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SEARCHING FOR NATIVE EGG-PARASITOIDS OF THE INVASIVE ALIEN SPECIES *HALYOMORPHA HALYS* STÅL (HETEROPTERA PENTATOMIDAE) IN SOUTHERN EUROPE

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Roversi P.F., Binazzi F., Marianelli L., Costi E., Maistrello L., Sabbatini Peverieri G. – Searching for native egg-parasitoids of the invasive alien species *Halyomorpha halys* Stål (Heteroptera Pentatomidae) in Southern Europe.

Halyomorpha halys is an invasive species, originating from East Asia, which was accidentally introduced in some areas of North America and Europe, where severe damages to different vegetable crops and fruit plants were recorded. Several studies in different countries focused on the possibility to apply biological control by means of egg-parasitoids. The main egg-parasitoids in the original area are unfortunately non-specific and thus the application of classical biological control is questionable. On the other hand, the possibility of using augmentative biological control by native egg-parasitoids able to exploit the new host is an interesting possibility in both Europe and the USA. In a preliminary assay, frozen egg masses of *H. halys* were exposed in the field in Central Italy. Results showed that some eggs were exploited by parasitoids belonging to the species *Anastatus bifasciatus* and *Ooencyrtus telenomicida*. Therefore, different native egg-parasitoids species widespread in Europe were tested in the laboratory in order to explore their ability to parasitize *H. halys* eggs. To this end, no-choice tests were carried out in climatic chambers (26°C, 70%RH, 16:8 L:D) in order to evaluate the parasitization potential of females collected in the wild. Conducted tests pointed out that *A. bifasciatus* and especially *O. telenomicida* may be potential candidates for the biological control of *H. halys*. In particular, *O. telenomicida* was able to parasitize the 35.56% of the exposed host eggs in the lab tests. New adults successfully emerged from the 22.92% of the eggs, and on the whole, this egg-parasitoid caused a significant hatching reduction of the *H. halys* eggs (more than 70%). Conversely, *Telenomus chloropus* and *Ooencyrtus pityocampae* were less promising species as biocontrol agents of this agricultural pest. Preliminary trials in pear orchards consisting of field releases of laboratory-reared specimens of *O. telenomicida,* one of the potential candidates for biological control, showed that this parasitoid is actually able to discover egg masses of *H. halys* (sentinel frozen egg masses) and to successfully parasitize them.

KEY WORDS: pest control, biological control, Brown Marmorated Stink Bug, natural enemy.

INTRODUCTION

Halyomorpha halys Stål (Heteroptera, Pentatomidae) is a polyphagous and herbivore insect species originating from East Asia, which became invasive in many regions of the World. The first records in the field, outside its original area, were reported in Pennsylvania USA, in 2001 (HOEBEKE and CARTER, 2003). In Europe*, H. halys* was officially recorded for the first time in Switzerland in 2007 (WERMELINGER *et al.*, 2008) while, in Italy, it was observed in 2012 in Modena (Emilia Romagna Region, Northern Italy) (MAISTRELLO *et al.*, 2014). Subsequently, further records were also reported in other regions of Northern Italy (Lombardy, Piedmont, Veneto and Friuli Venezia Giulia) and Central Italy (Tuscany) (BARISELLI *et al*., 2016; MAISTRELLO *et al.* 2016; SFR REGIONE TOSCANA, 2016; SFR REGIONE FVG, 2015). Genetic analyses showed that Italian populations may have reached the mainland following two different routes, the first from Switzerland and the second from Asia and/or North America (CESARI *et al.*, 2015). In all the new invaded regions *H. halys* spread more or less rapidly, becoming, worldwide, a serious pest due to its capacity to damage field and vegetable crops and tree fruit orchards (HAYE *et al*., 2015a; HOEBEKE & CARTER, 2003). Soybean, peaches, pears, apples, cherries are only some examples of strongly damaged plants (BERNON, 2004). *H. halys* feeds on the green parts of plants even if the most severe damages are generally recorded on fruits, which are often rendered unmarketable. Females lay their eggs during the growing season on the underside of plant leaves, in monolayer egg masses with approximately 28 eggs per batch (BERNON, 2004; RICE *et al*., 2014). Moreover, it is able to develop one, two or more generations per year, depending mainly on temperature (LEE *et al*. 2013). So far, control methods of this invasive species have been based on non-selective chemicals, which, however, led to contradictory results in terms of efficacy (LEE *et al*., 2013; HERLIHY *et al.*, 2016). Aggregation pheromones are also available even though they are suitable only for population monitoring, but not for mass trapping or sex disruption (LESKEY *et al*., 2012; HERLIHY *et al*., 2016). Therefore, more attention has been recently paid to biological control strategies and different studies have been performed in the USA, Europe and Asia (HAYE *et al*., 2015b; HERLIHY *et al*., 2016; RICE *et al*., 2014; OGBURN *et al.*, 2016). Investigations conducted in the field of classical biological control emphasized the potential use

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of egg-parasitoids native to the original area of the pest (TALAMAS *et al*., 2015, LARA *et al*., 2016). However, the most interesting candidates of the *H. halys* egg-parasitoid complex [*i.e. Trissolcus japonicus* Ashmead and *Trissolcus cultratus* Mayr (Hymenoptera, Platygastridae)] did not show any host specificity (HAYE *et al*., 2015b). The risk of using generalist species (with more or less host fidelity) in classical biological control is well known by experts and institutions (e.g. FAO, EPPO, IPPC), and many recommendations have been given for a careful use (SIMBERLOFF & STILING, 1996; MESSING & WRIGHT, 2006; VAN LENTEREN *et al*., 2003). Therefore, more recently, the focus has been on exploring the possibility to use native egg-parasitoids to control invasive species through augmentative approaches (SIVINSKI, 2013; COLLIER & VAN STEENWYK, 2004; CHAILLEUX *et al*., 2012). Studies aimed at selecting potential candidates from among native egg-parasitoids, to control *H. halys*, have been performed in Europe and in USA (HAYE *et al*., 2015b; HERLIHY *et al*., 2016). Regrettably, much more remains to be done and further investigations in different countries are required in order to search for an effective biocontrol agent to be used in augmentative control programs. Despite these difficulties, biological control is still regarded as "the most environmentally safe and economically profitable pest management method" (VAN LENTEREN, 2012). Moreover, biocontrol agents of *H. halys* could be also considered as a "complex" of more species (HAYE *et al*., 2015b). In fact, as in a "joint venture", they might play a significant role in the control of *H. halys*, and represent a key element of IPM strategies.

In the present work, native egg-parasitoids collected in Central Italy were investigated in order to evaluate their ability to exploit eggs of *H. halys*. In particular, the aim was to found effective native natural enemy/ies of this invasive pest to be used in augmentative biological control programs.

MATERIALS AND METHODS

ORIGIN OF THE INSECTS AND REARING PROCEDURE

In June 2016, a *H. halys* laboratory colony was established by field collection of adults and juveniles on hedgerows (*Laurus nobilis*, *Prunus laurocerasus*) and fruit trees (*Pyrus communis*, *Ficus carica*, *Prunus domestica*, *Prunus avium*) in Tuscany (Central Italy). Adult specimens were laboratory reared in insect cages (30x30x70 cm) with screen mesh and provided with hand paper stripes as ovipositioning substrate; fresh beans of *Phaseolus vulgaris* and seeds of *Arachis hypogaea* were offered as food and replaced three times per week, while a water source was added through moistened cotton. Eggs and juveniles of all stages were reared in small transparent plastic boxes (24x18x6 cm) with a screen mesh for ventilation. Fresh beans of *P. vulgaris* were offered as food that was replaced three times per week. Once young individuals reached the adult stage, they were transferred from the plastic boxes into the insect cages. Egg masses of *H. halys* were daily removed, producing a set of fresh egg masses (< 24h old) to use in the tests. Insect rearing was performed in an environmentally controlled rearing room at 26°C and 16:8 L:D photoperiod.

In order to obtain suitable egg-parasitoid species to test in the present work, eggs of *Dolycoris baccarum* L. (Heteroptera, Pentatomidae) and *Gonocerus juniperi* (Herrich-Schaeffer) (Heteroptera, Coreidae) were collected during June-July 2016 on *Juniperus communis* and *Corylus avellana* in uncultivated areas of Tuscany (mixed flora in the herbaceous layer, with bushes and trees of *Spartium junceum, J. communis, C. avellana, Acer campestris, Cupressus sempervirens*) not yet known to be infested by *H. halys*. Field-collected eggs were reared individually in glass tubes (15 cm long, 2 cm Ø) closed at both ends with screen mesh and located in climatic chambers at 26°C, 70%RH and 16:8 L:D (standard rearing and test conditions adopted in the present work), until egg hatching or egg-parasitoids emergence. Emerged egg-parasitoids were identified, sexed and reared in glass tubes where they were fed with pure honey *ad libitum*. From field collected heteropteran eggs, the following egg-parasitoids emerged: *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera, Encyrtidae), *Telenomus chloropus* (Thomson) (Hymenoptera, Platygastridae) and *Anastatus bifasciatus* (Geoffroy) (Hymenoptera, Eupelimdae). From additional eggs of *Thaumatopoea pityocampa* (Den. et Shiff.) (Lepidoptera, Thaumetopoeidae) collected in mixed *Pinus* spp. stands in Tuscany, specimens of *Ooencyrtus pityocampae* (Mercet) (Hymenoptera, Encyrtidae) were obtained.

EXPERIMENTAL PROCEDURES FOR LABORATORY TESTS

In collected egg-parasitoid species that displayed sexual behavior (*i.e. O. telenomicida*, *T. chloropus* and *A. bifasciatus*), newly hatched females were housed individually in a glass tube and paired each with a male of the same species and origin for mating. Females and males were maintained at standard conditions in a climatic chamber for 5 days and fed with honey until the beginning of the tests. In egg-parasitoid species which display a parthenogenetic reproduction behaviour (*O. pityocampae*), newly hatched females which emerged from the field-collected lepidopterans eggs, were housed individually for 5 days and reared in the same way, as described above, until the beginning of the test.

Parasitization ability of single field collected egg parasitoids, was tested in a no-choice experiment, offering each female a single batch of fresh *H. halys* eggs (<24h old) for 24h in climatic chambers at standard conditions; only masses with 26-30 eggs were used in the tests. After parasitization, each egg-batch was removed from the test tube, reared at standard conditions in a separate glass tube and daily checked until eggs hatched or egg-parasitoids emerged. In the tests the following parameters were recorded: n. of hatched eggs (n. of eggs from which a nymph of *H. halys* emerged), n. of parasitized eggs (n. of eggs with signs of parasitization as described below), n. of successfully parasitized eggs (n. of eggs from which at least one egg-parasitoid successfully emerged), n. of eggs with no host hatching and no egg-parasitoid emergence, n. of emerged egg-parasitoids, their sex ratio [♀/(♀+♂)] and their juvenile development time (in days). By careful inspection of *H. halys* eggs exposed to the egg-parasitoid females, some specific signs revealing the occurrence of parasitization were observed. In particular, in both *Ooencyrtus* species one or more egg stalks protruding outside the host chorion were visible, while in *A. bifasciatus,* a dark spot on the egg chorion where the female inserted the ovipositor was detected; moreover, parasitized host eggs generally turned to black few days after parasitization (MAPLE, 1947; VIGGIANI, 1994). All tests were replicated 10 times per each egg-parasitoid species.

FIELD EXPOSURE OF FROZEN *H. HALYS* EGGS IN NON-INFESTED SITES

Freeze-killed sentinel eggs, were exposed in Tuscany in non-infested areas, with the purpose of investigating the ability of native egg-parasitoids to detect and parasitize them in the field. Fresh egg masses of *H. halys* (n. 20 masses of 26.05 eggs on overage) were collected from the rearing units in the laboratory, freeze-killed at -80°C (Haier® ULT Freezer DW86L388) and then stored for 7-14 days. Several studies stressed that, generally, freezing host eggs at low and/or ultra-low temperatures does not significantly affect the egg-parasitoid capacity to complete their development, and frozen egg masses, including those of *H. halys*, are often used in several studies as sentinel eggs (Binazzi *et al*. 2015a; Haye *et al*., 2015b; Sabbatini Peverieri *et al*., 2015; Herlihy *et al*., 2016). However, it is also known that the use of sentinel eggs, even if not frozen, can underestimate rates of parasitism and species complex (Jones *et al.,* 2014).

In this work frozen eggs have been exposed for 3 days in the field in the same sites where egg masses of other heteropterans had been collected to obtain the egg parasitoids (described above). *H. halys* frozen eggs have been exposed once a month from May to August 2016, using each time 5 egg masses (see Results for details). Once removed from the field, the egg masses were reared at standard conditions in climatic chambers until egg-parasitoids emerged. The emerged egg-parasitoids were then identified, counted and sexed.

Ability of a lab-reared egg-parasitoid to parasitize eggs in an *H. halys* infested site

In an area of Modena Province in the Emilia-Romagna Region (Northern Italy), known to be infested by *H. halys* (Maistrello *et al*., 2016), 6 organic pear orchards were selected at the end of August 2016, in order to perform a preliminary tests in the field using the most promising egg-parasitoid selected in the present work. The scope was to investigate the ability of the selected egg-parasitoid to detect and parasitize under field conditions, the eggs of *H. halys.* In a field study performed throughout the previous two years using fresh sentinel egg masses in the same area of tests, only *A. bifasciatus* had emerged (Costi *et al.,* 2016; Maistrello, pers. comm.).

In order to carry out the trials, fresh egg masses of *H. halys* laid by females on paper stripes were collected from the rearing unit and treated in the same way we described above (n. 90 egg masses of 26.34 eggs on average). Before freezing at -80°C, each egg mass had been individually fastened by staples on a green cardboard (1.5 cm x 8 cm).

On September 13, 2016, in 4 pear orchards of the 6 previously selected, egg masses were exposed directly on pear trees in a central area of the orchard, while in the other 2 orchards, egg masses were exposed on the hedgerows close to the pears (see Results) for details; hedgerows were set at a distance of approximately 3m from the first row of pear trees in the orchard). In the experimental design of each of the first 4 sites, 4 pear trees were selected in a square, where each tree was located at one edge of the figure and a fifth tree was in the middle of it. On the whole, pear trees belonged to three different and consecutive plant rows of the orchard. In the other 2 sites, in mixed hedgerows, *H. halys* egg masses were set on 5 plants along the border, at a distance of 2m between trees. In total, 30 plants were selected for the tests, 5 plants per each site.

In the field, cardboards bearing *H. halys* egg masses were fastened on the underside of plant leaves by wire paper clips. On each of the selected plants (in both the pear orchards and the hedgerows), 3 egg masses were fastened, one at each of the following heights from the soil: 1m, 1.5m and 2m. In every site, the selected egg-parasitoid were released from adequate dispensers soon after the egg masses had been fixed on the plants. The egg-parasitoids dispensers consisted of the same type of glass tubes used for rearing in the laboratory, and were fastened at 1.5m in height from the soil on the trunk or main stem of each plant. Leaves bearing *H. halys* egg masses were approximately at 50 cm from the release dispenser. Field exposure of *H. halys* was designed so has to remove them after 2 days from the egg-parasitoid release (Herlihy *et al*., 2016). Then, removed eggs were reared under standard conditions in a climatic chamber until egg-parasitoids emergence.

The data obtained in the present work (see Results) suggested that *O. telenomicida* might be a valid candidate for the field control of *H. halys*. Hence this species was selected in order to plan the further field releases trials. In fact, *O. telenomicida* is a polyphagous species that can be easily reared on many different hosts in the laboratory, among these, *Graphosoma lineatum* L. (Heteroptera Pentatomidae) (Roversi, pers. comm.). Therefore, a colony of *O. telenomicida* was established in the laboratory and reared in a climatic chamber at standard conditions using *G. lineatum* eggs; host egg masses for the egg-parasitoid were obtained from a permanent laboratory colony previously established for different scientific purposes (Binazzi *et al.,* 2015b). Specimens of *O. telenomicida* used in the field-release experiment were 5-10 days old and they had been fed with honey *ad libitum* until the release. Egg-parasitoids were released at a rate of 30 females and 30 males per plant, for a total of 1.800 specimens divided into 30 release units, one dispenser per selected plant.

Statistical analysis

Data were tested for normality with the Shapiro-Wilk test (Zar, 2010). Transformations were then applied but failed to normalize the data. Data were then analysed by Kruskal–Wallis *H* test and pairwise comparisons carried out by Mann-Whitney *U* test (with Bonferroni correction) (Zar, 2010). Statistical procedures were performed by the statistical software SPSS 20.0.0.

RESULTS

Performance of the egg-parasitoids in the laboratory tests

The ability to parasitize *H. halys* shown by the different egg-parasitoids in laboratory no-choice tests was highly variable and only in few cases, parasitization was successful, with the emergence of new adults.

H. halys egg masses exposed to egg-parasitoids and those serving as control (not exposed to any egg-parasitoid) had an average of 27.38 (±0.21 SE) eggs/batch, and differences in the number of eggs per batch among the different groups, were not statistically significant (H = 6.427; df = 4; P = 0.166).

In the tests carried out with females of *O. telenomicida*, 35.56% (±5.86 SE) of the exposed *H. halys* eggs were parasitized and the 22.92% (±4.35 SE) of the total eggs exposed successfully produced egg-parasitoid offspring after the exposure (Table 1). The 37.33% (±8.69 SE) of the exposed eggs did not hatch, nor they produced any egg-parasitoid, whereas only the 27.11% (±8.63 SE) successfully hatched with the emergence of *H. halys* nymphs. Among the replicates, the maximum parasitization rate was 55.56%, while the maximum rate of eggs from which no *H. halys* nymphs emerged (unviable eggs and /or killed by the egg-parasitoid) was 100%. On the whole, from the 272 eggs of *H. halys* offered to the 10 females of *O.*

Table 1 – Parasitization of *Anastatus bifasciatus*, *Ooencyrtus telenomicida*, *Ooencyrtus pityocampae* and *Telenomus chloropus* on eggs of *Halyomorpha halys* under laboratory conditions (26°C, 70% RH, 16:8 L:D).

Egg-parasitoid species	n. of eggs/ batch[1]	% of hatched eggs[a]	% of parasitized eggs [a]	% of host eggs with parasitoid emergence[a]	% of eggs with no host hatching and no parasitoid emergence[a]	n. of emerged parasitoids/ total n. of exposed *H. halys* eggs	Sex ratio % [♀/(♀+♂)]	n. of females producing offspring/ tot. females tested	Female develop-ment time (days)	Male develop-ment time (days)
Anastatus bifasciatus	27.80 (±0.25) a	65.38 (±9.62) a	20.36 (±8.59) ab	15.00 (±6.79) ab	14.26 (±5.08) ab	42/278	32.50 (±13.51)	4/10	21.43 (±0.19)	20.67 (±0.18)
Ooencyrtus telenomicida	27.20 (±0.42) a	27.11 (±8.63) a	35.56 (±5.86) a	22.92 (±4.35) a	37.33 (±8.69) a	111/272	72.89 (±9.02)	9/10	15.02 (±0.14)	14.58 (±0.13)
Ooencyrtus pityocampae	27.70 (±0.42) a	44.88 (±9.20) a	20.88 (±7.02) ab	0.00 b	34.25 (±7.66) a	0/277	-	0/10	-	-
Telenomus chloropus	26.30 (±0.80) a	59.02 (±10.72) ab	5.91 (±5.53) b	2.58 (±2.21) b	35.07 (±8.48) a	7/263	100	2/10	15.25 (±0.11)	-
Control *H. halys* eggs without egg-parasitoids	27.90 (±0.10) a	93.56 (±1.75) b	-	-	6.45 (±1.75) b	-	-	-	-	-

[1] Within columns, percentages (±SE) followed by different letters are significantly different (P< 0.05).

telenomicida, 111 adults successfully emerged from the parasitized eggs, with a mean of 11.10 (±2.25 SE) adults per egg batch. Remarkably, all the tested females with only one exception accepted *H. halys* eggs as host. Offspring of *O. telenomicida* had a strong female biased sex ratio, 72.89% (±9.02 SE) and development time was of 15.02 (±0.14 SE) days for females and 14.58 (±0.13 SE) days for males.

In tests conducted exposing *H. halys* eggs to *A. bifasciatus* females, the 20.36% (±8.59 SE) of the eggs were parasitized and the 15.00% (±6.79 SE) successfully produced offspring. The percentage of *H. halys* eggs that successfully hatched was relatively high, 65.38% (±9.62 SE), while the eggs with no host hatching and no egg-parasitoid emergence were the 14.26% (±5.08 SE). In single cases, parasitization ability reached the 64.29% of the offered eggs and considering the total number of eggs that did not produce any *H. halys* nymph, the host mortality reached the 78.57% of the egg mass. On the whole, the 278 *H. halys* eggs exposed to the 10 females during the test, produced 42 egg-parasitoid adults, with a strong male-biased sex ratio, 32.50% (±13.51 SE). Only 4 females accepted the egg masses, producing offspring from *H. halys* eggs. Juvenile development time was shorter in males, 20.67 (±0.18 SE) days, than in females, 21.43 (±0.19 SE) days.

In the tests conducted with *T. chloropus*, a mean of 59.02% (±10.72 SE) of the offered eggs hatched, while the 35.07% (±8.48 SE) of them failed to hatch and did not produce any egg-parasitoid. Only the 5.91% (±5.53 SE) of the exposed *H. halys* eggs were parasitized by females and successful emergence of new egg-parasitoids occurred from the 2.58% (±2.21 SE) of eggs. Moreover, only two of the tested parental females accepted *H. halys* eggs for oviposition. In some cases, the presence of a *T. chlorpous* female in the test tube led to 100% mortality of *H. halys* eggs. However, only very few individuals and only females emerged from *H. halys* eggs; the adult stage was reached in 15.25 (±0.11 SE) days.

In the tests with *O. pityocampae*, the 44.88% (±9.20 SE) of the *H. halys* exposed eggs hatched. Although the 20.88% (±7.02 SE) of them was parasitized, no egg-parasitoid offspring emerged. *H. halys* eggs that did not hatch were the 34.25% (±7.66 SE).

Unexposed control eggs of *H. halys* had a mean hatching rate of 93.56% (±1.75 SE) and significant differences were observed among the egg hatching rates of the different groups (H = 22.674; df = 4; n = 50; P < 0.0001): when egg-parasitoids were present (with the exception of *T. chloropus*), *H. halys* eggs hatched at lower rate than the control one (see Table 1).

Percentages of parasitized *H. halys* eggs were significantly different among the tested egg-parasitoid species (H = 7.905; df = 3; n= 40; P = 0.043): *O. telenomicida* parasitized more eggs than *T. chloropus*. As regards the successfully parasitized eggs, differences were also significant (H = 17.60; df = 3; n= 40; P = 0.0001). In particular, the proportion of eggs from which at least one egg-parasitoid emerged, was higher in *O. telenomicida* than in all the other species except for *A. bifasciatus*. Moreover, the percentage of *H. halys* eggs with no host hatching, and no egg-parasitoid emergence, was also significantly different among the tested species (H = 16.771; df = 4: n=50; P = 0.001). In fact, in this trial, both *Ooencyrtus* species and *T. chloropus* caused a host egg mortality higher than that recorded in the untreated control.

Field exposure of frozen *H. halys* eggs in a non-infested area

As regards frozen eggs exposed in the field, data recorded on parasitization revealed that 9 of the exposed *H. halys* egg masses (n. 25 in total) were effectively detected by native egg-parasitoids, and that 59 eggs were successfully parasitized (Table 2). The 72 emerged egg-parasitoids belonged to the species *A. bifasciatus* and *O. telenomicida*, with the second species being clearly more numerous than the first one. Remarkably, in the light of the available data, *A. bifasciatus* seems to be more frequent in the first half of the growing season, while *O. telenomicida* appears to be predominant in the second half.

Table 2 – Egg-parasitoids emerged from *Halyomorpha halys* frozen eggs exposed in the field for 3 days in 2016 in a non-infested site (uncultivated area in Central Italy, see text for details on site description).

Date of *H. halys* eggs exposure	Mean n. of *H. halys* exposed eggs/egg masses	n. of egg masses parasitized/total n. egg masses exposed	Mean % of parasitized eggs on the total number of eggs available in detected egg masses	Total n. of emerged parasitoids	Emerged parasitoid species
15th May	26.6	1/5	10.71	3 (1♀, 2♂)	*Anastatus bifasciatus*
15th June	27.0	2/5	9.4	4 (1♀, 3♂)	*Anastatus bifasciatus*
19th July	25.6	2/5	14.3	14 (11♀, 3♂)	*Ooencyrtus telenomicida*
10th August	25.0	3/5	44.5	51 (42♀, 9♂)	*Ooencyrtus telenomicida*

ABILITY OF LABORATORY REARED EGG-PARASITOIDS TO PARASITIZE FROZEN *H. HALYS* EGGS IN AN INFESTED SITE

Despite the short time of exposure, *O. telenomicida* was able to detect the eggs of *H. halys* in the environment (on both pears and hedgerows) and to parasitize them (Table 3). Parasitized egg masses were recorded in 4 of the 6 study sites, thought only from 3 sites progeny was obtained. Number of detected egg masses and number of parasitized eggs were low, even if in single discovered egg masses, parasitized eggs reached the 25% of the total available eggs. From parasitized *H. halys* eggs, 15 adults (14 females and 1 male) successfully emerged.

DISCUSSION

In the present work, the egg-parasitoids obtained from field collections of native pentatomid and lepidopteran eggs were tested against *H. halys*. They all belonged to well-known generalist species: *A. bifasciatus*, *O. telenomicida*, *O. pityocampae* and *T. chloropus* (HUANG & NOYES, 1994; BATTISTI *et al*., 1988; VIGGIANI, 1994; TRJAPITZIN, 1988). Moreover, all these field-collected species could be reared in the laboratory in a sufficient number to perform the tests.

HAYE *et al.* (2015b) assumed that generalist egg-parasitoids of pentatomids would be the most likely candidates to successfully develop on *H. halys*, considering that they evolved effective strategies to overcome defense responses of a wide range of hosts (VINSON, 1990). In several cases, particularly in a medium/long-term perspective, native egg-parasitoids were reported to adapt to alien species exploiting them as new hosts. A recent example in Europe is the case of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae), the chestnut gall wasp: after a decade from its accidental introduction, several cases were reported of parasitoids shifting from oak gall wasps to the chestnut gall wasp (QUACCHIA *et al.,* 2013; MATOŠEVIĆ & MELIKA, 2013). That being said, it is often difficult to predict how much time is required to obtain an effective and economically sound natural control of invasive species by native organisms. To date, as regards *H. halys*, even if its introduction is suspected to have occurred in Europe before 2004, there is little evidence of field parasitization by native parasitoids. HAYE *et al*. (2015b) observed parasitization of field-exposed frozen eggs by *A. bifasciatus* and *T. cultratus.* In addition, field parasitization of fresh egg masses by *A. bifasciatus* was also reported in Northern Italy (COSTI *et al.*, 2016). Likewise, results consistent with previous studies were also found in this work. Conversely, in the USA, where *H. halys* was introduced in the early 90's, several different species of egg-parasitoids, including *Ooencyrtus* species, have adapted to the new host under field conditions (ABRAM *et al*., 2014; JONES *et al*., 2014; HERLIHY *et al*., 2016; CORNELIUS *et al*., 2016). However, so far, parasitization rates observed in the field have been extremely low for an effective control of the pest.

In Europe, HAYE *et al.* (2015b) tested in the laboratory different species of native egg-parasitoids (belonging to the genera *Trissolcus*, *Telenomus*, *Anastatus*), with the aim to select a candidate for augmentative biological control. These studies highlighted that *A. bifasciatus* is the most promising one and research on it is currently in progress. Nevertheless, the possibility that in other regions/habitats the parasitoid complex and the parasitization level of *H. halys* may be different from those so far observed cannot be excluded. In the present work, the focus was on *O. telenomicida*, a species that together with *A. bifasciatus* might play a key role as potential biocontrol agent of *H. halys*. Moreover, in

Table 3 – Parasitization of frozen eggs of *Halyomorpha halys* in a 2-day field trial in an infested site (pear orchards in Central Italy, 13th-15th September 2016) with the release of *Ooencyrtus telenomicida* laboratory reared specimens (see text for details).

Site	Plants	Mean n. of *H. halys* exposed eggs/egg masses	N. of parasitized egg masses/total n. of exposed egg masses	Mean % of parasitized eggs on the total number of eggs available (in detected egg masses)	Total n. of emerged parasitoids
SPA (orchard)	*Pyrus communis*	26.27	1/15	25.00	1 (1♀)
RI (orchard)	*Pyrus communis*	26.67	0/15	-	-
CO (orchard)	*Pyrus communis*	27.53	2/15	13.99	10 (10♀)
COS (hedgerow)	*Populus alba, Carpinus betulus, Cornus mas*	26.27	1/15	25.00	4 (3♀, 1♂)
VA (orchard)	*Pyrus communis*	25.27	1/15	4.00	0
VAS (hedgerow)	*Acer campestris*	25.07	0/15	-	-

accordance with Haye *et al.* (2015b), the possibility to select a strain of the potential biocontrol agents specifically adapted to *H. halys*, may be an interesting perspective in augmentative releases of mass-produced specimens. In this context, *O. telenomicida* might be a valid candidate. Since it is widespread from Europe to China, and it is well adapted to biogeographical regions with a Mediterranean climate (Samra *et al.*, 2015). On the other hand, the potential use of both *A. bifasciatus* and *O. telenomicida* in a simultaneous release is more questionable, since a partial overlapping of parasitization (*i.e.* hyperpasitism) might occur. In fact, *O. telenomicida* is a well-known hyperparasitoid and in *A. bifasciatus* the same behavior has been reported too (Viggiani, 1994; Catalán & Verdú, 2005; Cusumano *et al.*, 2011).

Conversely, *T. chloropus* was of minor interest since its parasitization ability resulted to be much lower than that observed in *A. bifasciatus* and *O. telenomicida.* Moreover, in contrast with the observations conducted in the present work, Haye *et al.* (2015b) observed no offspring production in *T. chloropus* tested in laboratory on fresh host eggs. A reasonable explanation might be that different local populations of the same egg-parasitoid species could have a slightly different ability to exploit this pest. This is also the case of *T. cultratus*, which in China appears to be one of the most important control agents of *H. halys* beside *T. japonicus*, while in Europe it shows extremely low levels of parasitization, under both laboratory and field conditions (Haye *et al.*, 2015b).

On the whole, the most important finding was that *A. bifasciatus* and *O. telenomicida* showed a good capacity to successfully emerge from parasitized eggs of *H. halys*, *i.e.* parasitized host eggs producing viable offspring. In *O. telenomicida*, the development of juveniles took approximately 15 days in females and males. This result is consistent with data presented by Catalán & Verdú (2005) and Stefanescu *et al.* (2003), where this egg-parasitoid developed respectively on *Nezara viridula* L. (Heteroptera, Pentatomidae) and *Iphiclides podalirius feisthamelii* (Duponchel) (Lepidoptera: Papilionidae); data on sex ratio are also quite similar to those reported by the cited Authors. Though further research is necessary, data collected in the present work lead to speculate that the new host of *O. telenomicida* should not considerably affect its reproductive ability. *O. telenomicida* showed for each tested individual, a relatively high parasitization ability and in some cases the presence of adult females in the test tubes was associated with a 100% mortality of *H. halys* eggs. It is also worth mentioning that beside the parasitization ability, this egg-parasitoid is also known for its host-feeding behavior, which can occur in the dead host (Cusumano *et al*., 2012). As it was observed by Haye *et al.* (2015b), *A. bifasciatus* is also capable to exploit the new host, even if a fairly higher parasitization ability was reported compared with that recorded here.

Conversely, egg-parasitoids tested here and in Haye *et al.* (2015b), even when they parasitized the host masses, generally failed to develop on fresh host eggs, while only in few cases successfully emerged from frozen host eggs. Abram *et al*. (2016) argued that in inundative biological control, the capacity to target and kill a maximum number of host eggs is more relevant than the ability to successfully emerge from them. In fact, parasitoids can contribute to the egg mortality of a pest in several ways such as probing eggs with the ovipositor, feeding on them or disrupting the embryogenesis. All these factors, individually or combined, may eventually lead to egg abortion (Jervis & Kidd, 1986; Abram *et al*., 2016). This aspect should be particularly considered in the new host association, where due to a lack of co-evolutionary history, native egg-parasitoids may try to parasitize eggs with different results, such as the killing of the eggs, even without a successful emergence of new adults. This could be also the case of unhatched eggs of *H. halys* reported by Abram *et al.* (2014) and Haye *et al.* (2015b). Similar findings were observed in the present study in the *O. pityocampae* test, where no offspring were produced but more than half of the exposed *H. halys* eggs failed to hatch in presence of the egg-parasitoid, and females parasitized effectively 1/5 of the offered eggs.

However, all results in the present work were obtained in laboratory using no-choice tests, in which specimens were constrained within the test units. This is a severe limitation and transferring knowledge from the laboratory directly to the field may be complex while potential success difficult to predict. Therefore, further accurate studies in semi-field and field conditions are required to define the effective ability of the egg-parasitoid to detect target host eggs in the environment. Moreover, even if parasitization occurs at high levels, habitat fidelity of the control agent cannot be ignored and should be further investigated. Herlihy *et al.*, (2016) focused on habitat fidelity of different egg-parasitoids: certain species were indeed more attracted to herbaceous vegetation, while others searched preferentially for habitats characterized by woody flora. Nonetheless, if, and what kind of environments are attractive to *O. telenomicida* or *A. bifasciatus*, is still a matter of further investigations. In fact, in the laboratory, single females of *O. telenomicida* and *A. bifasciatus* were able to parasitize relatively high percentages of eggs in an egg-mass, but the actual exploitation capacity of a batch in the field remains questionable.

In the present work, *O. telenomicida* was preferred to *A. bifasciatus* as potential biocontrol agent of *H. halys* and hence used in the field tests, on the basis of the following criteria: i) in laboratory, this species can be easily reared on different hosts (also on small-size ones, at least compared with those commonly exploited by *A. bifasciatus*); ii) parasitoid productivity for each single host egg of *H. halys*/*G. lineatum* (or other suitable hosts) is higher. In fact from an egg of *H. halys*/*G. lineatum*, 2-3 *O. telenomicida* specimens may emerge due to their the reduced body size compared to that of *A. bifasciatus*, (in bigger host eggs up to 6 specimens) (Stefanescu *et al.*, 2003); iii) the development time is faster (15 days vs. the 3 weeks required by *A. bifasciatus*); iv) the will to diversify the research topics, optimizing general resources: in fact *A. bifasciatus* is already under investigation in an international project funded by the European Union (Haye *et al.*, 2015b).

Data obtained in the field trial (in pear orchards) designed to control *H. halys* by releasing laboratory reared *O. telenomicida* (on *G. lineatum* as host) showed that, despite the short period of host-egg exposure, this egg-parasitoid was able to detect and parasitize the target host, not only on pears, but also on hedgerows of mixed plant species. Some females of *O. telenomicida* were able to recognize eggs of *H. halys*, even though, in general, the parasitization parameters evidenced so far a low ability to detect eggs and parasitize them. This might be due to different factors: i) the reduced time of host exposure (higher parasitization levels could be expected after a longer exposure); ii) the non adequate timing of the trial, probably too late to meet the optimal conditions of the egg-parasitoid (data obtained in the present work showed that a high activity of *O. telenomicida* can be expected in the middle of August, while field tests were carried out in the middle of September); iii) the non optimal condition of sentinel eggs (even if frozen host eggs appeared suitable in tests conducted here and in other studies); vi) an excessively low egg-parasitoid/host ratio. Regarding the last, it is known that the release rate in an augmentative biological control program is a key factor in the success of a control strategy (Crowder, 2007; Collier & Van Steenwyk, 2004). Nonetheless, the results obtained in this preliminary field trial with *O. telenomicida* are interesting and research is in progress in order to establish key parameters, such as those proposed by Bin and Vinson (1991): discovery efficiency, exploitation efficiency and parasitoid impact. Moreover, there are also some grounds for optimism, since studies of Abram *et al.* (2014), Herlihy *et al.* (2014), Jones *et al.*, 2014, Haye *et al.* (2015b), Ogburn *et al.* (2016), showed that fresh and frozen field-exposed sentinel eggs of *H. halys* were effectively detected by several native egg-parasitoids in both the USA and Europe. The cited studies focused on several egg-parasitoid species included in the genera *Anastatus, Trissolcus, Telenomus* and *Ooencyrtus*, whose importance is variable at local scale. Nevertheless, so far, natural parasitization levels in the field have been generally very low and the control by other natural agents, such as predators, even if reported in many circumstances, is not sufficient to actually limit this pest (Ogburn *et al.,* 2016).

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