AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Occurrence and impact of *Agrilus* spp. and associated egg parasitoids in hazel groves of Northwest Italy

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/133258> since 2016-01-13T15:45:07Z

Published version:

DOI:10.1111/jen.12057

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

UNIVERSITÀ DEGLI STUDI DI TORINO

This is the accepted version of the following article:

Moraglio S.T., Corte M., Tavella L., 2013. Occurrence and impact of *Agrilus* spp. and associated egg parasitoids in hazel groves of Northwest Italy,

which has been published in final form at

Journal of Applied Entomology 137 (10): 761-772. DOI: 10.1111/jen.12057

Occurrence and impact of *Agrilus* spp. and associated egg-parasitoids in hazel groves of Northwest Italy

Silvia T. Moraglio¹, Maria Corte², Luciana Tavella¹

¹Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di Torino, via L. da Vinci 44, 10095 Grugliasco (TO), Italy

²CRReSO - Consorzio di Ricerca Sperimentazione e Divulgazione per l'Ortofrutticoltura Piemontese, Corso Nizza 21, 12100 Cuneo, Italy

Corresponding author: Luciana Tavella

tel. +39 011 6708533 – fax +39 011 6708535

e-mail luciana.tavella@unito.it

Short title: *Agrilus* spp. and egg-parasitoids in hazel groves

Abstract: In several hazel (*Corylus avellana* L.) groves in the area of Langhe (Piedmont, Northwest Italy), many hazel branches suddenly withered, and in some cases the whole tree died, with heavy economic losses for the farmers. Symptoms of jewel beetle attacks were detected on the trees. Eight *Agrilus* species were caught by traps from 2007 to 2009 in the surveyed hazel groves; among them only four species have been known to develop on hazel. On the traps, *A. olivicolor* Kiesenwetter was the most abundant species, while almost only *A. viridis* (L.) was sampled by plant beating from 2008 to 2010. Moreover, almost all adults emerged from field-collected hazel wood belonged to this latter species that proved to be the main responsible for the severe attacks on the hazel trees. *A. viridis* adults emerged from late May to late June, and generally lived until the end of August, while egg-masses were observed from late May to late July. The egg-parasitoid *Oobius zahaikovitshi* Trjapitzin was found in all of the investigated groves; adults emerged already from the first egg-masses collected on hazel trees in late May-early June. *O. zahaikovitshi* was able to largely reduce *A. viridis* populations, with a parasitism rate of more than 50% in some groves. Recent attacks of *A. viridis* were related to a long period of drought; however, appropriate agronomic practices to minimise the effects of water stress and to conserve natural enemies, such as *O. zahaikovitshi*, could be more effective to protect hazel groves against boring beetle attacks.

Keywords: *Agrilus viridis*, *Corylus avellana*, *Oobius zahaikovitshi*, life cycle, seasonal abundance

INTRODUCTION

With nearly 3000 described species, the genus *Agrilus* (Coleoptera: Buprestidae) is one of the largest in the world (Bellamy 2008; Jendek 2012). Jewel beetles are generally considered secondary pests, attacking dying or weakened trees, because larvae can be destroyed by sap flow pressure and plant defence mechanisms like wound callus formation in vigorous trees (Evans et al. 2004). However, some species are rated as primary pests, and are able to kill trees directly with an extensive larval feeding under the bark causing the girdling of the phloem system (Heering 1956; Evans et al. 2004). Examples of this in Europe are *Agrilus biguttatus* (F.), which is considered a factor in oak decline (Moraal and Hilszczanski 2000; Vansteenkiste et al. 2004), *A. suvorovi* Obenberger, responsible for severe damage to young poplar plantations (Arru 1961-62), and *A. viridis* (L.), whose outbreaks are fatal for beech (Heering 1956; Lakatos and Molnár 2009).

Although most *Agrilus* species are harmful for forestry, some species can damage fruit farming, such as *A. viridis*, which can also attack hazelnut trees (*Corylus avellana* L.) (Curletti et al. 2003). Many subspecies or ecological forms of *A. viridis*, with larvae developing in different host plants, have been described, including *A. viridis ignotus*, a subspecies developing exclusively on hazel, with females being green in colour, like males (Ciampolini and Ugolini 1975). Moreover, three subspecies of *A. viridis*, named as the beech variety, the birch variety (both belonging to *A. viridis* var. *fagi*) and the willow variety (*A. viridis* f. *typica*) were recently separated by molecular analysis (Bernhard et al. 2005). However, determination of species and subspecies of the so called *A. viridis*-complex is very difficult, because they are morphologically very similar or even indistinguishable, and their taxonomy has been frequently revised (Bernhard et al. 2005; Bellamy 2008; Jendek 2012). In fact, the identification keys proposed for Italian Buprestidae does not separate out the different *A. viridis* subspecies in relation to host plants (Curletti et al. 2003). In the 1980s, *A. viridis* caused the withering of hazelnut branches, and sometimes the death of whole trees, in the hazelnut growing area of Piedmont, Northwest Italy, but it is thought that the outbreaks were probably due to the weakening of trees caused by an unknown hazelnut disease at that time, as well as because of the plantation of hazelnut groves in inappropriate areas (Ciampolini and Ugolini 1975; Pellegrino and Mozzone 1985).

In Northwest Italy, *A. viridis* completes one generation in a year (only few individuals needed two years for their life cycle). In the spring, adults emerge from characteristic D-shape holes and feed on foliage, which is followed by females laying eggs in masses on the bark of the host plants. Larvae bore galleries into the bark, sometimes boring short channels to discharge the excess sap outside, at which point they reach the cambial layer and cause severe damage to the trees by interrupting the sap flow of many vessels. Larvae feed in the cambial layer during the majority of their

development, following which they go into the inner wood and come back to the bark only to construct the pupal cell. Larvae hibernate in individual cells at 5-10 mm under the bark and pupate in the following spring (Ciampolini and Ugolini 1975). The same life-cycle and behaviour was observed on beech in Germany (Heering 1956). Some biological traits of *A. viridis* are similar to those of other *Agrilus* species, e.g., *A. suvorovi* on poplar in Italy (Arru 1961-62).

Starting from the summer of 2006, in several hazel groves in the area of Langhe (Piedmont, Northwest Italy), many hazel branches suddenly withered and, in some cases, the whole tree died, with heavy economic losses for the farmers. On these branches, the typical emerging holes of the wood-boring beetle adults belonging to the family Buprestidae were detected, and the symptoms were very similar to those previously attributed to the species *A. viridis* (Ciampolini and Ugolini 1975; Pellegrino and Mozzone 1985). However, 49 *Agrilus* species have been recorded in Italy and five of them are reported to develop on hazel trees (Curletti et al. 2003). Therefore, from 2007 to 2010, research was conducted to verify the presence and abundance of *Agrilus* species in the hazel groves in the area of Langhe, and to assess the species responsible for the hazel damage. Moreover, the presence of an egg-parasitoid of the genus *Ooencyrtus* (Hymenoptera: Encyrtidae) developing on *A. viridis* eggs had been observed in a previous study (Ciampolini and Ugolini 1975), so research was also carried out to evaluate the abundance and efficacy of egg-parasitoids, with the aim to implement effective and environmentally-friendly control tactics.

MATERIALS AND METHODS

***Agrilus* species**

Four-year-field surveys were carried out in the Langhe hills (Piedmont, Northwest Italy) in hazel groves planted with the cultivar Tonda Gentile delle Langhe to assess presence and abundance of *Agrilus* species. From 2007 to 2009, *Agrilus* adults were sampled by yellow sticky traps (40 × 25 cm) in hazel groves that were showing clear symptoms of jewel beetle attacks, signified by the presence of egg-masses and D-shape exit holes on the bark, or withering branches (table 1). In 2007-2008, 10 traps were placed in each grove on plants showing severe symptoms above 1.5 m from the soil, whilst in 2009, five traps were placed in the upper part of the crown (about 3 m from the soil) and five traps in the lower part (about 1.5 m from the soil). The traps were changed every two weeks from late May to early August in 2007, and weekly between late April and late October in 2008 and April and early August in 2009. Traps, labelled in relation to their location in the grove, were transferred to the laboratory, where they were stored at -20°C until their examination. *Agrilus* adults captured were then unglued from the traps with solvent (SuperAvio®, Bessone, Italy; cyclohexane 90%, dichloropropane 10%) and preserved in 70% ethanol until they were identified using the keys of Curletti et al. (2003). To compare the *Agrilus* abundance in the groves and years,

total numbers of adults captured by traps of each location ($n = 10$ per grove) in the eight-week-period from early June to early August were analysed by one-way-ANOVA after testing them for homogeneity of variance (Levene) and normality (Kolmogorov-Smirnov); means were then separated by Tukey test ($P < 0.01$). Statistical analysis was performed using the software SPSS version 17.0 (SPSS).

From 2008 to 2010, adult sampling was carried out in several hazel groves (tables 1 and 3) from late May to late June, in order to assess distribution of *Agrilus* species in the hazelnut growing area of Piedmont. This was achieved by beating the foliage of four hazel trees per grove on a tarpaulin (5×5 m) placed on the ground early in the morning (5:00-6:00 am). Due to thanatosis, *Agrilus* adults fell on the tarpaulin, and were collected and preserved in 70% ethanol until their identification using the keys of Curletti et al. (2003). To compare *Agrilus* sampled by plant beating in the groves reported in table 1, mean numbers of adults per survey captured in each grove were analysed by one-way-ANOVA after testing them for homogeneity of variance (Levene) and normality (Shapiro-Wilk); means were then separated by Tukey test ($P < 0.01$). Statistical analysis was performed using the software SPSS version 17.0 (SPSS).

From 2008 to 2010, at the end of April, pruned branches with symptoms of jewel beetle attacks were collected from severely infested hazel groves (table 1). In addition, dead branches that had been cut and left on the ground for at least one year were collected in 2010. The branches were reduced to pieces of 60 cm in length, and then transferred to the laboratory, where they were separated by site in cardboard boxes ($40 \times 60 \times 25$ cm), with two transparent plastic tubes (2.8 cm diameter $\times$ 11.5 cm length) inserted in one side to ease the collection of insects. Inside the boxes, the branches were spaced from the sides of boxes with expanded polystyrene laths to allow adult emergence all around the branches. The boxes were then kept in the open air, with protection only from the rain and direct sun. Three times a week the insects obtained were removed from the plastic tubes, and *Agrilus* adults were immediately identified using the keys of Curletti et al. (2003).

***Agrilus viridis* biology**

During field surveys from late April to late September in 2007-2008, egg-masses were sampled on a weekly basis by visual inspection of the hazel branches in the surveyed groves (table 1). In 2007, hazel branches were randomly inspected in each grove for 60 min. In March 2008, two branches from five plants per grove with the presence of egg-masses were sandpapered down to a length of 1 m to remove the old egg-masses, and were marked with a ribbon. The cleaned portions of the branches were inspected on a weekly basis to find new egg-masses and assess the beginning and end of the oviposition period. In both years, all egg-masses were removed with a piece of bark and transferred to the laboratory, where they were put into individual glass tubes (16 mm

diameter $\times$ 80 mm length) closed with a cotton plug that was wetted periodically. Tubes were kept in climatic chambers (T $24 \pm 1^\circ\text{C}$, RH $65 \pm 5\%$) and checked daily to observe egg hatching and the emergence of *A. viridis* larvae and of possible egg-parasitoid adults.

In 2008, *A. viridis* adults that had emerged from the field-collected branches were kept in a Plexiglas box ($40 \times 40 \times 40\text{cm}$) in climatic chamber (T $24 \pm 1^\circ\text{C}$, RH $65 \pm 5\%$) with fresh hazel foliage as food and a little hazel branch for oviposition. Every two days the boxes were checked to count new egg-masses that had been laid on the branches and on the sidewalls of the boxes, and the hazel foliage and branches were replaced. All the egg-masses which were taken off from the box sidewalls easily and without damage were put into individual glass tubes and kept in climatic chambers as described above. They were checked daily to observe egg hatching and larval emergence.

***Agrilus viridis* egg-parasitism**

The presence and abundance of egg-parasitoids in the surveyed hazel groves (table 1) were assessed from 2007 to 2010. All field-collected egg-masses were put into individual glass tubes, and kept in climatic chambers (T $24 \pm 1^\circ\text{C}$, RH $65 \pm 5\%$). The egg-masses were checked daily to assess parasitoid biology in 2007 and 2008, and monthly to evaluate the egg-parasitism at the end of the season in 2009 and 2010.

In 2007, to assess their longevity, egg-parasitoid adults were reared in glass tubes (16 mm diameter $\times$ 80 mm length), closed with a cotton plug that was wetted periodically, provided with some drops of honey on a leaf, and kept in climatic chambers (T $24 \pm 1^\circ\text{C}$, RH $65 \pm 5\%$). When they died, they were preserved in 70% ethanol. In the autumn, all of the un-hatched egg-masses in the tubes were stored in a polystyrene box placed in the open air, protected from the rain and direct sun. In the following spring, they were transferred to climatic chambers and checked weekly to verify the possible emergence of overwintering egg-parasitoids.

At the end of the growing season, samples of the egg-masses collected from 2007 to 2010 were observed using a stereomicroscope to count empty and un-hatched eggs. The exit holes of the empty eggs were then examined to establish if they had been chewed by beetle larvae or by egg-parasitoid adults. It was shown that larvae chew irregular-shaped holes on the underside of the eggs in contact with the bark during eclosion, while parasitoid adults chew nearly circular holes on the upper egg surface (Ciampolini and Ugolini 1975; Liu et al. 2007). Moreover, the eggs that had contained *A. viridis* larvae were often identifiable as they were full of larval droppings. The unhatched eggs were further dissected to verify the presence of beetle larvae or egg-parasitoid adults or larvae.

To evaluate the efficacy of the egg-parasitoids, three indices (discovery efficiency, parasitoid efficiency and parasitoid impact) proposed by Bin and Vinson (1990) were applied after some

adjustments. The ‘discovery efficiency’ (*i.e.*, percentage of egg-masses containing parasitized eggs) was calculated as the number of egg-masses discovered by the parasitoid (at least one parasitized egg) over the total number of examined egg-masses. The ‘parasitoid efficiency’ was modified as mean parasitism for each discovered egg-mass, and expressed as mean percentage of parasitized eggs within each discovered egg-mass. Finally, the ‘parasitoid impact’, or parasitism rate, corresponded to the number of parasitized eggs over the total number of field collected eggs. Samples of the egg-parasitoids emerged from egg-masses were sent to the respective specialist for the specific identification. Voucher specimens were deposited at the DISAFA (Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Torino, Italy).

RESULTS

Agrilus species

Eight species of *Agrilus* were sampled with yellow sticky traps from 2007 to 2009 (table 2). The four species *A. sulcicollis* Lacordaire, *A. derasofasciatus* Lacordaire, *A. convexicollis* Redtenbacher and *A. cuprescens* Menetries were only captured occasionally in some groves and in some years, while the other four species *A. angustulus* (Illiger), *A. graminis* Gory et Laporte, *A. olivicolor* Kiesenwetter and *A. viridis* were found more frequently (table 2). On the traps, *A. olivicolor* was the most abundant species, even though the number of captured adults of all *Agrilus* species decreased from 2007 to 2009 in spite of the higher number of traps (table 2). No differences were found between captures for the traps placed in upper part or lower part of the crown in 2009 (data not shown). Therefore, adults captured by traps of each location in each grove and year from early June to early August (*i.e.* an eight-week-period) were cumulated, and statistical analysis showed that mean numbers per grove were significantly higher in 2007 than in 2008-2009 in all groves except the grove in Bosia (G1) in 2008 (ANOVA: df: 12, 117; F: 132.091; $P < 0.001$; n: 130).

On the contrary, *A. viridis* was the most abundant species, indeed almost the only one, sampled by hazel foliage beating from 2008 to 2010, even though this species was found in variable amounts in relation to the sites (table 3). Significantly higher mean numbers per survey were observed in the groves in Carrù (G8), and in Cravanzana (G4) in 2008 and 2009, whereas in 2010 mean numbers per survey were significantly lower also in these groves (ANOVA: df: 16, 48; F: 117.89; $P < 0.001$; n: 65) (table 3A). Only eight adults of *A. olivicolor* were collected using this method during the three year study (table 3). Moreover, *A. viridis* was the primary species obtained from the living hazel branches collected in the groves from 2008 to 2010, with 97% of the emerged adults belonging to this species, while other species were always fewer or absent (table 4). By contrast, other species were more represented from the dead branches collected in 2010: the emerged adults

belonged to *A. graminis* (29%), to *A. angustulus* (10%), to *A. olivicolor* (7%) but only 55% to *A. viridis* (table 4).

***Agrilus viridis* biology**

During field surveys, 526 egg-masses were collected in total: 455 in 2007 and 71 on the cleaned branches in 2008. In 2007, egg-masses were found from the last ten days of May to the second half of July. On average, 5.8 ± 0.2 ($n = 93$; min. 3, max. 11) eggs were contained in each egg-mass and 4.1 ± 0.2 ($n = 197$; min. 1, max. 12) larvae emerged. When larvae did not emerge all together from the same egg-mass, 3.4 ± 0.3 ($n = 66$; min. 1, max. 13) days occurred between the first and the last emergence. In 2008, egg-masses were found on the cleaned branches from the beginning of June to the end of July (figure 1). For oviposition, *A. viridis* females showed to prefer some hazel trees; indeed, in each grove at least 40% of egg-masses was found on one of the five trees inspected, while none or few egg-masses were observed on the other trees (data not shown). In a similar fashion to 2007, 6.2 ± 0.2 ($n = 48$; min. 3, max. 10) eggs were contained in each egg-mass on average, and 3.4 ± 0.7 ($n = 15$; min. 1, max. 9) larvae emerged. The last larvae emerged 7.5 ± 2.7 ($n = 4$; min. 2, max. 13) days after the first ones in cases where they did not all emerge together.

In the laboratory, adult emergence from the field-collected branches lasted for approximately one month during the three-year-period, *i.e.* from late May to late June in 2008 and 2010, and from mid-May to mid-June in 2009 (figure 2). Adult emergence in 2009 was earlier than in 2008 and 2010 (figure 2), probably due to the highest temperature registered in May in that year (data not shown). In 2008, the adults of *A. viridis* that emerged from the branches were reared, and most of them lived until the end of August, while a few lived until mid-September. Females started to oviposit approximately one month after their emergence (*i.e.* late June, about 20 days after the occurrence of the first egg-masses in the field), and continued until late July (figure 1). Each female ($n = 12$) laid an average of 12.2 egg-masses ($n = 146$), each containing 4.9 ± 0.4 ($n = 29$, min. 3, max. 11) eggs, from which 3.0 ± 0.5 ($n = 15$; min. 1, max. 8) larvae emerged, which was similar to that observed for field-collected egg-masses.

***Agrilus viridis* egg-parasitism**

From the egg-masses collected in the hazel groves from 2007 to 2010, one egg-parasitoid, *Oobius zahaikevitchi* Trjapitzin (Hymenoptera: Encyrtidae), was consistently identified. In 2007, from 35 out of 69 parasitized egg-masses, both beetle larvae and parasitoid adults were obtained. In these cases, the first *O. zahaikevitchi* adults emerged 12.7 ± 0.7 (min. 5, max. 24 days) days after the last *A. viridis* larvae from the same egg-mass, and the last adults emerged 3.9 ± 0.5 ($n = 23$; min. 1,

max. 10) days after the first ones. In the laboratory, *O. zahaikevitchi* adults showed a longevity of 17.9 ± 2.3 (n = 42; min. 2, max. 60) days.

The *A. viridis* larva and egg-parasitoid emerging trends in 2007 are shown in figure 3. Overall, after examining the egg-masses at the end of the season, *O. zahaikevitchi* was already found in egg-masses collected at the end of May in the hazel groves in Bonvicino (G2) and Camerana (G3), with a discovery efficiency on that sampling date of 46.2% (n = 39) and 55.6% (n = 9), respectively. In the other groves in Bosia (G1) and Cravanzana (G4), the parasitoid was present in egg-masses collected from the beginning of June. Also in 2008, *O. zahaikevitchi* adults started to emerge from the egg-masses collected at the beginning of June in all of the investigated groves, except for the grove in Cravanzana (G4), where no parasitoids were found throughout the season.

After the examination of the egg-masses sampled from 2007 to 2010, *O. zahaikevitchi* showed an overall parasitism rate of *A. viridis* eggs in the last three years of over 30%, but this rate fluctuated highly among the surveyed groves (table 5). Only in the hazel grove in Bonvicino (G2) did parasitoid activity appear to be stabilised, with discovery and mean parasitism efficiencies of about 70% and a parasitism rate of about 50% from 2008 to 2010, all of which were thought to be increased from 2007 (table 5). In the grove in Bosia (G1), parasitoid activity increased from 2007 to 2009 (parasitism rates of 8.6% and 61.5%, respectively) but it decreased again in 2010 (parasitism rate of 22.8%). In all other groves the parasitism rate was more variable during the sampling period (table 5).

DISCUSSION

Many subspecies or ecological forms of the so called *A. viridis*-complex, with larvae developing in different host plants, have been described (Ciampolini and Ugolini 1975; Bernhard et al. 2005). However, since they are morphologically very similar or even indistinguishable, and their taxonomy has been frequently revised (Bernhard et al. 2005; Bellamy 2008; Jendek 2012), for this study the identification key proposed for Italian Buprestidae was followed, which does not separate out the different *A. viridis* subspecies (Curletti et al. 2003).

The four *Agrilus* species that were only collected by the sticky traps occasionally, *A. sulcicollis*, *A. derasofasciatus*, *A. convexicollis* and *A. cuprescens* (table 2), are known to have different host plants, such as *Castanea* spp., *Euonymus* spp., *Fagus* spp., *Fraxinus* spp., *Ligustrum* spp., *Pistacia* spp., *Populus* spp., *Quercus* spp., *Rosa* spp., *Rubus* spp., *Syringa* spp., *Vitis* spp., but their larvae do not develop on hazel (Curletti et al. 2003). It is possible that they came from the surrounding woods and were accidentally trapped during their movements. Conversely, the larvae of the other four species, *A. angustulus*, *A. graminis*, *A. olivicolor* and *A. viridis* (table 2), are known to develop on hazel, as well as other plants (Curletti et al. 2003), explaining why adults of these species were

captured on sticky traps more frequently. Actually, amongst all of the species, the most abundant in the surveyed hazel groves were *A. olivicolor* and *A. viridis*, even though *A. olivicolor* was largely sampled by sticky traps whereas *A. viridis* was almost the only beetle collected by beating the hazel foliage. The two field-sampling methods showed different levels of effectiveness and conflicting results. However, the different spatial distribution of *A. olivicolor* and *A. viridis*, assessed in a study in Switzerland to verify the horizontal and vertical distribution of saproxylic beetles in forests (Wermelinger et al. 2007), was not considered a possible cause. In this study *A. viridis* was almost exclusively captured in the canopy and *A. olivicolor* was collected at all levels (ground, medium canopy, canopy), whereas, in field-surveys carried out in Piedmont, the numbers of adults caught by traps placed in the upper part and in the lower part of the crown in 2009 were not different, either for *A. viridis* or *A. olivicolor*. However, the difference between the two sampling methods could be explained by adult behaviour. *A. viridis* adults feed on hazel foliage, so they usually stay on the hazel crown (Ciampolini and Ugolini 1975) that is shaken by foliage beating. On the contrary, *A. olivicolor* adults probably feed on other plants, perhaps on flowers, so they could be more attracted to the yellow traps while they are flying in the groves. Therefore, the two sampling methods provide different information, and could be used for species-specific field sampling.

Moreover, *A. viridis* was the most abundant species obtained from the pruned hazel branches (table 5), proving to be able to attack and develop on weakened trees, especially on parts of living trees that are not healthy, ultimately leading to the death from the activity of large numbers of larvae (Evans et al. 2004; Lakatos and Molnár 2009). From the dead branches, the other three species known to develop on hazel were found more abundantly, but were still in numbers fewer than those documented for *A. viridis* (table 5). Therefore, this species was confirmed to be the main damaging jewel beetle for the hazel groves of Piedmont, as already previously observed (Ciampolini and Ugolini 1975; Pellegrino and Mozzone 1985).

The oviposition behaviour of *A. viridis* females was consistent with known jewel beetle biology. By observing the cleaned branches in 2008, females showed a preference for laying eggs on some hazel trees in spite of the presence of others, probably choosing the trees in several stressed conditions, in accordance with previously observed behaviour for *A. viridis* on hazel (Ciampolini and Ugolini 1975; Pellegrino and Mozzone 1985) and on beech (Heering 1956; Lakatos and Molnár 2009). This behaviour has also been seen for *A. anxius* Gori on birch (Anderson 1944), *A. suvorovi* on poplar (Arru 1961-62), *A. auriventris* Saunders on citrus (Ohgushi 1978), *A. bilineatus* (Weber) on oak (Muzika et al. 2000), and *A. biguttatus* (F.) on oak (Vansteenkiste et al. 2004). Even when the hazel trees did not show evident symptoms of water stress, it is thought that the *A. viridis* females probably felt the effects of a long period of reduced rainfall, as occurred in the area of Langhe between the years of 2003 and 2007 (data provided by Rete Agrometeorologica, Regione Piemonte,

Settore Fitosanitario). In fact, the attacks of wood boring beetles, including *Agrilus* spp., can be affected by several effects of drought on trees, such as spectral qualities, temperature, acoustic emissions, allelochemicals and volatile stress metabolites production (Mattson and Haack 1987). Similarly, the possibility that stressed oaks release volatiles that are attractive to *A. bilineatus* was hypothesized (Haack and Benjamin 1982).

Drought has been reported as the main factor that caused an outbreak of *A. viridis* in Germany, interrupting the sap flow of trees and decreasing the mortality of the borer larvae in the trunks (Heering 1956). At the time of outbreak, when a large number of *Agrilus* larvae develop in the increased number of suitable trees and many adults are present in the field, a mass attack may also inhibit the water supply in healthy trees, ensuring the larval survival and leading to the death of a large number of trees (Heering 1956; Arru 1961-62; Ciampolini and Ugolini 1975; Ohgushi 1978; Mattson and Haack 1987; Evans et al. 2004; Vansteenkiste et al. 2004; Delb 2006; Lakatos and Molnár 2006; Liu et al. 2007). The outbreaks can be limited naturally when climatic conditions change and relatively high levels of rainfall occur (Heering 1956; Arru 1961-62; Lakatos and Molnár 2006).

Concerning the egg-parasitoids, *O. zahaikovitshi* was the only species that emerged from the egg-masses of *A. viridis*. However, this species was found to be widespread, even with different population levels, in all of the surveyed groves during the four-year period. Moreover, *O. zahaikovitshi* showed an early and effective activity being already present in the eggs collected in the field at the beginning of the season, and was able to largely reduce *A. viridis* populations with a parasitism rate of more than 50% in some groves. *O. zahaikovitshi* is also reported as an egg-parasitoid of the harmful emerald ash borer *A. planipennis* Fairmaire (Taylor et al. 2012). Considering its impact on the *A. viridis* populations in the hazel groves of Piedmont, this egg-parasitoid could be a good candidate for the control of *A. planipennis*, as *Oobius agrili* Zhang and Huang that proved to be an important mortality factor in China (Liu et al. 2007) and it has been introduced into North America for the biological control of the emerald ash borer (Duan et al. 2011, 2012). Therefore, the natural occurrence and abundance of *O. zahaikovitshi* in the hazel groves of the area studied here has to be conserved both for its effective control of the native *A. viridis* and as a potential biological control agent of the invasive exotic *A. planipennis*. To this regard, it is crucial to adopt control tactics respectful of its presence whenever necessary.

Chemical treatments against adult beetles could be needed, as they are susceptible because they live outside the wood. However, the adult presence in the field has to be verified by accurate sampling via the foliage beating method. Moreover, it is fundamentally important not to use broad-spectrum insecticides, but selective ones, in order to reduce the side effects on the rich complex of natural enemies present in the hazel groves. Appropriate agronomic practices to minimise the effects of

water stress and to conserve natural enemies, such as *O. zahaikevitchi*, could be effective to protect hazel groves against the boring beetle attacks. Furthermore, *O. zahaikevitchi* is not the only natural enemy of *A. viridis* in the hazel groves of Piedmont. Many larval parasitoids against *A. viridis* have been reported in the literature (Kenis and Hilszczanski 2004), and several hymenopteran adults emerged from encaged hazel branches in this study. Therefore, further investigations should be performed in a more exhaustive study to fully assess their role in controlling *A. viridis* outbreaks.

ACKNOWLEDGEMENTS

We are grateful to Gianfranco Curletti of the Museo Civico di Storia Naturale of Carmagnola (Torino, Italy) for his help in the identification of *Agrilus* spp. and to Emilio Guerrieri of the Istituto per la Protezione delle Piante of CNR of Portici (Napoli, Italy) for the identification of egg-parasitoid. We are also grateful to the colleagues of DISAFA, CReSO (in particular Claudio Sonnati) and technical assistance for their help in field surveys. Research was supported by a grant from Regione Piemonte.

REFERENCES

- Anderson R F, 1944. The relation between host condition and attacks by the bronze birch borer. J. Econ. Entom. 37, 588-596.
- Arru G M, 1961-62. *Agrilus suvorovi populneus* Schaefer (Coleoptera Buprestidae) dannoso ai pioppi nell'Italia settentrionale. Bollettino di Zoologia agraria e di Bachicoltura, Serie II 4, 159-286.
- Bellamy, C L, 2008. A World Catalogue and Bibliography of the Jewel Beetles (Coleoptera: Buprestoidea). Volume 4: Agrilinae; Agrilina through Trachyini. Pensoft Publishers, Sofia-Moscow. Pensoft Series Faunistica 79, 1932-2684.
- Bernhard D, Fritsch G, Glöckner P, Wurst C, 2005. Molecular insights into speciation in the *Agrilus viridis*-complex and the genus *Trachys* (Coleoptera: Buprestidae). Eur. J. Entomol. 102, 599-605.
- Bin F, Vinson S B, 1990. Efficacy assessment in egg parasitoids (Hymenoptera): proposal for a unified terminology. In: Wajnberg E. and S.B. Vinson (eds), *Trichogramma* and Other Egg Parasitoids. 3rd International Symposium. Les Colloques de l'INRA 56, INRA Editions, Paris, pp.175–179.
- Ciampolini M, Ugolini A, 1975. Reperti sull'etologia dell'agrilò del nocciolo e mezzi di difesa. Informatore Fitopatologico 25(1), 21-27.

- Curletti G, Rastelli M, Rastelli S, Tassi F, 2003. Coleotteri Buprestidi d'Italia. CD-ROM. Museo Civico di Storia Naturale di Carmagnola (Torino)– Progetto Biodiversità (Roma), Italy. Piccole faune 1.
- Duan J J, Bauer L S, Ulyshen M D, Gould J R, Van Driesche R, 2011. Development of methods for the field evaluation of *Oobius agrili* (Hymenoptera: Encyrtidae) in North America, a newly introduced egg parasitoid of the emerald ash borer (Coleoptera: Buprestidae). Biol. Control 56, 170-174.
- Duan J J, Bauer L S, Hansen J A, Abell K J, Van Driesche R, 2012. An improved method for monitoring parasitism and establishment of *Oobius agrili* (Hymenoptera: Encyrtidae), an egg parasitoid introduced for biological control of the emerald ash borer (Coleoptera: Buprestidae) in North America. Biol. Control 60, 255–261.
- Delb H, 2006. The current practice of forest pest monitoring in the Southwest of Germany. Proceedings of the Workshop on Methodology of Forest Insect and Disease Survey in Central Europe, IUFRO Working Party, Gmunden, Austria: 86-99.
- Evans H F, Moraal L G, Pajares J A, 2004. Biology, ecology and economic importance of Buprestidae and Cerambycidae. In: Bark and wood boring insects in living trees in Europe: a synthesis. Ed. by Kluwer, Dordrecht, the Netherlands, 447-474.
- Haack R A, Benjamin D M, 1982. The biology and ecology of the twolined chestnut borer, *Agrilus bilineatus* (Coleoptera: Buprestidae), on oaks, *Quercus* spp., in Wisconsin. Can. Entomol. 114(5), 385-396.
- Heering H, 1956. Zur Biologie, Ökologie und zum Massenwechsel des Buchenpracht-Käfers (*Agrilus viridis* L.). II. Teil. Z. Angew. Entomol. 39, 76-114.
- Jendek E, 2012. Corrections to *Agrilus* related species-group names in the world catalogue of Bellamy and new substitute names for *Agrilus* species-group homonyms. ZooKeys 249, 61-76.
- Kenis M, Hilszczanski J, 2004. Natural enemies of Cerambycidae and Buprestidae in Europe. In: Bark and wood boring insects in living trees in Europe: a synthesis. Ed. by Kluwer, Dordrecht, the Netherlands, 475-498.
- Lakatos F, Molnár M, 2006. Mass dieback of beech (*Fagus sylvatica*) in Zala County. Proceedings of the Workshop on Methodology of Forest Insect and Disease Survey in Central Europe, IUFRO Working Party, Gmunden, Austria: 142-149.
- Lakatos F, Molnár M, 2009. Mass Mortality of Beech (*Fagus sylvatica* L.) in South-West Hungary. Acta Silvatica et Lignaria Hungarica 5, 75-82.
- Liu H, Bauer L S, Miller DL, Zhao T, Gao R, Song L, Luan Q, Jin R, Gao C, 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius*

- agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biol. Control 42, 61-71.
- Mattson W J, Haack R A, 1987. The role of drought in outbreaks of plant-eating insects. BioScience 37(2), 110-118.
- Moraal L G, Hilszczanski J, 2000. The oak buprestid beetle, *Agrilus biguttatus* (F.) (Col., Buprestidae), a recent factor in oak decline in Europe. J. Pest Sci. 73, 134-138.
- Muzika R M, Liebhold A M, Twery M J, 2000. Dynamics of two-lined chestnut borer *Agrilus bilineatus* as influenced by defoliation and selection thinning. Agr. For. Entomol. 2(4), 283-289.
- Ohgushi R, 1967. On an outbreak of the citrus flat-headed borer, *Agrilus auriventris* E. Saunders in Nagasaki prefecture. Researches on Population Ecology 9, 62-74.
- Pellegrino S, Mozzone G, 1985. Gravi danni di agrilo nei nocioleti piemontesi. Informatore Agrario 41(40), 68, 70.
- Taylor P B, Duan J J, Fuester R W, Hoddle M, Van Driesche R, 2012. Parasitoid guilds of *Agrilus* woodborers (Coleoptera: Buprestidae): their diversity and potential for use in biological control. Psyche, article ID 813929: 10 pages.
- Vansteenkiste D, Tirry L, Acker J, van Stevens M, 2004. Predispositions and symptoms of *Agrilus* borer attack in declining oak trees. Ann. For. Sci. 61, 815-823.
- Wermelinger B, Flückiger P F, Obrist M K, Duelli P, 2007. Horizontal and vertical distribution of saproxylic beetles (Col., Buprestidae, Cerambycidae, Scolytinae) across sections of forest edges. J. Appl. Entomol. 131(2), 104-114.

FIGURES

Figure 1. Oviposition of *Agrilus viridis* females under laboratory and field conditions in 2008.

Figure 2. Adult emergence of *Agrilus viridis* from living hazel branches collected in 2008-2010.

Figure 3. *Agrilus viridis* larvae and *Oobius zahaikovitshi* adults emerged from the egg-masses collected in the hazel groves in 2007.

TABLES

Table 1. Hazel groves in the area of Langhe (Piedmont, NW Italy) where *Agrilus* species were sampled by sticky traps (T), plant beating (P), collection of egg-masses (E) and collection of infested branches (B) from 2007 to 2010.

Grove	Locality	Planting year	2007	2008	2009	2010
G 1	Bosia (44°36'6"N; 8°8'56"E)	1993	T+E	T+P+E+B	T+P+E+B	P+E+B
G 2	Bonvicino (44°30'31"N; 8°2'14"E)	1999	T+E	T+P+E+B	T+P+E+B	P+E
G 3	Camerana (44°25'57"N; 8°9'39"E)	2000	T+E	T+E+B		
G 4	Cravanzana (44°35'3"N; 8°8'10"E)	1975	T+E	T+P+E+B		
G 5	Cravanzana (44°35'4"N; 8°7'37"E)	1992			T+P+E+B	P+B
G 6	Arguello (44°35'0.80"N; 8°6'22"E)	1999			T+P+E	E
G 7	Cortemilia (44°35'20"N; 8° 9'35"E)	1975		P	T+P+E	P+B
G 8	Carrù (44°29'20"N; 7°51'26"E)	1990		P	P	P+B

Table 2. Adults of *Agrilus* species sampled by yellow sticky traps in the hazel groves investigated from 2007 to 2009. Mean numbers per grove of total adults captured by traps of each location (n = 10 per grove) in the eight-week-period (from early June to early August) in each year were analysed by one-way-ANOVA ($P < 0.01$); values followed by the same letter are not significantly different (Tukey test, $P < 0.01$).

Species	2007				2008				2009				
	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4	G 1	G 2	G 5	G 6	G 7
subgenus <i>Anambus</i>													
<i>A. sulcicollis</i> Lacordaire, 1835	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>A. angustulus</i> (Illiger, 1803)	0	1	30	1	0	0	15	1	0	0	0	3	1
<i>A. graminis</i> Gory et Laporte, 1837	5	4	19	0	1	2	0	0	1	0	0	1	0
<i>A. derasofasciatus</i> Lacordaire, 1835	0	3	1	2	2	2	0	2	0	0	0	0	2
<i>A. olivicolor</i> Kiesenwetter, 1857	48	70	61	57	48	5	0	23	5	2	6	0	4
<i>A. convexicollis</i> Redtembacher, 1849	0	0	1	0	0	0	0	0	0	0	0	0	0
subgenus <i>Agrilus</i> s.str.													
<i>A. viridis</i> (Linnaeus, 1758)	2	23	9	1	6	6	0	4	3	1	1	8	3
<i>A. cuprescens</i> Menetries, 1832	0	0	1	0	0	0	0	0	0	0	0	0	0
total	55	104	122	60	57	16	15	30	9	3	7	12	11
total no. of traps	40	54	48	60	180	230	120	160	100	110	120	100	110
starting of surveys	early June	late May			late April				early May	late April	early May	late April	mid-June
end of surveys	early August				late October		late August		early August				
<i>Agrilus</i> adults captured from early June to early August	2007				2008				2009				
	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4	G 1	G 2	G 5	G 6	G 7
mean no. of adults	5.50 B	9.00 A	9.90 A	6.00 B	5.40 B	1.30 CD	0.40 D	2.80 C	0.80 D	0.30 D	0.70 D	0.40 D	0.80 D
standard error	0.40	0.33	0.48	0.33	0.34	0.30	0.16	0.25	0.25	0.15	0.26	0.22	0.25

Table 3. Adults of *Agrilus* species sampled by plant beating in several hazel groves, those described in table 1 (A) and others in different localities in Piedmont (NW Italy) (B) from 2008 to 2010. Blank spaces indicate no sampling made. Mean numbers of *A. viridis* ($\pm$ SE) per survey were analysed by one-way-ANOVA ($P < 0.01$); values followed by the same letter are not significantly different (Tukey test, $P < 0.01$). *A. v.* = *Agrilus viridis*; *A. o.* = *Agrilus olivicolor*.

A	2008				2009				2010			
Grove	No. of surveys	<i>A.v.</i>	<i>A.o.</i>	Mean no. of <i>A.v.</i>	No. of surveys	<i>A.v.</i>	<i>A.o.</i>	Mean no. of <i>A.v.</i>	No. of surveys	<i>A.v.</i>	<i>A.o.</i>	Mean no. of <i>A.v.</i>
G 1	4	4	0	1.00 EF (± 0.41)	3	9	0	3.00 DE (± 0.58)	4	3	0	0.75 EF (± 0.48)
G 2	3	0	0	0.00 F (± 0.00)	4	0	0	0.00 F (± 0.00)	5	5	0	1.00 EF (± 0.32)
G 4	3	19	0	6.33 C (± 0.33)								
G 5					4	16	0	4.00 D (± 0.41)	4	2	0	0.50 F (± 0.29)
G 6					3	4	0	1.33 EF (± 0.33)	3	2	0	0.67 F (± 0.33)
G 7	3	1	0	0.33 F (± 0.33)	4	4	1	1.33 EF (± 0.33)	5	1	2	0.20 F (± 0.20)
G 8	4	53	0	13.25 A (± 0.48)	5	54	0	10.80 B (± 0.37)	5	15	0	3.00 DE (± 0.45)

B	2008			2009			2010		
Locality	No. of surveys	<i>A. v.</i>	<i>A. o.</i>	No. of surveys	<i>A. v.</i>	<i>A. o.</i>	No. of surveys	<i>A. v.</i>	<i>A. o.</i>
Alba (CN)				1	0	0			
Albaretto Torre (CN)	2	0	0	3	0	0	2	4	0
Benevello (CN)	1	0	0				2	3	0
Borgomale (CN)	2	7	0	3	0	0	1	0	0
Bosia (CN)				2	1	0	4	0	0
Bossolasco (CN)	2	0	0				1	0	0
Bubbio (AT)							11	0	0
Busca (CN)				1	0	1	1	0	0
Cairo Montenotte (SV)				3	1	0	3	2	0
Calamandrana (AT)							2	0	0
Carrù (CN)				11	86	0	18	52	0
Casale Monferrato (AL)							1	0	0
Castelletto Stura (CN)							2	1	0
Castelletto Uzzone (CN)	1	0	0	2	0	0	4	1	0
Castellino Tanaro (CN)				3	0	0			
Castino (CN)	16	10	1	16	20	1	9	7	1
Cessole(CN)							4	0	0
Conzano (AL)							1	0	0
Corsione (AT)							3	0	0
Cortemilia (CN)				2	10	0	5	0	2
Cravanzana (CN)	8	9	0	10	21	0	0	0	0
Cuccaro (AL)							3	0	0
Diano d'Alba (CN)	1	0	0						
Dogliani (CN)							1	0	0
Farigliano (CN)							3	0	0
Feisoglio (CN)	3	5	0	1	8	0			
La Morra (CN)							1	0	0
Lequio Berria (CN)	2	3	0	2	1	0	2	1	0
Levice (CN)	1	0	0						
Lù Monferrato (AL)							9	3	0
Moasca (AT)							1	0	0
Mombello Monferrato (AL)				1	2	0			
Moncestino (AL)							3	0	0
Mondovì (CN)							3	0	0
Monte Valenza							1	0	0
Narzole (CN)							2	0	0
Ozzano Monferrato (AL)							1	0	0
Perletto (CN)	4	3	0	3	0	0			

Piozzo (CN)	3	3	0	3	1	0			
Rodello (CN)				1	0	0			
Santa Vittoria d'Alba (CN)							1	0	0
Sinio (CN)	2	2	0				1	0	0
Somano (CN)							19	10	0
Torre Bormida (CN)	1	0	0	3	1	1			
Torresina (CN)				2	0	0			
Vesime (AT)							2	0	0
total	66	119	1	96	239	4	153	112	3

Table 4. Adults of *Agrilus* species emerged from the hazel branches collected in the investigated groves from 2008 to 2010.

Species	Living wood								Dead wood		
	2008				2009			2010			2010
	G1	G2	G3	G4	G1	G2	G5	G1	G5	G8	G7
<i>A. angustulus</i>	3					1					4
<i>A. graminis</i>											12
<i>A. olivicolor</i>									1		3
<i>A. viridis</i>	27	9	1	2	66	9	2	16	20	9	23
total	30	9	1	2	66	10	2	16	21	9	42

Table 5. Discovery and parasitism efficiency of *Oobius zahaikewitschi* on egg-masses of *Agrilus viridis* in the investigated groves from 2007 to 2010.

Year	Grove	Egg-masses (no.)	Eggs (no.)	Discovery efficiency (%)	Parasitism efficiency (mean % $\pm$ SE)	Parasitoid impact (%)
2007	G 1	41	243	17.07	49.37 ($\pm$ 11.04)	8.64
	G 2	27	150	44.44	54.38 ($\pm$ 7.86)	20.67
	G 3	12	53	83.33	80.33 ($\pm$ 4.72)	67.92
	G 4	13	86	15.38	80.36 ($\pm$ 5.36)	10.47
	total (sample)	93	532	33.33		18.23
2008	G 1	20	115	55.00	50.10 ($\pm$ 7.91)	26.96
	G 2	17	105	76.47	80.90 ($\pm$ 8.78)	57.14
	G 3	8	57	50.00	44.64 ($\pm$ 14.62)	19.30
	G 4	3	19	0.00		0.00
	total	48	296	58.33		34.46
2009	G 1	25	130	80.00	79.77 ($\pm$ 4.33)	61.54
	G 2	54	292	77.78	68.05 ($\pm$ 4.46)	54.11
	G 5	9	50	66.67	61.33 ($\pm$ 5.64)	34.00
	G 6	89	475	34.83	67.39 ($\pm$ 4.96)	22.95
	G 7	25	130	80.00	80.69 ($\pm$ 2.12)	63.08
	total	202	1077	58.42		41.41
2010	G 1	57	303	33.33	77.57 ($\pm$ 5.23)	22.77
	G 2	125	784	65.60	69.22 ($\pm$ 3.10)	47.07
	G 6	79	485	34.18	61.15 ($\pm$ 4.79)	18.35
	total	264	1572	49.04		33.52