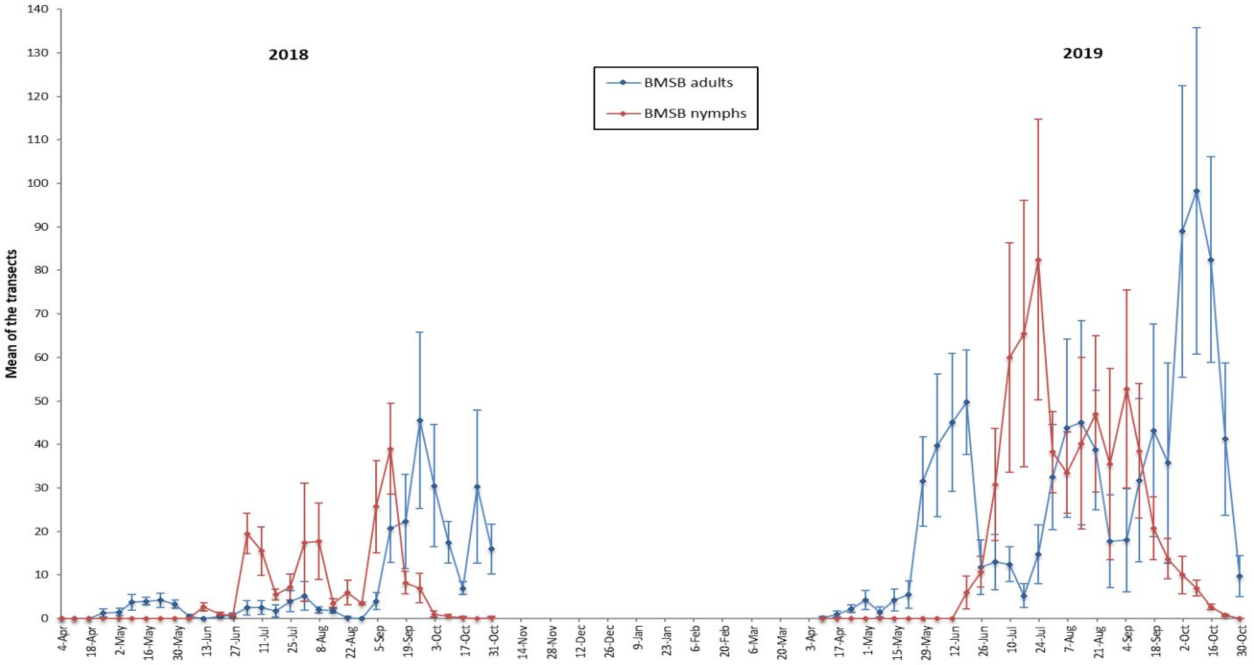

Fig. 1. Trend of BMSB weekly captures by pheromone-baited traps in kiwifruit in 2018-2019. Sites and cultivars were pooled. Dots indicate the means of insect caught in the four transects and vertical lines indicate the standard errors of the means. Adults are represented by solid blue line; mobile nymphs are represented by solid red line.

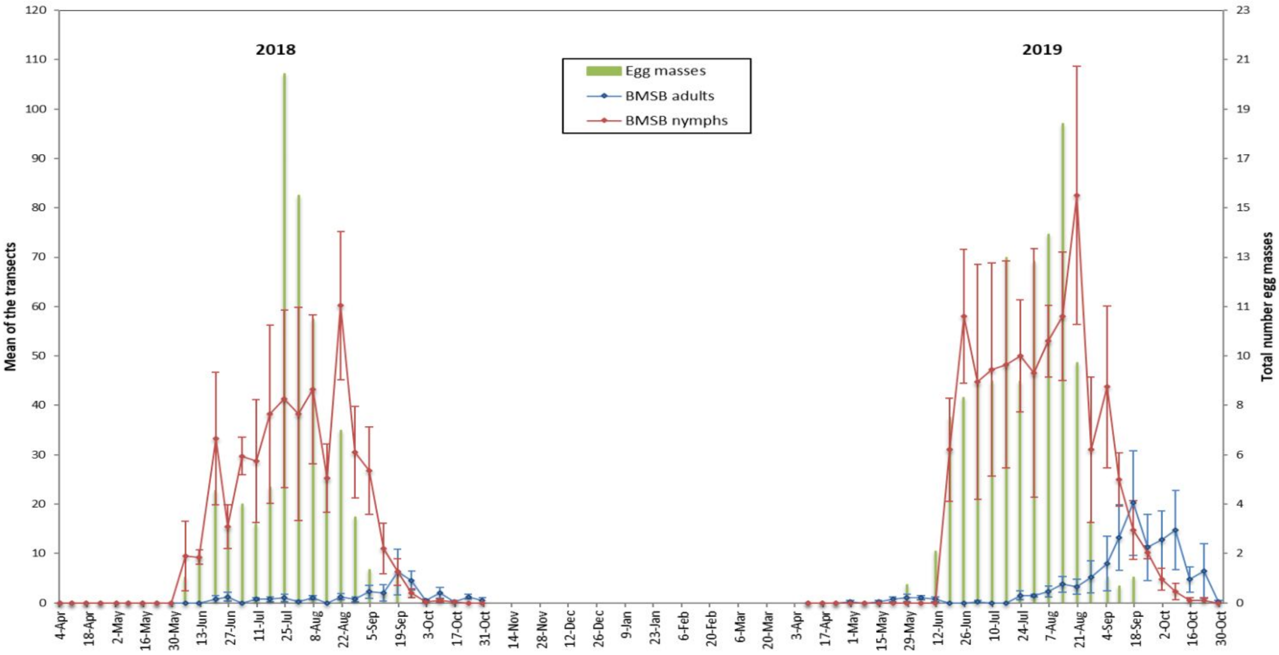

Fig.2.Trend of BMSB individuals observed weekly by visual samplings on kiwifruit in 2018-2019. Sites and cultivars were pooled. Dots indicate the means of insects observed in transects and vertical lines indicate the standard errors of the means. Adults are represented by solid blue line; mobile nymphs are represented by solid red line. The green columns show the total number of egg masses found per date.

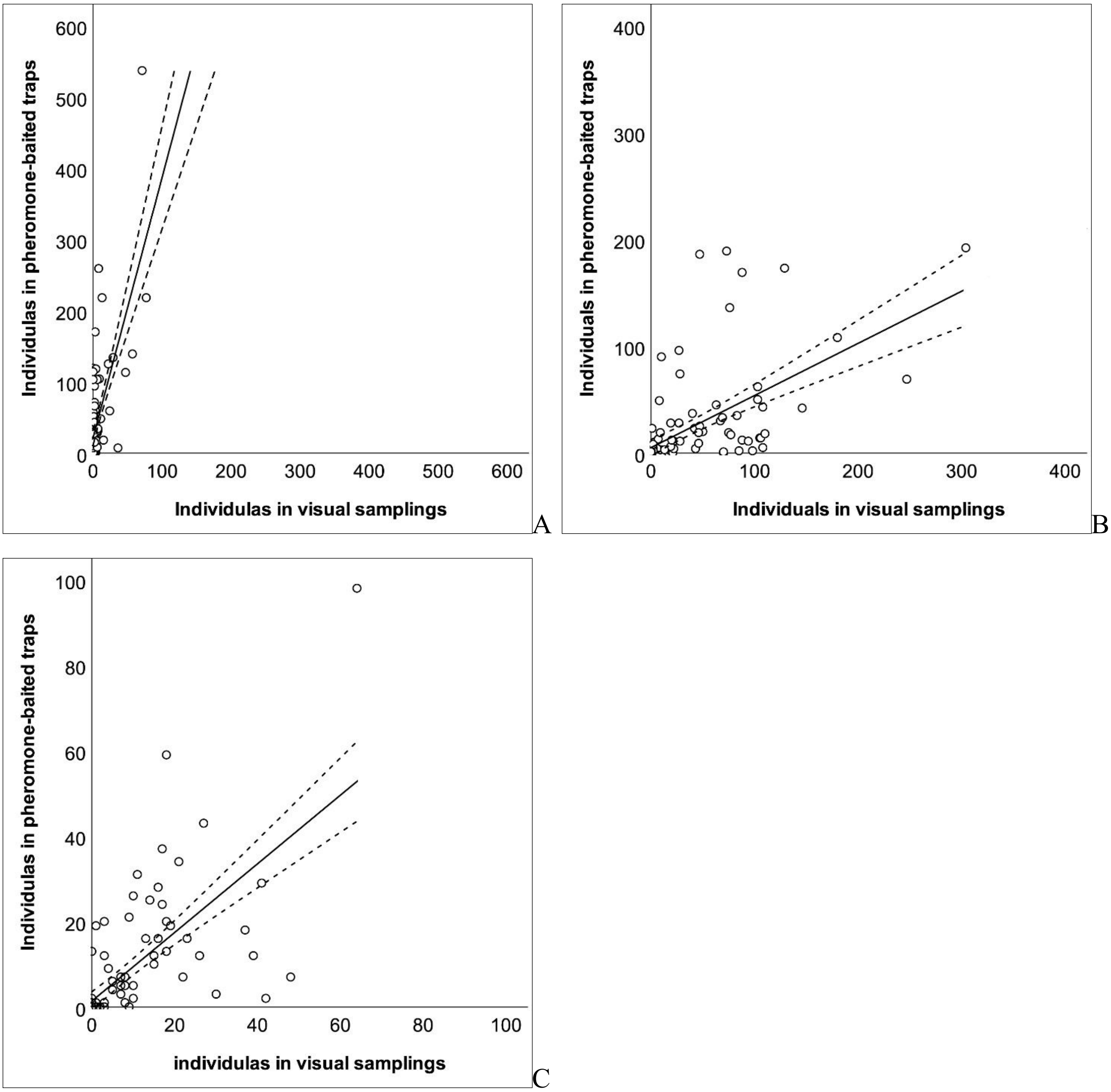

Fig.3. Correlation and 95% confidence intervals between the captures by pheromone-baited traps and visual samplings. A) BMSB adults; r = 0.56 (CI95%, 0.31 – 0.71) P< 0.001. B) small nymphs, r = 0.36 (CI95%, 0.20 – 0.54) P< 0.001. C) large nymphs, r = 0.57 (CI95%, 0.40 – 0.71) P< 0. 001.

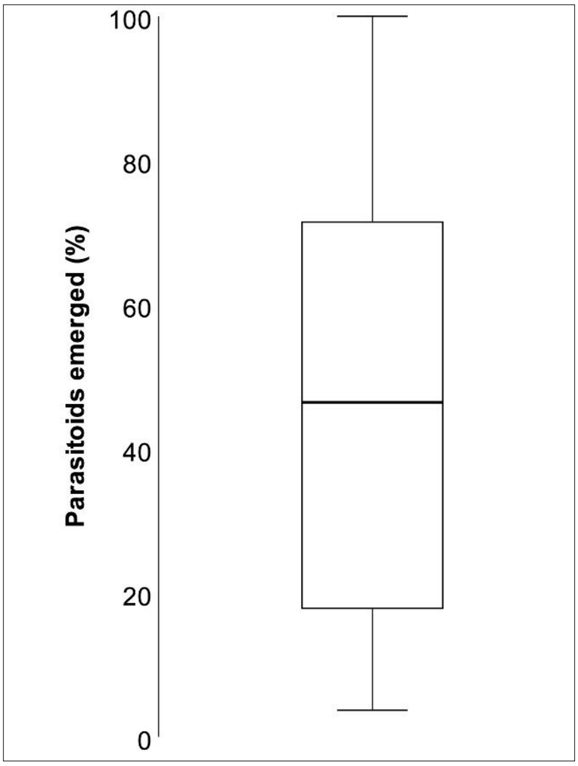

Fig. 4. Box plot showing the median (bold horizontal line), interquartile range (box) and total range (whiskers) of the exploitation efficiency by *Anastatus bifasciatus* of BMSB egg masses collected in 2018.

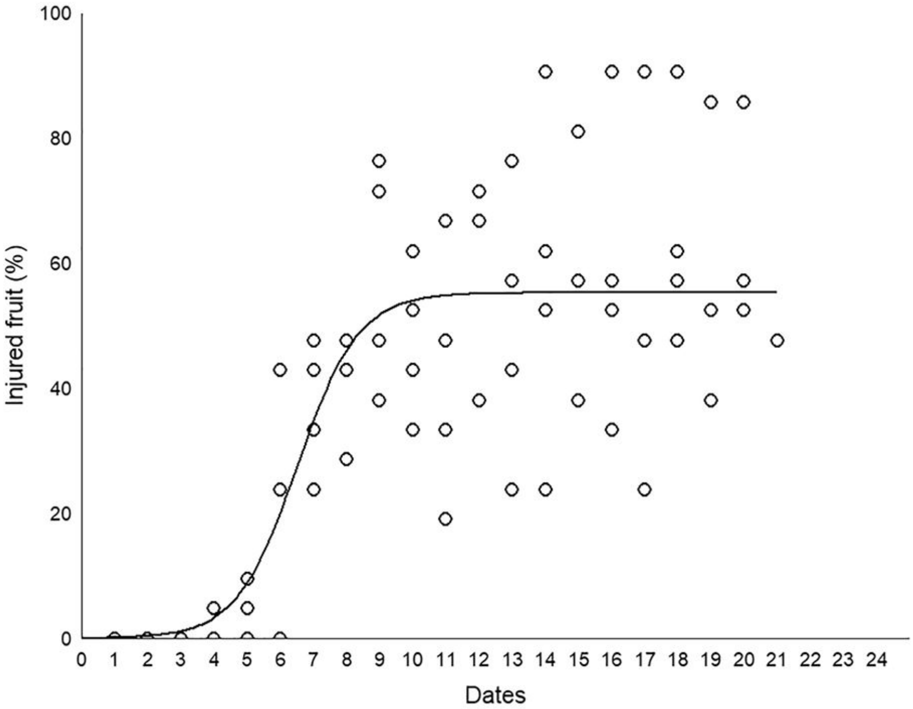

Fig. 5. Relationship between injured yellow-fleshed kiwifruit (%) and sampling week, using the model: $Y=A/(1+B*\rho^x)$. $R^2 = 0.59$, $P<0.01$ ($A=57.1$: $B=52.1$; $\rho=0.28$).

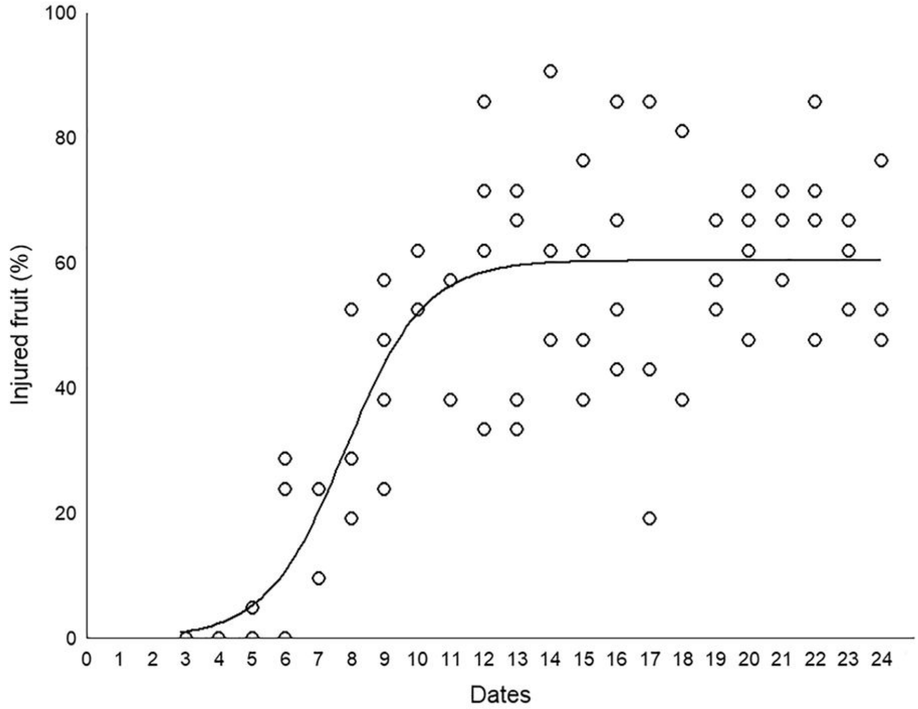

Fig. 6. Relationship between injured green-fleshed kiwifruit (%) and sampling week, using the model: $Y=A/(1+B^{*} \rho^{x})$. $R^2 = 0.67$, $P<0.01$ ($A=57.6$: $B=882.7$; $\rho=0.13$).

Table 1. Locations and characteristics of the sampled sites/transects of *Actinidia chinensis* var. *deliciosa* ‘Hayward’ and *Actinidia chinensis* var. *chinensis* ‘Zesy002’ orchards

Site	Insecticide treatment dates*	Cultivar	Vine Age (years)	Plantation density	Transect edge
Site 1 (44°16'35.77''N, 11°53'47.41''E)	July 26 and August 12 in 2018	Hayward	14	4.7x2.4 m	Wheat (2018); Fava beans (2019)
	July 25 and August 11 in 2019	Zesy002	4	4.7x2.4 m	Persimmon
Site 2 (44°17'37.45''N, 11°54'27.76''E)	July 18 and August 8 in 2018	Hayward	13	4.5x2.5 m	Kiwifruit
	July 17 and August 7 in 2019	Zesy002	3	4.8x2.5 m	Kiwifruit

* Trebon® UP 50 mL/hL (14.38 g etofenprox hL). The transects were not sprayed.

Table 2. Stinkbugs other than BMSB collected in the two years. The number of individuals of the different stages is shown

Year	Species	Life stages Adults	Nymphs	Egg masses
2018	*Nezara viridula*	58	191	14
	Rhaphigaster nebulosa	3		3
	Acrosternum heegeri	2		
	Graphosoma lineatum	7		
	Arma custos		4	
2019	*Nezara viridula*	56	169	21
	Palomena prasina	3		3
	Rhaphigaster nebulosa	1		
	Graphosoma lineatum	2		

Table 3. Discovery efficiency, successful parasitism, first instar BMSB nymphs pooling both sites and cultivars in 2018 and 2019. See Materials and Methods for parameter description. The mean egg number (±SE) per egg mass was 26.03±0.58 in 2018 and 27.26±0.14 in 2019.

Years	Discovery efficiency (%)	Successful parasitism (%)	First instar BMSB nymphs (%)
2018	40.63	20.41	44.42
2019	2.56	0.47	97.65

Table 4. Linear model of predictors of percentages of kiwifruit injured by BMSB. Confidence intervals and standard errors based on 1000 bootstrap samples.

	B	**SE**	**Confidence Intervals 95%**	**Standardized β**	**P**
Constant	-14.18	4.57	22.12 - 5.19		0.02
BBCH scale	11.58	1.07	9.42 - 13.75	0.46	<0.001
Actinidia variety	6.57	2.91	1.00 – 12.88	0.13	0.03
Total BMSBs per trap	0.22	0.03	0.16 – 0.28	0.41	<0.001

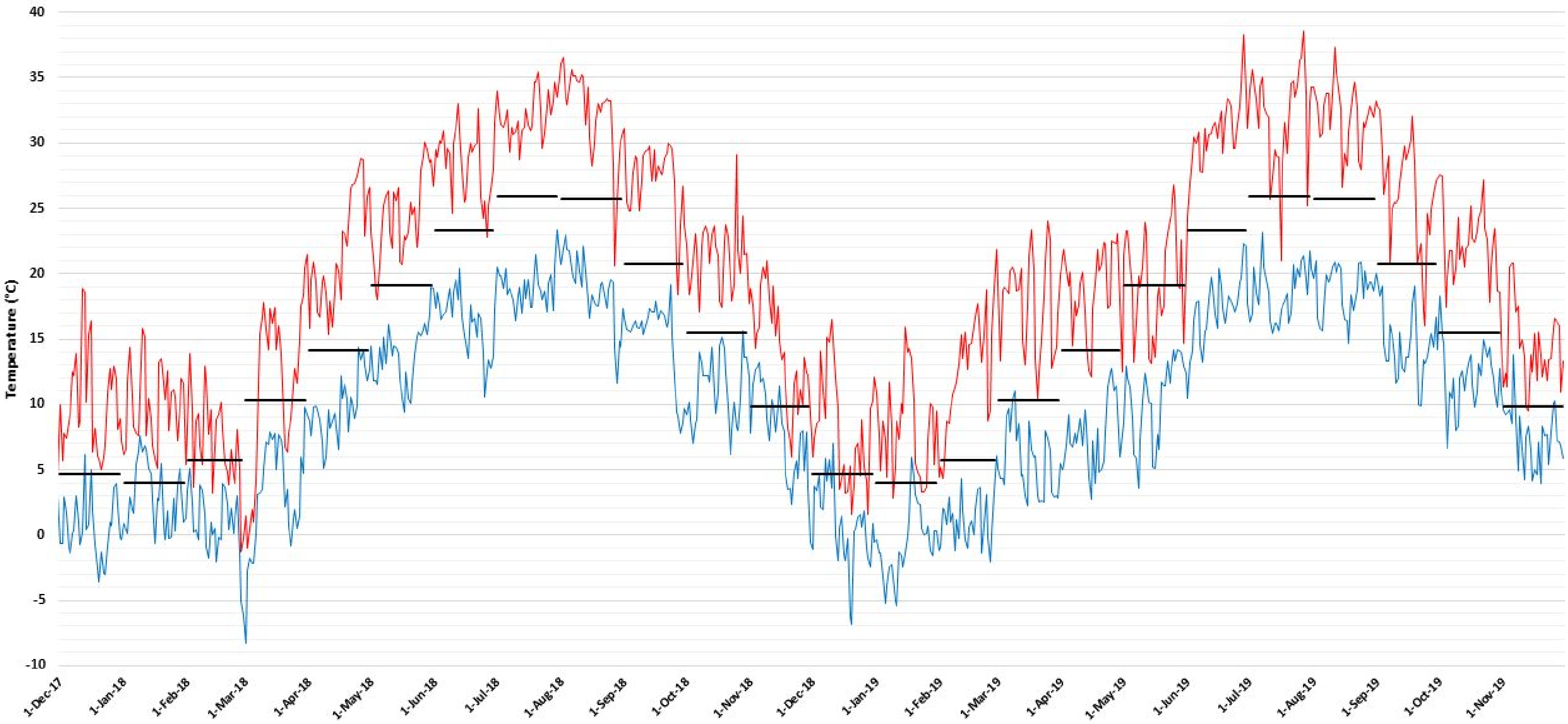

Fig. S1. Trend of daily maximum (solid red line) and minimum (solid blue line) temperatures measured 2 m above the ground in Faenza, Ravenna Province (Emilia-Romagna Region) from December 1 2017 to December 1 2019. The horizontal black bars show the average monthly temperature (from 1991 to 2019). (Data from Arpae Emilia-Romagna)

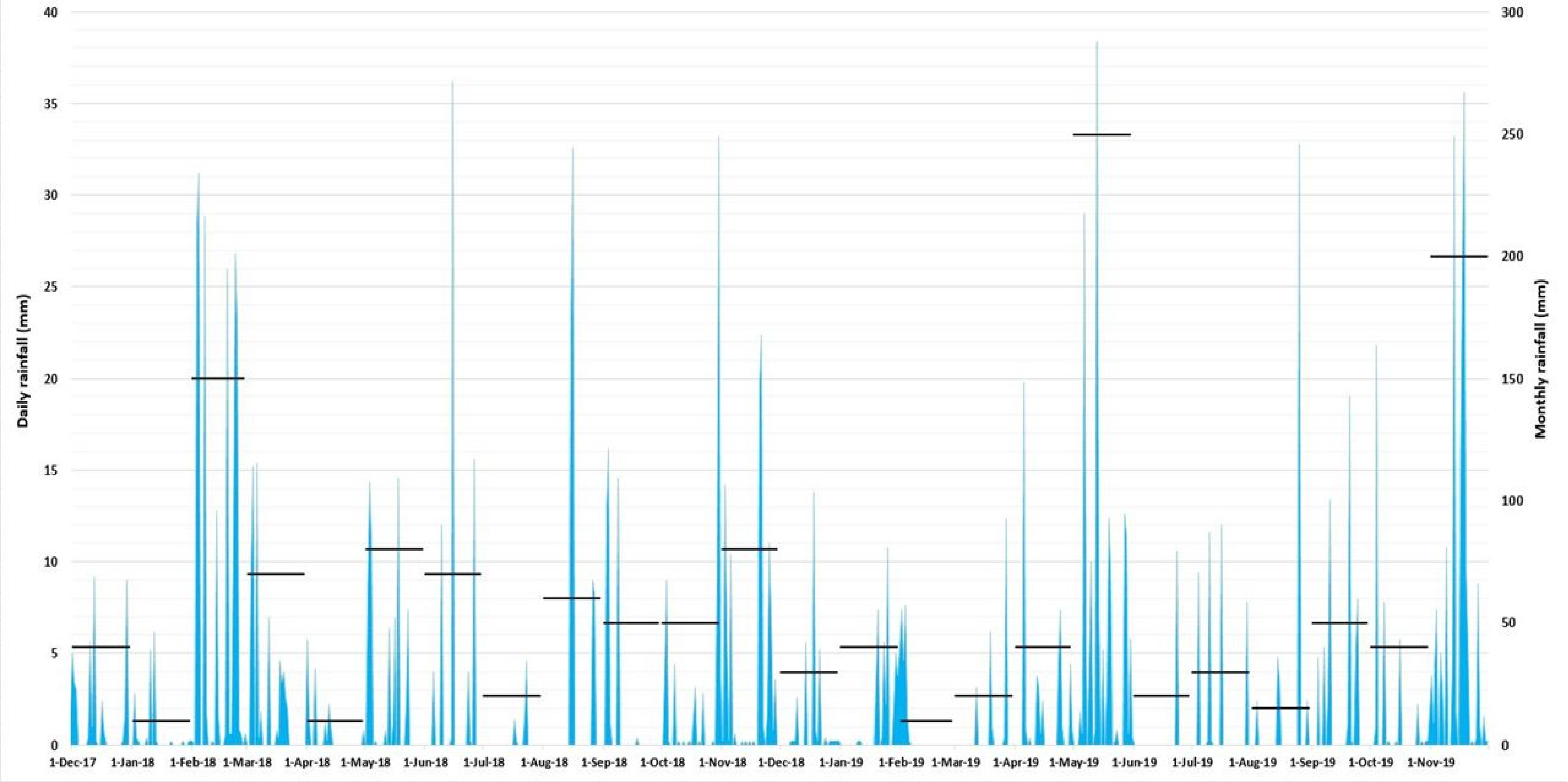

Fig. S2. Daily rainfall (mm, in blue) measured in Faenza, Province of Ravenna (Emilia-Romagna Region) from December 1 2017 to December 1 2019. The horizontal black bars show the cumulative monthly rainfall (mm, from 1991 to 2019). (Data from Arpae Emilia-Romagna)