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A 3-year survey on parasitism of *Halyomorpha halys* by egg parasitoids in northern Italy

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Abstract

A 3-year survey was carried out to assess the impact of egg parasitoids on *Halyomorpha halys* in northern Italy. In total, 1826 *H. halys* egg masses were collected in the 3 years, and parasitoid adults emerged from 12% of eggs in 2016 and 2017, and from 21% in 2018. *Anastatus bifasciatus* was the main species emerging from *H. halys* eggs at all sites and years, confirming its ability to develop on fresh *H. halys* eggs. Only a few adults of native scelionid species emerged: *Trissolcus kozlovi* (first record in Italy), *T. semistriatus*, *T. basalis* and *Telenomus turesis*. In addition, a few adults of the Nearctic hyperparasitoid *Acroclisoides solus* (first record in Europe) were obtained from *H. halys* eggs collected at different sites. In 2018, for the first time an adventive population of *Trissolcus japonicus* was also recorded at one site, where the parasitism rate by the parasitoid species complex was overall higher than in 2016 and 2017. An additional supplemental survey at other sites of northern Italy in 2018 revealed the presence of *T. japonicus* and *Trissolcus mitsukurii*. The distribution and abundance of *T. japonicus*, *T. mitsukurii* and *A. solus* should be further investigated. Their host range and their interaction with native egg parasitoids, especially with *A. bifasciatus*, should be also assessed to better understand their potential role in biological control of *H. halys*.

Keywords: Brown marmorated stink bug, *Anastatus bifasciatus*, *Trissolcus kozlovi*, *Trissolcus japonicus*, *Trissolcus mitsukurii*, *Acroclisoides solus*

Key message:

- Egg parasitoids emerged from 12 and 21% of *Halyomorpha halys* eggs collected in 2016-2017 and 2018, respectively.
- *Anastatus bifasciatus* was the predominant parasitoid species, while native scelionid species were only occasionally found emerging from H. halys eggs.
- *Trissolcus kozlovi* and *Acroclisoides solus* are recorded for the first time in Italy and Europe, respectively.

- In 2018, *Trissolcus japonicus* and *Trissolcus mitsukurii* were also found emerging from field-collected *H. halys* eggs.

Author Contribution Statement

LT, STM, MGP conceived and designed the research. GC, STM, MGP, SS and SV conducted the research. FT identified the parasitoids. MP performed molecular analysis. STM, LT and FT wrote the manuscript. All authors read and approved the final manuscript.

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a highly invasive harmful pest on several crops, as well as a nuisance pest in urban landscapes, when the adults invade human-made structures, often in very large numbers, to overwinter inside protected environments (Haye et al. 2015b). The species is native to East Asia, and it is now present in North America (the USA and Canada) and Europe, and most recently also in South America, in Chile (Leskey and Nielsen 2018). Moreover, other regions in both hemispheres with suitable climatic characteristics for the spread of *H. halys* are at risk of invasion (Zhe et al. 2017; Kriticos et al. 2017). In the invaded areas, *H. halys* has caused severe economic damage on many crops, among which pome and stone fruit, corn, hazelnut have been the most seriously affected (Rice et al. 2014; Maistrello et al. 2017; Bosco et al. 2018; Leskey and Nielsen 2018). Crop protection from *H. halys* damage mainly relies on intensive use of broad-spectrum insecticides, which does not always give a satisfactory control due to the pest's large host range, high mobility, and knockdown and recovery from many insecticides (Kuhar and Kamminga 2017; Leskey and Nielsen 2018). Their use also interferes with the largely adopted integrated pest management strategies, and leads to negative consequences on environment and human health. Therefore, research has been focused on generally more environmental-friendly control methods such as biological control.

In the native area, *H. halys* populations are attacked by a complex of egg parasitoids mainly belonging to Scelionidae and Eupelmidae (Lee et al. 2013). Within the egg parasitoid guild, *Trissolcus japonicus* (Ashmead) (syn. *T. halyomorphae* Yang; Talamas et al. 2013) is the predominant species, showing parasitism rates ranging from 50 to 80% in Beijink and Hebei provinces (China) (Yang et al. 2009, 2015; Zhang et al. 2017), and is therefore considered a good candidate for biological control of this pest. Since 2014, adventive populations of *T. japonicus* have been found in North America in Maryland (Talamas et al. 2015), and since then, its presence has been assessed in 10 states in the USA, and research is now going on to establish its impact on *H. halys* and on other native stink bugs in field (Milnes et al. 2016; Hedtrom et al. 2017; Morrison et al. 2018).

In Europe, classical biological control with the introduction of exotic biological control agents, such as *T. japonicus*, is currently not allowed, because of risks of potential non-target effects and restrictions due to the Nagoya protocol (Cock et al. 2010). In the meantime, several studies have been conducted in the invaded areas on native natural enemies, especially egg parasitoids. In North America and Europe, three principal groups of hymenopteran parasitoids attack *H. halys* eggs: Scelionidae (*Telenomus* spp., *Trissolcus* spp. and *Gryon* spp.), Eupelmidae (*Anastatus* spp.), and Encyrtidae (*Ooencyrtus* spp.) (Abram et al. 2017). The relative prevalence of different parasitoid species associated with *H. halys* eggs seems to be habitat dependent, since *Telenomus podisi* Ashmead is the most abundant species in field/vegetable crops, while *Anastatus* spp. and *Trissolcus* spp. are predominant in ornamental, forest, and seminatural/urban habitats (Abram et al. 2017). However, data have been collected using different sampling methods (e.g., collecting field laid egg masses or exposing fresh or frozen sentinel egg masses), and this can affect which parasitoid species are detected and their emergence rates (Abram et al. 2017). Specifically, sentinel egg masses seem to show lower parasitism levels, probably due to the absence of host-associated infochemical cues (Jones et al. 2014; Abram et al. 2017; Rondoni et al. 2017).

In a preliminary survey in NW Italy in 2015, a high concentration of *H. halys* egg masses was found on maple trees in a suburban landscape, and *Anastatus bifasciatus* (Geoffroy) emerged from 16% of the eggs (Abram et al. 2017). Therefore, this study aimed to assess if and which native egg parasitoids were able to adapt to the exotic stink bug and evaluate the parasitism rate of *H. halys* eggs in field conditions. To estimate the impact of egg parasitoids, *H. halys* egg masses were collected and examined in a 3-year survey in northern Italy, as sites located in suburban and agricultural landscapes not subject to insecticide pressure.

Materials and methods

Field collection on *H. halys* egg masses

Field surveys to assess the parasitism of *H. halys* eggs were conducted during a 3-year period from 2016 to 2018. In 2016 and 2017, *H. halys* egg masses were collected at 10 suburban sites in Piedmont (NW Italy) (Table 1). The sites were all characterized by the presence of maple (*Acer* spp.) not subject to any insecticide applications. Field surveys were carried out three times (every 2 weeks, from early August to early September) in 2016 and five times (two additional times compared to 2016, in mid-June and mid-July) in 2017. During surveys, canopies of maple between 1.5 and 2.5 m form the ground were inspected for the presence of egg masses of *H. halys* using a 4-step foldable aluminum ladder at each site for 30 min. Leaves with egg masses of *H. halys* as well as of other stink bugs were removed and transferred to the laboratory.

In 2018, field surveys were carried out at four sites in Piedmont (NW Italy), two of them already inspected in 2016 and 2017 (Table 1). The four sites were all characterized by the presence of high populations of *H. halys* and of various host plants besides maple, such ad ash (*Fraxinus excelsior* L.), walnut (*Juglans regia* L.), pear (*Pyrus communis* L.), dogwood (*Cornus sanguinea* L.), linden (*Tilia* spp.), hazelnut (*Corylus avellana* L.), bee-bee tree [*Tetradium daniellii* (Bennett) Hartley], tree of heaven [*Ailanthus altissima* (Miller) Swingle], Eurasian smoketree (*Cotinus coggyria* Scopoli), fig

(*Ficus carica* L.), corn (*Zea mays* L.) and black locust (*Robinia pseudoacacia* L.). Field surveys were carried out three or four times (in mid-June, mid-July, late August and mid-September) (Table 1). During surveys, canopies of all host plants between 1.0 and 2.5 m from the ground were inspected for the presence of egg masses of *H. halys* using a 4-step foldable aluminum ladder at each site for 4 h. Leaves with egg masses were removed and transferred to the laboratory. At the end of the season, in mid-September, an additional survey was carried out at six of the 10 sites inspected in 2016 and in 2017 and at further 23 sites in Piedmont and Lombardy (northern Italy), located at the foot of the Alps (sites in which at least one egg mass was found are reported in Table 1). All field-collected *H. halys* egg masses were transferred to the laboratory.

Laboratory rearing of *H. halys* egg masses

In laboratory, the field-collected egg masses were first separated into two groups: I) egg masses with at least one egg still closed and apparently viable, and II) completely vacated egg masses. The first ones were placed individually in plastic Petri dishes (60 mm diameter), and reared in controlled climate chambers at 24±1°C, 65±5% RH and 16:8 h L:D, until all nymphs and/or parasitoids emerged. The egg masses were checked every 2 days to record any emergence of bug nymphs and separated according to their taxa and sexed. For the most abundant taxa, sex ratio was also calculated as the mean percentage of female offspring for each egg mass from which the parasitoid species had emerged in laboratory. All parasitoids were then stored in 99% ethanol prior to identification.

In all 3 years, at the end of the season, all collected egg masses were inspected under a Leica stereo microscope S6D with a magnification up to 40× to assess the fate of all eggs. Following Morrison et al. (2016) and Jones et al. (2017), egg fate categories were assigned to individual eggs within each egg mass with some adjustments: (1) hatched, where *H. halys* emerged from the vacated egg, recognizable for the presence of at least one of these characteristics: attached open lid, egg buster, incision line of the lid (Javahery 1994; Yang et al. 2009); (2) parasitized, where parasitoid emergence had occurred, recognizable for different coloration and opening of the vacated egg: non incision line of the lid but a hole with irregular margins (Yang et al. 2009); (3) sucked, where the egg was empty and one or more stylet sheaths protruded the egg; (4) broken, where egg was empty and the chorion was broken in at least one place; (5) unhatched, where a direct cause of mortality could not be properly diagnosed.

Additionally, parasitized eggs from which parasitoids had emerged were ascribed to a parasitoid group based on some characteristics observed after parasitoid emergence in laboratory: (1) Eupelmidae and Pteromalidae leave larval waste and pupal exuviae fragments inside the egg that make the egg appear dark inside, and a hole with different irregular margins (Jones et al. 2017, personal observations) (group 1, ESM1); (2) Scelionidae leave the egg nearly empty with a creamy compact waste accumulated against the corion and some typical half-moon shaped residuals of chewed chorion around the exit hole (Yang et al. 2009) (group 2, ESM2).

Furthermore, to evaluate the overall efficacy of the emerged egg parasitoids, the three indices proposed by Bin and Vinson (1991) were applied at each survey in the 3 years. The 'discovery efficiency' (i.e., percentage of egg masses containing parasitized eggs) was calculated as the number of egg masses discovered by the parasitoid (at least one parasitized egg) over the total number of egg masses. The 'exploitation efficiency' was calculated as the number of parasitized eggs over the total number of eggs within the discovered egg masses. Finally, the 'parasitoid impact' or parasitism rate (i.e., percentage of parasitized eggs) was calculated as the number of parasitized eggs over the total number of field-collected eggs. In each year, the three indices were then compared among sampling dates via a binomial distribution model with a logit link function, using the general linear model (GLM) procedure of the software IBM SPSS® Statistics 25 (IBM Corp., NY, USA). Means were then separated at $P<0.05$ using the Bonferroni test under the GLM procedure.

Parasitoid identification and characterization

Ethanol-stored specimens were dried and glued on card points for morphological analyses. A Leitz large-field stereo microscope TS with magnification up to 160× and a spotlight Leica CLS 150× were used for morphological diagnosis. For Eupelmidae, individuals were identified using the keys proposed by Askew and Nieves-Aldrey (2004); for Pteromalidae, individuals were identified following the description of Grissell and Smith (2006) and compared with the holotype kindly provided by the National Museum of Natural Hystory, Smithsonian Institution (Washington, DC, USA). For Scelionidae, Mylar paper was used as filter to diffuse the spotlight and allow inspection of the specimens' microsculpture, which contains important features necessary for the species-level identification. *Telenomus* species were determined using the keys of Kozlov and Kononova (1983) and Johnson (1984), and *Trissolcus* species were identified using the keys by Talamas et al. (2017), Kononova (2014, 2015), and Kozlov and Lê (1977). Moreover, *Trissolcus* specimens were compared with pictures of holotype and paratypes made available via Specimage (specimage.osu.edu) by Norman Johnson (The Ohio State University, USA) and in Talamas et al. (2017). All the specimens used for morphological analysis were deposited in the Dipartimento di Scienze Agrarie, Forestali e Alimentari, Italy (DISAFA).

Molecular analyses were performed to confirm some morphological identifications, as a routine procedure, and also to characterize some parasitoids belonging to Scelionidae and Pteromalidae. Genomic DNA was extracted according to Gariepy et al. (2014), and the barcode region of the cytochrome oxidase I (COI) gene was amplified for Scelionidae using universal PCR primers for insects LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al 1994), and for Pteromalidae using the PCR primers LCO1490 and HCOOUT (5'-CCA GGT AAA ATT AAA ATA TAA ACT TC-3') (Carpenter 1999). As described in Stahl et al. (2019c), the PCR was performed in a 50 µl reaction volume: 2 µl of DNA, 37.9 µl molecular grade water, 5 µl 10X Quiagen PCR buffer, 3 µl dNTPs (25 mM each), 1.5 µl

$MgCl_2$, 0.2 µl of each primer (0.3 µM each), 0.2 µl *Taq* DNA Polymerase (Qiagen, Hilden, Germany). Thermocycling conditions were optimized to shorten reaction times and included initial denaturation at 94°C for 300 s, followed by 35 cycles of 94°C for 30 s, annealing at 52°C for 45 s and extension at 72°C for 60 s; then further 600 s at 72°C for final extension. PCR products were purified using a commercially available kit (QIAquick PCR Purification Kit, Qiagen Gmbh, Hilden, Germany) following the manufacturer's instructions and sequenced by a commercial service (Genechron S.r.l., Rome, Italy). The sequences were compared with the GenBank database using the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLASTn). All sequences that showed a similarity lower than 99% were deposited in the GenBank database. All residual DNA is archived at DISAFA.

Results

Field-collected egg masses

At the 10 sites surveyed in 2016 and 2017, a total of 671 and 436 *H. halys* egg masses (17,545 and 11,370 eggs) were collected, respectively (Table 2). Despite the higher number of surveys in 2017 (5) than in 2016 (3), an approximately similar number of egg masses was found at most of the 10 sites in the 2 years. In 2018, excluding the final survey of mid-September (sites from 13 to 24, Table 1), overall 469 and 155 *H. halys* egg masses (11,890 and 4035 eggs) were collected at the four sites surveyed three-four times and at the five sites surveyed at the end of the season, respectively (Tables 1, 2). Most *H. halys* egg masses were generally collected from mid-August to mid-September in the 3 years (Fig. 1).

Egg fate and parasitism rate

Overall more than 60% of *H. halys* eggs hatched or had already hatched in the first 2 years (64.5% and 62.9% in 2016 and 2017, respectively), while only 46.8% of eggs hatched or had already hatched in 2018, with the lowest value of 27.9% at site 6 (Table 2). At the same, the overall parasitoid impact was also similar in the first 2 years (12.7% and 11.8% in 2016 and in 2017, respectively), even if highly variable among the sites, while it was higher in 2018 (19.0%), with the highest value of 34.3% at site 6 (Table 2). In 2016 and in 2017, the total parasitism, including eggs from which parasitoids had emerged, was almost exclusively attributable to parasitoids of group 1 (i.e., Eupelmidae and Pteromalidae), with few emergences of parasitoids of group 2 (i.e., Scelionidae) at sites 4 and 5 (Table 2). In 2018, the parasitism due to group 1 was overall similar (13.6%), whereas that one due to group 2 increased to 5.4%, with the highest value of 15.6% at site 6 (Table 2). Percentages of sucked, broken and unhatched eggs were quite variables among sites and years (Table 2). Overall, the incidence of sucked eggs was low in the 3 years (0.4% in 2016, 3.0% in 2017, and 2.6% in 2018) (Table 2). The incidence of broken eggs was low in 2016 (1.7%), while it increased in 2017 (7.5%), and in 2018

(8.7%) (Table 2). The incidence of unhatched eggs was generally higher (20.8% in 2016, 14.8% in 2017 and 23.0% in 2018) (Table 2).

Although most egg masses were collected from mid-August to mid-September, the incidence of parasitized eggs was variable during the season (Fig. 1). The discovery efficiency was variable in the 3 years (30.1% in 2016, 23.0% in 2017, and 41.8% in 2018). However, while in 2016 and in 2017 no differences were found in relation to the period, in 2018 discovery efficiency was significantly higher in late August, mainly due to the site 6 (Table 3). The exploitation efficiency increased over the 3 years (39.8% in 2016, 44.3% in 2017, and 50.8% in 2018) (Table 3). The parasitoid impact showed the same trend as exploitation efficiency in 2016 and in 2017, whereas in 2018 increased throughout the season following the same trend of the discovery efficiency (Table 3). In particular, at site 6 the parasitoid impact increased from 1.3% in mid-June to 40.8% in mid-September.

Parasitoid species composition and abundance

In the laboratory, from egg masses collected at sites 1-12 (Table 1), 1548, 896 and 1215 hymenopteran adults emerged in 2016, 2017 and 2018, respectively, belonging to six species (Table 2). Among these parasitoid species, *A. bifasciatus* was the predominant species emerging from *H. halys* eggs in all years (97.5% in 2016, 99.4% in 2017 and 78.6% in 2018), and on all sites and dates (Tables 2 and 3, Fig. 1). In laboratory, *A. bifasciatus* emerged for 4-8 week period after nymph emergence and showed a mean percentage of female offspring for each egg mass of 63.9±1.45%. By contrast, few scelionids emerged in the 2 years: *Telenomus turesis* Walker at site 5 in 2016 and at site 11 in 2018 (0.1% of emerged parasitoids in both years); *Trissolcus kozlovi* Ryakhovskii only at sites 4 but always in all years (0.7% of emerged parasitoids in 2016, 0.4% in 2017, and 0.1% in 2018); *Trissolcus basalis* (Wollaston) and *Trissolcus semistriatus* (Nees von Esenbeck) [syn. *Trissolcus grandis* (Thomson): Talamas et al. 2017)] only at site 11 in 2018 (0.2% of emerged parasitoids for both species); *T. japonicus* in higher number on maple as well as on other plants only at site 6 in 2018 (20.8% of emerged parasitoids) (Table 2). The ratio between parasitoid *Acroclisoides solus* Grissell & Smith (Hymenoptera: Pteromalidae) emerged occasionally at site 10 in 2016 (1.7%) and at site 6 in 2017 (0.1%).

In the survey conducted at 23 sites in Piedmont and Lombardy in mid-September 2018, a total of 92 egg masses (2403 eggs) were collected at 12 sites (Table 1). In laboratory, 420 hymenopteran adults emerged, of which *A. bifasciatus* was the most abundant (50.0%), followed by *T. japonicus* (36.9%), *A. solus* (8.3%), and *Trissolcus mitsukurii* (Ashmead) (4.8%), which was found only in Lombardy (Fig. 3).

Parasitoid species characterization

Molecular analyses confirmed the identity of *T. japonicus* and *T. mitsukurii* emerged from *H. halys* (100% similarity with GenBank sequence, accession no. AB971832, and 99% similarity with

GenBank sequence, accession no. AB971831, respectively). Since sequences of *T. kozlovi* and *A. solus* were not present in the GenBank database, all sequences obtained from specimens identified as *T. kozlovi* and *A. solus* by morphological analyses were deposited into the GenBank database [MH521283 for *T. kozlovi* emerged from *H. halys*, MH521284 for *T. kozlovi* emerged from *Palomena prasina* L., MH521285 for *A. solus* emerged from *Arma custos* (F.)] (Table 4).

Discussion and conclusions

In the 3-year surveys (sites from 1 to 12, Table 1), parasitism rate of *H. halys* eggs, with successful native parasitoid emergence, was stable and overall lower than 20%, consistent with data from previous surveys reviewed by Abram et al. (2017). The predominant parasitoid species was the generalist *A. bifasciatus*, already known to be able to develop on *H. halys* viable eggs (Haye et al. 2015A; Roversi et al. 2016; Abram et al. 2017; Costi et al. 2019; Stahl et al. 2018, 2019a). Similarly, other species of the genus *Anastatus* have been found to develop on fresh *H. halys* eggs in the area of origin (Lee et al. 2013; Zhang et al. 2017) as well as in North America (Ogburn et al. 2016; Dieckhoff et al. 2017; Jones et al. 2017; Morrison et al. 2018). In our study, *A. bifasciatus* emerged from *H. halys* eggs collected at all sites and in all years, showing a wide distribution and suitability to be considered as a candidate for augmentative releases in Europe (Haye et al. 2015A; Stahl et al. 2018, 2019a). Moreover, female offspring of *A. bifasciatus* emerging from field-collected eggs was higher (over 60%) than what was obtained in the laboratory (Stahl et al. 2018), further confirming the suitability of the egg masses lais in the field. However, in the 3-year surveys, the overall impact of *A. bifasciatus* on *H. halys* eggs did not increase. Continuous augmentative releases of *A. bifasciatus* may accelerate local population growth, and increase parasitism of *H. halys* eggs, even if the current release strategy could not effectively suppress the pest (Stahl et al. 2018, 2019b).

Concerning the native scelionid species, very few individuals were found overall, consistent with previous studies demonstrating the inability of native parasitoids to develop on this exotic host (Haye et al. 2015a, Abram et al 2014). However, the four scelionid species obtained in our study were found for the first time to emerge from *H. halys* eggs laid in the field. Furthermore, the presence of *T. kozlovi* in Italy is reported for the first time. In particular, *T. kozlovi* is extremely similar to *T. japonicus* both morphologically (Talamas et al. 2017) and genetically (91% similarity), and laboratory trials are currently underway to confirm the ability of *T. kozlovi* to develop on fresh *H. halys* eggs. However, similarity to *A. bifasciatus*, the impact of *T. kozlovi* on *H. halys* eggs as well as its distribution did not increase in the 3 years, although it was found emerging from eggs of other bug species at different sites (data not shown here). This fact suggests a lower attractiveness and/or suitability of *H. halys* eggs for its development, even if higher than for other native scelionid species.

The discovery of adventive populations of the exotic *T. japonicus* in 2018 confirmed its presence in Italy, as observed in the same year by Sabbatini Peverieri et al. (2018). From its first record in Europe, in Switzerland in 2017 (Stahl et al. 2019c), the species could spread, according to its potential distribution (Avila and Charles 2018) and following *H. halys* presence. At the Piedmont site where *T. japonicus* was found for the first time on maple as well as on other plants in 2018 (site 6), comparing data of the 3 years show that the impact of the exotic parasitoid on *H. halys* eggs was additive to that of *A. bifasciatus*, which indeed increased in turn. This observation confirms that the two species con coexist and act synergistically in controlling *H. halys*, as predicted in the previous laboratory trials (Konopka et al. 2017b). Moreover, the presence of *T. japonicus* could be favorable for native scelionid species by providing them a lifeline as hyperparasitoids (Konopka et al. 2017a), but the interactions between the exotic and the native species should be evaluated in the following years. If the lifeline hypothesis was confirmed, a positive correlation between *T. japonicus* presence and successful development of native scelionid specie on *H. halys* eggs would be expected. Consequently, the spread and establishment of the exotic parasitoid could lead to an increased emergence rate of indigenous parasitoids from *H. halys* eggs. However, a molecular approach on single eggs, as developed by Gariepy et al. (2014, 2019) and Konopka et al. (2019), would be needed to distinguish if the native scelionid species behaved as hyperparasitoid of *T. japonicus* or as a direct parasitoid of *H. halys*. Furthermore, the impact of *T. japonicus* on non-target host species should be investigated especially in the field, as it was found emerging from other native pentatomid species in laboratory in the USA and Europe (Hedstrom et al. 2017; Botch and Delfosse 2018; Haye et al. 2019), as well as in field in China (Zhang et al. 2017). Further studies on *T. japonicus* distribution, spread and impact on *H. halys* and non-target pentatomid species, including zoophagous species, in our area are therefore needed. Similarly, distribution, spread and impact should be assessed also for *T. mitsukurii*, found emerging from *H. halys* egg masses collected in Lombardy, as well as in other regions by Sabbatini Peverieri et al. (2018). In Japan, this species is reported as the main egg parasitoid egg parasitoid of *H. halys* (Arakawa and Namura 2002) and *Nezara viridula* (L.) (Arakawa et al. 2004), which is also wide spread in Italy. Therefore, the potential for competition between *T. mitsukurii* and *T. basalis* on *N. viridula* eggs (Jones 1988; Nishimoto et al. 2015) needs to be taken in account. Moreover, *T. mitsukurii* was reported in Iran on eggs of bug species such as *Eurygaster integriceps* Puton, *Acrosternum arabicum* Wagner, *Acrosternum breviceps* (Jakovlev) and *Brachynema germari* Kolenati (Mohammadpour et al. 2016); therefore, its distribution and host range could be wider than supposed. Finally, the interactions among native and exotic parasitoids and stink bugs are further complicated by the presence of *A. solus*, which is known to be a hyperparasitoid of *Trissolcus* spp. (Grissell and Smith 2016; Gariepy et al. 2014). This species described in North America is here recorded in Europe for the first time. Its presence could represent a threat for both *T. japonicus* and *T. mitsukurii*, but potentially also for all the native species. In our study, it emerged not only from *H. halys* eggs but also from other pentatomid species, such as *A. custos* and *P. prasina*, collected at three sites in Piedmont and one in Lombardy, showing to be widespread in North Italy. Moreover, *Acroclisoides* sp. was recorded from *H. halys* eggs in China (Lee et al. 2013).

During the 3-year surveys, besides the parasitized eggs, also sucked and broken eggs of *H. halys* were recorded. These sucked and broken eggs are generally ascribed to the feeding activity of sucking and chewing predators, respectively, and from our collection data we could not attribute them to a specific predatory arthropod. Actually, few studies have been carried out on the impact of predators under field conditions; however, the incidence of sucked and broken eggs recorded in NW Italy was consistent with what was observed in another Italian area (Costi et al. 2019) as well as in North America, where the predation varied widely among the states and crops (Morrison et al. 2016; Ogburn et al. 2016).
In conclusion, during the 3 years, the impact of native parasitoid species on *H. halys* eggs did not increase. As expected, the most abundant species, *A. bifasciatus*, is a generalist parasitoid, with a wide host range and a limited population-level impact on the exotic host. In fact, indigenous parasitoids often fail to immediately adapt to the invasive species, but if subject to a strong selective pressure, they can evolve the capacity to develop successfully on the new host. This could be the case of native scelionid parasitoids, which can attack *H. halys* eggs, but fail to develop (Abram et al. 2014). However, this process may take a long time, and currently, after the discovery of adventive populations of *T. japonicus* and *T. mitsukurii*, the situation has been changing, as highlighted by the increased parasitism rate at the site where, together *A. bifasciatus*, also *T. japonicus* was detected in 2018. Long-term studies will be therefore necessary to assess the effects of the interaction between native and exotic parasitoids and hyperparasitoids on *H. halys* control.

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Compliance with ethical standards
Conflict of interest The authors declare that they have no conflict of interest.

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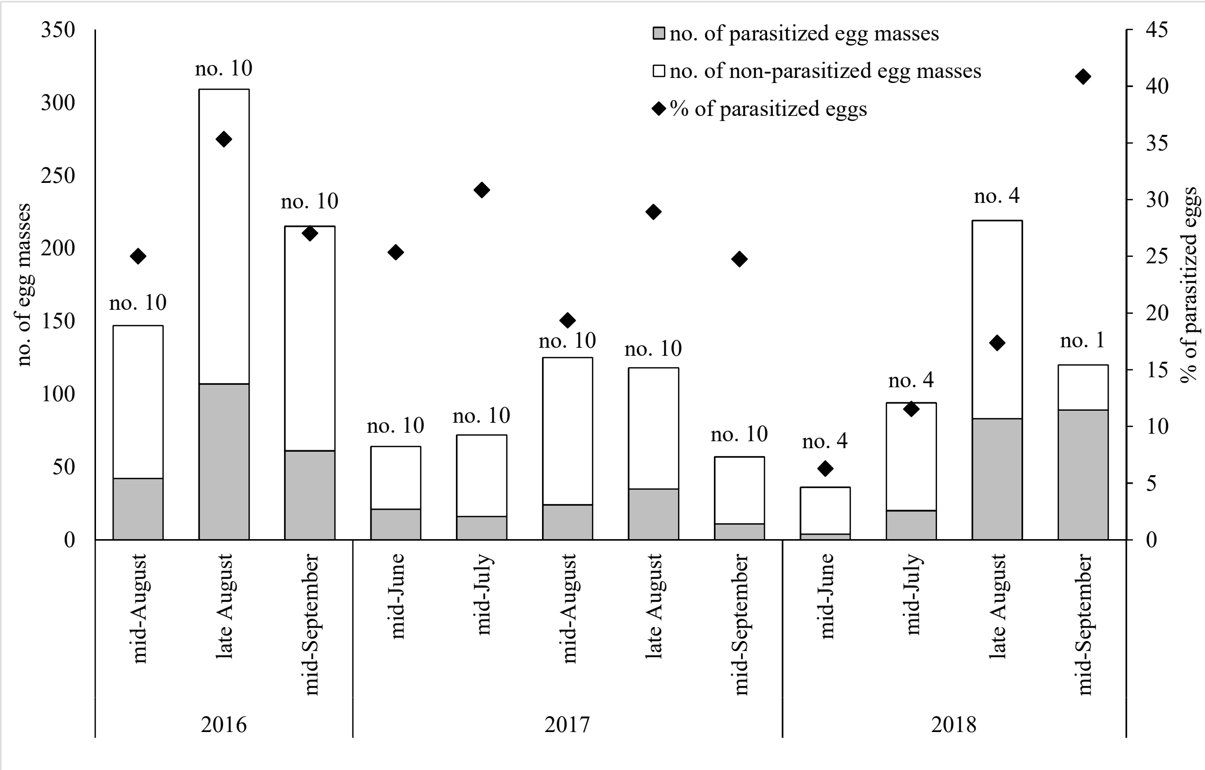

Figure 1. Total number of field-collected *Halyomorpha halys* egg masses, parasitized and non-parasitized ones, and respective parasitism rate of eggs per sampling date at the sites surveyed more than once in 2016, 2017, and 2018. Numbers of surveyed sites in each date are displayed above bars.

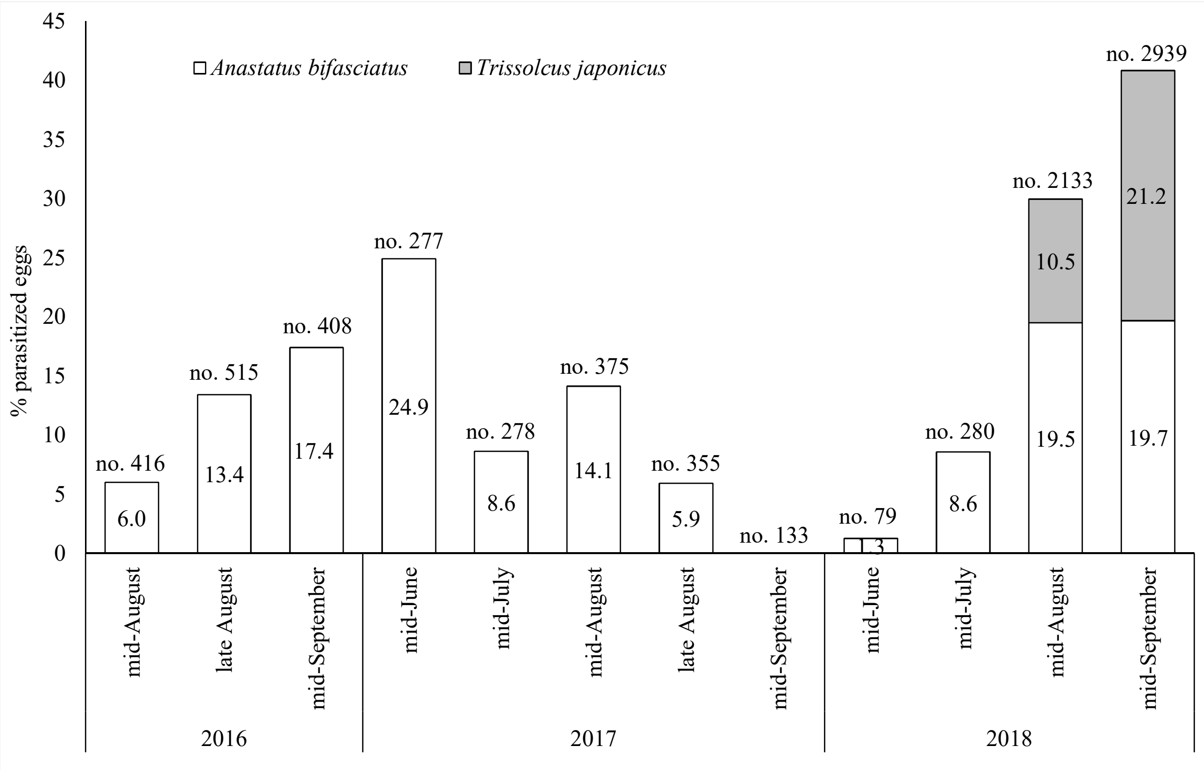

Figure 2. Parasitism rate of *Halyomorpha halys* eggs due to *Anastatus bifasciatus* and *Trissolcus japonicus* observed at site 6 in the three-year survey. Numbers of observed eggs in each date are displayed above bars.

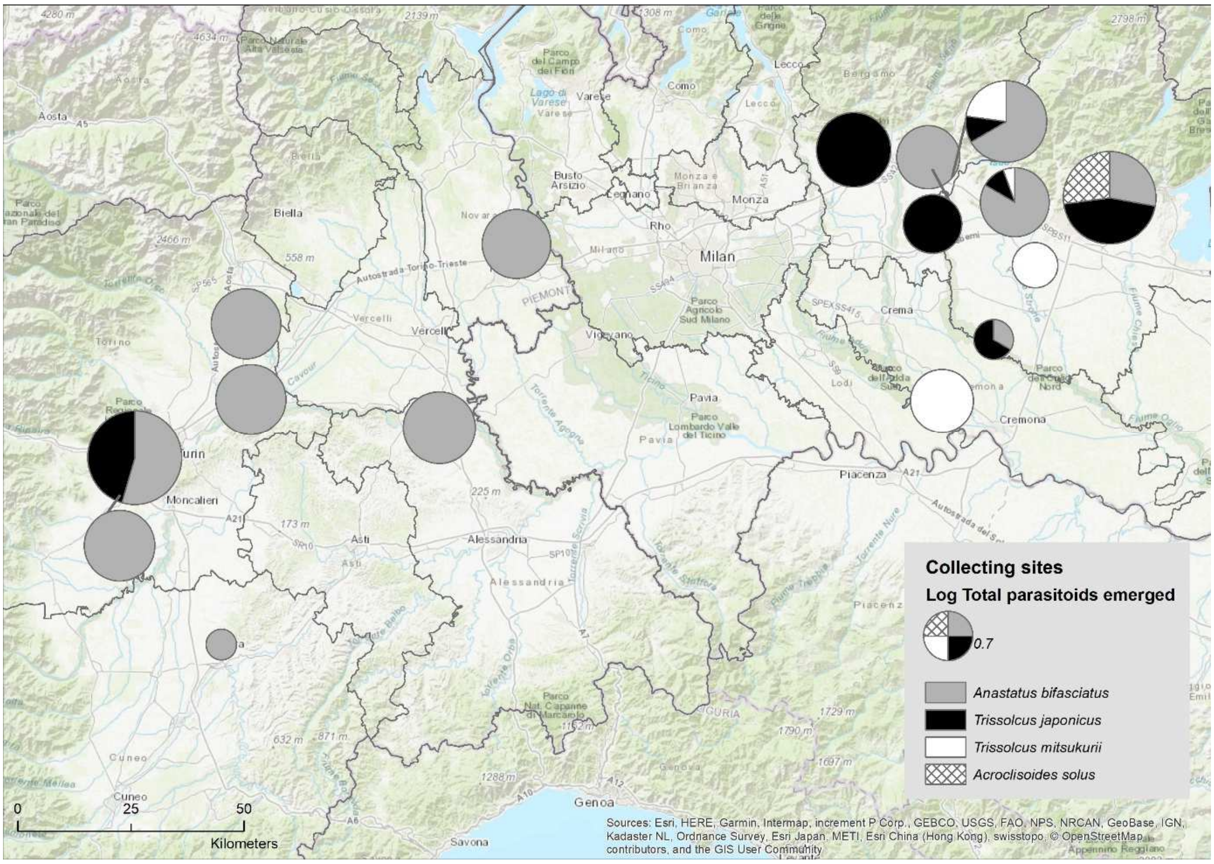

Figure 3. Proportions of emerged parasitoid species from field collected *Halyomorpha halys* eggs in the sites surveyed in mid-September 2018. Pie chart variation size is normalized by log.

Table 1. Sites in North Italy, where *Halyomorpha halys* egg masses were collected in 2016, in 2017 and in 2018.

Id	Site (province)	Coordinates	Altitude (m asl)	2016	2017	2018
1	Bra (CN)	44°42'23.6"N 7°50'31.9"E	286	3	5	1
2	Carrù (CN)	44°28'42.6"N 7°52'37.2"E	363	3	5	
3	Casale Monferrato (AL)	45°08'35.6"N 8°26'47.4"E	117	3	5	1
4	Cavour (TO)	44°46'52.4"N 7°22'59.2"E	295	3	5	3
5	Chivasso (TO)	45°11'42.8"N 7°54'54.6"E	182	3	5	1
6	Grugliasco (TO)	45°03'51.5"N 7°35'30.3"E	287	3	5	3
7	Manta (CN)	44°36'38.7"N 7°29'18.1"E	400	3	5	1
8	Orbassano (TO)	44°59'57.3"N 7°33'01.4"E	266	3	5	1
9	Trofarello (TO)	44°58'48.6"N 7°45'08.7"E	243	3	5	
10	Pinerolo (TO)	44°53'16.8"N 7°20'06.1"E	370	3	5	
11	Chieri (TO)	45°02'28.2"N 7°50'03.9"E	335			3
12	Narzole (CN)	44°36'58.7"N 7°51'56.0"E	300			3
13	Collegno (TO)	45°04'26.5"N 7°35'27.6"E	297			1
14	Cameri (NO)	45°30'31.7"N 8°39'41.7"E	166			1
15	Candia (NO)	45°19'05.5"N 7°53'58.9"E	232			1
16	Concesio (BS)	45°35'40.1"N 10°13'49.2"E	194			1
17	Fenili Belasi (BS)	45°28'27.6"N 10°07'44.9"E	98			1
18	Quinzano d'Oglio (BS)	45°18'54.1"N 10°00'32.1"E	54			1
19	Tagliuno (BG)	45°38'22.4"N 9°53'48.4"E	223			1
20	Grumello Cremonese (BG)	45°11'40.7"N 9°51'39.9"E	52			1
21	Bergamo (BG)	45°41'39.0"N 9°41'44.6"E	257			1
22	Chiuduno (BG)	45°39'13.3"N 9°50'28.9"E	223			1
23	Gorlago (BG)	45°40'42.7"N 9°49'47.0"E	242			1
24	Paderno Franciacorta (BS)	45°35'13.2"N 10°04'26.6"E	182			1

Table 2. Numbers of *Halyomorpha halys* egg masses and eggs collected at the 12 sites surveyed from 2016 to 2018, with respective percentages of hatched, parasitized (total, and attributable to the group 1, i.e. Eupelmidae and Pteromalidae families, or to the group 2, i.e. Scelionidae family), sucked, broken and unhatched eggs, and numbers and species of parasitoids emerged in laboratory.

Site	year (no. of surveys)	no. of egg masses	no. of eggs	% hatched	% parasitized (group 1; group 2)	% sucked	% broken	% unhatched	no. and species of parasitoids emerged in laboratory*
1	2016 (3)	74	1953	78.39	7.48 (7.48; 0.00)	0.00	1.08	13.06	72 *Ab*
	2017 (5)	63	1629	72.31	2.27 (2.27; 0.00)	2.52	8.90	14.00	16 *Ab*
	2018 (1)	10	282	78.01	0.35 (0.35; 0.00)	1.77	10.28	9.57	1 *Ab*
2	2016 (3)	17	472	65.04	14.83 (14.83; 0.00)	0.00	0.00	20.13	39 *Ab*
	2017 (5)	9	220	63.64	17.73 (17.73; 0.00)	0.00	8.18	10.45	28 *Ab*
3	2016 (3)	83	2195	59.09	18.50 (18.50; 0.00)	0.18	1.91	20.32	300 *Ab*
	2017 (5)	36	998	52.00	18.74 (18.74; 0.00)	10.52	2.51	16.23	110 *Ab*
	2018 (1)	24	649	46.53	10.63 (10.63; 0.00)	9.71	7.86	24.96	23 *Ab*
4	2016 (3)	81	2033	55.44	13.13 (12.59; 0.54)	1.23	4.57	25.63	149 *Ab*, 11 *Tk*, 19 *As*
	2017 (5)	64	1704	60.97	10.09 (7.34; 2.76)	0.00	17.90	11.03	92 *Ab*, 4 *Tk*
	2018 (3)	143	3588	55.52	10.67 (10.62; 0.06)	0.72	12.35	20.60	232 *Ab,* 1 *Tk*
5	2016 (3)	181	4847	66.45	9.53 (9.51; 0.02)	0.87	0.74	22.41	376 *Ab*, 1 *Tt*
	2017 (5)	19	490	65.92	11.02 (11.02; 0.00)	12.86	4.49	5.71	30 *Ab*
	2018 (1)	40	1059	52.69	12.18 (12.18; 0.00)	8.50	3.21	23.51	20 *Ab*
6	2016 (3)	54	1339	72.67	12.32 (12.32; 0.00)	0.00	1.19	13.82	69 *Ab*
	2017 (5)	53	1418	64.03	11.78 (11.78; 0.00)	2.47	5.22	16.50	139 *Ab*, 1 *As*
	2018 (4)	213	5431	27.88	34.32 (18.76; 15.56)	3.09	7.60	27.10	498 *Ab*, 253 *Tj*
7	2016 (3)	44	1070	63.36	16.64 (16.64; 0.00)	0.00	0.47	19.53	143 *Ab*
	2017 (5)	29	706	78.05	4.96 (4.96; 0.00)	3.54	1.70	11.76	21 *Ab*
	2018 (1)	5	119	59.66	14.29 (14.29; 0.00)	0.00	13.45	12.61	--
8	2016 (3)	74	1978	56.98	17.69 (17.69; 0.00)	0.05	2.53	22.75	252 *Ab*
	2017 (5)	86	2171	58.68	19.58 (19.58; 0.00)	1.52	5.39	14.83	306 *Ab*
	2018 (1)	76	1926	45.74	15.78 (15.42; 0.36)	1.82	8.10	28.71	21 *Ab*
9	2016 (3)	24	652	76.07	6.13 (6.13; 0.00)	0.15	2.91	14.72	24 *Ab*
	2017 (5)	43	1143	45.93	15.75 (15.75; 0.00)	3.67	4.90	29.75	19 *Ab*
10	2016 (3)	39	1006	54.67	14.02 (14.02; 0.00)	0.00	0.89	30.42	85 *Ab*, 8 *As*
	2017 (5)	34	891	77.55	5.50 (5.50; 0.00)	0.11	8.42	8.42	130 *Ab*
11	2018 (3)	88	2248	65.88	10.90 (10.63; 0.27)	1.29	6.43	16.19	144 *Ab*, 3 *Tb*, 2 *Ts*, 1 *Tt*
12	2018 (3)	25	623	69.34	3.53 (3.37; 0.16)	0.00	14.45	12.36	16 *Ab*

**Ab*: *Anastatus bifasciatus*, *Tk*: *Trissolus kozlovi*, *As*: *Acroclisoides solus*, *Tt*: *Telenomus turesis*, *Tj*: *Trissolcus japonicus*, *Tb*: *Trissolcus basalis*, *Ts*: *Trissolcus semistriatus*

Table 3. Mean (±SE) discovery efficiency, exploitation efficiency, and parasitoid impact on *Halyomorpha halys* egg masses collected in each sampling date (no. = sites) in the three-year survey. In each column, values followed by the same letter are not significantly different (Bonferroni test, $P < 0.05$, under GLM procedure with binomial distribution and logit link).

Date	2016 (no. = 9-10[a])			2017 (no. = 5-10[a])			2018 (no. = 3-4 [a])		
	Discovery efficiency (no.=10)	Exploitation efficiency (no.=9-10)	Parasitoid impact (no.=10)	Discovery efficiency (no.=7-10)	Exploitation efficiency (no.=5-9)	Parasitoid impact (no.=7-10)	Discovery efficiency (n=4)	Exploitation efficiency	Parasitoid impact (n=4)
Mid-June				(7) 25.46±8.29	(5) 71.72±6.43 a	(7) 18.99±6.67 a	14.58±7.12 b	(3) 47.02±22.02 ab	5.03±2.93 c
Mid-July				(9) 34.24±9.62	(9) 52.29±12.35 a	(9) 15.95±4.80 b	17.05±5.86 b	(3) 48.37±3.39 a	8.50±3.00 b
Mid-August	26.55±4.64	(9) 45.11±7.14 a	12.37±3.28 b	(10) 32.29±4.77	(8) 49.39±5.42 b	(10) 9.22±1.84 c			
Late August	36.78±4.57	(10) 44.45±4.63 a	17.13±3.08 a	(10) 29.36±6.41	(9) 39.52±5.84 c	(10) 11.25±2.15 bc	31.59±11.28 a	(4) 43.02±4.76 b	13.94±5.51 a
Mid-September	28.11±4.34	(10) 26.10±4.04 b	8.32±2.18 c	(9) 27.50±11.83	(5) 18.32±5.41 d	(9) 4.74±2.33 d			
Wald χ^2	2.950	102.392	139.568	6.318	151.281	242. 350	14.574	8.250	96.560
df	2	2	2	4	4	4	2	2	2
P	0.229	<0.001	<0.001	0.177	<0.001	<0.001	0.001	0.016	<0.001

[a]sites varied from 2 to 11 depending on the presence of egg masses (discovery efficiency and parasitoid impact) and on the presence of parasitized ones (exploitation efficiency).

Table 4. Sampling information and GenBank Accession Number for the deposited sequences generated from this study.

Species	sex	country	region	year of collection	host	GenBank Accession Number	ID code
Trissolcus kozlovi	m	Italy	Piedmont	2016	*Halyomorpha halys*	MH521283	DISAFA-draw1466-HYM-0482
Trissolcus kozlovi	f	Italy	Piedmont	2017	*Palomena prasina*	MH521284	DISAFA-draw1466-HYM-0481
Acroclisoides solus	f	Italy	Piedmont	2017	*Arma custos*	MH521285	DISAFA-draw1466-HYM-0480