

Attraction of different types of wood for adults of *Morimus asper* (Coleoptera, Cerambycidae)

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Abstract

Morimus asper reproduces mainly in freshly dead wood and, as a consequence, populations are affected by modern forestry practices. The taxon *M. funereus*, now incorporated into the species *M. asper*, is protected by the Habitats Directive (Council Directive 92/43/EEC) and its monitoring has received attention in recent years. Larvae of *M. asper* are polyphagous, but some studies indicate that adults prefer the wood of some tree species. Freshly cut log piles, which attract adults, have been proposed as a monitoring tool. For monitoring programmes, it is essential to select the most appropriate wood and therefore the attraction of different types of wood for adults of *M. asper* was investigated in two sites in northern Italian, using freshly cut log piles. The first experiment was carried out in the Nature Reserve Bosco della Fontana (a lowland forest, Mantua province), testing two autochthonous species (*Carpinus betulus* and *Fraxinus ornus*) and two allochthonous species (*Juglans nigra* and *Quercus rubra*). The second study was conducted in the Parco Naturale Regionale delle Prealpi Giulie (a mountain area, Udine province), employing *Fagus sylvatica*, *Fraxinus excelsior* and *Picea abies* (all autochthonous species). The population of this area belongs to the taxon formerly named *M. funereus*. In both sites, adults clearly preferred the wood of some particular species: *J. nigra* at Bosco della

Fontana and *F. sylvatica* in the mountain area. This is the first study which compared the attraction of several tree species and it showed that this selection is important for the monitoring of *M. asper*.

Keywords

Longhorn beetles, deadwood, monitoring, biodiversity, conservation, Northern Italy, Bosco della Fontana, Julian Prealps

Introduction

Biodiversity loss is altering the functioning of ecosystems (Cardinale et al. 2012) and also continues to be a concern in Europe (de Heer et al. 2005, Henle et al. 2007, Nieto and Alexander 2010). Modern forestry has substantially changed the species and age composition of forests over the last centuries (Bengtsson et al. 2000, Gossner et al. 2013) and these changes have resulted in loss of dead wood in terms of quantity, quality and dynamics with obvious consequences for forest biodiversity in general and particularly for those species associated with dead wood (Siitonen 2001, Stokland et al. 2012, Seibold et al. 2015). Saproxyllic beetles, i.e. species that depend on dead wood material at some stage of their life cycle (Speight 1989, Stokland et al. 2012), are reliable indicators of many aspects of dead-wood ecology (Stokland et al. 2012, Gossner et al. 2013). The presence of dead wood and saproxyllic Coleoptera in forests, woodlands and parklands is indicative of high quality mature habitats (Davies et al. 2008). Many saproxyllic beetles, occurring in Europe, are threatened (Nieto and Alexander 2010) due to continued habitat loss caused by logging and wood harvesting as well as the decline in veteran trees (Speight 1989, Nieto and Alexander 2010) and these concerns have led to 17 species of saproxyllic beetles being listed in the Annexes of the Habitats Directive (Council Directive 92/43/EEC). Article 11 of this Directive specifies that monitoring of the conservation status of these species is obligatory for all member States. For Italy, Trizzino et al. (2013) were the first to propose standard monitoring protocols for all arthropods listed in the Habitats Directive and, recently, a manual for the monitoring of all species and habitats of community interest was published for Italy. This manual provides methods and protocols recommended for the monitoring required by the Habitats Directive (Stoch and Genovesi 2016).

The Cerambycidae is one of the largest families of all Coleoptera and the largest family of saproxyllic beetles (Grimaldi and Engel 2005, New 2010); it comprises primary saproxyllics, hence initiating the decomposition process and preparing the substrate for colonisation by the secondary and tertiary saproxyllics (Hanks 1999, Stokland et al. 2012). Many authors have suggested that Cerambycidae might be particularly suited as indicators in forest ecosystems (Holland 2007, Ohsawa 2010, Hardersen et al. 2014). One species of this family, *Morimus asper* (Sulzer, 1776), has recently received particular attention. In the past the European populations of the genus *Morimus* were divided into five species (Reitter 1894, Dajoz 1976), but a recent study, based on COI and ITS2 gene sequences, found that all European and Turkish populations studied should be referred to as a single species, *M. asper* (Solano et al. 2013). This study confirmed the recent classification by several authors that *M. asper* and *M. fu-*

neruus belong to the same species (e.g., Sama and Löbl 2010, Sama and Rapuzzi 2011, Danilevsky 2015) and here the two taxa *asper* and *funereus* Mulsant, 1863 have been considered as subspecies of *M. asper*. This classification has implications for the conservation of this taxon, as *M. funereus* is listed in Annex II of the Habitats Directive and its status needs to be re-evaluated in the light of the new taxonomic results. For that purpose, a standard monitoring method for *M. asper* is urgently needed.

Probably the first method proposed for the monitoring of *M. asper* has been published by Vrezec et al. (2009), based on pitfall traps, placed in groups around fresh stumps. In contrast, Campanaro et al. (2011) suggested the use of freshly cut log piles as bait for monitoring and this method has also been proposed by Trizzino et al. (2013) and Bologna et al. (2016) for the monitoring of *M. asper*. Chiari et al. (2013) investigated this method in more detail and reported that it is reliable for detecting the presence and abundance of the target species and that occupancy probabilities increased with increasing volume of log piles. Hardersen et al. (2017) found that dead wood with a diameter of more than 12 cm was more attractive. Most authors concur that *M. asper* is a polyphagous species (e.g., Sturani 1981, Luce 1996, Sama 2002, Polak 2012) and this suggests that many tree species would be suitable for the monitoring. However, the data from Hardersen et al. (2017) suggested that the wood of two tree species tested did not have the same attraction for the adults of *M. asper*. Bărbuceanu et al. (2015) also reported that *M. asper* preferred certain tree species over others. As the freshly cut log piles are meant to function as bait for adults of *M. asper*, it was important to investigate the power of attraction of different types of wood and to analyse if the wood from different tree species were equally suitable for monitoring. The aim of this study was to test whether the choice of tree species affects the power of attraction of woodpiles for the adults of *M. asper*, investigating also allochthonous species. Two forests in northern Italy were investigated in two independent case studies.

Materials and methods

Study sites

Both study sites are located in northern Italy (Figure 1). The Nature Reserve “Bosco della Fontana” covers an area of 233 ha and is located in Central Northern Italy, Lombardy region (province of Mantua, municipality of Marmirolo). The reserve (altitude of 25 m a.s.l.) is part of the Natura 2000 network “IT20B0011 – Bosco Fontana”. The forest, which covers an area of about 198 ha, is mainly composed of *Carpinus betulus*, *Quercus robur* and *Q. cerris* and has been classified as belonging to the association *Polygonato multiflori – Quercetum roboris* Sartori 1984 (Campanaro et al. 2014). No wood has been extracted from the reserve since 1994 (Mason 2002) and this has led to large amounts dead wood being present (Travaglini et al. 2007). As a consequence, a large population of *M. asper* is present in the reserve (Hardersen et al. 2017) and it belongs to the nominate subspecies. The study sites were situated between 45.19961°N, 10.73476°E and 45.19848°N, 10.74199°E.

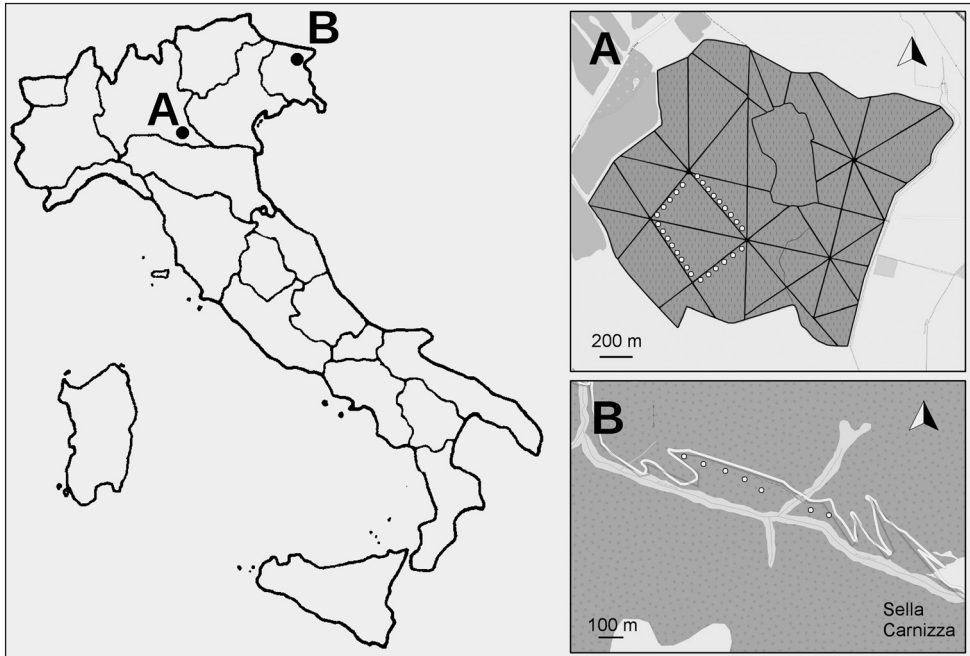


Figure 1. The two study areas, located in Bosco della Fontana (**A**) and in the Parco Naturale Regionale delle Prealpi Giulie (**B**) (northern Italy). White dots in A represent single log piles, white dots in B represent blocks of three log piles.

The Parco Naturale Regionale delle Prealpi Giulie (hereafter referred to as: Prealpi Giulie) was set up in 1996 and covers an area of about 10000 ha. It is located in North East Italy, in the Friuli Venezia Giulia region (province of Udine). The research was conducted in a forest of the municipality of Resia, locality Starmiza di Resia (between 46.343490°N, 13.299400°E and 46.341420°N, 13.307800°E) which is part of the Natura 2000 site “IT3320012 - Prealpi Giulie Settentrionali”. The study area was situated at a range of altitudes between 750 m and 850 m a.s.l. and was covered by almost pure stands of *Fagus sylvatica* which belong to the Illyrian *Fagus sylvatica* forests (Aremonio-Fagion). These were managed by shelterwood cutting and naturally occurring dead wood was removed, resulting in an almost pure stand of beech which was young, even-aged and poor in dead wood. The morphology of the local terrain was often very steep and, as a consequence, small scale landslides locally created mounds of dead wood. Additionally, dead wood accumulated over time in some small and isolated parts and these were not accessible by man. Results, obtained from preliminary research in 2014 and 2015 in this site, showed that the population density of *M. asper* was low, with a clustered structure (unpublished data) and that the expected effects of forest management and the observed population parameters of *M. asper* were concordant. Here the local population belonged to the subspecies *funereus*.

Building the freshly cut log piles

At Bosco della Fontana, freshly cut log piles (FCLP) were built from 26.01 to 21.03.2016, utilising trunks and branches with diameters from 13 to 45 cm of the following tree species: *Carpinus betulus*, *Fraxinus ornus*, *Juglans nigra* and *Quercus rubra*. All trees used came from the Nature Reserve. The wood was cut into logs with a length of 60 cm. These were stacked in 28 FCLPs (seven FCLPs for each tree species) and had a volume of approximately 0.3 m³. Each pile contained at least one log with a minimum diameter of 30 cm. The FCLPs were randomly ordered at the sides of forest roads, spaced at intervals of 50 m along these forest roads as indicated in Figure 1. In the Prealpi Giulie, the wood piles were built between 03.05 and 04.05.2016 from three tree species: *Fagus sylvatica*, *Picea abies* and *Fraxinus excelsior*. All trees utilised came from the Parco Naturale Regionale. The trunks used had diameters between 13 and 35 cm and were cut into logs with a length of 60 cm. As it was known that *M. asper* has a clustered distribution in the site investigated (unpublished data), a randomized block design was employed with distances of 85-215 m between the various blocks. The FCLPs were built in seven blocks and each block consisted of three piles built each from one of the three tree species. A total of 21 FCLPs were built along the transect indicated in Figure 1 and each had a volume of approximately 0.3 m³. Each pile contained at least one log with a minimum diameter of 26 cm. The average distance between blocks was approximately 115 m.

Searching for *M. asper*

The searches for adults of *M. asper*, requiring two people, consisted of a thorough inspection of the surface of each log of the FCLP, without dismantling the wood piles. Each specimen encountered was caught by hand and placed in a plastic holding container. Once the inspection of the FCLP had been concluded, all individuals were counted and sexed. Immediately after sexing, all *M. asper* were released on the same FCLP where they had been encountered. Phenology and peak activity of adults differed between the two study sites due to different climatic conditions: at Bosco della Fontana, the highest number of adults was present in April (Hardersen et al. 2017) whereas the highest number was observed in the Prealpi Giulie in June (unpublished data). Thus, at Bosco della Fontana, adults of *M. asper* were searched for once a week from 29.03 to 17.05.2016 (i.e. a total of eight samplings) between 20:00h and 22:30h (Table 1). In the Prealpi Giulie, adults of *M. asper* were searched for from 17.05 to 12.08.2016 between 18:00h and 20:00h. Here it had been planned to search each FCLP once a week, but bad weather (rain and low temperatures) forced the schedule to be modified (Table 1). Here, a total of 13 samplings were carried out.

Table 1. Survey dates and numbers of *M. asper* observed for the two study sites.

Bosco della Fontana		Prealpi Giulie	
Date	N. of <i>M. asper</i>	Date	N. of <i>M. asper</i>
29/03/16	18	17/05/16	0
05/04/16	45	25/05/16	3
12/04/16	66	01/06/16	1
19/04/16	48	10/06/16	5
26/04/16	36	21/06/16	6
03/05/16	65	25/06/16	6
10/05/16	59	30/06/16	6
17/05/16	48	04/07/16	5
		15/07/16	6
		21/07/16	8
		26/07/16	5
		04/08/16	0
		12/08/16	0

Statistical analysis

To compare the numbers of males and females over the sampling period, the Wilcoxon matched-pairs signed-ranks test was applied. To assess differences amongst the tree species in their attraction for *M. asper*, the data collected during all sampling dates were pooled for each site and compared with the Friedman's Test. This analysis was carried out separately for all specimens, males and females. Then a post-hoc Dunn's Test for Multiple Comparisons was applied. The tests were carried out with the software GraphPad InStat 3.1a for Macintosh.

Results

At Bosco della Fontana, a total of 385 individuals of *M. asper* were counted and, in the Prealpi Giulie, a total of 51 adults were observed (Table 1). This corresponded to an average of 1.7 *Morimus* adults on each FCLP during one survey for Bosco della Fontana and to an average of 0.19 *Morimus* adults on each FCLP during one survey for the Prealpi Giulie. Over the sampling period, the sex ratio was highly significantly in favour of males in both study areas (Bosco della Fontana: 318 males, 67 females, $P=0.008$, Wilcoxon matched-pairs signed-ranks test; Prealpi Giulie: 42 males, 9 females, $P=0.002$, Wilcoxon matched-pairs signed-ranks test).

At Bosco della Fontana, the number of adults, both males and females observed on the different types of wood, was significantly different (Friedman's Test, all adults, $Fr=22.1$, $P<0.0001$; males, $Fr=21.9$, $P<0.0001$; females, $Fr=12.2$, $P=0.007$). The wood of *J. nigra* and *Q. rubra* was significantly more attractive than that of *F. ornus*, with *C. betulus* being intermediary, considering all specimens, males and females, separately (Figure 2).

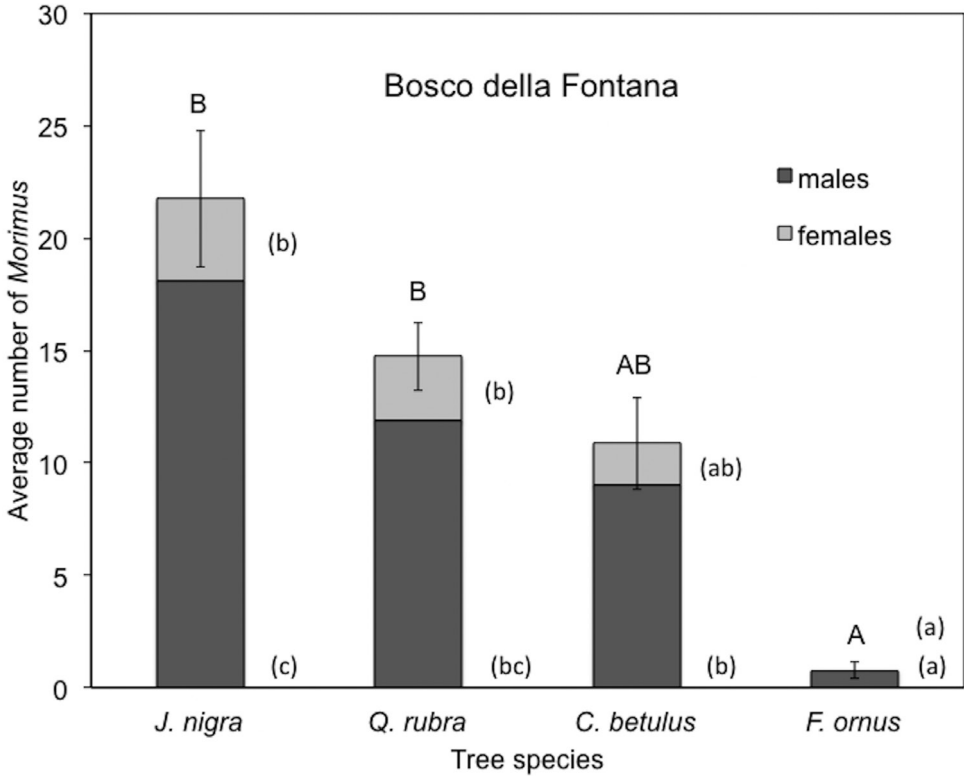


Figure 2. Bosco della Fontana. Average number (\pm SE) of adults of *M. asper* observed per sampling on the seven wood piles built from wood of different tree species. Different capital and small letters above columns (total adults) and on the right (males and females) indicate significant differences among wood types at the 0.01 and 0.05 levels, respectively (Dunn's Multiple Comparisons Test).

In the Prealpi Giulie, the number of individuals observed on the different types of wood was significantly different for all adults and for males (Friedman's Test: all adults, $F_r=10.6$, $P=0.005$); males, $F_r=10.2$, $P<0.006$). The number of female individuals observed followed the same pattern as for all adults and for males, but was not significant (Friedman's Test: females, $F_r=3.7$, $P=0.16$). The wood of *F. sylvatica* was significantly more attractive than that of *P. abies* with *F. excelsior* being intermediary, considering all adults and males (Figure 3).

Discussion

On the FCLPs of both sites investigated, adults of *M. asper* were observed regularly and permitted the monitoring of the local populations. These data are in line with the findings by Chiari et al. (2013) and Hardersen et al. (2017) that FCLPs are reliable for detecting the presence and abundance of the target species. In both sites, *M.*

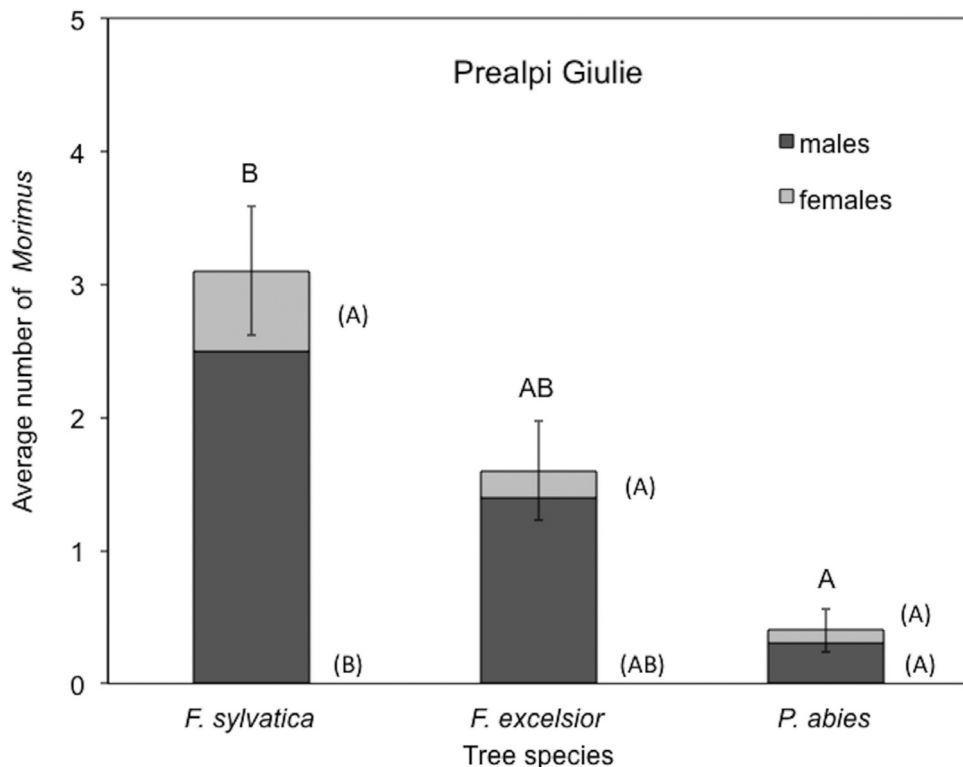


Figure 3. Prealpi Giulie. Average number (\pm SE) of adults of *M. asper* observed per sampling on the seven wood piles built from wood of different tree species. Different capital letters above columns (total adults) and on the right (males and females) indicate significant differences among wood types at the 0.01 level (Dunn's Multiple Comparisons Test).

asper males were recorded more frequently, as is commonly observed when surveying Cerambycidae (Lopez-Pantoja et al. 2008, Drag et al. 2011) and this also applies to *M. asper* (Chiari et al. 2013, Hardersen et al. 2017).

At Bosco della Fontana, the average number of *M. asper* observed on each wood pile was 1.7 during each sampling whereas, in the Prealpi Giulie, this number was only 0.19. Even though the two studies are not directly comparable, the large difference in the number of observed adults is indicative of different population densities. The high figure observed at Bosco della Fontana revealed a large population, probably as a direct consequence of the management actions which are aimed at the protection of dead wood in all its forms (Campanaro et al. 2014). In contrast, for the Prealpi Giulie, the data gathered indicated the presence of a small and patchy population of *M. asper*, in line with the current management of the forest investigated which is almost devoid of dead wood. The unfavourable conservation status of this protected beetle species is directly linked to the paucity of dead wood in this site of the Natura 2000 network and it would therefore be important to modify the management of these beech forests to

increase the amount of dead wood, as indicated by the plan for the conservation and development of the Park (Piano di conservazione e sviluppo del Parco Naturale delle Prealpi Giulie, Decreto del Presidente della Regione Friuli Venezia Giulia 20 marzo 2015 n. 062/Pres).

This is the first study which compared the power of attraction of several tree species and it showed that the selection of the species is important for the monitoring of *M. asper*. This study is also the first showing that some tree species attracted hardly any adults. These results were also consistent when males and females were considered separately. In the Prealpi Giulie, the results for females followed the same pattern as in all adults and in males, but they were not significant. This may have been caused by the low number of female individuals observed. The results confirmed that the wood of some tree species is more attractive to *M. asper* (Bărbuceanu et al. 2015, Hardersen et al. 2017). It was surprising that the two allochthonous species (*J. nigra*, *Q. rubra*) tested at Bosco della Fontana were most attractive for the adults of *M. asper*, as a preference for these non-native species cannot have evolved by natural selection. In contrast, the wood of the dominant species of Bosco della Fontana, *C. betulus*, attracted only 53% of adults when compared to *J. nigra* and the wood of the other autochthonous tree species was not attractive at all; *F. ornus* attracted only 3.7% of adults. These data are in line with the fact that the genus *Fraxinus* (or the family Oleaceae) is not mentioned as a host plant for this longhorn beetle (e.g., Romero-Samper and Bahillo 1993, Sama 2002, Dojnov et al. 2012).

In the Prealpi Giulie, the dominant tree species, *F. sylvatica*, attracted the largest number of adults of *M. asper* and these findings are in agreement with numerous authors who indicated that beech is one of the main host species (Romero-Samper and Bahillo 1993, Sama 2002, Polak 2012, Bărbuceanu et al. 2015) and with Fusu et al. (2015) who stated that beech is one of the preferred wood types by *M. asper*. In this case study, the second most attractive species was *F. excelsior*, a congener of *F. ornus*, which was the least attractive species at Bosco della Fontana. *Picea abies* was the least attractive species, even though *M. asper* was reported to complete its life-cycle in trunks of this tree species (Sturani 1981).

Adults of *M. asper* must be able to locate freshly dead wood, often a scarce resource, to reproduce. Once a tree has died, the decay process results in an extensive release of volatile organic compounds (Holighaus 2012) and host-plant recognition of insects depends on ratios of plant volatiles (Bruce et al. 2005). This has also been shown for adults of Cerambycidae (Paschen et al. 2012). For example, the composition of volatiles clearly separate hardwood from softwood species and, to some extent, individual species (Holighaus 2012). Even though no specific information is available on host location by *M. asper*, it seems likely that they seek out the preferred wood-types by means of volatile organic compounds.

The study revealed a further important aspect for the monitoring of *M. asper*, i.e. the weather. When a cold spell arrived in mid-late April in the province of Mantua, the number of adults observed almost halved. Similarly, in the Prealpi Giulie, late May and early June were characterised by particularly cold weather with frequent rain and the number of adults observed was low. These observations are in accordance with

Stanić et al. (1985) and Polak (2012) who reported that, at low temperatures (respectively below 12°C or 17°C), activity of *M. asper* is much reduced.

In summary, this study is the first which compared the attraction of numerous types of wood to adults of *M. asper* and showed that the wood of the tree species tested was not equally attractive. This finding is in line with Bărbuceanu et al. (2015) and Hardersen et al. (2017) who found that certain tree species are more attractive. Thus, when selecting wood for FCLPs for the monitoring of *M. asper*, it is important to choose wood which is attractive. Currently it can be said that the wood of *F. ornus* and *P. abies* should not be used for building wood piles. In contrast, the wood of the following trees attracted large numbers of adults: *J. nigra*, *Q. rubra*, *Q. robur* and *F. sylvatica* (Hardersen et al. 2017 and current study). While the wood of *C. betulus* and *F. excelsior* was moderately attractive for *M. asper*, monitoring could still be carried out. (Hardersen et al. 2017 and current study). For long-term monitoring programmes, it is essential to assess the long-term availability of the wood. It is also important that the wood of the selected tree species is freshly dead and has a diameter larger than 12 cm (Hardersen et al. 2017). This study, together with those by Chiari et al. (2013) and Hardersen et al. (2017), demonstrated that by investigating the factors which influence the selection of dead wood by the target species, the monitoring method for this elusive, localised and protected saproxylic species can be optimised.

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