



# Predatory ability of generalist predators on eggs, young nymphs and adults of the invasive *Halyomorpha halys* in southern Europe

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Received: 23 April 2020 / Accepted: 6 November 2020 / Published online: 13 November 2020  
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**Abstract** *Halyomorpha halys* (Stål, 1855) is an invasive pest causing serious damage to agricultural crops in Europe and the USA. Very little is known about *H. halys* predators in Europe. This survey evaluated the potential of generalist predators/omnivorous species by means of predation bioassays in tridimensional arenas, where the predator had to locate *H. halys* prey items on the leaves of a bean plant. Eleven species of different taxa were tested and the prey items consisted in fresh eggmasses, 1st and 2nd instar nymphs. One species was also tested against adults. Some predators were species commercially available as biocontrol agents against plant pests, other predators were wild, captured in habitats shared with *H. halys*. All tested specimens were starved 24 h

before starting the experiment. The survivorship of control prey items in predator-excluding cages was compared to that of predator treatment groups to determine the effect of predator presence. According to the results, the generalist species showed a quite low acceptance of *H. halys* prey items, since only two species caused 80% mortality on at least one item (*Eupholidoptera chabrieri* and *Rhynocoris iracundus*) and mortality due the other species never exceed 60%. Among commercially available species only *Adalia bipunctata* adults and *Chrysoperla carnea* larvae were effective, predated the eggs and 1st instar nymphs, respectively. Among the field collected specimens, the orthopteran *E. chabrieri* and the predatory hemipterans *R. iracundus*, *Nagusta goedelii* and *Himacerus mirmicoides* showed efficacy against 1st instar nymphs, *E. chabrieri* and *R. iracundus* showed efficacy against 2nd instar nymphs, whereas only *E. chabrieri* and *N. goedelii* predated the eggs. *R. iracundus* was also tested on the adults and successfully predated them. By identifying some of the species that can exploit *H. halys* as a suitable prey in southern Europe, the present investigation provides an important contribution for conservation biological control of this pest.

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Handling Editor: Eric Riddick.

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**Keywords** Brown marmorated stink bug · Conservation biocontrol · Predatory hemipterans · Predation bioassay

## Introduction

*Halyomorpha halys* (Stål, 1855) (Heteroptera, Pentatomidae) is an invasive insect native to eastern Asia, specifically China, Japan, Taiwan and South Korea (Leet et al. 2013; Haye et al. 2015a). Being a hitchhiker on inanimate objects, it has a high dispersion capacity facilitated by human activities and trade (Maistrello et al. 2018), that allows a fast colonization worldwide (Rice et al. 2014). It has been present in North America since 1996 (Hoebeke and Carter 2003; Hamilton 2009) and in April 2020 *H. halys* was reported in 46 states in the USA and in four provinces of Canada (Stopbmsb 2020). Most recently it was detected also in South America, specifically in Chile (Faúndez and Rider 2017). In the European continent, after the initial detection in Switzerland in 2004 (Haye et al. 2015a) the spread into the different countries was extremely fast. As of April 2020, *H. halys* is reported with established populations in 28 countries in Europe and along the Black Sea (Inaturalist 2020).

In Italy, the first official sighting occurred in 2012 in the province of Modena, Emilia Romagna (Maistrello et al. 2016). A crowdsourcing survey indicated a very fast spread all over the country and the surrounding main islands (Sicily, Sardinia, Corsica), and a model on its spatio-temporal dynamics tracked the possible date of entry back to 2009 (Maistrello et al. 2018). The genetic analysis of the samples collected over time shows that the Italian populations of *H. halys* have the highest biodiversity outside of its native Asia, as a result of multiple invasions that are still in progress (Cesari et al. 2018). In Italy, *H. halys* completes two generations per year with a partial overlap of the oviposition periods and contemporary presence of different development instars during summer. The reproductive potential shown in Italy is high, with on average 285 and 215 eggs each for the overwintering and first-generation females, respectively ( $R_0 = 24.04$  and  $5.44$ , respectively for the two generations) (Costi et al. 2017).

*Halyomorpha halys* is a polyphagous species with more than 300 host plants, including fruit trees, crops, vegetables, ornamental and spontaneous plants (Rice et al. 2014; Haye et al. 2015a). Damage occurs mainly to fruits and seeds as a consequence of the feeding activity with the piercing-sucking mouthparts, that cause scarring, malformations, suberifications, discolored areas and/or necrotic areas and brownish spots

that render agricultural products unmarketable (Rice et al. 2014) resulting in considerable economic losses. In the Midwest USA states in 2010, *H. halys* caused damages of over \$37 million for the apple trade (Leskey et al. 2012). In northern Italy, it became a key pest of fruit orchards just a few years after its discovery (Maistrello et al. 2017) and reliable estimates for 2019 report an overall economic impact of €588 million on northern Italy fruit production (pear, peach, apple, kiwi), with up to 80–100% yield losses in the orchards (CSO Italy 2020). Another feature that makes this pest difficult to manage is its high mobility, typical of all instars. Adults fly on average 2 km per day, but can also reach 116 km per day (Wiman et al. 2015; Lee and Leskey 2015), while the nymphs, from the second stage onwards, can walk over 20 m per day among the different plants (Lee et al. 2014).

Managing *H. halys* poses a big issue in the invaded regions, but no ultimate solution to efficiently manage this pest has been found yet. Currently, the management of this invasive pest relies on increased applications of broad-spectrum insecticides, thus disrupting previous IPM programs with further negative impact on the economy and the environment (Leskey et al. 2012; Maistrello et al. 2017).

Considering that in invaded areas there are no specific *H. halys* antagonists, field surveys were carried out, both in cultivated and unmanaged areas, exposing either fresh or frozen *H. halys* egg masses, to verify the potential of generalist antagonists. The eggs were both preyed and parasitized, usually in low percentage for both categories, and as regards to predators, the damages due to chewing mouthparts were on average greater than those due to a pierce-sucking apparatus (Ogburn et al. 2016; Cornelius et al. 2016; Abram et al. 2017; Shanovich et al. 2020). According to Biddinger et al. (2017) the wasp *Bicyrtes quadrifasciatus* (Say, 1824) (Hymenoptera: Crabonidae) uses the nymphs of *H. halys* to feed and rear their larvae. In the study by Morrison et al. (2017) spiders were considered, in particular those present in potential overwintering sites of *H. halys* and by studying what remained in the spider webs of Agelenidae, Pholcidae and Theridiidae that can feed on *H. halys*.

In the laboratory, potential predators have been tested on eggs by means of no choice tests. Tettigoniidae, Gryllidae, including *Acheta domesticus* (Linnaeus, 1758), Acrididae, like *Melanoplus femurrubrum* (De Geer, 1773), Forficulidae,

*Chrysoperla carnea* larvae (Stephens, 1836) (Neuroptera: Chrysopidae), Coccinellidae and Salticidae, like *Phidippus audax* (Hentz, 1845) are able to prey on the eggs of *H. halys* or reduce the percentage of hatching (Abram et al. 2014; Morrison et al. 2016; Pote and Nielsen 2017; Poley et al. 2018). In the study of Pote and Nielsen (2017), the predators were also tested on the first two juvenile instars of *H. halys* showing how predatory Hemiptera such as nabids and reduviids can prey on the first instar nymphs and how nabids and *Podisus maculiventris* (Say, 1832) manage to prey on the second instar nymphs. Arellano et al. (2019) tested *Euthyrhynchus floridanus* (Linnaeus, 1767) (Hemiptera: Pentatomidae) on all developmental instars including the adult stage, showing some potential for biological control.

Most of the studies focused on the potential of antagonists in North America, but very little is known about the predatory communities in the European continent. A three year field survey carried out in 2014–2016 using fresh sentinel egg masses in Emilia Romagna, (Italy) showed that impact of predation on eggs never exceeded 6% with similar percentages of damages due to chewing mouthparts and pierce-sucking mouthparts. However this type of survey did not allow identification of the predators (Costi et al. 2019). A laboratory study showed that the arboreal ant species *Crematogaster scutellaris* (Olivier, 1792) (Hymenoptera, Formicidae) cannot prey on fresh eggs of *H. halys* but is very efficient in preying all the nymphal instars (Castracani et al. 2017).

The aim of this study was to evaluate the predatory ability of solitary generalist insect predators commonly found in the Italian agroecosystems on the eggs and on the first two juvenile instars of *H. halys*. In this work, we tested both predatory species collected in the field, sharing the same habitat of *H. halys*, and species of predators already used for the biocontrol of other agricultural pests, such as aphids and mealybugs. Among the field collected predators, almost all native to Europe, we included also the invasive *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae), native to Asia and detected in Italy since the 2000s, which is considered an important predator of aphids in many crops (Roy et al. 2016).

## Materials and methods

### *Halyomorpha halys* rearing

*Halyomorpha halys* was reared in climatic chambers at 26 °C, 60% RH, with a L:D 16:8 photoperiod inside clear mesh cages (30 × 30 × 30cm, approximately 40 individuals per cage) with organic tomatoes, carrots, green bean pods and raw peanuts as food. A bottle cap with a water-soaked cotton swab was used as water supply. Separate cages were used for adults and nymphs. A bean plant (*Phaseolus vulgaris*) with at least two developed leaves and pieces of papers were placed in the adult cage for egg laying. Food, water and paper were replaced twice per week. Eggs were collected daily and transferred to Petri dishes with a bean pod and wet cotton. When the nymphs reached the second instar, they were transferred to the nymphs' cage.

Predators: (1) biocontrol agents available on the market

Artificially reared insect predators were obtained from a company specialized in production of biocontrol agents for plant pests (Bioplanet s.c.a., Cesena, Italy). The tested species and instars are reported in Table 1. Predators were delivered in plastic containers with dispersing material provided by the company. In the laboratory, they were kept in optimal conditions and fed on *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) eggs, also supplied by the same company.

Predators: (2) wild biocontrol agents, field collected

Wild insect predators were collected between April and August 2018 during specific sweep-net and tree-beating sessions (from 7h00 to 10h00 am) in four urban parks with mixed trees and shrubs in Reggio Emilia (northern Italy), where *H. halys* was abundant. Field-collected species are reported in Table 1 together with information on the collection method and sites. Predators were individually collected in 50 ml Falcon tubes and kept in a refrigerated bag. Once in the laboratory, the specimens were transferred to plastic cylindrical containers (diameter 11 cm, height 6 cm with a 5 cm diameter anti-aphid net on

**Table 1** Details on the tested predatory species, including their origin and, in the case of field-collected ones, the sampling technique and sites

Order	Family	Species	Life stage	Origin	Sampling technique	Sampling sites
Coleoptera	Coccinellidae	<i>Adalia bipunctata</i> (Linnaeus, 1758)	Adult	Artificially reared		
		<i>Harmonia axyridis</i> (Pallas, 1773)	Adult	Field	Tree beating	Middle of the tree
		<i>Cryptolaemus montrouzieri</i> (Mulsant, 1853)	Larvae	Artificially reared		
		<i>Cryptolaemus montrouzieri</i>	Adult	Artificially reared		
Dermoptera	Forficulidae	<i>Forficula auricularia</i> (Linnaeus, 1758)	Adult	Field	Tree beating	Top of tree canopy
Orthoptera	Tettigoniidae	<i>Eupholidoptera chabrieri</i> (Charpentier, 1825)	Adult	Field	Sweep net	Tall grass
Neuroptera	Crysopidae	<i>Chrysoperla carnea</i> (Stephens, 1836)	2nd instar larvae	Artificially reared		
Hemiptera	Anthocoridae	<i>Anthocoris nemoralis</i> (Fabricius, 1794)	Adult	Artificially reared		
	Nabidae	<i>Himacerus mirmicoides</i> (O. G. Costa, 1834)	Adult	Field	Sweep net	Alfalfa, tall grass
	Reduviidae	<i>Rhynocoris iracundus</i> (Poda, 1761)	Adult	Field	Tree beating and sweep net	Shrubs
		<i>Nagusta goedelii</i> (Kolenati, 1857)	Adult	Field	Tree beating	Top of tree canopy

the lid to allow airflow) kept at room temperature ( $23 \pm 1$  °C) and provided with a mixed diet that included 2nd instar larvae of *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae), 2nd instar larvae of *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae), field-collected aphids and *E. kuehniella* eggs. For the omnivorous species *F. auricularia* and *E. chabrieri*, diet included also carrots, green beans and peanuts. A bottle cap with a water-soaked cotton swab was used as water supply. Field-collected specimens were kept in these conditions for 1–3 days before the beginning of the predation test, in order to have at least four individuals of the same species ready. Preliminary trials showed that, after four days under these conditions, the survival of the field-collected specimens of all the tested species was higher than 90%.

#### Predation test

Experimental arenas consisted of transparent cylindrical plastic boxes (20 cm height, 10.5 cm diameter) with a 5 cm diameter anti-aphid net on the lid to allow airflow and one bean plant bearing at least two well-developed leaves, which was placed in the center of the box. Four *H. halys* prey items were considered: (1) one freshly laid (<24 h) egg mass with at least 21 eggs; (2) one hatched egg mass with at least 18 first instar nymphs (1–2 days old); (3) five second instar nymphs (1–2 days old); (4) two adults (one male and one female), only when *R. iracundus* was tested as predator. All prey items were collected from the laboratory rearing. Only the egg masses laid on paper were used for the trials. One prey item was randomly placed on one leaf of the plant. In the case of unhatched/hatched egg masses the paper support was clipped to the underside of the leaf. Each tested predator was randomly released, dropping it on the leaf of the plant from above after a 24 h starvation

period. The box was closed with the lid and placed inside in a climatic chamber 26 °C, 60% RH, with a L:D 16:8 photoperiod. After 48 h, the box was opened, and the status of the prey items was carefully checked under a stereomicroscope for signs of predation according to the predator's mouthparts, recording if they were damaged or dead. The unhatched egg masses were kept inside the climatic chamber at the previously described conditions for five days inside 7 cm diameter Petri dishes to check for emergence of nymphs. In case of eggs showing signs of predation, they were considered damaged if after five days the nymphs emerged, and dead if no nymphs emerged. In the case of nymphs and adults the ones that showed missing body parts or did not move after stimulation with a brush were considered damaged and dead, respectively. The status of the predator at the end of the trial was also recorded (alive/dead).

For each considered predatory species/instar, a minimum of eight replicates was performed for each type of prey item. For each replicate of each combination predator–prey item, a control replicate was carried out at the same time with the same procedure but without the presence of the predator. Each predator was tested only once. Trials were staggered due to predator availability.

### Statistical analysis

Generalized linear model (GLM) with a binomial error structure (logit link function) was used to compare the number of dead prey items and survivors between the treatment (simultaneous presence of predator and prey item) and the respective control (only prey items). For egg mortality, a comparison was made between the number of hatched eggs in treatment and control.

Four separate analyses were run on the different stages of *H. halys*: eggs, first instar nymphs, second instar nymphs and adults. In each analysis a single GLM with two factors (treatment and predator type) and their interaction was used. The only exception was the one on *H. halys* adults where only one predator (*R. iracundus*) was tested.

All statistical analyses were performed using R version 3.6.3 (R Core Team 2019). To assess the general significance of treatment, predatory type and their interaction, an analysis of deviance of the fitted model with Wald statistics  $\chi^2$  was performed. To find which predator type produces a significant statistical

difference in mortality between the two treatments, a multiple comparison approach was used (Bretz et al. 2011) using the `ghlt` function of the `mulcomp` package with ad-hoc set of contrasts. This procedure is conducted on the scale of the linear predictor (logit) and, assuming asymptotically normally distributed parameter estimates, produces z-values and p-values associated with this assumption (Hothorn et al. 2008).

In three cases a so-called “complete separation” was found in the data (zero survival or zero deaths in some treatment-predator type combinations). This is a well-known problem in GLM because it causes a substantial failure in the maximum likelihood procedure to estimate parameters (Lesaffre and Albert 1989). A suitable R package (`brglm2`) was used to deal with this problem.

## Results

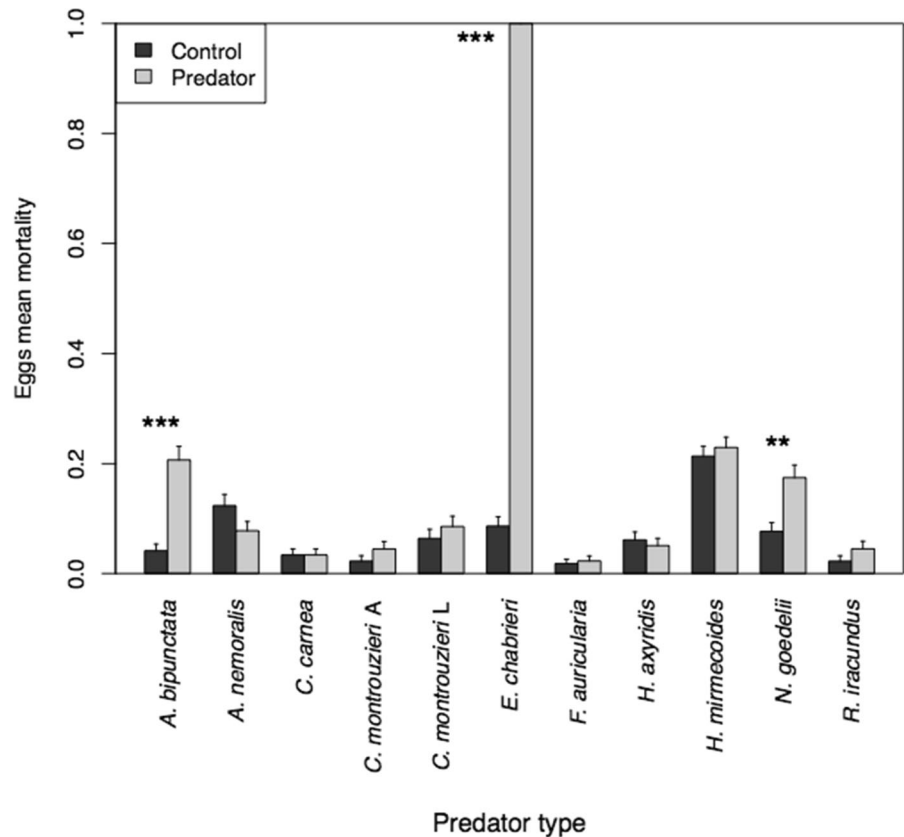
### Predation bioassay

As a preliminary result, a high survival of all predators was recorded in the experiments. Survival was often between 90 and 100% and just in the case of 2nd instar larvae of *C. carnea* the minimum predator survival was 86%.

For each type of prey, there were cases of complete separation. In the case of eggs, no eggs survived in the presence of *E. chabrieri*. In the case of N1 no deaths were recorded in the control of *N. goedelii* and for N2 no deaths were recorded in the presence of *H. axyridis*.

For each type of prey there is a significant general difference between the control and the presence of the predator (eggs:  $\chi^2 = 12.34$ ;  $df = 1$ ;  $p < 0.001$ . N1:  $\chi^2 = 78.42$ ;  $df = 1$ ;  $p < 0.001$ . N2:  $\chi^2 = 27.18$ ;  $df = 1$ ;  $p < 0.001$ ). A significant general variability emerges (Figs. 1, 2, 3) also among predators for all types of prey (eggs:  $\chi^2 = 926.90$ ;  $df = 10$ ;  $p < 0.001$ . N1:  $\chi^2 = 358.19$ ;  $df = 10$ ;  $p < 0.001$ ; N2:  $\chi^2 = 73.40$ ;  $df = 10$ ;  $p < 0.001$ ), and this variation affects both control and predator treatments. This underlying natural variability is captured only because the control treatment (absence of predator) was repeated for every predator treatment. More importantly, for the aim of this experiment, several significant differences on prey mortality were detected for the interactions (combinations) of predator and treatment in every prey type analysis (eggs:  $\chi^2 = 70.07$ ;  $df = 10$ ;  $p < 0.001$ . N1:

**Fig. 1** Comparison of the mean mortality of *Halyomorpha halys* eggs observed in the presence of the predator and in the relative control after 48 h. Error bars indicate SE. Asterisks indicate a significant difference in mortality between control and treatment (predator): \*\*\*p < 0.001; \*\*p < 0.01



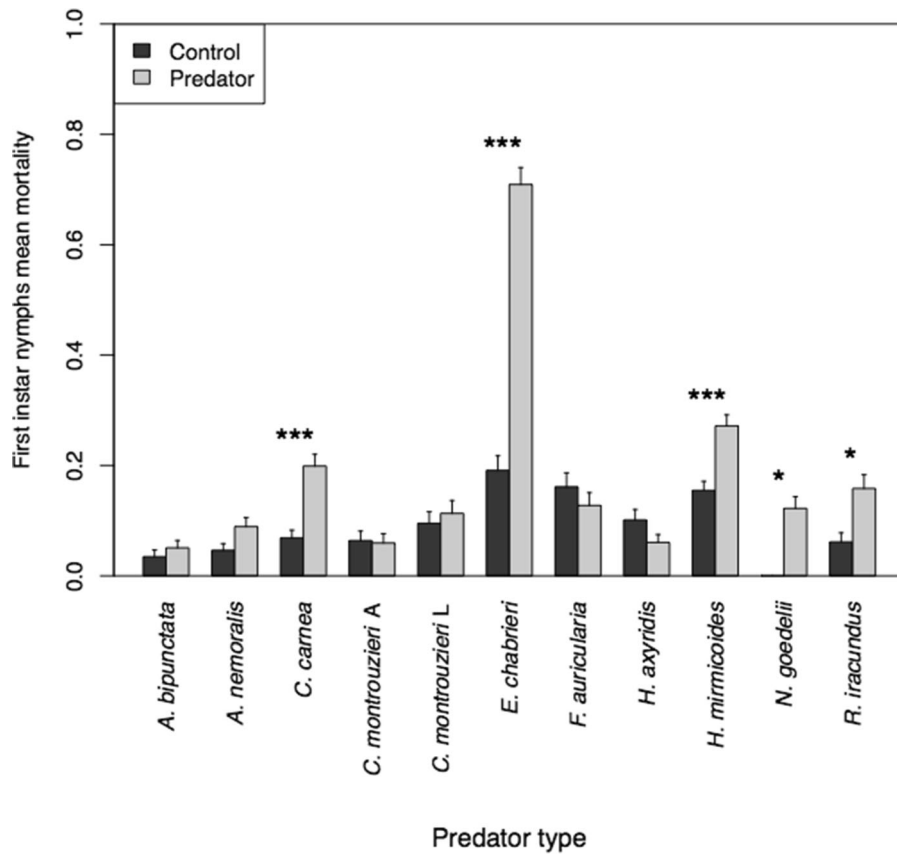
$\chi^2 = 96.06$ ;  $df = 10$ ;  $p = 0.001$ . N2:  $\chi^2 = 57.26$ ;  $df = 10$ ;  $p < 0.001$ ), indicating that, for the same type of prey, predators showed remarkable differences in efficacy (Figs. 1, 2, 3).

The number of hatched eggs was significantly reduced in presence of *A. bipunctata* (prey mortality treatment = 0.20; prey mortality control = 0.05;  $z$ -value = 5.22;  $p < 0.001$ ), *E. chabrieri* (prey mortality treatment = 1.00; prey mortality control = 0.09;  $z$ -value = 8.66;  $p < 0.001$ ) and *N. goedelii* (prey mortality treatment = 0.16; prey mortality control = 0.00;  $z$ -value = 3.35;  $p = 0.009$ ) (Fig. 1). When *F. auricularia* was tested, 4% of the eggs showed attempts of attack with mandibles. Nevertheless, this type of damage did not affect eggs hatching.

The survivorship of *H. halys* 1st instar nymphs was significantly reduced in presence of the 2nd instar larvae of *C. carnea* (prey mortality treatment = 0.20; prey mortality control = 0.08;  $z$ -value = 4.73;  $p < 0.001$ ), and the adults of *E. chabrieri* (prey mortality treatment = 0.71; prey mortality control = 0.20;  $z$ -value = 10.25;  $p < 0.001$ ), *H.*

*mirmecoides* (prey mortality treatment = 0.21; prey mortality control = 0.16;  $z$ -value = 4.41;  $p = 0.001$ ), *R. iracundus* (prey mortality treatment = 0.16; prey mortality control = 0.07;  $z$ -value = 3.04;  $p = 0.03$ ) and *N. goedelii* (prey mortality treatment = 0.13; prey mortality control = 0.00;  $z$ -value = 3.02;  $p = 0.02$ ) (Fig. 2). The survivorship of *H. halys* 2nd instar nymphs was significantly reduced in presence of adults of *E. chabrieri* (prey mortality treatment = 0.90; prey mortality control = 0.24;  $z$ -value = 5.76;  $p < 0.001$ ) and *R. iracundus* (prey mortality treatment = 0.80; prey mortality control = 0.14;  $z$ -value = 5.58;  $p < 0.001$ ) (Fig. 3). *R. iracundus*, the only predator tested on *H. halys* adults, significantly reduced their survivorship (prey mortality treatment = 0.44; prey mortality control = 0.00;  $z$ -value = 2.08;  $p = 0.03$ ).





**Fig. 2** Comparison of the mean mortality of *Halyomorpha halys* first instar nymphs observed in the presence of the predator and in the relative control after 48 h. Error bars indicate SE.

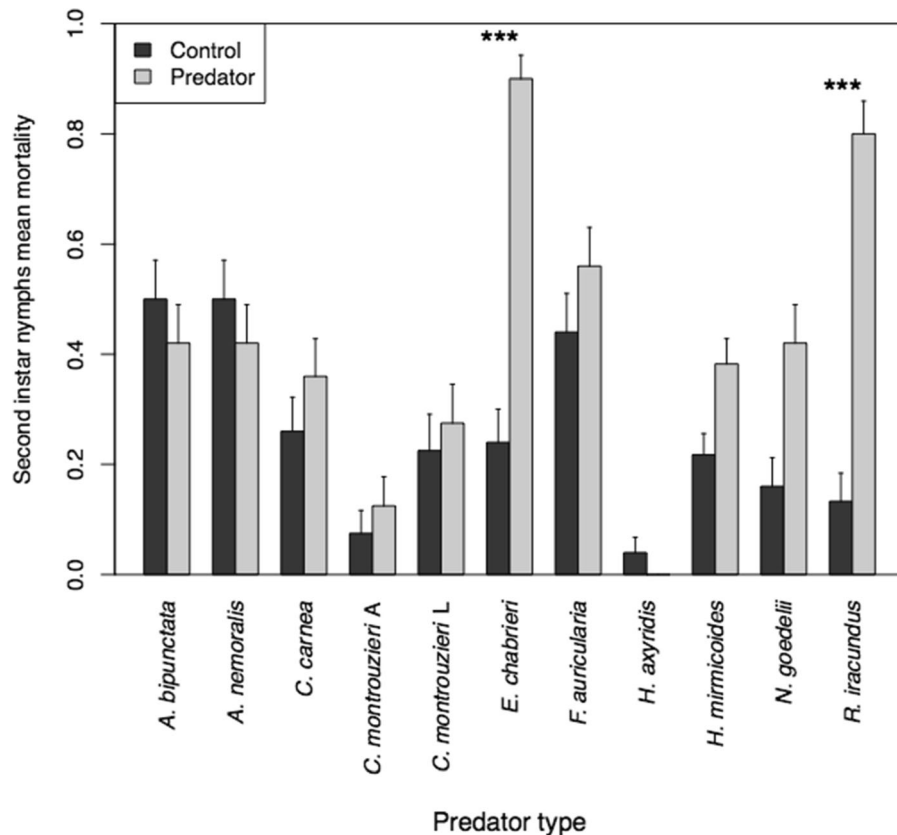
Asterisks indicate a significant difference in mortality between control and treatment (predator): \*\*\* $p < 0.001$ ; \* $p < 0.05$

**Discussion**

The present study is the first to screen in Europe the predatory potential of generalist insect predators on the invasive *H. halys*, considering both species commonly reared for biocontrol purpose of plant pests and species commonly found in the field, sharing the same habitat of the invasive pest. According to our results, although six out of ten predators significantly reduced the survival of at least one of the life stages of *H. halys* provided, only two species caused a mortality higher than 80% (*E. chabrieri* and *R. iracundus*) and in the other cases mortality of the prey items never exceeded 60%. Besides, four of the tested species showed no attempts of predation despite starving for 72 h in total (24 h food deprivation before the trial and 48 h of the assay). The low acceptance of the life stages of *H. halys* as prey that occurred in the laboratory during the forced feeding bioassays

suggests that in field conditions, where the predators have access to alternative prey items, these species are likely to have a quite low impact on the overall mortality of the invasive stinkbug. Although the present study is not exhaustive, as it did not include other species of arthropod generalists potentially effective in predating *H. halys* (i.e. Coleoptera Carabidae, other species of Orthoptera, spiders), it appears that results obtained with species typical of the European context are substantially similar to the ones obtained in North America (Morrison et al. 2016; Pote and Nielsen 2017).

Considering the candidates with chewing mouthparts, among the tested Coccinellidae only *A. bipunctata* showed significant predation, but only on the eggs of *H. halys*. The ability to consume *H. halys* eggs was observed in a laboratory study in a tridimensional arena only in adults of *Coccinella septempunctata* (Linnaeus, 1758) (Pote and Nielsen 2017). In Petri



**Fig. 3** Comparison of the mean mortality of *Halyomorpha halys* second instar nymphs observed in the presence of the predator and in the relative control after 48 h. Error bars indicate

SE. Asterisks indicate a significant difference in mortality between control and treatment (predator): \*\*\* $p < 0.001$

dish studies also *Coleomegilla maculata* (De Geer) adults (Morrison et al. 2016; Abram et al. 2014), *C. maculata* second instar larvae (Abram et al. 2014), and *H. axyridis* adults (Morrison et al. 2016) occasionally fed on the eggs. All the other tested coccinellids, that included also *C. montrouzieri*, were totally ineffective (Morrison et al. 2016; Pote and Nielsen 2017; Poley et al. 2018). In the laboratory study by Pote and Nielsen (2017) no predatory ability was observed on young *H. halys* nymphs by the adults of *C. septempunctata*, *C. maculata* and *H. axyridis*. Coccinellids are essential biocontrol agents of soft-bodied arthropods, especially aphids (Rutledge et al. 2004), but apparently only very few of them seem to have a role in predating eggs and nymphs of this invasive pentatomid, likely only under specific circumstances.

The dermapteran *F. auricularia* was not effective in predating the first and second instar nymphs, but it damaged the eggs, although without affecting their

survival. According to Poley et al. (2018), *F. auricularia* did not show significant predation on *H. halys* eggs in laboratory tests (only one specimen over ten tested was able to predate two eggs after five days), whereas in the field Forficulidae species appear to be the most efficient predators of *H. halys* sentinel egg masses (Poley 2017). Plant protection personnel inspecting fruit crops in northern Italy report similar observations (personal observation). According to Morrison et al. (2016), *H. halys* eggs were attacked by specimens of the family Forficulidae, showing incomplete chewing. However the species were not identified in their study. Forficulidae have been recognized as important biocontrol agents of insect pests in fruit orchards (Suckling et al. 2006) and it is therefore likely that some Dermaptera, which are typically omnivorous species, can exploit *H. halys* eggs as food source, but probably *F. auricularia* is not the most efficient predator for this prey item.



The tettigoniid *E. chabrieri* proved to be an excellent predator for all the prey items taken into consideration. In general, Orthoptera have always shown good predatory efficacy on eggs (Morrison et al. 2016; Pote and Nielsen 2017; Poley et al. 2018). In our trials *E. chabrieri* successfully predated the young nymphs, probably due to its large size (adults can be 2–3 cm in length). However, being an omnivore, during the predation test it also fed on the bean plant used as support.

The second instar larva of the neuropteran *C. carnea* was able to prey on the first instar nymphs, but not on the eggs. In the study of Abram et al. (2014), *C. carnea* negatively affected the survival of *H. halys* eggs, but in that case, both the egg mass and the predator were positioned inside a Petri dish (diameter 10 cm, height 2 cm). Our experiments were performed in more natural conditions, with the egg masses placed underneath a leaf, and this might have affected the ability of the predator to detect/attack the prey items.

Among the stylet-sucking predators, the anthocorid *A. nemoralis* did not reduce the survival of any of the prey items provided. Anthocorids are predators of small soft-bodied phytophagous arthropods (mites and insects) and their eggs, and some species are reared as biocontrol agents of plant pests (Lattin 1999). The genus *Orius* is known as an egg predator of *H. halys* in its native range (Lee et al. 2013). Previous studies by Morrison et al. (2016) and Poley et al. (2018) found that *O. insidiosus* can predate *H. halys* eggs, although at quite low rates. Despite having similar size (3 mm), *A. nemoralis* is not as efficient as *Orius sp.*, possibly because its mouthparts are too weak to penetrate the *H. halys* egg shell or due to different predatory behavior.

The nabid *H. mirmicoides* was effective in predated only the first instar nymphs. These results confirm the previous findings obtained on *Nabis sp.* by Pote and Nielsen (2017) and on unidentified nabids by Morrison et al. (2016), but in our case the identification of the predatory specimens was made at the species level, providing a better insight on the role of these predatory hemipterans.

Considering Reduviidae, *N. goedelii* significantly predated the eggs and the first instar nymphs, while *R. iracundus* proved effective against the first nymph instar and the second instar nymphs and the adults, but not on the eggs. *R. iracundus* predated more males (six out of eight) than females (three out of eight), probably

because *H. halys* males are generally smaller than females, so perhaps they are easier to be manipulated and successfully predated. The reduviid *Isyndus obscurus* (Dallas) is known to predate nymphs and adults of *H. halys* in native Japan (Lee et al. 2013). In the laboratory, American reduviid species were totally ineffective on eggs of *H. halys* (Morrison et al. 2016; Pote and Nielsen 2017). However *Arilus cristatus* (L.) and *Sinea spinipes* (Herrich-Schaeffer) significantly reduced the survival of first instar nymphs (Pote and Nielsen 2017). Other laboratory trials (Jones 2013) indicate that *A. cristatus* adults were the most efficient predators of *H. halys* adults, and that *A. cristatus* nymphs efficiently predated first, second and third instar nymphs. Our study confirms that predatory Heteroptera like some species of Nabidae and Reduviidae seem to play a significant role in consuming the nymphs and adults of *H. halys*.

The comparisons of performance among the predators that significantly reduced survival on at least one prey item indicated that *E. chabrieri* was the most effective, consuming all the eggs and predated 90% and about 60% of the second and first instar nymphs, respectively. Like the majority of the other predators, *R. iracundus* was not able to consume eggs, but it had an efficacy similar to that of *E. chabrieri* in predated second instar nymphs (80%), and successfully predated 43% of the adults. Therefore *E. chabrieri* and *R. iracundus* are the most effective predators of *H. halys* among the species considered in this study, possibly because they were the biggest in size and likely also with the strongest mouthparts.

Interestingly, eggs were the least predated item among the ones offered to the tested predators, as they were consumed only by the tettigoniid *E. chabrieri* and to a much lesser extent also by the coccinellid *A. bipunctata* and the reduviid *N. goedelii*. Very little/no consumption was recorded by the predators tested in the already cited studies of Abram et al. (2014), Pote and Nielsen (2017), Morrison et al. (2016) and Castracani et al. (2017). In our experiments, the egg masses were fresh, laid during the previous 24 h on a paper support, that was clipped underneath a leaf, in order to simulate the natural situation, and no observations were performed during the 48 h of exposure to the predators. Hypotheses to explain the unsuccessful *H. halys* egg predation include: (1) physical–mechanical factors: the mouthparts of the tested predators were either too weak or too small to affect the eggs,

due to their size and/or the toughness of the chorion. This is possibly the case of *F. auricularia*, which caused some damage, though not sufficient to prevent hatching; (2) chemical factors: the freshly laid eggs might lack semiochemicals that allow their recognition as suitable prey or possess repellent semiochemicals; (3) behavioral factors: eggs are immobile and are not detected/attacked by those predators that need the mobility of the prey to trigger the predatory behavior, like reduviids (Haridass et al. 1988); (4) the position of the egg mass underneath the leaf, that might prevent optimal access, manipulation, grasping/piercing of the eggs by some of the predators; (5) a combination of the previous. Further investigations on the physical/chemical egg features and behavioral studies could shed a light on these aspects.

In our experiment, the survivorship of second instar nymphs was usually lower than that of first instar ones and hypotheses to explain this include: (1) differences in the production of the defensive compounds typically emitted upon disturbance. Instar-specific compounds have already been demonstrated in other species of Pentatomidae (Borges and Aldrich 1992); (2) behavioral factors related to mobility cues: first instar nymphs have a very reduced mobility as they usually remain on the egg mass to acquire the symbionts necessary for their survival (Taylor et al. 2014). Thus, to some species of predators they might represent less suitable prey items compared to the second instar nymphs, which are very mobile (Lee et al. 2014).

In this work we demonstrated that the commercially available predators are not effective, except for *A. bipunctata*, that slightly decreased the survival of the eggs, and *C. carnea* that slightly decreased the survival of the first instar nymphs. Among the field-collected specimens, we showed that only one species proved to be able to predate all the developmental stages of *H. halys*, almost none attack the eggs but some species in the families Tettigonidae, Nabidae and Reduviidae can attack the young nymphs.

*Halyomorpha halys* usually lays its eggs under the leaves of shrubs and trees mainly at the top, and a predator capable of preying on both eggs and first instar nymphs and which during our sampling was found mainly on the treetops is *N. goedelii*, a Reduviid known to be a predator of small arthropods inhabiting deciduous trees (Dioli 2013). From the second instar onwards, the nymphs become very mobile and begin

walking up and down from plant to plant (Acebes-Doria et al. 2017). While on the trees, all nymphal instars can be predated by the arboreal ants *C. scutellaris* (Castracani et al. 2017). On their way down, both nymphs and adults can be intercepted by *R. iracundus*, found mainly in shrubs and in the lower parts of the trees (Rieger 1972). While moving on the ground from one plant to the other, the nymphs can be attacked by the nabid *H. mirmicoides* and the tettigonid *E. chabrieri*, typically found in the grassy areas. Egg masses could be occasionally predated/damaged by some Dermaptera and coccinellids. However they are better exploited by egg parasitoids and the predominant and most successful species in Europe is the generalist *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) (Haye et al. 2015b; Costi et al. 2019; Moraglio et al. 2020).

Thus, by identifying some of the species of the community of natural enemies that can exploit *H. halys* as a suitable prey in southern Europe, the present investigation provides an important contribution for conservation biological control of this invasive species. In this view, a significant implementation will be provided by more detailed field and laboratory investigations, such as a study on the gut content of the arthropod predators sharing the habitat with *H. halys* and surveys on the myrmecofauna of the agroecosystems affected by *H. halys*.

**Acknowledgements** The authors are grateful to Joshua Gearing for English editing. The work was partially funded by the Emilia Romagna region, within the Rural Development Plan 2014–2020 Op. 16.1.01-GO-PEI-Agri-FA 4B, Pr. «HALYS», coordinated by CRPV.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that there are no known conflicts of interest associated with this publication.

**Research involving human and/or animal rights** This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

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