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ORIGINAL CONTRIBUTION

Life history and geographical distribution of the walnut twig beetle, *Pityophthorus juglandis* (Coleoptera: Scolytinae), in southern Europe

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Abstract

In September 2013, the walnut twig beetle (WTB) *Pityophthorus juglandis* Blackman, a species native to Mexico and south-western USA, was recorded for the first time in Europe, in northern Italy. The collected adults were found to be vector of the fungus *Geosmithia morbida* Kolařik, Freeland, Utley & Tisserat, an aggressive pathogen causing thousand cankers disease in walnut (*Juglans* spp.). To determine the geographical distribution of the WTB and the main aspects of biology, phenology and voltinism, an intensive survey of the main walnut plantations near the site of the first finding was conducted in 2014. The beetles began to fly with a mean air temperature of about 18°C (mid-May) and continued until late October. Two partially overlapping generations occurred, with the second taking place in late September. The WTB was found in 14 of the 27 monitored walnut plantations. The infested sites were spread over four different non-contiguous administrative provinces belonging to two regions (Veneto and Lombardy) of northern Italy. The most distant infested plantations were about 130 km apart along a west–east gradient, and about 70 km along a north–south gradient. In this respect, the distribution area of the WTB in northern Italy may be prudently estimated at about 4200 km^2. Molecular analysis of the collected individuals showed no genetic differences among the six sampled *P. juglandis* populations, suggesting that a few individuals might have arrived in Italy through a single introduction event and then spread over the territory. Given the quick mortality of infested walnuts and the wide distribution area, eradication strategies appear unrealistic. Possible strategies of biological control or local chemical treatments must be investigated.

Introduction

Alien bark and ambrosia beetles are among the most dangerous forest pests worldwide (Brockerhoff et al. 2006a) and are recognized as one of the most successful groups of invasive species (Haack 2006; McCullough et al. 2006). Given that they can be transported within wood-packaging materials (McCullough et al. 2006; Zahid et al. 2008; Rassati et al. 2015a), fresh timber (Piel et al. 2008), wood chips (Flø et al. 2014) or woody plants (Liebhold et al. 2012; Eschen et al. 2015), they can easily elude phytosanitary inspections at points of entry (Brockerhoff et al. 2006b; Haack 2006). New alien species of bark and ambrosia beetles are intercepted and recorded in Europe every year (Kirkendall and Faccoli 2010), with an increasing trend positively related to the growth in international trade (Marini et al. 2011; Rassati et al. 2015b).

In September 2013, the walnut twig beetle (WTB) *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae, Scolytinae), a species native to Mexico and south-western USA (California, Arizona, and

New Mexico), was recorded for the first time in Europe, in northern Italy (Montecchio and Faccoli 2014). In both native and non-native regions of the USA, this species is associated with the fungus *Geosmithia morbida* Kolařik, Freeland, Utley & Tisserat (Ascomycota: Hypocreales: Bionectriaceae), an aggressive pathogen causing thousand cankers disease (TCD) in walnut (*Juglans* spp.) (Tisserat et al. 2009, 2011; Kolarík et al. 2011; Seybold et al. 2013). Similarly, the individuals of WTB collected in Italy were found to be vectors of *G. morbida* (Montecchio and Faccoli 2014). Although the first European WTB infestations were found on black walnut (*J. nigra*) plantations cultivated intensively for timber production (Montecchio and Faccoli 2014), a later survey also found WTB and TCD on English walnut trees (*J. regia*) (Montecchio et al. 2014).

The WTB is a minute (1.5–1.9 mm) bark beetle reproducing under the bark of many walnut species (Wood 1982; Faccoli 2015). In spring, adults colonize rough areas of bark at the base of twigs, but can also infest the underside of large branches and the warmer side of the trunk (Newton and Fowler 2009). In southern USA, larval development usually takes 6–8 weeks to complete generally two overlapping generations per year (Newton and Fowler 2009). The WTB can be observed flying from mid-April to late October, whereas winter is usually spent by the adults under the bark of host trees (Newton and Fowler 2009).

Although the nutritional value of the pathogen for beetle consumption is unknown, the *P. juglandis*–*G. morbida* association is very effective in killing the host tree. As in many other bark beetle species (Wood 1982), the WTB adults carry the *G. morbida* conidia on the elytra (Newton and Fowler 2009; Seybold et al. 2013) and introduce the fungus into trees during bark colonization and gallery formation (Tisserat et al. 2009). The primary infestation symptoms are canopy yellowing, leaf wilting, twig and branch dieback, and a large number of small bark cankers occurring mainly on twigs and small branches (Tisserat et al. 2009). In the early stages, *G. morbida* produces small, roughly circular cankers that develop around the WTB galleries and are usually not visible until a thin layer of the outer bark is removed (Tisserat et al. 2009; Grant et al. 2011). Even when leaf wilting is present, branches with numerous beetle galleries and cankers often show no outward appearance of bark damage, except for the beetle entrance holes, making detection of the disease difficult (Newton and Fowler 2009). With time the cankers expand, becoming more diffuse, and longitudinal sections collected through them reveal grey to brown discoloration of both phloem and outer bark (Montecchio and Faccoli 2014). Cambial discoloration occurs, however, only after extensive bark colonization by the fungus. In the advanced stages of decline, beetle galleries and associated cankers occur every 2 to 5 cm in the bark, and the cankers coalesce and girdle twigs and branches (Tisserat et al. 2009; Grant et al. 2011). Following insect infestation and pathogen phloem infection, trees die within a few months.

Since the mid-1990s, TCD has been responsible for widespread mortality of many walnut species in the USA (Randolph et al. 2013), where both the WTB and TCD have spread from south-western (Cranshaw 2011) to north-eastern states and the east coast following the national trade in infested timber (Newton and Fowler 2009; Jacobi et al. 2012; Seybold et al. 2013). The presence of the WTB and TCD in Europe is considered a serious threat to *J. nigra* and *J. regia*, which is also susceptible to the disease (Utley et al. 2013; Montecchio et al. 2014). During the last 90 years, in fact, several areas of southern Europe have been reforested with mixed tree species for wood production, and both black and English walnuts are now present in large proportions (Eichhorn et al. 2006). Nowadays, walnuts are economically, culturally and environmentally highly valued trees, being cultivated for fruits, timber and as traditional landscape trees (Eichhorn et al. 2006). The high susceptibility of *J. nigra* and *J. regia* to the WTB and TCD may hence have serious impacts on the landscape and economy of many European agricultural and forest areas.

Although no precise information is available, the importation of infested wood (with bark) from the USA is the most likely introduction pathway of the WTB into Europe. An intensive survey of the beetle populations was thus required both to assess the WTB distribution and to better understand the life traits of this species in the invaded environment. The first aim of this study was to conduct a systematic survey of the main walnut plantations occurring near the site of first finding to determine the extension of the infestation area. At the same time – as no information was available concerning the life history of the WTB in southern Europe – the second aim was to investigate the main aspects of biology, phenology and voltinism of the Italian populations of the WTB using both infested logs and traps. Finally, the third aim was to assess – by molecular markers applied on the adult beetles – the origin and the number of introductions of WTB in Italy.

Materials and Methods

WTB distribution

In September 2013, a WTB infestation was found in north-eastern Italy on black walnut trees growing in a timber production plantation (L1 in fig. S1 and table S1) (Montecchio and Faccoli 2014). A preliminary survey conducted in the following months discovered four other infested plantations (L2–L5) in the same area (Montecchio et al. 2014). To check the real distribution of the WTB in northern Italy, a specific survey was conducted in spring–summer 2014 in the main black walnut plantations in the region of the first finding (Veneto), and in two regions bordering east and west (Friuli Venezia Giulia and Lombardy, respectively) (fig. S1 and table S1). A total of 27 walnut plantations were selected to have a geographical distribution as homogeneous as possible. The presence of the WTB was investigated using a 12-black-multifunnel trap (Econex, Murcia, Spain) in each plantation (Seybold et al. 2012a). Traps were set up the last week of July and were hung in the middle of the plantations on a tree branch at about 2 m above the ground. Each trap was baited with a dispenser releasing a pheromone blend specific for the WTB (Contech Enterprises, Victoria, BC, Canada). The compound (400 mg) is adsorbed on an inert sponge inside the device and is released at a rate of about 1 mg/day at 20°C. The release rate is highly temperature dependent and generally doubles with every 5°C increase in temperature. With an average daily temperature of about 25°C, the dispenser lasts about 200 days. Traps were left in the field for 84 days (until the last week of October). All trapped individuals were identified morphologically (Wood 1982) and by genetic analyses (see Molecular analysis section).

WTB phenology and voltinism

Phenology and voltinism of the WTB were investigated in spring and summer 2014 by both tree sampling and pheromone traps. In each of the five walnut plantations where the WTB was originally found (L1–L5 in fig. S1 and table S1), three infested black walnut trees – that is with bark showing colonization symptoms such as penetration holes – were cut down and logged in February. Five infested branches per tree, approximately 35–40 cm in length and 3–4 cm in diameter, were placed singly in aerated transparent glass tubes (50 cm long, 10 cm diameter) for adult emergence. Both ends of the tubes were covered with a fine metal mesh screen to retain the emerged beetles until checking and collection. Tubes were then stored outdoors, piled horizontally on an open iron shelf that was placed under a plastic roof to protect it from rain and direct sunshine, while ensuring good ventilation and thermal exchange. Before being placed in the tubes, the cut surfaces of the logs were sealed with paraffin to reduce drying. Tubes were checked and emptied once per week until no more insects were found. Emerged beetles were collected from the tubes, counted and sexed according to Wood (1982).

During the second half of May 2014, a 12-black-multifunnel trap (Econex, Murcia, Spain) baited with the specific aggregation pheromone described above was set up in the same five walnut plantations (L1–L5) where infested trees had been sampled in February. Traps were checked every week until the end of October of the same year, and all trapped species were identified morphologically.

Air temperature was recorded hourly using data loggers (HOBO Temp) placed in the monitored walnut plantations.

Molecular analysis

Six WTB adults (three males and three females) among those emerged from the branches collected in six of the 27 monitored sites (L1–L5 and L27) were selected, for a total of 36 individuals. Four sites (L2–L5) were closely distributed around the walnut plantation where the WTB was found for the first time (L1), while the sixth site (L27) was more distant (fig. S1). Individuals captured with traps, instead, remained in the collector cups for about three months, and their conditions were not good enough to allow genetic analysis. The WTB adults emerged from the tree branches were individually stored at −80°C until DNA extraction. Nucleic acid extraction was performed for all the individuals following a *salting out* protocol (Patwary et al. 1994). Two fragments of the cytochrome oxidase 1 (cox1) were then amplified using universal primers for animal mitochondrial DNA. The 5′ region of this gene, generally used as barcode for species identification (Hebert et al. 2003), was amplified using the primers LCO1490 and HCO2198 (Folmer et al. 1994), whereas the 3′ region was obtained with the primers C1-J-2185 and TL2-N-3014 (Simon et al. 2006).

All amplifications were performed in 20 μl reactions (1x PCR Go Taq Flexi buffer (Promega, Madison, WI, USA), 2.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.2 μM for each primer, 0.5 U of Taq polymerase (Promega), 2 μl DNA template). Thermal cycling conditions for the cox1 fragments were 5 min at 96°C, followed by 35 cycles of 96°C for 45 s, 50°C for 45 s and 72°C for 1 min, with a final extension of 72°C for 5 min.

Polymerase Chain Reaction products were purified using exonuclease and Antarctic phosphatase (New England Biolabs, Ipswich, MA, USA) and sequenced at the BMR Genomics Service (Padova, Italy). Sequences obtained were then compared and identified through a nucleotide BLAST analysis (http://blast.ncbi.nlm.nih.gov) on the GenBank database.

Results

WTB distribution

A total of 4473 individuals of WTB were captured using traps, among which about 66% were females. The male:female ratio of the adults emerged from the logs was instead 1 : 0.8. WTB was found in 51% (14) of the 27 monitored walnut plantations (table S1). The infested sites were spread over four non-contiguous administrative provinces (Vicenza, Treviso, Padova, and Mantova) belonging to two regions (Veneto and Lombardy) (fig. S1). The most distant infested plantations were located at about 130 km from each other along a west–east gradient, and at about 70 km along a south–north gradient (fig. S1). In this respect, the distribution area of the WTB in northern Italy in 2014 may be prudently estimated as about 4200 km^2. All monitored sites within a radius of about 30 km around the first recorded infested plantation (L1) were found to be positive for the WTB presence (province of Vicenza and partially of Treviso, table S1 and fig. S1). Interestingly, the most western infested plantation – in Lombardy (Marmirolo) – was isolated from all the others.

Walnut twig beetle captures varied widely depending on the monitored plantation (table S1). The localities where the WTB and the infestation symptoms were recorded in 2013 showed high population densities, with hundreds or even thousands of individuals per trap. Instead, most of the walnut plantations where the insect was found for the first time in 2014 showed very few captures, in some cases only one or two individuals per trap (table S1). Only in two localities where WTB infestations were newly discovered in 2014 (L15 and L20) the population density, that is the number of trapped individuals, was similar to that of the populations discovered in 2013. In addition, the walnuts growing in the plantations found infested for the first time in 2014 did not show specific symptoms of insect infestations or fungal infection, suggesting the occurrence of a very recent site colonization. However, during preliminary observations made in spring and summer 2015, the WTB was found again in all the sites where it had previously been recorded in 2013 and 2014, suggesting that this beetle species has permanent populations in all these localities.

WTB phenology and voltinism

In spring 2014, the first adult beetles were trapped and emerged from the logs with a mean air temperature of about 18°C (second half of May) (fig. 1). A second and more consistent emergence peak was recorded about 4–5 weeks later, at the beginning of July (Fig. 1). Two new trapping peaks occurred about three months later, at the beginning of September and beginning of October respectively (Fig. 1). The time elapsed between the May and July peaks (about one month) was too short to allow the full development of a new generation. In addition beetle emergence from logs showed a temporal trend consistent with trap captures (fig. 1) suggesting that two partially overlapping generations occurred – one from May to September and the other from July to October – each lasting about 12 weeks. In northern Italy the WTB overwinters as mature larvae, pupae and young adults under the bark of trees infested in late summer.

After insect emergence, the infested logs were debarked to investigate the characteristics of the reproductive systems of the WTB. Males excavated small nuptial chambers under the bark where they

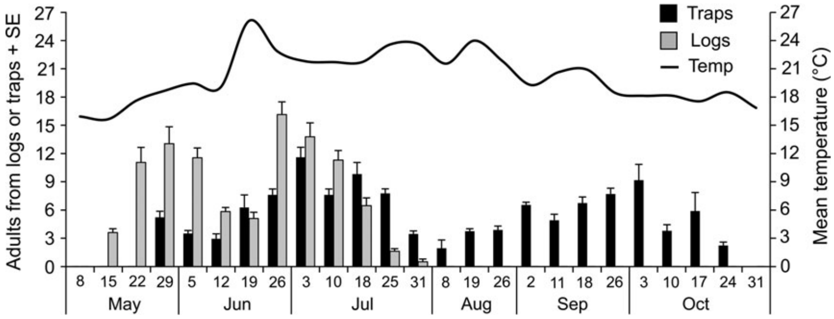

Fig. 1 Phenology and voltinism of the WTB populations monitored in northern Italy in spring–summer 2014 in relation to the mean daily temperature. The mean number of adults (+SE) was calculated considering either the adults emerged from infested walnut logs (grey bars) or the adults caught using pheromone traps (black bars).

were reached by 3–8 females, usually 4–5. After mating, females bored short tunnels in the phloem where eggs were laid along both sides of the galleries. Egg galleries usually radiated transversely (across the wood grain) from the nuptial chamber, while larval galleries were directed longitudinally (with the wood grain). The mean colonization density was about 16.5 new emerging adults per square decimeter of infested bark, although there was a wide variability among different localities.

Molecular analysis

In total, 36 individuals of the WTB were sequenced, six for each of the six walnut plantations from which logs were collected. Sequencing of the 5′ region and 3′ region of cox1 gene yielded a 607-bp-long and a 724-bp-long fragment, respectively. The analysis of both fragments showed no differences among the sequences of the insects collected from different sites. The BLAST comparison of the 5′ fragment showed that there is a 100% identity of this fragment with *P. juglandis* haplotype H2 (GenBank ID) from Rugman-Jones et al. (2015). For the 3′ fragment, no homologous sequences of *P. juglandis* were present in GenBank.

Discussion

For three consecutive years (2013–2015), stable populations of the WTB were observed in different regions of northern Italy, where the species may produce up to two partially overlapping generations per year and can survive the relatively cold winter as mature larvae, pupae and young adults under the bark of trees infested in late summer. These results suggest that the WTB is well acclimated in southern Europe.

The survey conducted with pheromone traps discovered the presence of the WTB in many walnut plantations which were originally not recorded as infested. In this respect, the WTB population density – reported as number of trapped adults – largely varied among the monitored plantations, from a few to thousands of individuals per trap. The localities where the WTB was recorded for the first time in 2013 showed the highest captures and the most evident symptoms of infestation and tree decay. Instead, most of the walnut plantations where the WTB was found for the first time in 2014 showed very low captures and no infestation symptoms on trees. These data suggest that the WTB may infest the same plantations for several years with progressively increasing populations that then expand to other walnut trees growing close to the first core infestation. This hypothesis is corroborated by the presence of the WTB in 2014 in all monitored sites within a radius of about 30 km around the infested plantation recorded first in 2013. The fact that in the following years the WTB was found again both in all sites infested in 2013 and in many other new plantations, suggests that this beetle species has permanent and expanding populations.

The WTB has historically been a minor pest of walnuts and has always been considered of negligible importance on native hosts (Bright 1981; Graves et al. 2009; Leslie et al. 2010; Seybold et al. 2012b). For these reasons, the life history of this species received little investigation prior to the last 10–15 years. Our results concerning phenology and voltinism of the WTB in northern Italy show that adults start to fly in spring with a mean air temperature of about 18°C and continue until late October. In this respect, the mean air temperature gives important information about the period of insect emergence and flight (*i.e.* insects captured in traps), and hence about phenology and voltinism. The seasonality of trap captures indicates that the WTB activity is driven by air temperature – for instance, it decreases significantly as daily mean temperatures drop below about 18°C (fig. 1) – supporting the results obtained by Chen and Seybold (2014). The number of emergence peaks observed in 2014 from both infested logs and pheromone traps, as well as their temporal distribution over spring and summer, suggests that the WTB has two partially overlapping generations in northern Italy: one from May to September and the other from July to October. This conclusion is supported by the occurrence of two cohorts of adults emerging from the same infested logs at two different times (in May and July). The first group, emerging in mid–late May, is composed of insects overwintering as adults that are ready to emerge as soon as the spring mean temperature reaches at least 18°C (fig. 1). These individuals begin the first generation that will conclude in early September. The newly emerged adults start a second generation composed of individuals overwintering as larvae of various instars and requiring some additional spring development before to emerge in July of the following year. The second cohort of emerging adults instead appears later (end-June, early July), beginning a generation that will produce only overwintering adults (October) – because there is no time for starting a second generation – and ready to emerge in May of the following year. Our data are consistent with recent observations in the USA (Cranshaw and Tisserat 2012; Nix 2013; Audley 2015). For example, in Colorado, both larvae and adults can be observed in logs during winter, suggesting a continuous breeding in spring and summer producing overlapping generations. Furthermore, spring emergence of overwintering adults has been observed first in late April (Cranshaw and Tisserat 2012), that is a little earlier than we observed in our study. However – similar to that observed in northern Italy – a second peak in flight activity occurs in mid-July and declines by early autumn. This means that two-three generations are normally produced in Colorado, which overlap considerably (Cranshaw and Tisserat 2012). The WTB life history is considerably different in warmer climates. For example, in eastern Tennessee and Virginia flight activity was recorded until the beginning of December and January, respectively, with larvae, pupae and adults occurring throughout the winter (Nix 2013). Similar studies conducted in California showed that overwintering instars include larvae in various stages of development and adult flights may begin in March (Cranshaw and Tisserat 2012). Seybold et al. (2012a) highlighted that WTB flight activity peaks at temperatures between 23 and 24°C and stops at temperatures below 17–18°C. Hence, the only month WTB flight did not occur in California was December (Seybold et al. 2012b). In southern USA, larval development is shorter taking only 6–8 weeks (Cranshaw and Tisserat 2012).

In summer 2015, both pest and pathogen were also found in a black walnut plantation of Piedmont (Rondissone, province of Turin), more than 320 km west of the first recorded site (Bosio G and Gullino C, unpublished data). The latter infested plantation is isolated from the others, and specific molecular analyses are still in progress to investigate the possible affinity of this population with those sampled in north-eastern Italy. The WTB is hence distributed in isolated populations occurring in three contiguous regions of northern Italy (Piedmont, Lombardy, and Veneto) along an east–west pathway about 400 km long. As observed in the USA (Seybold et al. 2012c), the non-continuous distribution of the WTB can be related to the spotted presence of walnut trees and plantations in the landscape and to the intensity of human-mediated movement of walnut logs and barked wood products. However, how and when the WTB reached Italy is still an open question. According to the spread mechanisms observed in North America (Seybold et al. 2012c), the most likely pathway of introduction of the WTB in Europe may be the importation of fresh infested walnut wood and timber (with bark) from the USA, where this species is already present. We do not actually know whether the WTB reached Italy directly from the USA or first passed through other European countries, but the lack of new finding records from other areas of Europe suggests that it arrived directly from the USA.

Although collected from different localities, the analyzed individuals of WTB showed no genetic variability among populations. The fact that no differences have been found in the cox1 fragments suggests that all the analysed populations may derive from a single introduction. The only haplotype found in northern Italy corresponds to the haplotype H2 found by Rugman-Jones et al. (2015), which is the second most common haplotype in the WTB native range and present in almost all states except Arizona and New Mexico (Rugman-Jones et al. 2015). This could be due to the founder effect, that is the reduced genetic variation that occurs when a population is established by a single or a few individuals (Gillespie and Roderick 2014), suggesting that the WTB may have arrived in Italy through a single introduction event and with few individuals. This is a common pattern recorded for invasive species (*e.g.* Rubinoff et al. 2010; Cifuentes et al. 2011). However, in this study we performed only preliminary genetic analyses with a limited number of tested individuals and genes, so caution should be taken regarding the hypothesis of single or multiple introductions. A larger number of samples associated with a finer scale analysis (e.g. by microsatellites) would hence be needed to obtain more information about the number and origin of introduction events in Italy. Using next-generation sequencing, Hadziabdic et al. (2015) recently developed and characterized microsatellite loci specific for the WTB which will be useful to investigate genetic diversity and population structure of this species across a widespread distribution including both North America and Europe, and to clarify the introduction pathways of this invasive pest across countries and continents.

Many walnut species and their hybrids are susceptible to the WTB (Wood and Bright 1992; Seybold et al. 2012d; Serdani et al. 2013; Utley et al. 2013). They can be present naturally in the landscape, cultivated either for fruits or for timber, or as ornamental trees. The WTB is not a particularly aggressive species, and susceptibility is similar among different walnut species. Susceptibility to TCD, instead, varies greatly among species – with *J. nigra* being the most susceptible – and even among individuals of the same tree species (Tisserat et al. 2011; Freeland et al. 2012; Utley et al. 2013). Both insect and disease were reported for the first time in Europe in 2013 on both black (*J. nigra*) and English (*J. regia*) walnuts (Montecchio and Faccoli 2014; Montecchio et al. 2014), and promptly added to the EPPO (European and Mediterranean Plant Protection Organization) Alert List. In 2014, the Veneto region issued an official 'Decree of compulsory control' (updated in 2015) including both the identification of the 'infested quarantine area' (periodically updated according to new findings) and a list of specific control measures. In the same year, EPPO started the Pest Risk Analysis (PRA) procedure, which is still in progress.

Every year an increasing number of new invasive alien insects are recorded as established in both Europe and North America (Work et al. 2005; Kirkendall and Faccoli 2010; Rassati et al. 2015a,b). This trend is expected to continue with the increase in international trade which will intensify the number and frequency of new arrivals (Levine and D'Antonio 2003; Hulme 2009; Marini et al. 2011). For instance, two other Nearctic insects feeding on *Juglans* recently invaded Italy: the walnut husk fly *Rhagoletis completa* Cresson (Diptera: Tephritidae) (Aluja et al. 2011), and the leafminer *Coptodisca lucifluella* (Clemens) (Lepidoptera: Heliozelidae), collected on both *J. regia* and *J. nigra* (Bernardo et al. 2012). Due to its widespread distribution and according to the lack of genetic differentiation found among populations, we may assume that the WTB arrived in Italy several years ago by a single introduction event, although major damage has only been recorded since summer 2013 (Montecchio and Faccoli 2014; Montecchio et al. 2014). Given the widespread presence and its rapid reproduction and dispersal, the WTB might quickly increase its abundance and distribution in Italy and other European countries. Accordingly, damage by the WTB will probably increase in the near future, leading to a gradual decline of walnut health and a progressive reduction in the number of black walnut plantations. Based on the high mortality recorded in the field and the large number of infested plantations, the application of eradication protocols seems to be an unrealistic solution. Possible strategies of biological control or local chemical treatments have to be carefully assessed.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Distribution map of the monitored walnut plantations occurring in Veneto, Friuli Venezia Giulia, and Lombardy regions (NE Italy). Black circles indicate WTB absence; grey squares indicate WTB presence; white triangles indicate infested sites where the WTB phenology was monitored. All monitored sites within a radius of about 30 km around the first recorded infested plantation (L1) were positive for WTB (large circle around site L1).

Table S1. Location and characteristics of the walnut plantations where WTB presence was monitored with pheromone traps in 2014.