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## Halyomorpha halys, a serious threat for hazelnut in newly invaded areas

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- 1 Halyomorpha halys, a serious threat for hazelnut in newly invaded areas 2 3 Lara Bosco, Silvia T. Moraglio, Luciana Tavella 4 5 Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), ULF Entomologia 6 Generale e Applicata, University of Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy 7 8 Corresponding author: Luciana Tavella 9 Phone +39 011 6708533, fax +39 011 6708535, email luciana.tavella@unito.it 10 11 Abstract 12 Following its first detection, *Halyomorpha halys* has become a key pest in many crops in NW 13 Italy. In this area, one of the most important crops is hazelnut, in which the species can cause 14 severe damage through feeding on nuts. Therefore, semi-field trials were carried out in NW 15 Italy to compare the harmfulness of *H. halys* with that of the local hazelnut bug species, such 16 as Gonocerus acuteangulatus, Nezara viridula, and Palomena prasina. Additionally, a 2-year 17 field survey was conducted in hazel groves in NW Italy and W Georgia, another important 18 hazelnut cropping area, to assess the presence and abundance of the new invasive species and 19 to evaluate the damage at harvest. Monitoring was carried out by plant beating and by 20 commercial traps throughout the growing season. In semi-field trials, H. halys was the most 21 harmful species, causing the highest damage in kernels, and was able to survive and 22 reproduce at higher rates. During field surveys in NW Italy, H. halvs was sampled in groves 23 late in the season in 2015 and, with higher populations, throughout the season in 2016. In W 24 Georgia, bug population levels consistently increased in the 2-year period, resulting in a 25 significant increase of damage at harvest in 2016. A similar trend is hence expected also in 26 NW Italy in the following years. Moreover, data on individuals collected in different points of 27 the hazelnut groves confirmed the border-driven behavior of this pest, leading to 28 consideration of potential integrated pest management solutions. 29 30 Key words: Brown marmorated stink bug, Semi-field trials, Field surveys, Damage 31 evaluation, NW Italy, W Georgia 32 33 Key message: 34
  - *Halyomorpha halys* has become a serious pest in NW Italy.

35	٠	In semi-field trials, H. halys was more harmful than common hazelnut pest bug
36		species because of greater damage on kernels and maximum survival and reproductive
37		rates.
38	•	In field surveys, in NW Italy H. halys was scarcely found in 2015-2016, whereas in W
39		Georgia its population consistently increased in 2016, resulting in significantly higher
40		damage.
41	•	A similar trend is hence expected on hazelnut in NW Italy in the following years.
42		
43		

#### 44 Introduction

- 45 Different bug species belonging to the families Coreidae and Pentatomidae (Hemiptera:
- 46 Heteroptera) are responsible for economic damage to hazelnut in the main producing areas of
- 47 Europe and Turkey (Moraglio et al. 2014; Tuncer et al. 2014; Erper et al. 2016). Adults and
- 48 nymphs can cause different types of damage in relation to the period when they feed on the
- 49 nuts: early attacks during nut development, i.e., in May-early June, lead to an increase of seed
- 50 abortion and early nut drop, while later attacks during kernel growth, i.e., in mid-June-
- 51 August, determine symptoms on kernels, such as whitish or brownish spots, spongy tissue,
- 52 and surface depression (Boselli 1932; Tavella et al. 2001a, 2003). Damage on kernels result
- 53 from an injection of saliva rich of enzymes during feeding, which changes kernel composition
- 54 (Vaccino et al. 2008; Memoli et al. 2017). Currently, *Gonocerus acuteangulatus* (Goeze)

55 (Hemiptera: Coreidae), Nezara viridula (L.), and Palomena prasina (L.) (Hemiptera:

- 56 Pentatomidae) are the primary insect pests of hazelnut in Europe and Turkey (Tuncer et al.
- 57 2005; Romero et al. 2009; Moraglio et al. 2014).
- 58 Besides the well-known hazelnut bugs, the recent invasion of the exotic brown marmorated
- 59 stink bug Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) represents a serious threat to
- 60 hazelnut in Europe and Turkey, as already observed in North America, where this species has
- 61 also been producing economic losses in hazelnut crops. In semi-field trials carried out in
- 62 Oregon, *H. halys* caused damage on different hazelnut cultivars throughout the entire period
- 63 of nut and kernel development (Hedstrom et al. 2014). Therefore, one purpose of our study
- 64 was to investigate the potential of *H. halys* to damage the local hazelnut cultivar in Piedmont
- 65 (NW Italy), in comparison with the main indigenous bug species, such as the most widespread
- 66 *G. acuteangulatus* and *P. prasina* (Sonnati et al. 2009) and the occasionally found *N. viridula*
- 67 (authors' personal observation).
- 68 Since the first report of *H. halys* in Europe more than 10 years ago, the species has recently
- 69 become an important fruit pest in many agroecosystems (Haye et al. 2015b). In Italy, the
- round second hazelnut-producing country worldwide (data of 2014, FAOSTAT 2017), the exotic
- 71 pest was first recorded in the Emilia Romagna in 2012 (Maistrello et al. 2013) and in the
- 72 Piedmont in 2013 (Pansa et al. 2013). In this latter region of NW Italy, where hazelnut is
- 73 widely cultivated and still increasing, *H. halys* has been causing severe damage and high
- reconomic losses in apple, nectarine, pear, and nashi pear (Pansa et al. 2015; Candian et al.
- unpublished data) as well as in corn (Rancati et al. 2017). By contrast, only a few data are
- 76 available about the presence and abundance of *H. halys* in Georgia (Gapon 2016), the third
- 77 hazelnut-producing country worldwide after Turkey and Italy (data of 2014, FAOSTAT

- 78 2017). Hazelnut is an important traditional nut crop in different Georgian zones, such as the
- regions Abkhazia, Adjara, Samegrelo, Guria, Imereti, and Kakheti (Mirotadze et al. 2009).
- 80 Therefore, another purpose of this study was to assess the presence, abundance, and
- 81 harmfulness of *H. halys* by plant beating, use of traps, and damage evaluation in commercial
- 82 hazel groves in NW Italy, in the Piedmont region, and in W Georgia, in the Samegrelo region,
- 83 during 2015-2016.
- 84 The overall objectives of this study are: (1) comparing the damage caused by different
- 85 hazelnut bugs, (2) comparing their survival and reproduction on hazelnut under semi-field
- 86 conditions, (3) assessing the presence and abundance of *H. halys*, and (4) evaluating the
- 87 damage to hazelnut production due to bug feeding in two important growing regions in Italy
- 88 and Georgia.
- 89

## 90 Material and method

# 91 Harmfulness of H. halys in comparison with other hazelnut bug species

- 92 In 2015, semi-field trials were carried out in a commercial hazel grove untreated with
- 93 insecticides, located in Magliano Alfieri (province of Cuneo, 44°45'46.50"N, 8° 3'13.56"E,
- 94 205 m a.s.l.), planted with the local cultivar Tonda Gentile delle Langhe. In the trials, the
- 95 following four species were tested: the newly introduced *H. halys* and the commonly present
- 96 *G. acuteangulatus*, *N. viridula*, and *P. prasina*.
- 97 Insect collection and rearing
- 98 Starting from April, adults and nymphs of the tested bug species were collected from different
- 99 wild or cultivated host plants [*Rhamnus cathartica* (L.) (Rhamnaceae), *Cornus sanguinea* L.
- 100 (Cornaceae), Crataegus monogyna Jacq., Prunus spp., and Rosa spp. (Rosaceae) for G.
- 101 acuteangulatus and P. prasina, Sambucus nigra L. (Caprifoliaceae) for N. viridula; peach and
- 102 pear for *H. halys*]. Field-collected adults and nymphs were then transferred to the laboratory
- 103 where they were reared in net cages  $(930 \times 475 \times 475 \text{ mm})$  (MegaView Science Co., Ltd,
- 104 Taichung City, Taiwan) containing broad bean seedlings [Vicia faba L. (Fabaceae)], fresh
- fruits, and shelled hazelnuts. Mass rearing was performed in climatic chambers at  $24 \pm 1^{\circ}C$
- 106 and RH 65  $\pm$  5%, with an L/D of 16:8 h.
- 107 Hazel branch caging and insect insertion
- 108 To prevent insect damage to nuts in early June, 300 hazel branches with at least four clusters
- 109 of fruits were caged inside sleeve cages of polythene net (730 mm in length, 170 mm in
- 110 diameter, and 60-mesh grid) [Artes Politecnica, Schio (VI), Italy], supported by an inner
- 111 cylinder of a plastic net (310 mm in length, 165 mm in diameter). On the tree, each cage was

112 closed with a twist tie proximally at one end around the branch and distally at the other end

113 over the top of the branch.

- 114 Single specimens of the tested bug species were inserted in the cages on July 3, i.e., when
- 115 nuts of the cultivar Tonda Gentile delle Langhe have reached the final volume and kernels are
- in an advanced stage of development (Guidone et al. 2007), enabling the bugs to cause greater
- 117 damage (Tavella et al. 2001b, 2003). Specifically, 30 males and 30 females of *H. halys*, *G.*
- acuteangulatus, and N. viridula and 60 nymphs (fourth- and fifth-instar nymphs) of P.
- 119 *prasina* were individually encaged in 240 cages and left until harvest time, i.e., mid-August.
- 120 For *P. prasina*, late instars nymphs were used, since adults were not available in the required
- 121 numbers. Out of 60 remaining cages, 30 cages were discarded since the isolated branches
- 122 were found dried out or had only few hazelnuts due to the physiological fruit drop, while in
- 123 the other 30 cages, no insects were inserted to obtain data for undamaged hazelnuts.
- 124 Damage evaluation at harvest
- 125 On August 4, just before the harvest time, all the cages were removed and brought to the
- 126 laboratory, where the nuts contained in each cage were collected separately and stored at 5°C
- 127 until they were shelled. The kernels were examined to detect any bug damage symptoms (i.e.,
- 128 alterations evident only on the seed, consisting of whitish or brownish spots, spongy tissue,
- 129 surface depressions). Damage assessment was done following the procedure generally used
- 130 by nut processing industries. First, the kernels were separated into two categories: with
- 131 evident symptoms of bug damage on the surface (external damage) and without any
- 132 symptoms on the surface. The nuts with the external damage are usually immediately
- 133 discarded by processing industry. Subsequently, the latter kernels were cut into four parts to
- 134 detect the presence of possible internal symptoms, not evident on the surface, and separated
- 135 into two further categories: with internal symptoms of bug damage (internal damage) and
- 136 healthy. All damaged kernels were further divided into two categories: with spongy and only
- 137 white tissue (white) or with brown tissue or both white and brown tissues (brown). The brown
- 138 category in particular affects the commercial quality of kernels in industrial chain processes.
- 139 Additionally, shriveled and rotten or moldy kernels were also recorded, as well as blank nuts.
- 140 The cages containing dried-out branches or only blank nuts were discarded.
- 141 Proportions of damaged kernels in total kernels and of kernel showing external and brown
- 142 symptoms within damaged kernels in each cage were compared among species via a binomial
- 143 distribution model with a logit link function, using the general linear model (GLM) procedure
- 144 of the software IBM SPSS<sup>®</sup> Statistics 22 (IBM Corp., NY, USA). The two outcomes of each
- 145 of the three binomial analysis were: (1) damaged versus non-damaged kernels, (2) kernels

- 146 showing external versus internal damage within all damaged kernels, and (3) kernels showing
- 147 brown versus white damage within all damaged kernels. Means were then separated at
- 148 P < 0.05 using the Bonferroni test under the GLM procedure.
- 149 Insect survival in the cages

150 During the trials, the insects were checked weekly for survival. All dead specimens were 151 recorded and replaced with new ones of the same species and sex. After their removal and 152 transfer to the laboratory on August 4, the cages were inspected to check for insect survival 153 and the presence of eggs and nymphs. Percentages of replacement (i.e., the insects introduced 154 to replace the dead ones) were arcsine square-root-transformed and analyzed with one-way 155 ANOVA, using the sex/stage (i.e., male, female, or female with offspring) within the same 156 species as the main factor. Subsequently, when any difference in mortality was found between 157 sex/stage within the species, species was used as the main factor. Means were separated at 158 P < 0.05 using Tukey's test. Total numbers of nymphs per each cage containing offspring 159 were analyzed with one-way ANOVA, using species as the main factor. Means were then separated at P < 0.05 using Tukey's test. The software IBM SPSS<sup>®</sup> Statistics 22 (IBM Corp., 160 161 NY, USA) was used for all analyses.

162

### 163 Field monitoring of bugs and damage evaluation

164 To assess the presence and abundance of bug species, a 2-year (2015–2016) field survey was 165 carried out in five commercial hazel groves, of which three were located in NW Italy 166 (Piedmont region) and two in W Georgia (Samegrelo region). In these two regions, both 167 grove size and the agroecosystem were different. In NW Italy, the three groves were 168 integrated in a heterogeneous landscape, composed of various cultivations, such as cherry, 169 peach, and pear orchards, vineyards, vegetable crops. On the borders of these groves, there 170 were wild plants well known as host plants for hazel bugs, such as C. sanguinea, Prunus spp., and Pvrus spp. In addition, favorite hosts of the bugs, in particular of G. acuteangulatus (i.e., 171 172 C. sanguinea, R. cathartica, and Rosa canina L.) (Schaefer and Mitchell 1983, Werner 2007, 173 Moraglio et al. 2014), had been planted in four groups in the corners of each orchard in 2014. 174 In W Georgia, the two groves were integrated in a simplified landscape (i.e., a large hazelnut 175 cropping area of about 300 ha), surrounded by other hazelnut groves, and characterized by the 176 presence of wild Cornus sp., Rubus sp., and Cryptomeria japonica (Thunb. ex L.f.) D. Don on 177 some borders. Sites, hazelnut cultivars, pest management, and wild plants grown in the 178 surroundings of the groves are reported in Table 1.

179 Monitoring of bugs by plant beating and traps

180 In the surveyed groves, the presence and abundance of *H. halys* and other bug species were

- assessed on groups of hazel trees in different positions of the grove, by plant beating, and byusing traps.
- 183 Plant beating consisted of scouting the branches of the selected plants over a white nylon
- tarpaulin  $(2.5 \times 2.2 \text{ m})$  placed on the ground, collecting and counting all the fallen bugs. It
- 185 was performed early in the morning (5:00-7:00 am) to prevent adults from flying away. In
- 186 NW Italy, monitoring by plant beating was carried out on July 10 and 27 in 2015 and on July
- 187 1, 15, and 29 in 2016. In each grove, the sampling was performed on groups of hazel trees
- 188 located in the center (one and two groups in 2015 and 2016, respectively) and along the
- borders (four groups, i.e., one per border). In 2015 and in 2016, each group consisted of three
- 190 plants (total of 15 plants) and four plants (total of 24 plants), respectively. In W Georgia,
- 191 monitoring by plant beating was carried out on June 10, July 9, and August 5 in 2015 and on
- 192 June 15 and 28, July 13 and 27, and August 9 in 2016. Due to the large size of the groves (see
- 193 Table 1), the sampling was performed on groups of trees located at the following three
- 194 distances from the border with attractive plants: 5 m (i.e., border area), 100 m, 200 m (i.e.,
- 195 central area). At each distance, three groups of plants were selected, each consisting of two
- 196 plants (total of 18 plants) and of four plants (total of 36 plants) in 2015 and 2016,
- 197 respectively.
- 198 The presence of *H. halys* was also assessed by using commercially available traps, i.e.
- 199 Rescue<sup>®</sup> (Sterling International, Inc., Spokane, WA, USA) traps in 2015, and Alpha Scents<sup>®</sup>
- 200 (Alpha Scents, Inc., West Linn, OR, USA) in 2016. The traps were always baited with Alpha
- 201 Scents<sup>®</sup> lures [methyl (E, E, Z)-2,4,6-decatrienoate, *EEZ*-MDT) and used in both years in NW
- 202 Italy and only in 2016 in W Georgia. In particular, one trap in NW Italy and two traps in W
- 203 Georgia were placed along the border of each surveyed grove in mid-June and checked every
- 204 2 weeks until mid-November.
- 205 Mean numbers of bugs collected per plant and per survey were compared in each grove
- 206 between the 2 years; in each region across all groves, the same data were compared on the
- 207 basis of the position of plants (i.e., on the border or in the center in NW Italy, at different
- 208 distance from the border in W Georgia). Data were analyzed with a Poisson distribution
- 209 model with a log link function using the GLM procedure of the software IBM SPSS<sup>®</sup>
- 210 Statistics 22 (IBM Corp., NY, USA). Means were then separated at P < 0.05 using the
- 211 Bonferroni test under the GLM procedure.
- 212 Damage evaluation at harvest

- 213 At harvest time, a sample of 1 kg of hazelnuts was manually collected beneath each surveyed
- tree at each position in the five groves. In 2015, additional nut samples were collected from a
- 215 further central group of hazel trees in groves 1 and 2 in NW Italy. In the laboratory, hazelnuts
- 216 were shelled and 300 intact kernels were randomly chosen from each plant and checked for
- 217 bug damage as described above. As with numbers of bugs collected per plant, also mean
- 218 proportions of damaged kernels per plant were compared in each grove between the 2 years
- and in each region overall the groves on the basis of the position of plants with a binomial
- 220 distribution model (outcomes: damaged versus non-damaged kernels) with a logit link
- 221 function using the general linear model (GLM) procedure of the software IBM SPSS®
- Statistics 22 (IBM Corp., NY, USA). Means were then separated at P < 0.05 using the
- 223 Bonferroni test under the GLM procedure.
- 224

# 225 Results

# 226 Harmfulness of H. halys in comparison with other hazelnut bug species

- 227 The mean percentages of kernels exhibiting symptoms due to bug feeding were significantly 228 higher in the cages with all the tested species than in the control; in particular, the values were 229 significantly higher for *H. halys* (75% of analyzed kernels) in comparison with *N. viridula* (57%), P. prasina (39%), and G. acuteangulatus (39%) (Table 2). For all the tested species, 230 231 no significant differences were found by comparing the percentages of kernels damaged by 232 males and females within the species (data not shown). Among damaged kernels, H. halys 233 and G. acuteangulatus showed significantly higher percentages of external symptoms (81 and 234 78%, respectively) in comparison with the other two species, and G. acuteangulatus showed
- significantly higher percentages of brown tissue (93%) in comparison with the other species.
- 236 The mean percentages of shriveled kernels were significantly higher for *H. halys* and *N.*
- viridula (20% of analyzed kernels), followed by *P. prasina* (10%) and *G. acuteangulatus*
- 238 (5%), which did not differ significantly from the control (6%). The highest mean percentages
- of rotten and/or moldy kernels were observed in the cages with *H. halys* (10% of analyzed
- 240 kernels); G. acuteangulatus (5%) and N. viridula (6%) showed values not significantly
- 241 different, while *P. prasina* (1%) did not differ significantly from the control (2%) (Table 2).
- Some females of *H. halys* (n = 24), *G. acuteangulatus* (n = 8), and *N. viridula* (n = 11) laid
- 243 eggs inside the cages, and the offspring could survive and develop (Table 3). Such ovipositing
- females were mostly alive throughout the trials (n = 23, 4, 10 for *H. halys*, *G. acuteangulatus*
- and *N. viridula*, respectively) or replaced only once in the first survey (n = 1, 4, 1 for *H. halys*,
- 246 *G. acuteangulatus* and *N. viridula*, respectively). For *P. prasina*, since the individuals were

- 247 caged as late instar nymphs, adults emerging into the cages could not mate, and consequently
- females could not oviposit. The mean number of nymphs per female was significantly higher
- for *N. viridula* (32.2) compared to *H. halys* (19.6) and *G. acuteangulatus* (12.3) (Table 3).
- 250 The mean percentages of replacement were not significantly different in the cages with males,
- females, and females plus nymphs of the same species for *H. halys* (ANOVA: P = 0.126;
- 252  $F_{2;9} = 2.636$ ), *G. acuteangulatus* (ANOVA: P = 0.393;  $F_{2;9} = 1.039$ ), and *N. viridula*
- 253 (ANOVA: P = 0.70;  $F_{2;9} = 3.624$ ) or with nymphs, females, and males for *P. prasina*
- 254 (ANOVA: P = 0.564;  $F_{2,9} = 0.610$ ) (Table 3). Nevertheless, significant differences were found
- between the mean replacement percentages of the four species. In particular, the lowest and
- 256 highest rates of insects replaced during surveys were observed for *H. halys* and for *G*.
- 257 *acuteangulatus*, respectively (Table 3).
- 258

# 259 Field monitoring of bugs and damage evaluation

- 260 Monitoring of bugs by plant beating and traps
- In NW Italy, *H. halys* was not found in the samples taken by plant beating in 2015 (0%;
- 262 n = 134), while it represented 3% of total collected bugs in 2016 (n = 272). In particular,
- adults and nymphs of this species were collected in mid- and late July in a group of plants on
- the border near the attractive plants planted in 2014, in both groves 1 and 3. The other bug
- species were *G. acuteangulatus*, *N. viridula*, and *P. prasina*, which were sampled by plant
- beating in variable amounts in the surveyed groves. For all Italian sites, average numbers of
- bugs per plant were  $1.48 \pm 0.65$  in 2015 and  $2.01 \pm 1.22$  in 2016 (Table 4). By using traps, in
- 268 2015, specimens of *H. halys* were captured only in grove 3, namely, eight nymphs on
- 269 September 15 and one adult plus 59 nymphs on October 8. In 2016, higher numbers of
- 270 specimens were collected in groves 1 and 3 from late August or early September, respectively
- 271 (Fig. 1). High numbers of insects were trapped during October; the numbers then decreased
- during early (grove 1) or late November (grove 3) (Fig. 1).
- In W Georgia, *H. halys* represented 67 and 84% of total sampled bugs in 2015 (n = 45) and in
- 274 2016 (n = 829), respectively. Specimens of this species were collected on hazel starting from
- July 9 in 2015 and from June 15 in 2016. Overall, the 45% of total collected specimens were
- 276 nymphs (n = 735). Average numbers of bugs per plant were  $0.46 \pm 0.06$  in 2015 and
- $2.83 \pm 1.43$  in 2016 (Table 5). Therefore, there was an evident increase of bugs between the 2
- 278 years, essentially corresponding to the increase of *H. halys* compared to the other bug species
- 279 (Table 5). In particular, the following three species were also collected on hazelnut in W
- 280 Georgia: G. acuteangulatus (17 and 2% of total bugs in 2015 and 2016, respectively), N.

- viridula (7 and 12% of total bugs in 2015 and 2016, respectively), and P. prasina (7 and 1%
- of total bugs in 2015 and 2016, respectively). By traps (placed only in 2016), the first
- specimens of *H. halys* were captured in late June (grove 4) and in mid-July (grove 5) (Fig. 1).
- 284 Then, in grove 4, the captures suddenly increased between late July and early September;
- afterward, they started to decrease. In grove 5, the highest mean values of insects were
- recorded in early September, while in the previous dates, on average 11.5 bugs per trap were
- captured (Fig. 1).
- 288 Population levels were variable in relation to the plant position in the orchards. In NW Italy,
- the mean numbers of bugs per plant were significantly higher in the groups of plants on the
- borders than in the center of the surveyed hazel groves, in both 2015 and 2016 (Table 4). In
- 291 W Georgia, a marked gradient of population levels was observed, starting from the border
- towards the center of the orchards, especially in 2016, when the mean values were
- significantly higher than in 2015 (Table 5).
- 294 Damage evaluation at harvest
- 295 In NW Italy, the percentages of kernels exhibiting symptoms due to bug feeding in the three
- hazel groves surveyed in both years ranged, on average, between 7.8 and 19.4% (Table 4).
- 297 Consistent with the results of bugs sampled by plant beating, the kernels collected from the
- groups of plants located in the center of the orchard were significantly less damaged than
- those collected along the borders (Table 4).
- 300 In W Georgia, the mean percentages of kernels showing symptoms of damage caused by bugs
- increased strongly from 2015 to 2016, from 4.3 to 51.3% in grove 4 and from 1.4 to 26.6% in
- 302 grove 5 (Table 5). In 2016, the highest percentages of damage were recorded in kernels
- 303 collected from the groups of plants along the border, and a marked gradient from the border
- 304 toward the center of the orchard was evident (Table 5).
- 305

### **306 Discussion and Conclusions**

- 307 In the semi-field trials, the symptoms on kernels resulting from *H. halys* feeding were similar
- 308 to those caused by the other tested species as well as to those observed in similar trials
- 309 performed on local cultivars in Oregon (Hedstrom et al. 2014). Nevertheless, in our trials, the
- 310 newly introduced species was the most harmful one in comparison with the common hazelnut
- 311 bugs. Among the categories of symptoms (i.e., external or internal damage, white or brown
- 312 tissues), we defined within damaged kernels, *H. halys* caused mainly external damage,
- 313 together with *G. acuteangulatus*, and brown tissue symptoms, and such categories particularly
- 314 affect the commercial quality of kernels, especially in nuts used in processed foods. In the

- 315 cages with *H. halys* and with *N. viridula*, the highest percentages of shriveled hazelnuts were
- also found, probably due to feeding during kernel expansion (Hedstrom et al. 2014).
- 317 Additionally, in the cages with *H. halys*, the highest percentages of moldy and/or rotten
- 318 hazelnuts were recorded; hence, an indirect damage due to secondary pathogenic infections
- 319 through the punctures on kernels and/or to transmission with the feeding stylets cannot be
- 320 excluded (Rice et al. 2014). The role played by *H. halys* on this indirect damage needs to be
- 321 further investigated, as well as its contribution to the increase of blank nuts when it feeds on
- 322 hazelnuts before shell and kernel expansion (Hedstrom et al. 2014). In our trials, blank nuts
- 323 were not considered, since bugs fed on hazelnuts from kernel expansion to the harvest,
- 324 therefore resulting in kernel malformations and/or spongy or corky symptoms.
- 325 In NW Italy, from its first report in 2013, *H. halys* has significantly spread, becoming
- 326 worrisome, and caused economic losses in stone and pome fruits as well as cereals (Pansa et
- al. 2013, 2015; Rancati et al. 2017), whereas it was rarely observed on hazelnut until 2015.
- 328 During field surveys in 2015, few adults and nymphs were sampled in the groves, while their
- numbers increased in 2016; however, the damage at harvest was similar in the 2 years and
- 330 probably caused by the other bug species. The augmentation of infestations on hazelnut in the
- following growing seasons is expected, as suggested by the outbreaks of *H. halys* already
- 332 observed in the early 2017 growing season (personal observation).
- 333 In W Georgia, *H. halys* was first observed in the hazelnut groves in 2015, and the proportion
- of the field-collected specimens (69% of total sampled hazel bugs) was by far greater than
- that observed in NW Italy. However, during the 2016 season, populations were significantly
- 336 higher than in the previous season, which is consistent with the first outbreaks of the exotic
- 337 species recorded in Georgia in the same year (Gapon 2016). In fact, in our study, the first
- 338 serious damage on hazelnut was reported in 2016, with mean percentages of damaged kernels
- of 51 and 27% in 2016 versus 4 and 1% in 2015, in groves 4 and 5, respectively.
- 340 The high percentages of nymphs found through plant beating indicate that hazelnut can serve
- both as feeding and reproductive host for *H. halys*, while on other host plants, only or mostly
- adults are observed (Acebes-Doria et al. 2016; Zobel et al. 2016). In addition, *H. halys*
- 343 showed the highest percentages of survivors as well as of females laying eggs in the cages,
- 344 further suggesting the suitability of hazelnut as a host plant.
- 345 Across all field surveys, irrespective of the grove, region, and year, the highest numbers of
- 346 bugs per plant, and also of *H. halys*, were collected in the groups of plants along the borders
- 347 of the orchards compared to those in the center. Even considering the high heterogeneity of
- 348 the two areas, this result confirms that the invading population of *H. halys* establishes on wild

- 349 or cultivated host plants grown in the surroundings and migrates into the hazel crop area
- 350 throughout the season, as already reported for other crops (Leskey et al. 2012; Rice et al.
- 351 2014). This perimeter-driven behavior could be exploited by using perimeter applications of
- insecticides to control *H. halys* in the borders, as suggested for other fruit crops (Blaauw et al.
- 353 2015). To increase efficiency, the perimeter treatment could be coupled with the use of
- attractive plants (e.g., grown on the borders of the grove not used for vehicle movement) to
- 355 manage the pest populations outside the crop (Mathews et al. 2017; Nielsen et al. 2016;
- 356 Soergel et al. 2015).
- 357 Commercially available traps and lures can be useful tools for monitoring and detection,
- 358 decision making, and management strategies. In our study captures varied substantially
- among sites during the sampling period, but generally peaked in the late season (mid-August
- 360 to early October), consistently with other studies carried out using commercially available
- 361 lures (combination of aggregation pheromone *EEZ*-MDT) (Weber et al. 2017). Only in one
- 362 orchard in W Georgia, earlier captures we recorded from mid-June come from overwintering
- 363 populations. Differences in the captures may reflect the size of the overwintering population
- in the general vicinity of the traps (Bergh et al. 2017), but earlier peaks are also typical of
- 365 higher population years (Weber et al. 2017), as well as affected by lure composition. Both
- 366 these factors can be at the base of the significant increase in 2017 of early captures by traps on
- 367 hazelnut in both NW Italy and W Georgia (personal observation). Therefore, the traps can
- also be used in hazelnut crops to develop and apply thresholds to trigger management
- 369 decisions which ultimately reduce insecticide usage.
- 370 Broad-spectrum insecticides can provide an effective control of *H. halys*, but only if they are
- applied directly on the insects (Kuhar and Kamminga 2017; Leskey et al. 2012; Rice et al.
- 372 2014). Therefore, multiple applications are needed for bug control throughout the season,
- 373 nullifying the integrated pest management programs largely adopted in NW Italy. Moreover,
- 374 due to the scarcity of active ingredients authorized on hazelnut crops in Italy, the repeated
- 375 application of insecticides such as pyrethroids would be particularly detrimental for secondary
- 376 pest outbreaks (e.g., spider mites, aphids, mealybugs) and for the community of beneficial
- 377 species (AliNiazee, 1998). Additionally, in some areas (e.g., in Georgia and Turkey), few
- 378 local farms are equipped for the application of pesticides at the level required for a successful
- 379 management of *H. halys*. Moreover, chemical treatments are not always feasible due to
- 380 several factors such as strongly sloping land, non-rational management of plantations (e.g.
- 381 irregular tree spacing, high number of branches per plant, uncontrolled growth of suckers),
- 382 and excessive tree height and canopy density.

383	In a perspective of reduction or avoidance of chemicals and preservation of a relatively stable
384	agroecosystem, such as the hazelnut orchard, alternative pest management strategies should
385	be further developed and tailored for the peculiar characteristics of the considered hazel
386	groves (geographical area, size of the orchards, surroundings, agroecosystem traits, etc.). As
387	observed in apple orchards (Morrison et al. 2016), the attract-and-kill strategy, using a few
388	trees intensively baited with pheromone and sprayed on a weekly basis with insecticides
389	efficacious against H. halys, could reduce the area treated with an insecticide. This strategy
390	should be evaluated also for hazelnut, even if it is currently not cost effective due to the high
391	costs of the intensive usage of lures (Weber et al. 2017). Moreover, as a long-term solution,
392	biological control with the natural enemy complex of <i>H. halys</i> , native or introduced, could
393	play a role in managing this pest, especially in organic farming systems. In particular,
394	hymenopteran egg parasitoids within the genera Anastatus (Eupelmidae), Ooencyrtus
395	(Encyrtidae), Trissolcus, and Telenomus (Scelionidae) are under evaluation for H. halys
396	control (Dieckhoff et al. 2017; Haye et al. 2015a; Herlihy et al. 2016; Ogburn et al. 2016).
397	
398	Author Contribution
399	LT conceived and designed the research. LB and STM conducted the experiments, analyzed
400	the data, and wrote the manuscript. All authors read and approved the final manuscript.
401	
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406	
407	Compliance with ethical standards
408	Conflict of Interest The authors declare that they have no conflict of interest.
409	
410	

# **Tables and Figures**

- **Table 1** Localization and characteristics of the five hazel groves surveyed in NW Italy

# 414 (Piedmont) and W Georgia (Samegrelo) during 2015 and 2016

Hazel	Location	Cultivar/	Size (m <sup>2</sup> )	Bordering host plants
grove		pest management		
1	Italy, Piedmont, Asti	TGdL <sup>a</sup> /	4950	Cornus sanguinea
	(44°54'35.59"N, 8°15'43.26"E; 129 m a.s.l.)	organic		
2	Italy, Piedmont, Cossano Belbo (CN)	TGdL/	5527	Prunus avium
	(44°38'27.53"N, 8°11'54.06"E; 460 m a.s.l.)	conventional		Prunus persica
				Pyrus malus
3	Italy, Piedmont, Magliano Alfieri (AT)	TGdL/	3200	Cornus sanguinea
	(44°45'46.50"N, 8° 3'13.56"E; 205 m a.s.l.)	no insecticide		
		treatments		
4	Georgia, Samegrelo, Chitatskari	Tonda di Giffoni/	71200	Cornus sp.
	(42°27'18.21"N, 41°52'18.68"E; 60 m a.s.l.)	conventional		Rubus sp.
				Cryptomeria japonica
5	Georgia, Samegrelo, Chitatskari	Anakliuri/	183200	Cornus sp.
	(42°26'50.0"N, 41°51'52.41"E; 60 m a.s.l.)	conventional		Rubus sp.
				Cryptomeria japonica

415 <sup>a</sup>: Tonda Gentile delle Langhe cultivar

# 417 **Table 2** Mean percentages ( $\pm$ SE) of kernels damaged by bugs, shriveled, and moldy per cage in the presence of the tested bug species and in the 418 absence of insects (control)

Species	No. of	No. of	% Of total	% On total dar	naged kernels <sup>d</sup>	% Of other defe	cts
	cages <sup>a</sup>	nuts <sup>b</sup>	damaged kernels <sup>c</sup>	External	Brown	Shriveled	Moldy/rotten
Halyomorpha halys	54	532	74.82±4.14 <b>a</b>	80.53±3.24 <b>a</b>	87.69±2.24 <b>b</b>	19.60±3.06 <b>a</b>	10.22±2.44 <b>a</b>
Gonocerus acuteangulatus	53	580	39.07±4.49 c	78.02±4.28 <b>a</b>	92.59±3.42 a	5.08±1.80 c	5.50±1.39 ab
Nezara viridula	58	579	57.03±4.31 <b>b</b>	61.67±4.21 <b>b</b>	80.39±3.43 b	19.73±3.96 <b>a</b>	6.03±1.71 <b>b</b>
Palomena prasina	59	568	39.49±3.94 c	56.79±5.01 <b>b</b>	84.72±3.91 <b>b</b>	10.26±2.75 <b>b</b>	0.75±0.33 c
Control	30	295	$0.00{\pm}0.00$ d	-	-	5.91±3.68 c	1.56±1.23 c
GLM	Wald $\chi^2$		81708.480	72.323	18.988	69.459	38.313
	df		3	3	3	4	4
	Р		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

419 In each column, values followed by the same letter are not significantly different (Bonferroni test, P < 0.05, under GLM procedure with binomial

420 distribution and logit link)

- 421 <sup>a</sup>Total number of cages (excluding cages containing only blank nuts)
- 422 <sup>b</sup>Total number of analyzed kernels
- 423 °% Of total damaged kernels on total number of analyzed kernels.

424 <sup>d%</sup> Of kernels showing external (versus internal) and brown (versus white) symptoms on total damaged kernels

- 425 **Table 3** Numbers of cages with females, females plus nymphs (i.e., females laying eggs in the
- 426 cages), males, and nymphs of the tested bug species: mean numbers ( $\pm$  SE) per female of
- 427 nymphs developed in the cages (offspring): percentages of the specimens replaced during
- 428 weekly surveys (% of replacement, mean  $\pm$  SE per survey) and of the specimens alive
- 429 throughout the trials, therefore never replaced (% of survival), in 2015

Species	Stage/sex	No.	Offspring	% Of	% Of survival	
Species			(mean per cage)	replacement		
Halyomorpha	females + nymphs	24	19.58±1.62 b	$1.04 \pm 0.01$	95.83	
halys	females	7		$14.29 \pm 0.06$	42.86	
	males	29		5.17±0.03	75.86	
	total	60		4.58±0.02 a	80.00	
Gonocerus	females + nymphs	8	12.25±3.73 b	$15.63 \pm 0.06$	50.00	
acuteangulatus	females	24		$26.04 \pm 0.06$	41.67	
	males	26		$17.31 \pm 0.03$	65.38	
	total	58		20.69±0.04 b	53.45	
Nezara viridula	females + nymphs	11	32.18±5.20 a	4.55±0.03	81.82	
	females	19		$10.27 \pm 0.03$	62.50	
	males	29		$15.52 \pm 0.04$	55.17	
	total	59		11.83±0.03 ab	62.50	
Palomena	nymphs	5		12.50±0.13	50.00	
prasina	nymphs (emerg. female)	29		6.29±0.04	77.78	
	nymphs (emerg. male)	27		$13.89 \pm 0.04$	55.56	
	total	61		10.06±0.03 ab	65.52	
ANOVA	F		7.797	4.199		
	df		2; 40	3; 12		
	Р		0.001	0.030		

430 In each column, values in bold followed by the same letter are not significantly different

431 (Tukey's test, P < 0.05)

432

Grove/	Mean n. of bugs per plant per date		GLM			Mean % of damaged kernels per		GLM		
position	(% of <i>H. haly</i>	(% of <i>H. halys</i> )				plant				
	2015	2016	Wald $\chi^2$	df	Р	2015	2016	Wald $\chi^2$	df	Р
Grove										
1	$2.77 \pm 0.96$	1.04±0.23 (5.33%)	37.596	1	< 0.001	19.42±2.76	19.44±2.55	0.002	1	0.966
2	0.70±0.24	$0.47{\pm}0.11$	2.011	1	0.156	7.82±1.13	16.47±2.60	242.383	1	< 0.001
3	0.97±0.26	4.53±0.98	58.702	1	< 0.001	13.98±1.18	$14.58 \pm 1.58$	0.664	1	0.415
Position										
Border	$1.78 \pm 0.43$	2.03±0.36				6.51±0.74	5.66±0.61			
Centre	$0.28 \pm 0.14$	$0.47{\pm}0.11$				2.60±0.30	$3.07 \pm 0.58$			
GLM										
Wald $\chi^2$	16.581	54.411				368.105	314.177			
df	1	1				1	1			
Р	< 0.001	< 0.001				< 0.001	< 0.001			

Table 4 Mean numbers ( $\pm$  SE) of hazel bugs total collected per plant and per date and mean percentages ( $\pm$  SE) of damaged kernels per plant

(n = 300) in each grove, on the borders, and in the center across all three hazel groves surveyed in NW Italy (Piedmont) during 2015 and 2016

436 GLM procedure was performed with Poisson distribution and log link for number of bugs, with binomial distribution and logit link for

437 percentage of damaged kernels

438

440 **Table 5** Mean numbers ( $\pm$  SE) of hazel bugs total collected per plant and per date and percentages ( $\pm$  SE) of damaged kernels by bugs per plant,

Grove/	No. bugs per plant per date (% of <i>H. halys</i> )		GLM			% Of damaged	% Of damaged kernels			
Position <sup>a</sup>	2015	2016	Wald $\chi^2$	df	Р	2015	2016	Wald $\chi^2$	df	Р
Grove										
4	0.46±0.16 (68.42%)	1.44±0.38 (89.27%)	11.963	1	< 0.001	$1.43 \pm 0.17$	26.63±2.36	1301.845	1	< 0.001
5	0.46±0.19 (65.38%)	4.22±2.10 (82.53%)	50.578	1	0.001	4.25±0.51	51.31±2.84	3338.852	1	< 0.001
Position										
5 m	0.89±0.23	6.50±3.06 a				3.58±0.77 a	55.78±3.49 a			
100 m	0.83±0.23	1.63±0.59 b				2.39±0.30 b	33.28±4.10 b			
200 m	$0.00{\pm}0.00$	0.38±0.15 c				3.00±0.82 ab	27.85±1.71 c			
GLM										
Wald $\chi^2$	1.445	117.020				17.070	972.285			
df	1	2				2	2			
Р	0.229	< 0.001				< 0.001	< 0.001			

441 in each grove, and overall in the different groups of plants in the two hazel groves surveyed in W Georgia (Samegrelo) during 2015 and 2016

442 In each column, values per position followed by the same letter are not significantly different (Bonferroni test, P < 0.05, under GLM procedure).

443 GLM procedure was performed with Poisson distribution and log link for number of bugs, with binomial distribution and logit link for

444 percentage of damaged kernels





447 **Fig. 1** Numbers of adults of *Halyomorpha halys* captured per pheromone trap in the hazel

448 groves 1 and 3 in NW Italy (Piedmont) and 4 and 5 in W Georgia (Samegrelo) during 2016. In

the x-axis, the Roman numerals I and II referred to the first and the second half of the month,

450 respectively. <sup>1</sup>Missing data for grove 5; <sup>2</sup>missing data for grove 4

445

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