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CYCLE: XXXIII

PREVENTION AND MANAGEMENT OF *VESPA VELUTINA* SPREAD IN EUROPE

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Summary

The Asian yellow-legged hornet *Vespa velutina* is an invasive species introduced in France in 2004, that has rapidly colonised other European countries, including Italy since 2012. *V. velutina* preys on other insects for rearing its brood, with a specialisation towards the European honey bee *Apis mellifera*, a species that has not evolved defensive strategies against this hornet. Moreover, the size of *V. velutina* colonies, together with their distribution and density in the environment, are causing great concern to citizens, for the associated possibility of stings that could lead, in some cases, to fatal events. As a result, the diffusion of *V. velutina* could generate multiple negative impacts in the invaded countries by: *i)* contributing to pollinator decline and to honey bee colony losses; *ii)* affecting the ecosystem services provided by pollinators; *iii)* competing with native species; *iv)* generating economic impacts for the beekeepers and the society, the latter in relation to the costs of management practices; *v)* creating concern among citizens. Considering the issues posed by this invasive species of Union concern, European countries must develop and implement procedures for preventing its spread and for limiting its impacts, for the application of the EU Reg. 1143/2014.

To date, the mostly feasible measures that could be used to achieve these results are the establishment of monitoring networks to rapidly detect the presence of the species and the timely detection of hornet colonies. Both measures could be improved by deepening the current knowledge on the species and by developing new tools and strategies for monitoring *V. velutina* and locating nests before the beginning of the reproductive phase of the colonies. These are the aims that drove the activities of this PhD research project, with the perspective of providing new knowledge and tools for improving the management of the species in Europe.

A review has been prepared for summarising the diffusion of *V. velutina* in Europe, its impacts, and the options for preventing its spread or for managing established populations. A pluriannual monitoring activity in the invaded areas of Italy allowed to gather accurate data on *V. velutina* distribution. These data were analysed to understand the environmental and species-dependent characteristics mostly correlated to the presence of *V. velutina* nests. These information were modelled for forecasting the diffusion of the species in consecutive years. This predictive approach could be used for establishing surveillance areas around outbreaks or over the border of its expanding range, for limiting the spread of the species. Moreover, the effectiveness and selectiveness of monitoring traps and baits customarily used for assessing the presence of *V. velutina* were compared and evaluated, to understand the efficacy in trapping the target species and the consequences on native insects.

New tools and technologies have been tested for understanding their efficacy in early detecting *V. velutina* colonies. The use of thermal IR cameras proved to be a viable technique for detecting nests of *V. velutina*, even during the summer season. Performance and limitations were described in relation to environmental and operative characteristics. The harmonic radar tracking of flying hornets is an innovative and viable method that could be used for detecting nests of the species. Results have demonstrated its applicability in complex landscapes, with various landcover characteristics (open terrain as well as woodlands and urban areas) and slope degrees. This technology could be used for managing *V. velutina* outbreaks but could also be adapted to other contexts, for example for understanding the movements and ecosystem interactions of several flying insects, thus leading to new discoveries in the field of insect science.

Finally, the effect of the presence of *V. velutina* on native wasp species (*Vespa crabro*, *Vespula vulgaris*, *Vespula germanica*) has been evaluated in an invaded area of Italy. Results highlighted that *V. velutina* has not caused a relevant alteration to the abundance and distribution of native wasps, thus the effects of competition are restrained, at least in areas recently colonised by this species.

The combination of these findings has contributed to advance the knowledge on the biology and ecology of this species and on its impacts. Such knowledge could be used to improve the strategies that should be developed in Europe for preventing its establishment and for managing expanding populations.

1.

Introduction

Bees and other pollinators are an essential component of biodiversity, and are fundamental for the maintenance of ecosystem stability. By searching for nectar, they significantly contribute to pollen dispersal and plant reproduction (Waser & Ollerton 2006). Bees are known to contribute approximately 80% of pollination of entomophilous plants (Gill et al. 2012), which are plants that rely on insects for the dispersion of pollen. Several of these plants are cultivated for food production, like apples just to name one, making pollination a global essential service for human well-being (Potts et al. 2016).

Honey bees and other pollinators are threatened globally due to multiple disturbing factors (Brown & Paxton 2009, Potts et al. 2010) such as habitat loss, degradation and fragmentation (Goulson et al. 2008), incorrect use of pesticides (Gill et al. 2012, Rundlöf et al. 2015), climate change (Memmott et al. 2007), diffusion of parasites and pathologies (Fürst et al. 2014). Invasive alien species are globally considered as the second major threat to biodiversity, and their introduction and spread may represent an additional factor that could worsen the conservation status of pollinators (Brook et al. 2008).

The Asian yellow-legged hornet *Vespa velutina* (Lepeletier 1836) is the perfect example of an invasive alien species able to threaten native pollinators. Introduced in Europe in 2004, this species is nowadays colonizing several countries (Monceau et al. 2014a) including Italy since 2012 (Demichelis et al. 2014, Porporato et al. 2014, Bertolino et al. 2016). Due to its intensive predation activity towards the European honey bee *Apis mellifera* (Monceau et al. 2013a) and its wide predation spectrum, which includes other Hymenoptera (Apidae, Vespidae), Diptera and other species (Rome et al. 2011), *V. velutina* represents a new threat for honey bee colonies and native pollinator communities. Moreover, the presence of nests in areas frequented by people (Monceau & Thiéry 2017), together with the size reached by the colonies (Rome et al. 2015), generates concern among citizens (Sumner et al. 2018) and in some cases could lead to stings and fatal events (Feàs Sanchez & Charles 2019).

For containing its spread and impacts, the European Commission has included *V. velutina* in the “black-list” of invasive alien species of Union concern (Reg. EU 1141/2016). This imply that European countries should adopt strategies and procedures for: *i)* prevent the establishment of new populations; *ii)* rapidly detect and remove new populations; *iii)* control or manage widespread populations for containing or limiting their impacts (Reg. EU 1143/2014).

This framework has driven the activities of the LIFE STOPVESPA project, an initiative, coordinated by the University of Turin, for controlling the spread of *V. velutina* in Italy, which has also been awarded with the European Bee Award 2020 for the best project that developed innovative solutions for protecting bees, pollinators and biodiversity more in general. Indeed, this project established an Early Warning and Rapid Response System for limiting the spread and impacts of *V. velutina*, thanks also to the development of new technologies for detecting nests, such as the harmonic radar for tracking flying hornets back to their colonies.

The PhD research project on “Prevention and management of *Vespa velutina* spread in Europe” is based on the activities developed in Italy by the LIFE STOPVESPA project, and aims to investigate the biology, ecology and spread modalities of *V. velutina* in the invaded area, to better understand the consequences of the invasion and develop tools and strategies that could increase the efficiency of management practices. The research activities allowed the preparation of a collection of six manuscripts, which are presented as individual chapters of this PhD thesis.

- Chapter 2 “***Vespa velutina*: an alien driver of honey bee colony losses**”, a review on the species with a focus on its impacts and on the options for monitoring and controlling its spread.

- Chapter 3 “**Establishing surveillance areas for tackling the invasion of *Vespa velutina* in outbreaks and over the border of its expanding range**”, an analysis of occurrence data for forecasting the spread of the species and establish buffer areas for improving monitoring and control activities.
- Chapter 4 “**Viability of thermal imaging in detecting nests of the invasive hornet *Vespa velutina***”, in which the performance and limitation of thermal IR cameras in detecting nests of *V. velutina* are described.
- Chapter 5 “**Tracking flying insects in complex environments by means of a harmonic radar**”, in which it is described the application of an innovative tracking technique for locating the position of *V. velutina* nests, by the real-time tracking of hornets that are flying from honey bee colonies to their nests.
- Chapter 6 “**Effectiveness and selectiveness of traps and baits for catching the invasive hornet *Vespa velutina***”, in which the performance of different combinations of traps and baits commonly used for monitoring wasps and hornets are compared, together with an evaluation of side effects on native species.
- Chapter 7 “**Introduced *Vespa velutina* does not replace native *Vespa crabro* and *Vespula* species in Italy**”, in which the consequences of *V. velutina* invasion towards native wasps are explored in an invaded area of Italy.

In parallel to the preparation of these manuscripts, findings and results achieved during the PhD research project were shared with the scientific community at national and international conferences, in the form of oral presentations and posters. A comprehensive list of these activities is available in Annex I.

In addition, the acquired knowledge in the management of *V. velutina* populations has been shared with laypersons, stakeholders, and administrations, to promote the transferability of scientific findings for improving the management procedures of the species in Europe. The prepared documents are listed and synthetically described in Annex II.

2.

Vespa velutina: an alien driver of honey bee colony losses

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Abstract: *Vespa velutina*, or Asian yellow-legged hornet, was accidentally introduced from China to other parts of the world: South Korea in 2003, Europe in 2004, and Japan in 2012. *V. velutina* represents a serious threat to native pollinators. It is known to be a fierce predator of honey bees, but can also hunt wild bees, native wasps, and other flying insects. When *V. velutina* colonies are developed, many hornets capture foraging bees which are coming back to their hives, causing an increase in homing failure and paralysis of foraging thus leading to colony collapse. The hornets may enter weak beehives to prey on brood and pillage honey. Unlike *Apis cerana*, *Apis mellifera* is unable to cope with the predation pressure of *V. velutina*. Monitoring the spread of an invasive alien species is crucial to plan appropriate management actions and activities to limit the expansion of the species. In addition, an early detection of *V. velutina* in areas far away from the expansion front allows a rapid response aimed to remove these isolated populations before the settlement of the species. Where *V. velutina* is now established, control measures to prevent colony losses must be implemented with an integrated pest management approach.

Keywords: *Vespa velutina*; alien driver; honey bee; damage; pollinator

2.1 Introduction

Invasive alien species have always been a risk to ecosystems. They are a serious obstacle to the conservation of biodiversity, both globally and locally, as their stabilization and spread in new environments break the pre-existing balances. By coming into contact with a new environment, alien species can lead to a gradual degradation and alteration of the new habitat and the decline of indigenous species, until in some cases some of them become extinct (Atkinson 1996, Gandhi & Herms 2010, Lever 2010).

The Asian yellow-legged hornet (*Vespa velutina nigrithorax*, Du Buysson 1905) is a social wasp, belonging to one of the 11 subspecies (Van der Vecht 1956, 1959, Archer 1994) of *V. velutina* originally present in Continental Asia (Perrard et al. 2014), where it is native to subtropical and temperate areas of Indo-China (Archer 1994, 2012).

The species established itself in non-native countries such as South Korea in 2003 (Choi et al. 2012) in the southern port town of Busan, and Japan, on Tsushima Island in 2012 (Sakay & Takahashi 2014, Ueno 2014), in Kitakyushu City on Kyushu Island in 2015 (Minoshima et al. 2015) and on Iki Island in 2017 (Takahashi et al. 2019). Arrived in France probably in 2004 along with garden pots imported from China (Haxaire et al. 2006, Villemant et al. 2006), the species spread to neighbouring countries. From France it reached the Navarra province and Basque country (Spain) in 2010 (Castro & Pagola-Carte 2010, Lopéz et al. 2011), Galicia (Rodríguez-Flores et al. 2019) and Catalunya (Pujade-Villar et al. 2013) in 2012, Majorca Island (Spain) in 2015 (Leza et al. 2018, 2019), the Minho province (Portugal) in 2011 (Grosso-Silva & Maia 2012), and Flobecq in the Hainaut province (Belgium) in 2011 (Rome et al. 2012). In 2012, the Asian yellow-legged hornet was detected for the first time in Italy in the Liguria Region (Demichelis et al. 2014); afterwards, the hornet started to spread in this region mainly along the coastline (Porporato et al. 2014, Bertolino et al. 2016, Lioy et al. 2019). In Piedmont Region (Italy) arrived in 2013 (Porporato et al. 2014, Bertolino et al. 2016). It was detected also in Veneto and Lombardy Regions (Italy) between 2016 and 2017, with no more reports in the following years, and in Tuscany Region (Italy) in 2017. By 2017, the species had colonized an area of at least 1,110 km^2 in Italy (Lioy et al. 2019). *V. velutina* was firstly recorded in Germany in 2014 and a nest was found in Büchelberg (Rheinland-Pfalz, Witt 2015). In 2016, few hornets were found in the United Kingdom, and in 2017 also in Netherland, Switzerland (UK National Bee Unit 2016), and Scotland (Budge et al. 2017), Fig. 1.

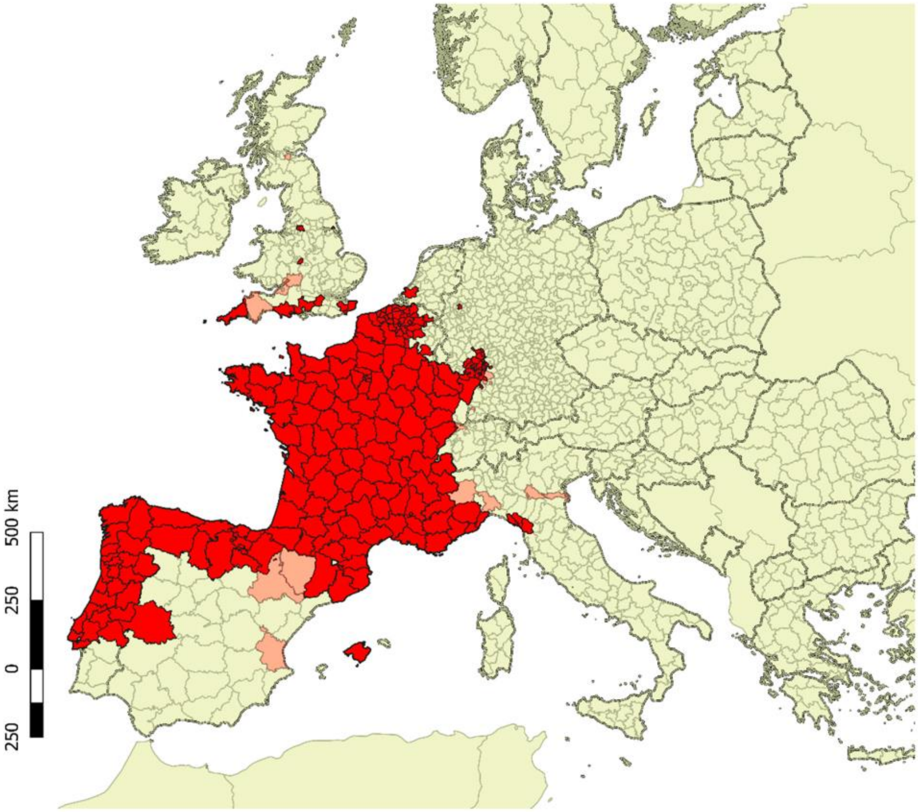

Figure 1. Presence of *V. velutina* in Europe estimated from several sources. Red areas indicate districts where hornets are established or have been reported in 2018 and 2019. Light-red areas show districts where hornets or nests have been exclusively spotted in the past until year 2017.

The spread of *V. velutina* in Europe and in non-native Asian regions seems to respect the predicted climatic suitability maps modelled by Villemant et al. (2011a). Global warming could worsen the current situation (Barbet-Massin et al. 2013). This hypothesis was confirmed by Rodríguez-Flores et al. (2019): high minimum temperatures, dew temperature, relative humidity and low maximum temperatures favour the occurrence and spread of *V. velutina*. These conditions are common in coastal areas and can promote the rapid dispersal of this pest.

V. velutina creates considerable damage to the environment and beekeeping activities. For this reason, the species has been included by European Union in the black-list of invasive alien species (Reg. EU 1141/2016) for which it is mandatory to develop surveillance plans and actions to limit its spread as well as control and containment strategies. The Japanese Ministry of the Environment added this hornet to the list of invasive alien species in 2015 (Kishi & Goka 2017).

2.2 Biology

The colony of *V. velutina* is started by a single inseminated queen that builds, using fibrous substances of plants origin and saliva, a primary nest after overwintering, typically in April, thus producing the first workers. During the warm season, they enlarge the primary nest (which has an approximate size of 4-15 cm) directly or build a secondary nest normally on treetops (Rodríguez-Flores et al. 2019). Nests have normally a circular shape and can grow up to 100 cm in diameter, containing several thousands of hornets. Rome et al. (2015) report up to 13,300 adults and 563 new queens from a single nest. At the end of the summer, reproductive individuals emerge and mate; the colonies generally collapse in late autumn or winter, while newly-mated queens search for a place where they can overwinter and, the following year, they start a new cycle (Archer 2012, Monceau et al. 2014a, Rome et al. 2015, Lioy et al. 2019), Fig. 2.

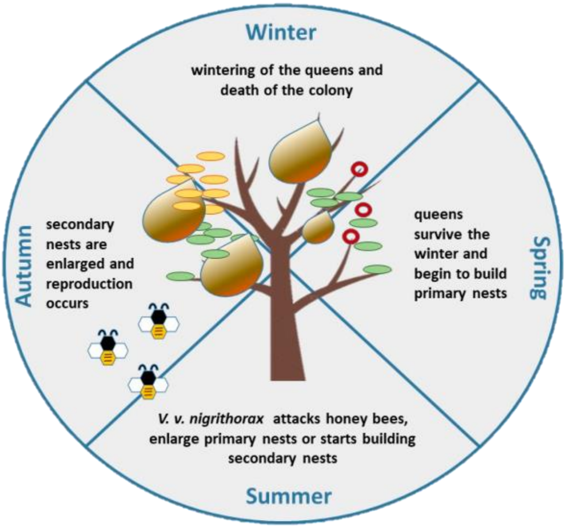

Figure 2. Life cycle of *V. velutina*.

Hornets use olfactory stimuli to search for long-distance food sources, especially with regard to the localization of honey bee colonies, but the nature of these stimuli is not yet entirely clear (Couto et al. 2014, Wang et al. 2016, Laurino & Porporato 2017).

The components of the hive, which attract the most attention of *V. velutina*, have been the subject of study for some years. Hornets are strongly attracted by the odour of some hive products, especially pollen and honey (Couto et al. 2014). A laboratory study showed that *V. velutina* workers can use both visual and olfactory cues to locate honey bees (Wang et al. 2014).

Honey bees attract the attention of the hornets thanks to the production of geraniol, a component of the aggregation pheromone of the colony. Less effective than pollen and honey, but still attractive is the royal jelly, thanks to the presence of homovanillyl alcohol (HVA) and methyl-4hydrobenzoate (HOB), substances that are part of the pheromone produced by the honey bee queens, but also present in the royal jelly. Betaocimene emitted by larvae also produces olfactory stimuli that are very attractive to hornets (Couto et al. 2014).

The pheromones produced by the hornet colony’s components are being studied for their possible use in biological control techniques (Turchi & Derijard 2018). Couto et al. (2016), in a neurobiological works on *V. velutina*, showed the presence of several microstructures in the antennal lobe of the males, which are probably linked to sex pheromones. Recently Wen et al. (2017) announced the isolation of the sex pheromones from *V. velutina* queens. Cheng et al. (2017) proved that *V. velutina* uses sting venom volatiles as an alarm pheromone.

2.3 Impacts

In Europe, *V. velutina* is considered invasive, both for its expansion capabilities at European scale (Fournier et al. 2017, Robinet et al. 2017, Barbet-Massin et al. 2018) and the impacts that it could produce by preying on honey bees and native insects (Beggs et al. 2011, Monceau et al. 2013b, 2014a, 2014b). The species can cause serious damage and imbalances to biodiversity and ecosystems in areas where it has been introduced. This is aided by *V. velutina*’s high reproductive rate, high dispersal ability, broad diet, wide habitat preference, superior competitive ability, and most importantly, multiple mating of its queens (Moller 1996, Martin 2017, Takahashi et al. 2019).

Although the species is not considered in Europe more dangerous than native hornets and wasps (De Haro et al. 2010), it can cause problems to human-health and several accidents and some fatal events were recorded (Feàs Sanchez & Charles 2019). Moreover, by frequently establishing colonial nests in urban areas, *V. velutina* could generate social impacts due to citizens’ perception of fear of possible stings (Liu et al. 2015, Tabar et al. 2015, Sumner et al. 2018). *V. velutina* causes multiple threats, however the extent of the impacts produced has not yet been documented and quantified exhaustively. Despite this lack, it is possible to assess the components and/or the activities that are affected or may be impacted by this hornet. From the analysis of the research conducted so far in Europe it is possible to reckon three major negative impacts that the species may create.

1. Economic threats: loss of honey bee colonies and decreasing of beekeeping products; cost for control activities and nests removal.
2. Ecological threats: impacts on biodiversity; impacts on pollination activity; competition with native species.
3. Public health threats: potential risk for citizens.

2.3.1 Damage to beekeeping

The hornets hunt foraging honey bees returning to their colonies by hovering in front of the hive entrance, grabbing the honey bees in flight, and killing them with their jaws. The thorax is then selected, for the high protein content provided by the flight muscles, and transported to the nest for feeding the larvae.

The intense predatory activity of *V. velutina* towards honey bees can generate a decrease in the strength of the honey bee colonies and the subsequent collapse of the family. This is due to the increase in the number of foraging bees which do not come back in their hive with a consequent increase of the overall probability of homing failure and finally with the disruption of colony foraging activities, which leads to complete foraging paralysis (Requier et al. 2019). This primarily generates economic damage to the beekeeping sector, as well as a decrease in the number of honey bees in the environment, resulting in a decline in the ecosystem pollination service.

In some European regions, the predation of *V. velutina* has resulted in the loss of almost 50% of bee families. In the south-west of France, beekeepers reported losses of between 30% and 80% of honey bee colonies, resulting in poor production of honey and other beehive products. In 2010 in Gironde (France), due to *V. velutina*, the Union Nationale pour l’Apiculture Francaise declared that 30% of bee colonies were destroyed or weakened (Monceau & Thiery 2016). In Western Liguria (Italy) the authors of the present review (unpublished results) have found an increase of 18% in winter colony losses in areas where *V. velutina* is not controlled.

The costs incurred both for the implementation of public information campaigns and for the destruction of *V. velutina* nests are relevant economic issues. In 2011 in France, the beekeeper organization Groupement de Dèfense Sanitaire des Abeilles (GDSA) coordinated the destruction of more than 1,000 nests in Aquitania, while a private company destroyed about 500 nests in the Toulouse area. The total cost of these interventions can be quantified to more than 165,000 euros.

Leza et al. (2019) demonstrated that the presence of *V. velutina* produces an increase of oxidative stress in honey bee workers under field conditions. This leads to a higher expression and activity of antioxidant enzymes and mitochondrial-related genes and higher lipid oxidative damage in the individuals of the colony exposed to this predator. Other authors reported that other stressors, like herbicides or migratory management, could increase lipid peroxidation in honey bees (Helmer et al. 2015) suggesting that these situations, along with the presence of *V. velutina*, could affect honey bees' health (Leza et al. 2019).

The apiaries are a very attractive source of food for *V. velutina*, because there is a high concentration of honey bees. Studies performed in France have demonstrated that in urbanized environments, where the concentration of apiaries is high, the diet of *V. velutina* is composed for almost 70% of honey bees and other similar species (Apoidea, Rome et al. 2011). Monceau et al. (2014b) monitored the predation of *V. velutina* on apiaries. In an apiary with six beehives, in the sampling period they caught a total of 360 workers, and most of these visited the apiary daily. This indicates that once the species has identified an important protein source such as an apiary, it visits the site every day, probably because of a greater success of predation. Of the six beehives monitored in the season, one was completely destroyed, while in the other five the size of the colonies halved. In addition, five *V. velutina* nests were discovered within 1 km from the experimental apiary; so, it is likely that apiaries were attacked by individuals from different colonies.

The colonies of *Apis mellifera* manifest a certain defensive ability towards *Vespa crabro*, the European native hornet, but fail to implement effective defensive behaviours towards *V. velutina*, having had no opportunity to co-evolve with this predator. In the Asian regions, where the local bee species, *Apis cerana*, has co-evolved with *V. velutina*, the honey bees have instead developed very effective defence techniques, resulting in suffocation and heating of the predator (balling), and the formation of a compact agglomeration of honey bees on the flying board (Ken et al. 2005). *A. mellifera* is also able to curl up hornets that rest on the flying board of hives, but this behaviour does not reach large percentages of success.

At the end of the summer, when the colonies of *V. velutina* are very populous, hornets can get to besiege the hives and penetrate inside them annihilating the colonies of honey bees. The use of doors with passages less than 5.5 mm in diameter can prevent the entrance of hornets and delay the definitive collapse of the colonies, but if the beekeeper does not intervene to eliminate the hornets, the honey bees cannot get out and the colony is destined to collapse (Fig. 3).

Figure 3. *V. velutina* in hunting activity in front of a hive (left) and heavy attack of hornets on the flying board of a hive (right).

As it often happens in many species of insects, climatic conditions, especially temperature and humidity, affect the predator's activities. In the case of *V. velutina*, the increase in the efficiency of predation, which is most evident in the middle hours of the day, would be the result of an increase in temperatures and the level of solar radiation (Laurino & Porporato 2017).

2.3.2 Impact on ecosystem

The predatory activity of *V. velutina* has a negative impact on insect communities, reducing their abundance and may cause damage to local biodiversity even at the ecosystem level. The predation pressure known in apiaries since the month of July may have similar effects also on other pollinating insects, creating a decline in pollination effectiveness. In fact, besides honey bees, *V. velutina* preys on other Hymenoptera, including different species of wild bees and other Vespidae (wasps in general), but also Diptera (flies and mosquitoes), Lepidoptera (butterflies and moths) and other insects. Species preyed upon by *V. velutina* and their proportion varies according to the prey availability in the environment. A French study showed that in an urban environment, *V. velutina* preys mostly honey bees and other Apoidea (66% of the diet), while in a woodland environment, bees and other Apoidea drops to 33% and Diptera increases to 32% (Rome et al. 2011, Fig. 4).

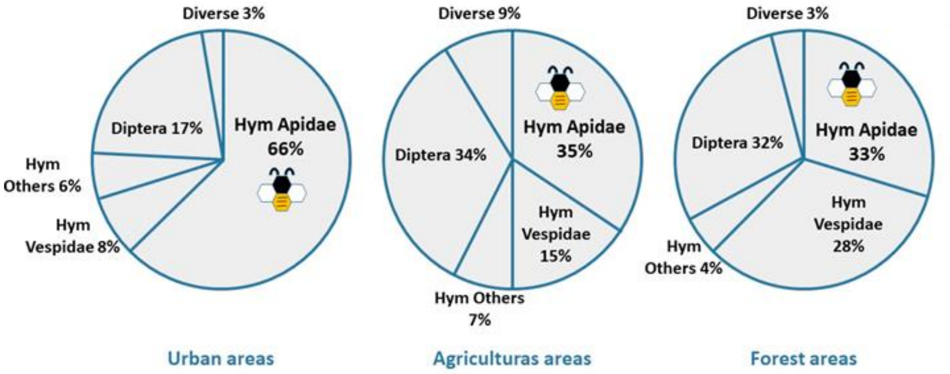

Figure 4. *V. velutina* prey spectrum: preliminary results in three different environments (Rome et al. 2011).

2.4 Monitoring and Surveillance Systems

Monitoring the spread of an invasive alien species is crucial to plan appropriate management actions and activities to limit its expansion. Only monitoring and surveillance strategies permit to assess the presence of the species on the territory and identify the areas of expansion or new invasive outbreaks. Since *V. velutina* is particularly attracted by honey bees, it is important to involve beekeepers and beekeeper associations to maximize the efficacy of monitoring strategies, together with the contribution of all interested citizens.

An early detection of *V. velutina* in areas far away from the expansion front allows to perform a rapid response aimed to remove these isolated populations before the settlement of the species (Genovesi et al. 2010). In fact, *V. velutina* queens might be accidentally transported by human activities in very remote areas, where these insects can give rise to new colonies and populations (Bertolino et al. 2016). An early warning and rapid response system (EWRRS) for *V. velutina* is based on three key moments (Fig. 5).

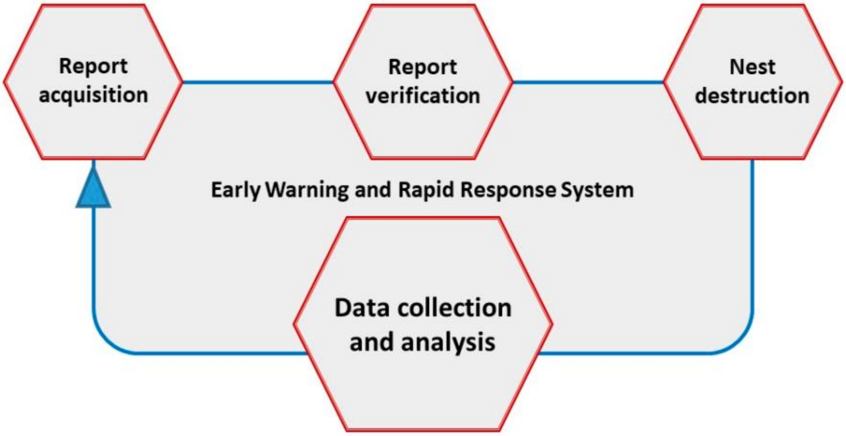

Figure 5. Early warning and rapid response system for *V. velutina*.

Results of EWRRS are the rapid detection of the species and the readiness of intervention, which increases the probability of destroying the colonies before the birth and the mating of the future founder queens. This increases the probability of success in the containment of *V. velutina*. Key aspects of an effective management strategy are: simplicity of the procedures; rapid intervention; exportability on a national and international scale; economic sustainability.

Different monitoring methods for *V. velutina* exist, such as the direct observation of hornets in apiaries or on flowers and the use of traps. Several trap models have been proposed to catch *V. velutina* adults; basically, they can be reduced to bottle, funnel, and sticky traps or to a combination of them.

In addition to commercial baits, many types of self-produced carbohydrate or protein baits can be used. Sugar based baits include beer, vinegar, grenadine, acetic acid, fermented honeycomb juice, honey, different type of sweeteners mixtures, etc. (Turchi & Derijard 2018). They are better used between February and May, so to catch the founder queens when they begin the construction of the primary nests, and from August until November, to detect the presence of the species in new areas or to catch the reproductive adults. Demichelis et al. (2014) recommend the use of lager beer (0.33 litres with 4.7% alcohol), because it is attractive for the hornets, inexpensive, and selective towards honey bees (Fig. 6).

During colony development, between June and August, protein baits (meat, fish, etc.) can be used. In addition, Rodríguez-Flores et al. (2019) highlighted that elevation and meteorological factors influence the effectiveness of bait trapping.

Figure 6. Bottle trap with lager beer (Demichelis et al. 2014).

2.5 Control

Control invasive alien species is difficult and expensive in the long term; therefore, every effort should be undertaken to prevent their establishment and diffusion in new areas. The many methods developed to control *V. velutina* in Europe and in Asia outside of its native range have been previously reviewed (Beggs et al. 2011, Choi et al. 2012, Monceau et al. 2014a, Kishi & Goka 2017, Turchi & Derijard 2018).

Until now, no single control method has proved to be fully effective, but the coordinated use of several methods under an integrated pest management approach should greatly reduce the impact of *V. velutina* on honey bees and on the environment (Fig. 7).

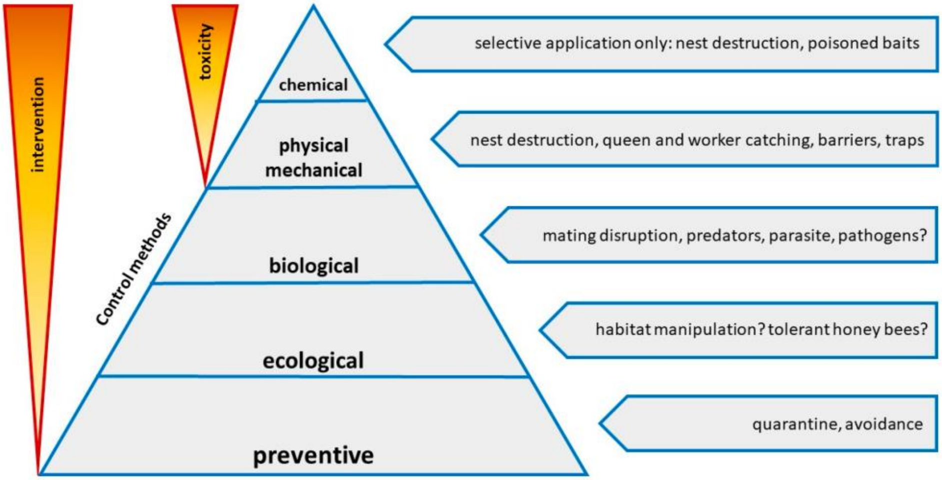

Figure 7. Integrated pest management pyramid showing available or possible methods to control *V. velutina*, from the low intervention and not toxic preventive methods to the high impact chemical methods that implies the use of toxic insecticides.

Control efforts may be implemented against adult hornets or nests. In the first case, the baited traps used for monitoring purposes can be used either for controlling purposes, but traps are at the moment not selective enough to prevent extensive captures of non-target insects, with possible extensive impacts to native species (Rojas-Nossa et al. 2018, Requier et al. 2019). Other types of control techniques are used or tested in Europe: bucket poisoned baits, passive traps, electric traps, electric harps, badminton rackets, beehive muzzles, nest gunshot, or the use of the hornet workers as poison carriers (Turchi & Derijard 2018).

The detection and destruction of the nests of *V. velutina* is currently the most effective control method, especially when the nests themselves are destroyed before the reproductive phase of the colony which normally occurs in early September. In any case, it is important to search for and destroy active nests in all stages from foundation to winter (Feàs Sanchez & Charles 2019). Once a nest has been located, it must be destroyed in a complete way, paying special attention to killing the queen, the majority of the workers, and all the brood present in the combs.

The discovered nests should be immediately destroyed by people with specific training and equipped with suitable personal protective clothes and the necessary tools. The methods of intervention are various in relation to the place where the nests are built and the size of the colonies.

Nests are generally treated with insecticides for hornets and wasps, using also special extendable rods capable of reaching nests that are in high positions (Fig. 8).

Figure 8. Different methods used to destroy the nests depending on their position.

The control of the populations of *V. velutina* is hindered by the difficulty of finding all nests. Embryo nests are small and difficult to be observed; later the nests, although very voluminous, are often difficult to be located since covered by tree canopy. In fact, *V. velutina* can build nests in several environments, such as natural, rural, and urban areas, and on different substrates (Rodríguez-Flores et al. 2019). They can be found on trees, shrubs, roofs or balconies of houses, inside gaps, but also in soil cavities or on rocky substrates (Fig. 9).

Figure 9. Primary nest built under a roof canopy (left) and secondary nest (right) built on *Acacia dealbata* tree in Liguria Region (Italy).

2.5.1 Nest detection

Several techniques are currently available to locate hornet nests, but they are all tedious, extremely labour consuming, and/or expensive (Turchi & Derijard 2018).

The triangulation involves capturing at least three specimens and their subsequent release from various locations, in order to record the direction of their flight; if the hornets tend to return in a straight direction, there is a good chance that the three directions they took would intersect at a point that will correspond to nest position (Blot 2008, Leza et al. 2018). The on-view tracking of tagged hornets, which are made more evident with a feather or a cotton thread, has been suggested. The drone-assisted nest tracking, a theoretical study that uses several drones equipped with cameras capable of analysis an image of a hornet marked with a thread carrying a fluorescent Styrofoam ball, has recently been published (Reynaud & Guérin-Lassous 2016). Alternatively to these observational methods, it is possible to locate the nests by using equipment developed in recent years.

The infrared thermal imaging camera has been tested in Portugal, in UK (Kennedy et al. 2018, Semmence 2018), and in Italy (Lioy et al. 2020a, Bortolotti et al. 2016).

Radio-telemetry has been implemented in UK as a tool for tracking hornets back to their nests and provided an efficient mean of finding nests in complex environments (Kennedy et al. 2018).

A European LIFE project (LIFE STOPVESPA) has just been completed to contain the spread of *V. velutina* in Italy by implementing an EWRRS (www.vespavelutina.eu). For this reason, two prototypes of harmonic entomological radars able to track the flight of hornets in real time and quickly locate the position of nests were developed by the Polytechnic University of Turin (Italy). The radars are capable to follow the flight of hornets equipped with a passive transponder (tag). The radar emits a series of short pulses at a given frequency (9.41 GHz), which are then retransmitted at a double frequency (for this reason, it is called ‘harmonic’) by the tag fixed on the thorax of the hornets (Milanesio et al. 2016, 2017, Fig. 10). The tracks obtained allow to follow the path of the hornets and locate the nests. Thanks to the high transmission power, a wide maximum operating distance of 490 m was obtained (Maggiora et al. 2019).

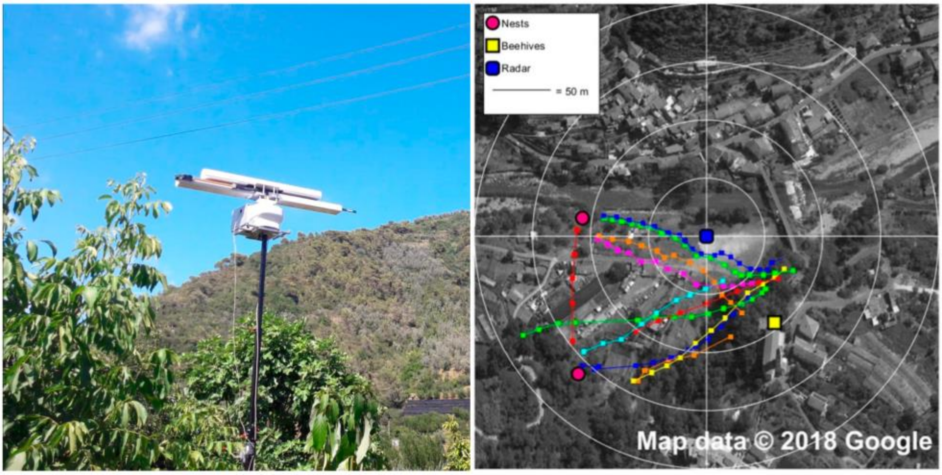

Figure 10. Harmonic radar able to follow the flight of hornets equipped with a passive transponder (left). On the map, the tracks obtained allowed to follow the path of the hornets and locate the nests (right).

2.5.2 Biological control

Identifying organisms capable of parasitizing hornets could allow the selection of potential control agents, always remembering that they could also be transmitted to native species. At the moment, there are no known effective enemies or adversities in Europe that could be used for the biological control of *V. velutina*. The main limitation for a biological control programme is the lack of knowledge on the biology and ecology of *V. velutina* in both the native and the invaded territory.

In Asia *V. velutina* is parasitized by *Bareogonalos jezoensis* (Hymenoptera: Trigonalidae), but its use in biological control methods cannot be considered as a fighting agent in Europe since it would also parasitize other species of wasps or other insects. Before any use of parasites or other exotic biological agents, preventive and rigorous verifications are always necessary to rule out any effects on other native species (Yamane 2014).

In France, larval forms of the *Conops vesicularis* (Diptera: Conopidae) were found, inside the abdomen of some individuals of *V. velutina,* which resulted in their death (Darrouzet et al. 2014, Requier et al. 2019). Spradbery (1973) reports that adults of *C. vesicularis* can wait at the entrance of the nest for homing workers, attack them and oviposit their eggs. So, individuals of *V. velutina* may be parasitized by this species in the environment, during foraging activity, or near the nest. However, the effectiveness of *C. vesicularis* as a biological agent for controlling *V. velutina* populations appears limited.

Another potential parasite of *V. velutina* has been confirmed by Villemant et al. (2015), with the discovery of *Pheromermis vesparum* (Nematoda: Mermithidae). This parasite was found in adult specimens of *V. velutina* in France on two occasions, in November 2012 at Dompierre-sur-Besbre, and in January 2013 in Issigeac. However, even in this case, its effectiveness as biological control agent appears limited, as they are the only two cases of nematodes found on *V. velutina* specimens throughout Europe.

In France, an entomopathogenic fungus (*Beauveria bassiana*) has been described to infect the common wasp *Vespula vulgaris* (Harris et al. 2000). French researchers are studying if it is therefore likely to infect other hornets such as *V. velutina*. Poidatz et al. (2018a) described *Metarhizium robertsii* as potential biological control agents against the invasive hornet *V. velutina*.

Some species of mammals (such as *Meles meles*) and birds (such as *Garrulus glandarius*, *Merops apiaster*, *Parus major*, *Pica pica*, *Sitta europaea*, and *Gallus gallus domesticus*) can prey upon *V. velutina*, but the predatory activity carried out by these animals is essentially sporadic and not enough to limit the population of the hornet; *Pernis apivorus* was also reported to exploit active *V. velutina* nests (Laurino & Porporato 2017, Macià et al. 2019).

2.6 Conclusions

The alien species *V. velutina*, since its accidental introduction in France, has now successfully colonized several European countries. In the areas in which it has settled, it has become clear that it has a negative effect on *A. mellifera*, documented by the numerous losses of honey bee colonies reported by beekeepers. Hornets concentrate their predation activity on the honey bee colonies as they provide an abundant and continuous source of food. *V. velutina*, however, hunts numerous other insects present in the environment and, among them, wild bees, attacking them while they are on the flowers for collecting nectar and pollen. This activity removes from the environment insects that play a very important ecosystem role. In fact, by visiting cultivated and spontaneous plants, wild pollinators guarantee not only the production of seeds and fruits of economic interest, but also the biodiversity of spontaneous plants.

Recent studies have shown that in Europe, and in other continents, pollinating insects and honey bees are in decline due to a combination of multiple factors. There is no doubt that the accidental introduction of the invasive predator *V. velutina* can aggravate the situation. In fact, the Asian yellow-legged hornet has widely contributed to the decrease in the colonies of honey bees, weakening them to such an extent that they collapse and are more susceptible to parasites, viruses, and fungi.

Given the fundamental role of natural pollinators, studies are currently under way to ascertain the real effects of *V. velutina* on wild bee populations and, more generally, on the environment in the newly introduced areas.

The introduction of an invasive exotic species, as well as causing damage to the ecosystem and biodiversity, can also generate a great deal of damage to the economy. *V. velutina* has a negative impact, particularly on agriculture. The disappearance of honey bee colonies leads to the loss of bee products, putting the beekeeping industry in the position to be out of the market due to the lack of production and/or the rising of production costs. This type of economic impact is currently the most studied and can be easily expressed in monetary values. The reduction in production yields of crops, as a result of the reduction of pollinators in general, is not quantifiable at the moment.

3.

Establishing surveillance areas for tackling the invasion of *Vespa velutina* in outbreaks and over the border of its expanding range

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Abstract: The Asian yellow-legged hornet *Vespa velutina* is an invasive alien species in many areas of the world. In Europe, it is considered a species of Union concern and national authorities have to establish surveillance plans, early warning and rapid response systems or control plans. These strategies customarily require the assessment of the areas that could be colonised beyond outbreaks or expanding ranges, so as to establish efficient containment protocols. The hornet is spreading through a mix of natural diffusion and human-mediated transportation. Despite the latter dispersion mode is hardly predictable, natural diffusion could be modelled from nest data of consecutive years. The aim of this work is to develop a procedure to predict the spread of the Asian yellow-legged hornet in the short term, in order to increase the efficiency of control plans to restrain the diffusion of this species. We used data on the mean distances of colonial nests between years to evaluate the probability of Asian yellow-legged hornet dispersal around the areas where the species is present. The distribution of nests in Italy was mainly explained by elevation (95% of nests located within 521 m a.s.l.) and distance from source sites (previous years' colonies; 95% within 1.4–6.2 km). The diffusion models developed with these two variables forecast, with good accuracy, the spread of the species in the short term: 98–100% of nests were found within the predicted area of expansion. A similar approach can be applied in areas invaded by the Asian yellow-legged hornet, in particular beyond new outbreaks and over the border of its expanding range, to implement strategies for its containment. The spatial application of the models allows the establishment of buffer areas where monitoring and control efforts can be allocated on the basis of the likelihood of the species spreading at progressively greater distances.

Keywords: Asian yellow-legged hornet; invasive species; control plans; monitoring; nest distance; predictive models

3.1 Introduction

Implementing cost-effective management plans for invasive alien species requires the development of tools that can improve the performance of control activities. A control plan should foresee different stages, including assessment of feasibility, implementation, monitoring and evaluation of the results (Braysher 1993, Bertolino et al. 2005). Monitoring is a necessary step to both verify whether control activities are effective and to provide feedbacks to improve management strategies (Braysher 1993, Bertolino & Viterbi 2010). Prioritisation to support a cost-effective allocation of resources is part of decision-making in species management (McGeoch et al. 2016). When the goal of the management plan is the containment of a species, it is necessary to evaluate where the species is most likely to spread in the short term, in order to better localise control activities. This requires assessing which areas should be surveyed and the intensity of the monitoring activity that should be allocated in each area (Hauser & McCarthy 2009).

Modelling procedures are customarily used to predict the spatial dynamics of invasive species dispersal over time. Models are built by fitting empirical data into mathematical functions or using field data to simulate population dynamics to be spatially projected (Sharov & Liebhold 1998, Gilbert et al. 2004, Shatz et al. 2016). While these procedures represent a powerful tool to provide information to improve management strategies, they require good knowledge about the ecology and dispersal abilities of the target species and are mainly used for simulations at large scales (Hastings et al. 2005).

The Asian yellow-legged hornet (*Vespa velutina* Lepeletier, 1836) is a social wasp, native to tropical and subtropical areas of Indo-China (Archer 1994, 2012). The species established itself in non-native countries such as France (Haxaire et al. 2006), South Korea (Choi et al. 2012) and Japan (Ueno 2014). From France, the species spread to neighbouring countries (Grosso-Silva & Maia 2012, Rome et al. 2013, Bertolino et al. 2016). In Italy, the Asian yellow-legged hornet was detected for the first time in Liguria in 2012 (Demichelis et al. 2014); afterwards, the hornet started to spread in this region mainly along the coastline (Porporato et al. 2014, Bertolino et al. 2016) and, in 2017, the species had colonised an area of at least 1,110 km^2 (Lioy et al. 2018). In Europe, the species is considered invasive, both for its expansion capabilities at European scale (Fournier et al. 2017, Robinet et al. 2017, Barbet-Massin et al. 2018) and the impacts that it could produce by preying on honey bees and native insects (Beggs et al. 2011, Monceau et al. 2013b, 2014a). Although the presence of the species is not considered a problem for human-health (De Haro et al. 2010), by frequently establishing colonial nests in urban areas, the Asian yellow-legged hornet could generate social impacts due to citizens' perception of fear of possible stings, which could lead to thousands of phone calls from people asking for the destruction of the nests (Liu et al. 2015, Tabar et al. 2015, Sumner et al. 2018). Moreover, the management of phone calls and the maintenance of control activities lead to significant economic costs (Robinet et al. 2017). For these reasons, attempts to control this species have been undertaken in many countries since its early stage of invasion (Monceau et al. 2014a, Bertolino et al. 2016, Rodríguez-Flores et al. 2018). Its recent inclusion in the European list of invasive alien species of Union concern (Reg. EU 1141/2016) requires Member states to implement surveillance protocols and control strategies.

The colony of the Asian yellow-legged hornet is initiated by a single inseminated queen that builds a primary nest after overwintering, thus producing the first workers. Afterwards, during the warm season, they enlarge the primary nest or build a secondary nest; with time, nests grow up to a sphere of about 50–100 cm in diameter, containing several thousands of hornets. From September onwards, reproductive animals emerge and mate; in late autumn or winter, all the colonies die, while newly-mated queens search for a place where they can overwinter and, the following year, they start a new cycle (Archer 2012, Monceau et al. 2014a, Rome et al. 2015). As for many other arthropods, invasions may proceed in smooth advances of the main front or in jumps. In the first case, species spread by natural dispersal of animals, giving rise to a diffusion-like process (Suarez et al. 2001). Conversely, jumps usually occur when the dispersal is human-mediated (Hastings et al. 2005, Homans & Horie 2011). In the case of the Asian yellow-legged hornet, this happens usually by the accidental movement of goods (e.g. straw, soil, timber) that contain dormant overwintering queens or by active adults travelling as hitchhikers on vehicles, though long-distance active dispersal could not be excluded in many cases (Marris et al. 2011, Bertolino et al. 2016, Robinet et al. 2017). Human-mediated transportation is hardly predictable and therefore only a large-scale monitoring system could allow the rapid finding of new sites of invasions. On the contrary, the natural dispersal could be forecast with observational data of presence recorded year by year. Distances covered by Asian yellow-legged hornets to establish new colonies are not known. Although queens are considered efficient flyers, published studies that demonstrate in the field the flying abilities of new founder queens to disperse from their original colony and create their own colonies are, however, still lacking. Population spread rate has been estimated in some countries and values are non-consistent, suggesting that spread rate could be different case-by-case, for example, depending on environmental and morphological characteristics of the invaded area. Robinet et al. (2017) estimated a mean spread rate of the population of 78 km/year (range between 75–112 km/year) in France, Bertolino et al. (2016) a mean spread rate of 18 km/year in Italy and Choi et al. (2012) a diffusion of 10–20 km/year in

South Korea. Sauvard et al. (2018) tested the flying abilities of workers in laboratory conditions throughout flight mill experiments; they demonstrated that workers are able to fly on average from 10 km to 30 km per flight test. This does not mean that workers in the field actually keep these flying values, since, in natural conditions, they are not forced to fly up to their maximum limit. It is likely that queens are also efficient flyers, but is not probable that queens in dispersion will travel to their maximum flight limit, but will probably stop to build their new colonies where they find a suitable spot (cost-benefit behaviour).

Habitat suitability and the possible spread of the Asian yellow-legged hornet in Europe have already been modelled at large scales with different approaches (Ibáñez-Justicia & Loomans 2011, Villemant et al. 2011a, Fournier et al. 2017, Robinet et al. 2017, Keeling et al. 2017). Some of these models have recently been validated and the prediction has proved to be adequate for real occurrence data (Barbet-Massin et al. 2018). However, if large scale modelling (i.e. European level) allows understanding long-term potential distribution of the species, their use in control activities is limited, since control plans are developed locally based on nest dynamics and distribution. A detailed description of Asian yellow-legged hornet nest dynamics has been reported and modelled for a municipality in France (Franklin et al. 2017, Monceau & Thiery 2017); however, the scenario of Andernos, in which the species has established a viable population and reached high-density values, could be different from new invaded areas of other European countries.

Though the fast spread of the Asian yellow-legged hornet in Europe clearly shows that control activities have been generally ineffective, modelling scenarios indicate that increasing the percentage of removed nests could slow down the spread rate (Robinet et al. 2017). Currently, control plans for the Asian yellow-legged hornet are based on finding and destroying the maximum number of nests, ideally all, present in the managed area before the dispersal of the new queens later in the year. Therefore, an efficient monitoring system must be established to locate colonial nests. This should consider not only the present known range of the species, but also an external buffer zone where it is likely that founder queens could disperse and establish new colonies in the short term. Customarily, the monitoring effort is high at the front of a species expansion and decreases with the distance. How fast it decreases is often connected with the species spread rate and human-resource availability. In the case of *V. velutina*, however, an optimal allocation of the effort could be established with information on the likelihood of nests being built at progressive distances from the frontline. With this information, the monitoring effort in an area could be calibrated with the likelihood of dispersal, increasing the cost-efficiency of the monitoring scheme.

The aim of this study is to create an adaptive predictive model of expansion for the Asian yellow-legged hornet, which could be applied in any new invaded areas to both predict the hornet natural expansion and to allocate the available monitoring and control resources, based on species colonisation probabilities. We used data on the mean distances of colonial nests between years to infer the likelihood that queens will naturally spread the year after at a certain distance from the invasion front. This approach allows modelling species spread with no need for taking account of local characteristics (e.g. environmental characteristics, climatic conditions, carrying capacity) in the perspective of establishing early warning and rapid response systems for this species in new invaded areas.

3.2 Materials and Methods

The western part of Liguria, where many nests are discovered every year, is the main Italian district colonised by the Asian yellow-legged hornet (Bertolino et al. 2016). The species has been detected in this area since 2012: *i)* a male was trapped in Loano at about 70 km from the French border (Demichelis et al. 2014), but no nests were detected in the following 5 years in this area; *ii)* one hornet was trapped in Ventimiglia at about 2 km from the French border. First nests were discovered in 2013 (Porporato et al. 2014) in some municipalities near France (5 nests in the cities of Dolceacqua, Vallecrosia and Bordighera). The species has also been observed in eastern Liguria, Piedmont, Lombardy, Veneto and Tuscany, but here, observations were scanty and only few nests were reported (Lioy et al. 2018). Therefore, the main colonised area of Liguria has been selected as the study area for the development of the predictive model.

The analysis is based on verified nest positions collected during four years (2014–2017), considering both nests discovered in spring during the foundation phase, which represents a small proportion of the data (2–3% of the total nests discovered in each year) and developed nests discovered later in the season (data available as Table S3; 2013 nests were not included due to the small dimension of the sample size). Since nests are difficult to detect, in particular before the fall of the leaves, a great effort was dedicated in creating an enlarged monitoring network, including multiple sources of information. Nests were reported by: *i)* citizens and beekeepers; *ii)* firefighters, civil defence teams and local authorities that received reports from citizens; *iii)* a network of more than 1,000 beekeepers with 1,638 monitoring stations established in a wider area of Liguria and Piedmont (Fig. S3). Nests were also actively searched for by monitoring teams of the LIFE STOPVESPA project involved in field surveys. These teams were *i)* verifying the reported nests, *ii)* verifying the presence of hornets in apiaries and searching for nearby nests and *iii)* actively monitoring the environment, searching for nests also with the use of binoculars. The teams were also active during autumn and the beginning of winter; this allowed the detection of additional nests that might have been hidden by tree leaves in the previous months. The teams' activity was fundamental to discover nests further away from urban areas and not frequented by people. Dissemination activities with hunters and fishermen allowed the involvement of people who frequented different environments, increasing the possibilities to detect nests in natural areas or riverbeds. Data were aggregated by year and analysed with R and QGIS (QGIS Development Team 2019, R Core Team 2019).

For each year, the area colonised by the Asian yellow-legged hornet has been estimated by a range analysis, with the kernel method of the R's package *adehabitatHR* (Calenge 2006). The limits of the estimated ranges of each year were used as a starting point to evaluate the areas at different likelihood of colonisation in the subsequent year. Outlier nests, located in Liguria distant from the main colonised area, were treated as potential further source of diffusion in addition to the border of the expanding range.

In a natural diffusion process, queens which found new colonial nests in one year originated from nests of the year before (source sites). The set of these measures can be used as a forecast of distances where the nests could be found the following year. Accordingly, a nearest-neighbour analysis was used to estimate the distances between nests of each year from source sites of the previous years. We then used these measures to develop a probability model of the distances where queens could establish their nests in the following year. From the estimated distances, a probability plot was constructed respectively for years 2015, 2016 and 2017. A non-linear regression analysis was used to estimate the equations with the best fitting for the data. These equations were used in QGIS to build the model: *i)* a grid with 100 m × 100 m cells was overlapped to the area outside the Asian yellow-legged hornet's range of a single year; *ii)* the distances between the centroids of each cell and the nearest source site was calculated and the species probability of colonisation for each cell of the grid was estimated according to the previous equation on nests distances from sources. This process was repeated for each year, to create predictive models for years 2016, 2017 and 2018.

Asian yellow-legged hornet's nests in Italy are not distributed with uniformity along the elevation (Fig. 1). Therefore the ranges, estimated applying the predictive models, were clipped at three different altitudes (700, 900 and 1,200 m a.s.l.), thereby producing three different scenarios for each year. The criteria that guided the selection of these limits are: 99% of the nests were found within 700 m a.s.l.; only one nest was discovered at 906 m a.s.l. in Piedmont (Porporato et al. 2014); adult hornets have been reported up to 1,200 m a.s.l.

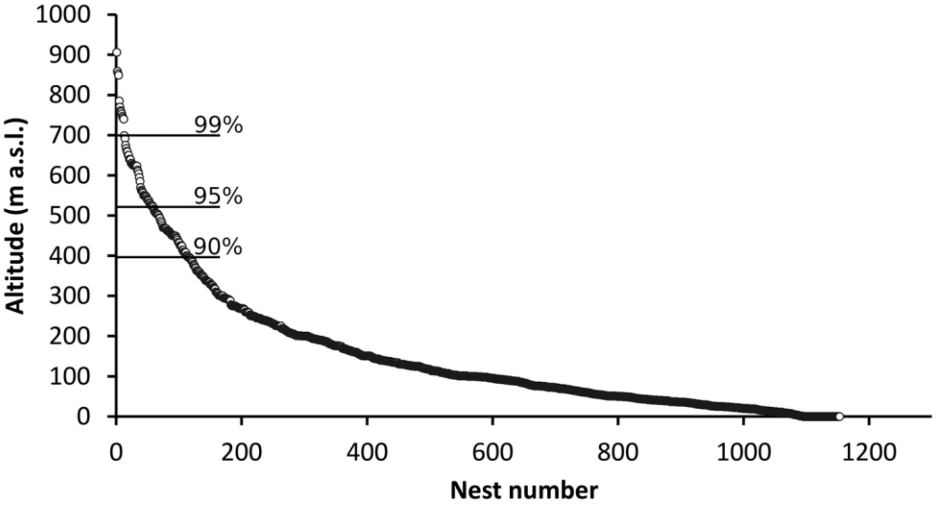


Figure 1. Distribution of Asian yellow-legged hornet nests along the altitude gradient: most of the nests are at low altitude, 90% of them within 396 m, 95% within 521 m and 99% within 699 m a.s.l. Nests were discovered up to 906 m a.s.l.

The predictive models for years 2016 and 2017 were validated comparing the probabilities of colonisation associated with the position of nests (i.e. position of the nest found in that year) for their respective years against the probabilities associated to pseudo-presence data, which are points randomly positioned in the areas of colonisation predicted by the models. A ROC analysis (Fielding & Bell 1997) that allowed the calculation of the area under the ROC function (AUC) was used for the validation procedure (Sing et al. 2005).

To further evaluate the importance of elevation and distance of nests from source sites when modelling the Asian yellow-legged hornet expansion, a generalized linear model (GLM) with binomial distribution and logit link function was used to compare the variables associated with 1,130 points of presence (nests' positions) and 1,130 random points of pseudo-absence. Five variables (one species-dependent and four environmental) were selected as explanatory variables of the GLM: *i)* distance of nests from source sites (nests of the previous year), which is the species-dependent variable that has been hypothesised as the main explanatory variable; *ii)* elevation upon the sea level; *iii)* surface aspect, grouped in the eight corresponding factors of 45° each (north, north-east, east and so on); *iv)* distance between nests and water resources; *v)* land cover (Regione Liguria 2015, 1:10.000). Nine macro-categories were identified for the land cover variable, on the basis of main environmental characteristics of the study area: urbanised, agricultural, woodlands, riparian areas, coastal areas, alpine grasslands, vineyards and olive groves, greenhouses, other environments. GLM results were compared with AIC in order to select the best model. Climate conditions were not considered because they do not change considerably in short distances, while data on carrying capacity, according to habitat suitability, are not available.

3.3 Results

The nearest-neighbour analysis highlighted that nests of the Asian yellow-legged hornets were mostly located within short distances from source sites: 50% of nests were found within 203–668 m from nests of the previous years and 95% within 1.4–6.2 km (Table 1). Few nests were found at greater distances from source sites, up to about 11 km in 2015–2016, but only at 3.5 km in 2017.

Table 1. Maximum distance of nests from source sites (nests of the previous years) grouped in proportion intervals for years 2015, 2016 and 2017.

Proportion of nests (%)	**Distance from source sites (m)**		
	2015	**2016**	**2017**
50	668	411	203
75	1,852	864	450
90	3,222	1,637	924
95	6,211	2,633	1,372
100	10,912	11,162	3,513

The probability of finding Asian yellow-legged hornet nests over the limits of its colonisation range consequently decreases rapidly with increasing distances from source sites (Fig. 2). The trends were explained by logarithmic functions (2015: $R^2 = 0.97$, $F_{1,230} = 7504$, $p < 0.001$; 2016: $R^2 = 0.94$, $F_{1,484} = 7738$, $p < 0.001$; 2017: $R^2 = 0.92$, $F_{1,411} = 4330$, $p < 0.001$).

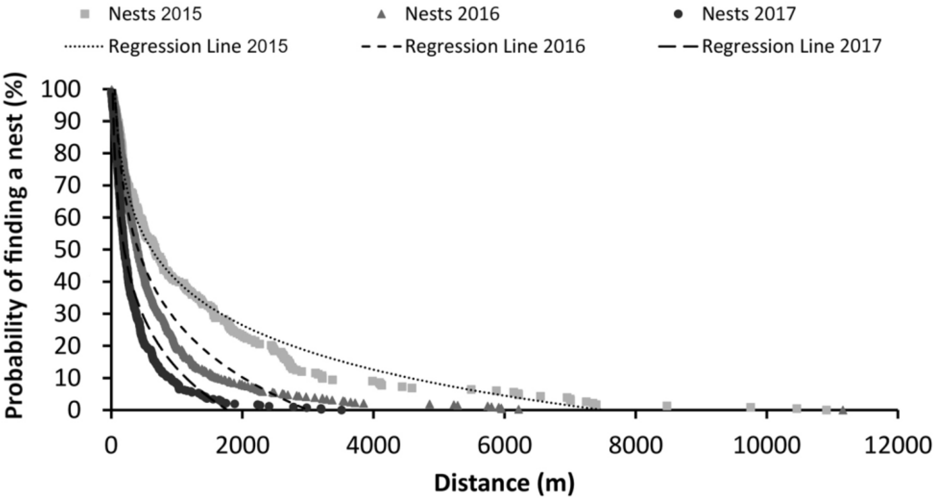
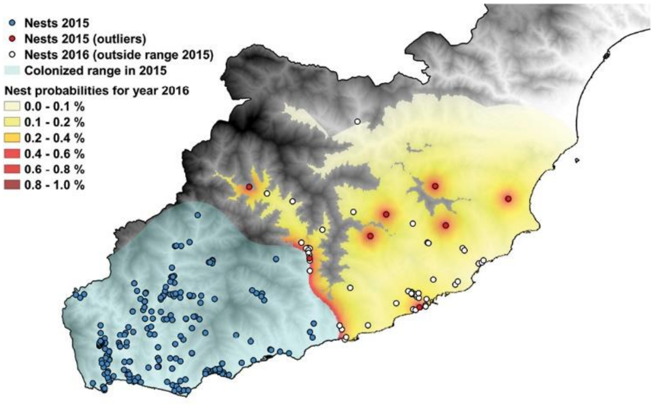
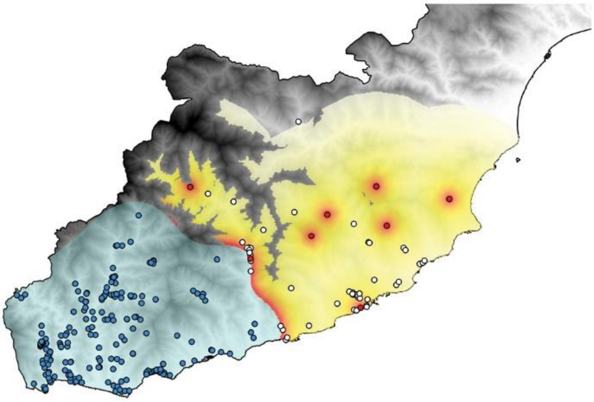


Figure 2. Nests distances from source of diffusion of the previous years: the distance of nests from a possible source of diffusion is given on the x-axis, while the probabilities to find a nest on the y-axis. The lines represent the logarithmic regression models of the data (regression line 2015: $y = -0.2 \ln(x) + 1.785$, $R^2 = 0.97$; regression line 2016: $y = -0.25 \ln(x) + 2.0057$, $R^2 = 0.94$; regression line 2017: $y = -0.227 \ln(x) + 1.6967$, $R^2 = 0.92$).

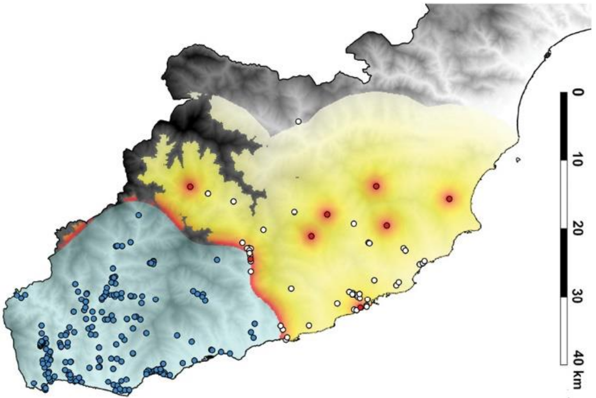
The spatial application of the probabilistic models, developed to predict the expansion of the Asian yellow-legged hornet in 2016, is reported in Fig. 3 for the three altitudinal ranges. Similar maps for 2017 and 2018 are reported in Fig. S1 and S2, respectively. For each model, the amount of area at different level of probability of colonisation has been estimated in probability intervals (Table 2 for 2016 and Table S1 and Table S2 for 2017 and 2018).



Predictive Model for 2016 - clip at 700 m a.s.l.



Predictive Model for 2016 - clip at 900 m a.s.l.



Predictive Model for 2016 - clip at 1,200 m a.s.l.

Figure 3. Predictive model of expansion for year 2016 clipped at three different altitude thresholds (700 m, 900 m and 1,200 m a.s.l.). Blue dots indicate nests of year 2015 inside the continuous range, red dots nests of 2015 outside the continuous range. For 2016, only nests outside the 2015 range are reported (white). Coloured areas from red to light yellow indicate progressively less probability of colonisation in 2016.

Table 2. Predictive models of year 2016: areas to be monitored for each probabilities range of colonisation by the Asian yellow-legged hornet. The areas of the three elevation scenarios are reported: A) 700 m a.s.l; B) 900 m a.s.l; C) 1,200 m a.s.l.

Probabilities range (%)	Area A (km^2)	Area B (km^2)	Area C (km^2)
90–100	0.04	0.04	0.08
80–90	0.07	0.10	0.16
70–80	0.21	0.23	0.33
60–70	0.30	0.38	0.68
50–60	1.15	1.32	2.16
40–50	3.50	4.04	5.91
30–40	13.97	15.03	19.77
20–30	59.67	68.02	81.47
10–20	220.48	258.38	296.23
0–10	232.61	263.37	283.05
Total	532.00	610.91	689.84

The predictive models for years 2016 and 2017 have been tested with the position of nests actually discovered in those years. Of the nests located in 2016 outside the range of the previous year, 98% were included in the predicted areas of expansion of the two scenarios at 900 m and 1,200 m a.s.l. and all the nests in 2017 were included in the predicted areas of the three scenarios. The analysis of the area under the ROC function highlights a difference between probabilities associated with nests' position and probabilities associated with pseudo-presence data, therefore each model predicts quite well the spread of the Asian yellow-legged hornet (2016: $AUC_{700\ m} = 0.78$, $AUC_{900\ m} = 0.78$, $AUC_{1200\ m} = 0.77$; 2017: $AUC_{700\ m} = 0.88$, $AUC_{900\ m} = 0.88$, $AUC_{1200\ m} = 0.88$).

The GLM analysis, which better explains the presence of hornet colonies in relation to species-dependent and environmental variables, takes into account all the considered explanatory variables and the interaction between the elevation and the distance between nests and source sites (Nagelkerke's pseudo-$R^2 = 0.60$). The variables that contribute more to the model are elevation, source distance and the interaction between these two variables (Fig. 4).

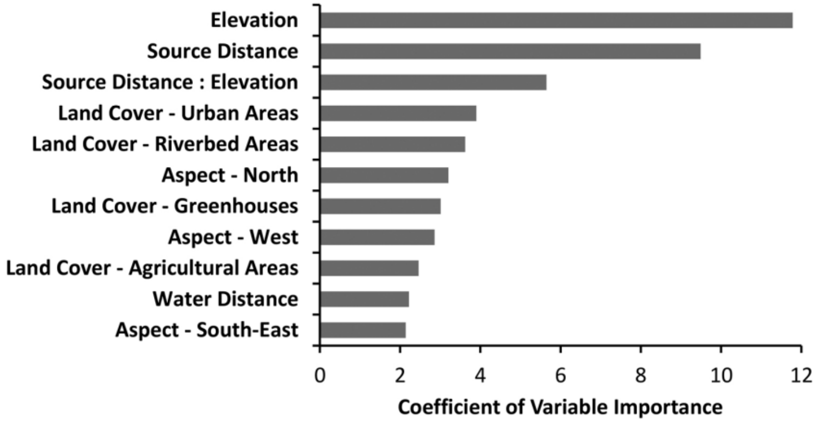


Figure 4. Coefficient scores for the explanatory variables of the GLM analysis on presence/pseudo-absence data: elevation, source distance and their interaction are the variables that contribute more to explaining spatial distribution of nests.

3.4 Discussion

The effective management of spreading invasive species requires the development of monitoring systems able to detect new areas colonised by the species in the short term, in order to timely extend control activities. We developed a system to evaluate the probability of Asian yellow-legged hornet dispersal around the area where the species is present, with a progressively lower likelihood of colonisation by the species at increasing distances. The model was built with GIS software and a database with coordinates of nests located in each year. Measures of the distances of nests found in one year from a possible source of diffusion (nests of the previous year) were used to build likelihood percentages of spread at progressive distances in the subsequent year. Comparison of nest locations with pseudo-presence data confirmed that both altitude and distance from possible source sites were main factors explaining the distribution of nests. Furthermore, our predictive models were tested in two years with real data (i.e. locations of nests found during control activities). In 2016 and 2017, 98–100% of Asian yellow-legged hornet nests were found within the predicted area of expansion, supporting the validity of our modelling approach. With this method, data routinely collected during monitoring and control activities of Asian yellow-legged hornet populations could be used as a feedback to increase the effectiveness of management strategies, allocating the available resources in relation to the probabilities of spread in the short term.

Of the nests reported in Liguria, more than a half were located within 1 km from nests of the previous year, about 90% within few kilometres (0.9–3.3 km) and nearly all within 11 km. These data indicate that new queens, despite their probable great flying ability, mostly build new colonies at short distances from their nests of origin and only few nests will be located at greater distances, due to natural diffusion on long distances or, more probably, to human mediated transportation. These reduced distances are in accordance with the spread of the species in Italy (18.3 ± 3.3 km/year, Bertolino et al. 2016), which is much lower than in France (78 km/year, Robinet et al. 2017). This means that local characteristics may drive species distribution and expansion; consequently, control approaches should be adaptive to local nest distributions that are a proxy of local characteristics.

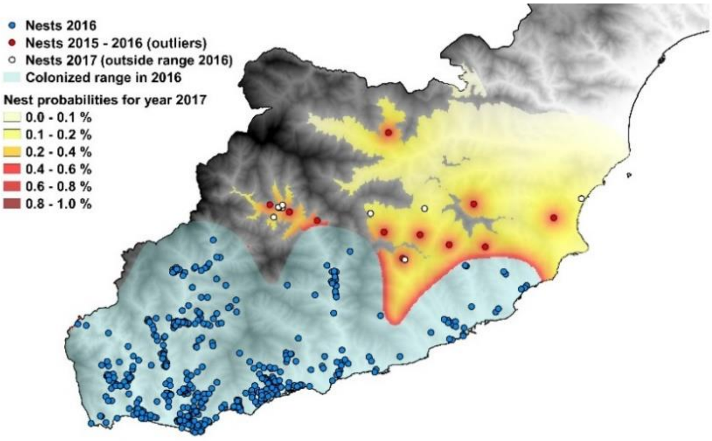
The data on nests' distribution collected in these years in Italy suggest that nests are not randomly distributed in the study area, but follows aggregative patterns. This is normal in spreading populations, where areas firstly colonised by the species act as source sites for nearby areas, which are at lower densities. This is the contest where our modelling technique can be used to improve control strategies. On the contrary, areas colonised over many years by the Asian yellow-legged hornet, such as the municipality of Andernos in France, have different local nest dynamics and, after the initial phase of invasion, nests became randomly distributed (Monceau and Thiery 2017). In this French municipality, the species reached a very high density in 2014 of 12.26 nests per km^2 with an average distance to the nearest nest of 153 m (95% confidence interval 143–163 m). This contest of high densities is completely different from the scenarios of new outbreaks or spreading populations. In the case of established populations, a control strategy that aims to limit or reduce the impact of the species should be developed. In case of new outbreaks or spreading populations, the control strategies should foresee the development of early warning and rapid response systems for the early detection of nests or containment plans, as suggested by the EU (Reg. EU 1143/2014) or as performed after the invasion of Majorca in the Balearic Islands (Leza et al. 2018) or Great Britain (Defra 2017). For example, the contingency plan developed for Great Britain requires the establishment of demarcated areas (buffer areas) nearby the sites of invasion after the presence of the Asian yellow-legged hornet has been confirmed. The early warning and rapid response approach support the need to develop a predictive model of expansion in the short-term using data collected locally: the protocol here proposed can be easily adapted and used to increase the efficiency of the monitoring activity. Intensive monitoring and control activities in a buffer area around the range of the species or new invasion outbreaks, allocated considering the different likelihood of colonisation, might therefore allow cost-effective use of the available resources. In this regard, the situation in Liguria is ideal for developing a control strategy that foresees the identification of buffer areas to monitor with different intensity, because the species is spreading mainly through a corridor along the coastline from West to East, with the sea to the South and mountains that might act as a partial barrier to the North (Bertolino et al. 2016). These characteristics could constrain the spread of the Asian yellow-legged hornet, thus reducing the areas that should be covered and increasing the possibility for effective monitoring. Therefore, morphologic characteristics of the environment should be considered when exporting this approach in other European areas, since monitoring and control effectiveness could be maximised by the presence of limiting factors or could be reduced by their absence.

Arthropods may jump long distances when the dispersal is human-mediated (Hastings et al. 2005, Homans & Horie 2011). An important implication of the possibility for a species to cross long distances is that it can overcome barriers, established to contain the species within the present range. For instance, nests of the Asian yellow-legged hornet have been recorded in Europe, tens and even hundreds of kilometres away from the invasion front, thus suggesting an accidental human transportation of founders (Rome et al. 2009, Bertolino et al. 2016, Robinet et al. 2017). In 2016, only one nest was found in Veneto at about 270 km from the invaded areas in Italy, while, in 2017, some adults were observed at 140 and 170 km, respectively in the eastern part of Liguria and northern Tuscany (Lioy et al. 2018). In previous years, animals and nests were found at several tens and up to 150 km from possible sources of diffusion (Bertolino et al. 2016). Identifying natural dispersal from human-mediated transportation is not always easy. However, even considering some of long-distance reports as resulting from natural dispersal would not change the validity of our simulation. In fact, we were interested in building an information system that could help to plan the yearly optimal allocation of the monitoring effort, covering an area of possible expansion from the continuous range of the species. Of course, a comprehensive management strategy also requires the development of plans to find and manage sub-populations found even at considerable distances from the expansion front. This is what is usually foreseen in the surveillance protocol of an early warning and rapid response system (Britton et al. 2010, Homans & Horie 2011), a protocol that has been established in Italy by the development of a wide monitoring network with the collaboration of the beekeepers (Fig. S3). Ideally, such surveillance system should allow the location of Asian yellow-legged hornet nests, established from long-distance dispersal or human-mediated transportation of queens. In case of detection of new propagules, our data-informed process could help in establishing an intensive monitoring network to locate and destroy nests before a new invasion starts, as well as with the use of new technologies as the tracking of hornets with harmonic radars (Milanesio et al. 2016, 2017) or radio-telemetry (Kennedy et al. 2018).

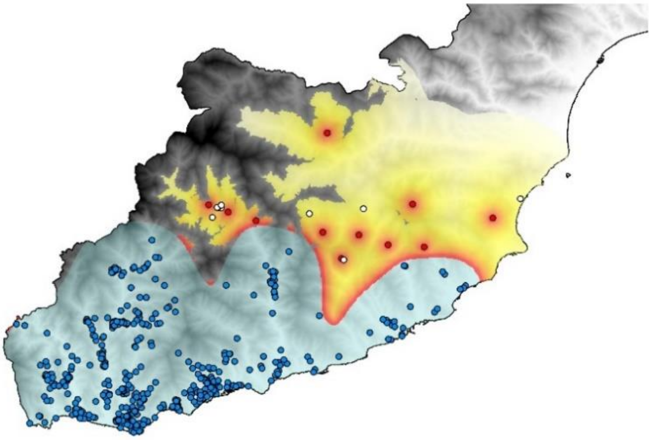
An aspect that must be considered is the bias in nest detection, since tree leaves often hide *V. velutina* colonies. For this reason, a wide monitoring network has been developed, as well as for areas not colonised by the species and for nearby regions and multiple sources of information have been considered (citizens, beekeepers, firefighter teams, monitoring teams, …). Monitoring teams also continued to work in the field during autumn and winter, detecting nests that might have been previously covered by tree canopies.

The method here proposed allows the assessment of the proportion of landscape that should be surveyed over the front of the spreading range of an invasive social insect species and the intensity of the monitoring activity allocated at progressive distances. It only requires the availability of nest locations in successive years, which are a proxy of other local (either climate or environmental) characteristics, and can be improved by increasing the efficiency of data collection. This approach is different from other modelling techniques, such as climatic or habitat models widely used for invasive species (Beaumont et al. 2009, Di Febbraro et al. 2016), including the Asian yellow-legged hornet (Ibáñez-Justicia & Loomans 2011, Villemant et al. 2011a, Balmori 2015, Fournier et al. 2017, Keeling et al. 2017, Robinet et al. 2017). In fact, these models estimate the areas that could be invaded in the future, comparing climatic or habitat characteristics of such areas with niche requirements of the species, but their use in short-term management strategies is limited. This is because many of these models extrapolate the parameters from other areas with different characteristics or because they are produced at large scales, while species management is usually implemented at more local scales. These approaches are extremely important when the aim is to understand the consequences of invasion in the long term and at European level. Instead, our method estimates the likelihood of colonisation of new areas by the species in the short term, from one year to another and for the studied population, important information that could be used to improve the efficiency of local management plans for the Asian yellow-legged hornet and other similar species that build colonial nests.

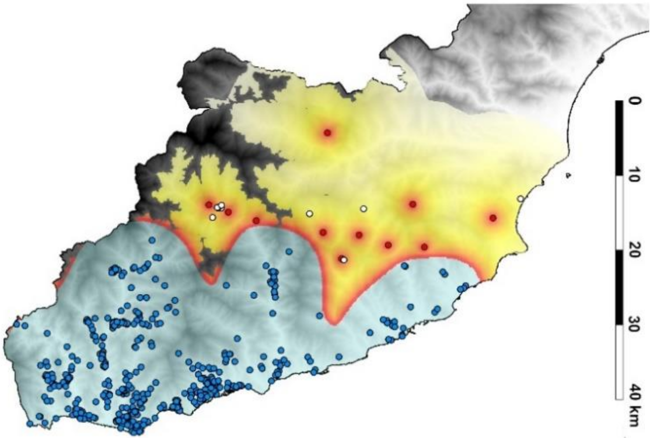
Supplementary material



Predictive Model for 2017 - clip at 700 m a.s.l.

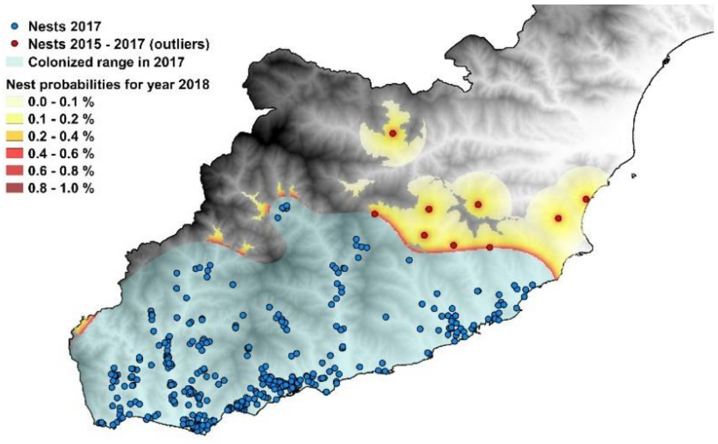


Predictive Model for 2017 - clip at 900 m a.s.l.

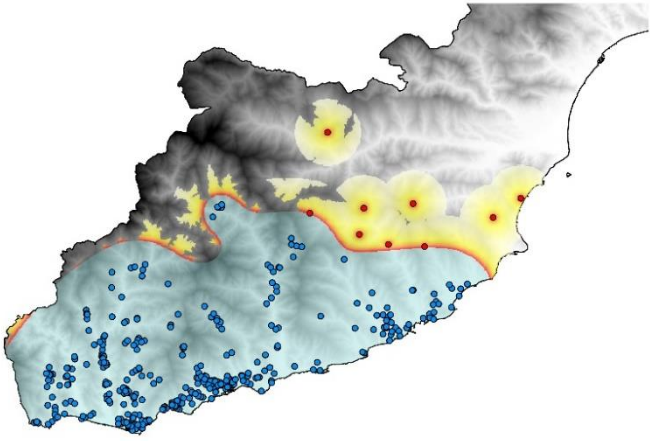


Predictive Model for 2017 - clip at 1,200 m a.s.l.

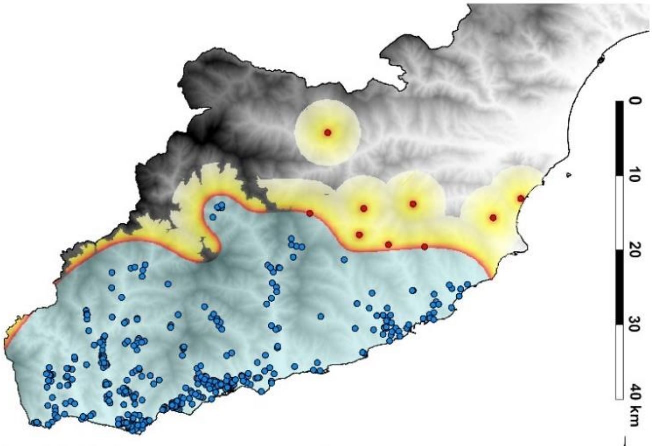
Figure S1. Predictive model of expansion for year 2017.



Predictive Model for 2018 - clip at 700 m a.s.l.



Predictive Model for 2018 - clip at 900 m a.s.l.



Predictive Model for 2018 - clip at 1,200 m a.s.l.

Figure S2. Predictive model of expansion for year 2018.

Figure S3. Monitoring network developed by LIFE STOPVESPA project in Liguria and Piedmont regions (Italy), with the involvement of 1,240 beekeepers and citizens that collaborated to monitor the presence of *V. velutina* in 1,638 monitoring stations with hornet-baited traps or observation in apiaries.

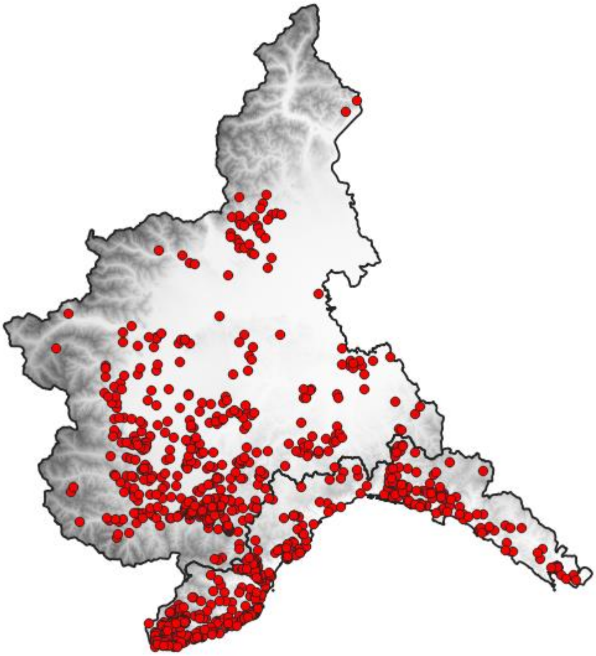


Table S1. Predictive models of year 2017: areas to be monitored for each probabilities range of colonization by the Asian yellow-legged hornet. The areas of the three elevation scenarios are reported: A) 700 m a.s.l; B) 900 m a.s.l; C) 1,200 m a.s.l.

Probabilities range (%)	Area A (km^2)	Area B (km^2)	Area C (km^2)
90 - 100	0.05	0.07	0.10
80 - 90	0.15	0.23	0.32
70 - 80	0.33	0.39	0.52
60 - 70	0.89	1.11	1.48
50 - 60	2.47	3.05	4.14
40 - 50	7.55	8.88	11.52
30 - 40	25.52	28.97	34.25
20 - 30	83.15	98.23	107.74
10 - 20	136.72	172.17	210.09
0 - 10	161.12	186.06	235.39
Total	417.95	499.16	605.55

Table S2. Predictive models of year 2018: areas to be monitored for each probabilities range of colonization by the Asian yellow-legged hornet. The areas of the three elevation scenarios are reported: A) 700 m a.s.l; B) 900 m a.s.l; C) 1,200 m a.s.l.

Probabilities range (%)	Area A (km^2)	Area B (km^2)	Area C (km^2)
90 - 100	0.07	0.09	0.11
80 - 90	0.07	0.10	0.14
70 - 80	0.17	0.27	0.33
60 - 70	0.61	0.89	1.09
50 - 60	1.13	1.72	2.14
40 - 50	2.80	3.86	4.61
30 - 40	7.12	9.51	11.79
20 - 30	18.68	22.90	29.77
10 - 20	50.79	61.13	74.25
0 - 10	97.01	124.44	153.87
Total	178.45	224.91	278.10

Table S3. Database of *Vespa velutina* nests discovered in Liguria region (Italy) in the period 2013–2017.

https://doi.org/10.3897/neobiota.46.33099.suppl6

4.

Viability of thermal imaging in detecting nests of the invasive hornet *Vespa velutina*

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Abstract: *Vespa velutina* is an invasive hornet species that is colonising Europe, generating considerable impacts on honey bees, beekeeping and biodiversity. Control and early warning strategies for this species are mainly based on monitoring plans and procedures of nest detection and destruction. Technological tools (harmonic radar, radio-telemetry) have been developed to increase the probabilities of nest detection in new outbreaks. Since hornets are able to regulate nest temperature, thermography may represent an additional technique that may be used, both alone or in support to other techniques.

In this study, the viability of thermal imaging in detecting nests of *V. velutina* was evaluated in controlled conditions. The influence of different environmental and operative variables (time of the day, presence/absence of leaves covering the nest, distance between the nest and the operator) were tested on three nests detected during August 2018 in Italy. All the nests were detectable by thermal imaging, but environmental and operative variables affect their detectability. The temperature difference between the nests and the surrounding reaches its maximum before sunrise and without a tree canopy covering the nests. Although nests were visible in some cases from 30 m, the detectability was higher at shorter distances, even if this variable may also depend on infrared camera resolution. An increase in the environmental temperature also generates a decrease of nest detectability. Although some limitations could occur, these results show the applicability of thermography in detecting *V. velutina* nests before the beginning of the reproductive phase, and consequently its potentiality in control strategies.

Keywords: alien species; Asian yellow-legged hornet; monitoring; nest detection; remote sensing; thermography

4.1 Introduction

Biological invasions are an increasingly common phenomenon that in some cases may trigger several environmental and socio-economic problems (Kettunen et al. 2008, Stout & Morales 2009, Scalera 2010, Vilà et al. 2010). Social insects are among the most efficient invaders and are able to tamper in many ways with the ecological equilibrium of the invaded areas (Beggs et al. 2011). A recent example is the case of the Asian yellow-legged hornet (*Vespa velutina*) in Europe. The hornet began its spread in France in 2004 from a few or even a single mated queen (Arca et al. 2015) that arrived through freight transport. Nowadays, it can be found in several countries of western Europe (Robinet et al. 2019), where it represents a threat to honey bees and native insect species (Monceau et al. 2014a).

The negative impacts of *V. velutina* in invaded areas have led to an increase in studies concerning its biology, behaviour, ecology and spread dynamic (Monceau et al. 2013a, 2014b, Monceau & Thiéry 2017, Lioy et al. 2019, Robinet et al. 2019, Laurino et al. 2020), although there are still several ongoing aspects to be investigated. Concerning the containment of the invasion, the early detection and destruction of nests is, at the moment, the most effective approach to prevent the establishment and spread of the species in new invaded outbreaks, or to decrease its impacts in colonised areas (Turchi & Derijard 2018). Therefore, considerable efforts were spent in order to develop viable protocols to spot hornet nests.

A traditional strategy consists of attracting foraging hornets to specific feeding points with protein or sugar baits and then carefully look to their flight route in order to locate the nest (Leza et al. 2018). This method involves a considerable employment of staff and its efficacy probably depends on terrain conditions, hornet density, nest position and experience of the operators.

Researchers have also dedicated increasing efforts in new technical tracking tools specifically designed for following foraging hornets. Radio tracking (Kennedy et al. 2018) and harmonic radar tracking (Milanesio et al. 2016, 2017, Maggiora et al. 2019) are very promising technologies which gives a valuable outcome in detecting hornet colonies. Nevertheless, these techniques require specific equipment and trained staff in hornet manipulation, although the major issue for the application of these strategies is spotting the exact position of the nest in the trees. In fact, the hornets usually build their nests in the tree canopies, hidden by foliage at a considerable height (Monceau et al. 2014a, Rome et al. 2015), making their localisation difficult and time consuming, even if the tracking method allows to get in a radius of a few meters from the nest.

Other tools may be used to spot nests or to support tracking methods especially in finding the exact location of nests in tree crowns, such as thermal cameras. Thermography with thermal cameras is an imaging method based upon the detection of the infrared waves that every object and body emit, according to their inherent properties and temperature. Detectability of an object is proportional to the temperature difference existing between the object and the surrounding environment. This technology provides a rapid and non-invasive scanning tool that has been applied in many fields, such as physiological, medical, agricultural and natural science (Kastberger & Stachl 2003, Mangus et al. 2016, Osroosh et al. 2018). Thermal imaging cameras were also used for spotting wild animals (Focardi et al. 2001, Cilulko et al. 2013), other colonial insects such as bumble bees (Roberts & Osborne 2019) and in the detection of insect pests in agricultural products (Al-doski et al. 2016).

It is well known that social insects are able to control the temperature of their nests in order to ensure a favourable environment for themselves and their brood through social homeostasis (Schmolz & Lamprecht 2004), and honey bees, wasps and hornets are no exceptions (Kastberger & Stachl 2003, Kovac & Stabentheiner

2012). Several species of hornets tend to maintain the nest temperature around 28-30°C by altering their own metabolism, helped by the insulating properties of the nest envelope (Stabentheiner & Schmaranzer 1987, Martin 1990). Therefore, it is possible to assume a temperature difference between the nest and the surrounding environment.

The use of a thermal imaging camera, especially at specific times of the day such as early morning or late evening, may help in detecting the hornet nests as thermal anomalies against the background of tree canopies. Unlike honey bees, wasp and hornet colonies have an annual development cycle which starts from a solitary founder queen and then the number of workers increases during the season. Therefore, a colony in its early stage displays a limited ability for thermoregulation and only large colonies are able to maintain an optimum temperature (Martin 1990, Schmolz & Lamprecht 2004). Assuming that this is also true for *V. velutina*, the fully grown developed nests are the most suited to be revealed by thermography.

The use of thermal imaging for detecting *V. velutina* nests has been previously tested in Portugal, UK (Semmence 2018) and Italy (Bortolotti et al. 2016), however results on the feasibility, potential and limitations of this method have, to our knowledge, never been published to date. This study represents a first effort in describing the viability of thermal imaging camera in detecting *V. velutina* nests. Performance and limitations of the proposed method are described in relation to different environmental and operative conditions, such as the time of the day, the distance between the nest and the operator, and the presence of a tree canopy in front of the nest.

4.2 Materials and Methods

4.2.1 Infrared camera features

Experiments were performed to assess the possible use of thermal images in detecting nests of the hornet *V. velutina*. Thermal images were taken using the Avio Advanced Thermo TVS-500E infrared (IR) camera. This IR camera operates in the spectral range of 8-14 μm wavelengths with a spatial resolution of 320×240 px. All thermal images were processed using the GORATEC Thermography Studio software.

4.2.2 Data acquisition

Thermal images of nests were taken during August 2018 in the village of Calvo (N 43.82994, E 7.55702), part of the municipality of Ventimiglia (IM) in Liguria (Northwest of Italy), where *V. velutina* has been established since 2013. In this area, three different active nests of the species were located in tree crowns, at an approximate height between 5–8 metres from the ground. One nest was located on a holm oak tree (*Quercus ilex*, nest number one) while two other nests were located on olive trees (*Olea europaea*, nest number two and three). Nests were sampled for an overall period of five days (from the 8th to the 23rd of August) with the IR camera. A total of 56 thermal images were taken during the sampling period. The operator was forced to adopt a simple random sampling scheme instead of a stratified sampling design, due to the limited availability of the IR camera, the limited access possibility to the area where nest number one was located (private area) and the detection of nest number three after the beginning of the sampling. For each nest, the operator took several thermal images from the ground at early morning (from 6:00 to 8:00 am) and at evening (from 6:00 to 8:00 pm), and from different distances (from 5 to 40 m), recording time and measuring distances with a laser rangefinder. Thermal images were also taken from standpoints in which the nest was screened by a tree canopy. The environmental temperature was recorded for each thermal image using the integrated thermometer equipped in the IR camera. Afterwards, a qualitative score of nest visibility was assigned by the operator to each thermal image: not visible (1), poorly visible (2) and clearly visible (3).

4.2.3 Detectability estimation

The IR camera provides a temperature value for each pixel of the image; however, since the emissivity (effectiveness in emitting energy as thermal radiation) of the nests is not known, the monitored temperature cannot be used as an absolute value. Therefore, the temperature difference between the nest and its surrounding represents an index for comparing thermal images of several nests. By means of the IR camera software, the maximum value of temperature was extracted for each nest, selecting with a polygon the area of the pictures containing the nest. The same criteria was then used around each nest to obtain the corresponding mean temperature of the surrounding environment (excluding pixels of the sky that return temperature values equal to the lower limit of the thermal scale). The difference between maximum temperature value in nest area ($T_{max.nest}$) and average temperature value of the surrounding environment ($T_{avg.surrounding}$) divided by this last variable, named Thermal Detectability Index (*TDI*), was used as a parameter of nest detectability.

$$TDI = \frac{T_{max.nest} - T_{avg.surrounding}}{T_{avg.surrounding}}$$

The correlation between *TDI* and the qualitative visibility score values estimated in the field by the operator was tested with a Linear Regression Analysis, to evaluate if this parameter may represent a reliable index of nest detectability. Then, a Linear Mixed Model (LMM) was used to evaluate the effect of the following variables on nest detectability (*TDI*): (1) time of the day (evening/morning), (2) distance between the nest and the operator performing the sampling and (3) presence/absence of a tree canopy in front of the nest. Due to the presence of multiple nests, the identification code of the nest was included as a random factor of the LMM. Moreover, a square root transformation was adopted to *TDI*, in accordance to the Box-Cox Lambda value of an equivalent linear model.

4.3 Results

The Linear Regression Analysis highlighted a positive correlation between *TDI* and the qualitative visibility score values ($F_{1,54} = 53.62$, $P < 0.001$, $R^2 = 0.50$), confirming that *TDI* is a reliable index of nest detectability. All the three sampled nests were detectable by the thermal imaging camera (Fig. 1), despite one nest being significantly more visible than the others (ANOVA: $F_{2,53} = 10.34$, $P < 0.001$, Fig. 2b), and this explains the importance of the nest as a random factor of the LMM.

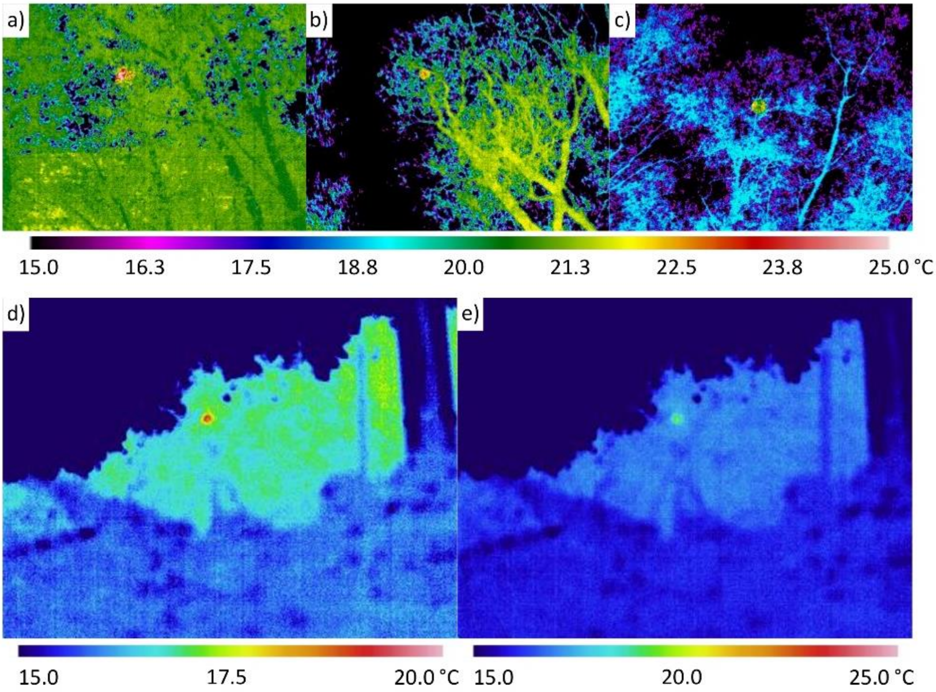

Figure 1. Application of thermal imaging for detecting *V. velutina* nests: a) nest number one; b) nest number two; c) nest number three; d) nest number one in the morning at 30 m from the operator; e) nest number one in the evening at 30 m from the operator.

Environmental and operative conditions influence nest detectability (Fig. 2), and this is confirmed by the results of the LMM analysis (Table 1, Table 2 and Fig. 3).

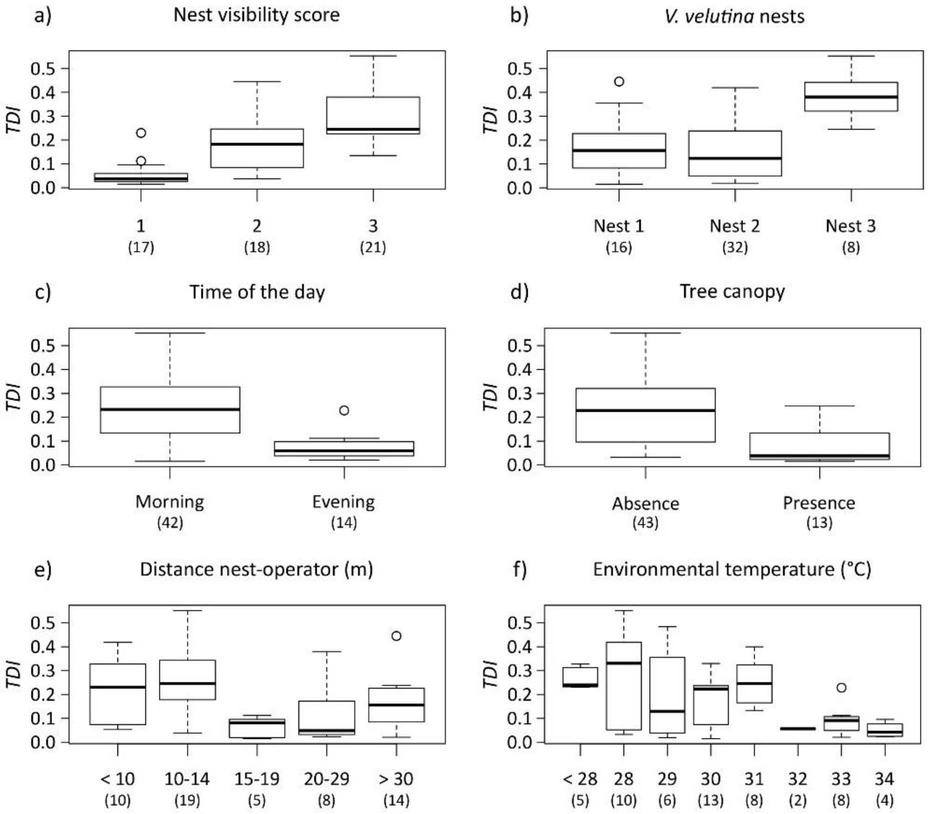

Figure 2. Boxplot of the Thermal Detectability Index (*TDI*) in relation to the following variables: a) qualitative score of nest visibility (1 = not visible; 2 = poorly visible; 3 = clearly visible); b) *V. velutina* nests; c) time of the day when the sampling was performed (morning/evening); d) presence/absence of a tree canopy in front of the nest; e) distance of the nest from the operator (m); f) environmental temperature (°C). Sample size for each factor level is reported in brackets.

Table 1. ANOVA table of the fixed effects of the LMM analysis on nest detectability (*TDI*): all the considered variables significantly affect nest detectability.

Variables	*numDF*	*denDF*	*F*
(Intercept)	1	50	67.67 ***
Time of the day	1	50	17.47 ***
Tree canopy	1	50	15.52 ***
Distance nest-operator	1	50	4.92 *

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2. Coefficients of the fixed effects of the LMM analysis on nest detectability (*TDI*): the time of the day and the tree canopy are categorical variables with two levels (respectively morning/evening and presence/absence) while distance nest-operator is a continuous variable.

Variables	*Estimate*	*SE*	*DF*	*T*
(Intercept)	0.586	0.067	50	8.779***
Time of the day (evening)	- 0.125	0.038	50	- 3.284**
Tree canopy (presence)	- 0.144	0.037	50	- 3.852***
Distance nest-operator	- 0.005	0.002	50	- 2.218*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The presence of a tree canopy in front of the nest significantly decreases its detectability (presence of tree canopy: *EMMs* = 0.29, *SE* = 0.06, 95% *CI* = 0.02-0.57; absence of tree canopy: *EMMs* = 0.44, *SE* = 0.06, 95% *CI* = 0.20-0.68). In addition, nests are more detectable during the morning than during the evening (evening: *EMMs* = 0.30, *SE* = 0.06, 95% *CI* = 0.03-0.58; morning: *EMMs* = 0.43, *SE* = 0.06, 95% *CI* = 0.19-0.67). An increase in the distance between the nest and the operator reduces nest detectability (Table 2), although some nests were still visible at more than 30 m in favourable conditions. A decrease in *TDI* was observed (Fig. 2f) when the environmental temperature reached values that were approximately the average temperature of the combs in hornet's nests (Martin 1990).

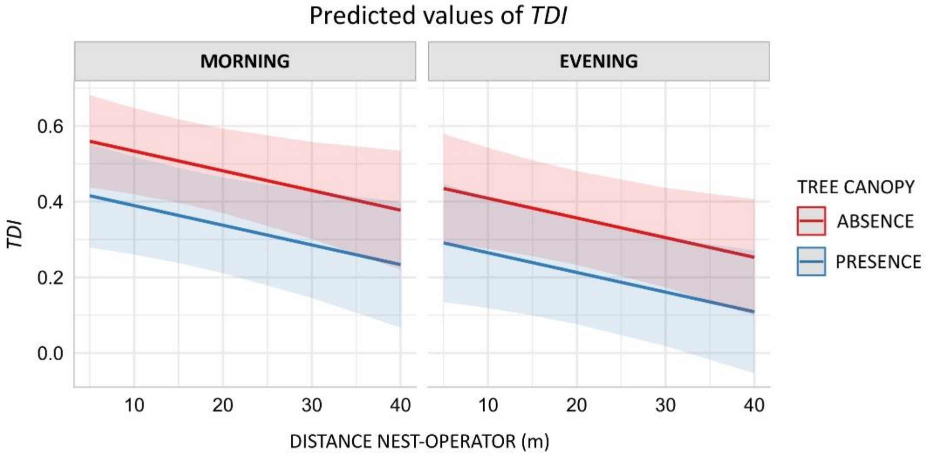

Figure 3. Predicted values of the Thermal Detectability Index (*TDI*) in relation to the three variables of the LMM analysis: the time of the day (morning on the left and evening on the right); the distance between the nest and the operator in metres (on the x-axis); the presence (blue) or absence (red) of a tree canopy.

4.4 Discussion

This study represents a first effort in describing the viability, potential and limitations of the use of the thermal imaging camera in detecting nests of the invasive hornet *V. velutina*. Although this study was carried out in unfavourable climatic conditions for the use of thermography, i.e. one of hottest months of the year for Italy (August), the provided results give evidence of the applicability of thermal cameras in spotting nests of this invasive hornet. This experiment demonstrate that nests may be detected in summer before the beginning of the reproductive phase of the colony, which generally starts with the emerging of gynes (potential queens) during the month of September (Monceau et al. 2014a, Rome et al. 2015). Therefore, thermal imaging may be profitably used to support monitoring activities and early nest detection of *V. velutina* or other invasive colonial species with a similar aboveground nesting behaviour.

Nevertheless, environmental and operative conditions could decrease nest detectability. The presence of a tree canopy in front of the nest is one of the most limiting factors, preventing nest detection even at close range. Therefore, it is important to accurately monitor the tree canopy from different perspectives. The use of other searching techniques as triangulation (Leza et al. 2018), radio tracking (Kennedy et al. 2018) or harmonic radar tracking (Milanesio et al. 2016, 2017, Maggiora et al. 2019) could scale down the potential area of nest location, then allowing the IR camera to explore a reduced area from different viewpoints. On the other hand, if no physical obstacle covers the nest, thermal imaging is effective even from distances of tens of metres, in spite of plays of light and shadows that may not allow the identification of nests by sight.

The increase of the environmental temperature during the day may limit nest detectability, due to the higher air temperature and the presence of sunrays on the foliage of the trees. Moreover, *V. velutina* is predominantly diurnal (Perrard et al. 2009, Poidatz et al. 2018b); since nest temperature is positively correlated with the number of individuals inside the nest (Schmolz & Lamprecht 2004), it can be assumed that the difference in temperature between the nest and its surroundings (*TDI*) is at its maximum before sunrise, when all the hornets are inside the nest and the environmental temperature of the surroundings reach its minimum. On the contrary, detectability decreases after sunrise, when the environmental temperature reaches values similar to the inside temperature of hornet’s nests (Martin 1990). This could represent a limit in the use of IR cameras in southern countries of Europe characterised by high temperature values during the summer, while in cooler countries this variable may have less influence on nest detectability.

The distance between the nest and the operator performing the sampling seems to influence nest detectability, but this effect could be related to the resolution of the IR camera used for this study (320×240 px). Since IR cameras with a higher resolution are available on the market (e.g. 1024×768 px), it is possible to hypothesize that the effect of this variable may decrease with a higher quality equipment, with a consequent increase in nest detectability.

This study provides inedited results on the viability and limitations of the use of IR cameras in detecting nests of the invasive hornet *V. velutina*. Further surveys are required in order to evaluate the detection probability of this technique in non-controlled conditions, a crucial assessment for the inclusion of thermal imaging cameras into management strategies for *V. velutina*. Moreover, a survey in different European countries, which are characterised by different environmental and operative conditions, longer samplings over time and IR cameras with different resolutions are fundamental for comparing the efficiency of this method between countries and understanding the influence of other variables that may limit or increase nest detectability (i.e. season, weather conditions, nest dimension). The use of thermal imaging coupled with other nest detection techniques, with a broader range, will in any case help to improve nest detection strategies to contrast the establishment and spread of *V. velutina* in new invaded areas, or even other invasive colonial species with a similar nesting behaviour.

5.

Tracking flying insects in complex environments by means of a harmonic radar

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Abstract: An innovative scanning harmonic radar has been recently developed for tracking insects in complex natural landscapes. This easily movable technology has been tested on an invasive hornet species (*Vespa velutina*) for detecting the position of their nests in the environment, in the framework of an early detection strategy. The new model of harmonic radar proved to be effective in tracking hornets either in woodland, urban areas, or in hilly environments, with a maximum detection range of ~300 m in complex landscapes. Furthermore, this technology could provide information on several unknown aspects of insect's ecology and biology. In this case, the mean foraging range of *V. velutina* (~400 m with a maximum value of 786 m) and flying features of foraging (flying speed of ~6.7 m s^{-1}) and homing hornets (~4.1 m s^{-1}) were discovered for the first time in non-controlled conditions.

Keywords: Asian yellow-legged hornet; *Vespa velutina*; invasive species; insect tracking; harmonic radar; nest detection

5.1 Introduction

Understanding the movement of animal species is crucial for advancing our knowledge on several topics of species biology, ecology, social behaviour or ecophysiology, and more in general perceive the role of individuals and populations in the ecosystems (Kays et al. 2015). This knowledge could be useful for planning conservation schemes for native species but also for understanding the movements of invasive species in the introduced environments, in order to establish appropriate management strategies (Lennox et al. 2016). Approaches for tracking animal species vary according to taxa, species size and the environment in which they are tracked (Daniel Kissling et al. 2014, Kays et al. 2015). Due to their small dimension, insects are among the most difficult groups to be tracked, but they also represent a significant portion of terrestrial biodiversity that should be properly investigated (Mora et al. 2011, Stork 2018).

Since the last century, several technologies have been developed and improved to follow the movement of insects in the environment. Based on the presence or absence of batteries, they can be roughly divided into two main categories: *i)* active systems like radio telemetry (Daniel Kissling et al. 2014) or *ii)* passive systems that require the use of radar technologies (Chapman et al. 2004, Drake & Reynolds 2012), e.g. vertical-looking radars for studying insect migration, scanning harmonic radars for low-altitude studies, harmonic direction finders for low-range applications. Among radar techniques, scanning harmonic radars (Riley & Smith 2002) allow to understand the movement of insects in the environment for several hundred metres, by applying light passive transponders (tags with a weight generally between 1-15 mg) on the insects that reflect the radar signal minimising environmental interference (clutter). However, this technology has been used mainly in flat and simple environments or flying arenas, for tracking and studying species of different characteristics and sizes such as honey bees (Riley et al. 1996, 2005, Capaldi et al. 2000), bumblebees (Osborne et al. 1999, Makinson et al. 2019), butterflies (Ovaskainen et al. 2008), moths or flies (Chapman et al. 2004, Drake & Reynolds 2012).

The scanning harmonic radar technique has been recently improved to overcome one of its main limiting factors, which is the operability in complex and hilly landscapes (Milanesio et al. 2016, 2017). The latest enhancement of this technology permitted to further extend the tracking range up to about 500 m of radius from the radar position in flat terrain conditions (Maggiora et al. 2019). This innovative scanning harmonic radar has been used with success for tracking the flight of *Vespa velutina* in Italy (Maggiora et al. 2019), an invasive hornet species, which is colonising several countries of Europe and Asia, and is able to generate negative impacts on honey bee colonies, native insect communities and social issues related to the presence of nests in the environment (Laurino et al. 2020).

Here we describe the performance of this new scanning harmonic radar in tracking insects in complex environments, by analysing its application in the detection of nests of *V. velutina* as a case study. Performances of the harmonic radar are evaluated in terms of:

i) success in tracking *V. velutina* workers from the apiaries, where hornets are hunting honey bees, to their nests;
ii) length of the tracks in relation to the environmental characteristics in which the harmonic radar is operating;
iii) length of the tracks recorded with the harmonic radar in relation to other traditional tracking techniques, such as the visual tracking and triangulation of flying directions (Leza et al. 2018).

Moreover, we highlight how the harmonic radar tracking could be used for understanding unknown aspects of several insect species in natural and non-controlled conditions, in this case the flying characteristics (e.g. ground speed) and the distances of *V. velutina* colonies from apiaries where hornets were preying on honey bees (foraging range).

Hornets were tracked in Italy in nine localities of Liguria (Table S1) with different characteristics in terms of land cover (open terrains, urban areas and woodlands), road density, elevation, slope degree and *V. velutina* density. Four of these localities were new invasive outbreaks where the species was present at low densities and the predation pressure on honey bee colonies was restrained, while the other localities were inside the area that had been colonised by the species since 2014 (Bertolino et al. 2016). Hornets have been tagged and tracked with the harmonic radar and the transponders previously described by the authors (Maggiora et al. 2019).

5.2 Materials and Methods

5.2.1 Study areas

The technique of the harmonic radar tracking has been applied in nine different localities of Liguria (Italy), in the framework of the control activities developed to contain the spread of *V. velutina* in this region (Lioy et al. 2019, Laurino et al. 2020). Four of these study areas (Ameglia, Arcola, Riccò del Golfo in La Spezia district and Finale Ligure in Savona district) were new invasive outbreaks characterised by a low nest density of *V. velutina* and low predation pressure on honey bee colonies. The other five study areas of Imperia district (Camporosso, Dolceacqua, Ospedaletti, and the two villages of Calvo and Latte in the municipality of Ventimiglia) were located inside the colonised range of the species (Bertolino et al. 2016), and are characterised by a high nest density and an intensive predation pressure on honey bee colonies (Table S1).

5.2.2 Tracking technique for nest detection

The harmonic radar and the tags that have been used for tracking the flight of *V. velutina* were designed and developed ad-hoc for following insects in complex environments; their technical characteristics have been previously described by the authors (Maggiora et al. 2019). At the beginning of a new tracking session, worker hornets are trapped, usually in apiaries when preying honey bees, and the transponders are attached on their thorax by means of an orthodontic glue, without anesthetising the insects. Subsequently, hornets are released from the tagging location and are immediately able to resume their activity, such as flying and preying on honey bees. The whole tagging procedure requires less than one minute per hornet. Tag weight (15 mg) is approximately 4-7% of the weight of *V. velutina* workers (mean worker's weight changes over the season between 189 mg and 386 mg, Rome et al. 2015).

The harmonic radar then records independently all the tracks of flying hornets that are inside its detection range. The real-time analysis of the recorded tracks allows understanding the main flying directions. If the nest of *V. velutina* is located outside of the maximum detection range of the radar (about 500 m in flat terrain, Maggiora et al. 2019) or behind physical obstacles, the harmonic radar is moved according to the flying directions of the hornets; this operation is repeated until the position of the nest is determined. The area where the nest is located is highlighted by the presence of several tracks that converge or begin from the same site. The visual inspection of the area permits the exact detection of the position of the nest.

The total number of tagged hornets was recorded for each tracking session, together with the radar operation time, the number of radar movements per session, the number of detected nests per session and the minimum distance between the nests and the apiaries where hornets were hunting honey bees (Table S2).

5.2.3 Tracking lengths and environmental characteristics

The main parameter permitting to estimate the performance of the harmonic radar in different natural and complex environments is the length of the tracks of tagged insects. In order to obtain this parameter, fixes (hornets detected by the harmonic radar at each radar's rotation) were extracted for each tracking session and uploaded on a GIS software (QGIS Development Team 2019). Afterwards, consecutive fixes of the same track were connected with the shortest line, so to obtain hornet tracks and calculate their length.

The length of the tracks in each fix position (n = 2,580) was modelled with a GLMM (see data analysis) to evaluate the effect of environmental features (land cover, elevation above sea level, slope gradient, road density). The land cover layer was obtained through a photo interpretation of satellite images and classification in three macro-levels: open terrains (landscapes predominantly characterised by open areas, such as fields), urban areas (matrices formed by buildings/roads) and woodlands (matrices formed by forests). Elevation above sea level and slope degree were obtained by a Digital Elevation Model (resolution of 20 m).

5.2.4 Visual tracking of flying hornets

The length of the tracks recorded by the harmonic radar was compared with the length of the tracks recorded when adopting a traditional technique for tracking insects, such as the visual tracking and triangulation of flying directions (Leza et al. 2018). In six of the nine localities where the harmonic radar tracking has been applied, an operator was waiting near a honey bee colony till one *V. velutina* worker catches a honey bee. Subsequently, after the hornet disjoined the most energetic parts of its prey (the thorax, Perrard et al. 2009), the operator visually tracked the flight of the hornet when flying back to its nest, by means of a binocular and by recording with a GPS the position where the hornet disappeared from view. In some cases (n = 4), common flying routes were identified, and we were able to resume the visual tracking with other hornets from the previous disappearance position. Finally, GPS positions were uploaded on a GIS software to calculate the length of the tracks with this traditional technique.

5.2.5 Estimation of *Vespa velutina* ground flying speed

Harmonic radar tracking allows to estimate the ground flying speed of *V. velutina*, by analysing the distance between each recorded position at consecutive radar rotations. Giving that the time of each radar rotation is fixed (3 s), it is possible to estimate the hornet's speed between each detection (Drake & Reynolds 2012).

The ground flying speed of *V. velutina* has been estimated in the three localities of La Spezia district, due to the availability of a subsample of clear tracks with consecutive detections per each rotation of the radar and good weather conditions. Furthermore, based on their direction, tracks were classified in homing tracks (*H)*, which belong to hornets flying from the apiary to the nest, and foraging tracks (*F*), which belong to hornets flying towards the apiary for hunting honey bees. Data on wind speed and direction were obtained from weather stations close to the study areas.

5.2.6 Data analysis

Data analyses were performed with the software R (R Core Team 2019). Environmental characteristics of the localities were analysed with a Principal Component Analysis (PCA; package *factoextra*), to understand affinities between study areas and correlations between the considered variables. The length of the tracks between localities recorded with the harmonic radar were compared with the Kruskal-Wallis and Dunn tests with Bonferroni correction, while the flying speed between foraging and homing hornets was compared with Wilcoxon rank-sum test (two-tailed).

Generalized Linear Mixed Models (GLMM; package *lme4*) with gamma distribution and log link function were used to assess *i)* the influence of environmental variables on the length of the tracks and *ii)* compare tracking methods between study areas. The locality has been included as a random effect of both models, while slope degree as an uncorrelated random slope for the GLMM analysis on environmental characteristics. In both cases, continues variable were standardized, and multi-collinearity of environmental variables has been taken into account by calculating the Variance Inflation Factor ($VIF_{elevation}$ = 1.5, VIF_{slope} = 1.5, $VIF_{road\_distance}$ = 1.0).

5.3 Results and Discussion

5.3.1 Tracking success

The harmonic radar tracking allowed to discover the position of several *V. velutina* nests (*n* = 11) in six of the nine localities, with an efficiency of 75% in outbreaks and 60% in colonised areas (Table S1). Similar success rates (63%) of nest detection have been obtained with radio telemetry in other sites (Kennedy et al. 2018). The tracking session in the outbreak of Arcola (La Spezia district) is reported as an example (Fig. 1), while the results from the other localities are available in the supplementary material (Figs. S1-S11).

Nests of *V. velutina* detected with the harmonic radar tracking were located at a mean distance of 395 ± 208 m (*M* ± *SD*, *n* = 10) from the apiaries where hornets were preying on honey bees (min = 72 m, max = 786 m, Table S2), confirming the previous hypothesis on the possible foraging range of *V. velutina* (Monceau et al. 2014a, Poidatz et al. 2018b). When nests were detected, a mean of 35 ± 20 hornets were tagged (overall tagged hornets in the nine localities: *n* = 657). In these cases, the harmonic radar was used for 11 ± 4 hours (overall hours of radar operation time: *n* = 190) from a mean of 3 ± 2 positions (overall radar positions: *n* = 47), with a maximum of seven positions for the same tracking session (Table S2). The movement of the radar was necessary to overcome physical obstacles (e.g. hills); to reduce handling time or prevent this issue, the scanning harmonic radar could be easily mounted on a van or alternatively more harmonic radar units could be used simultaneously.

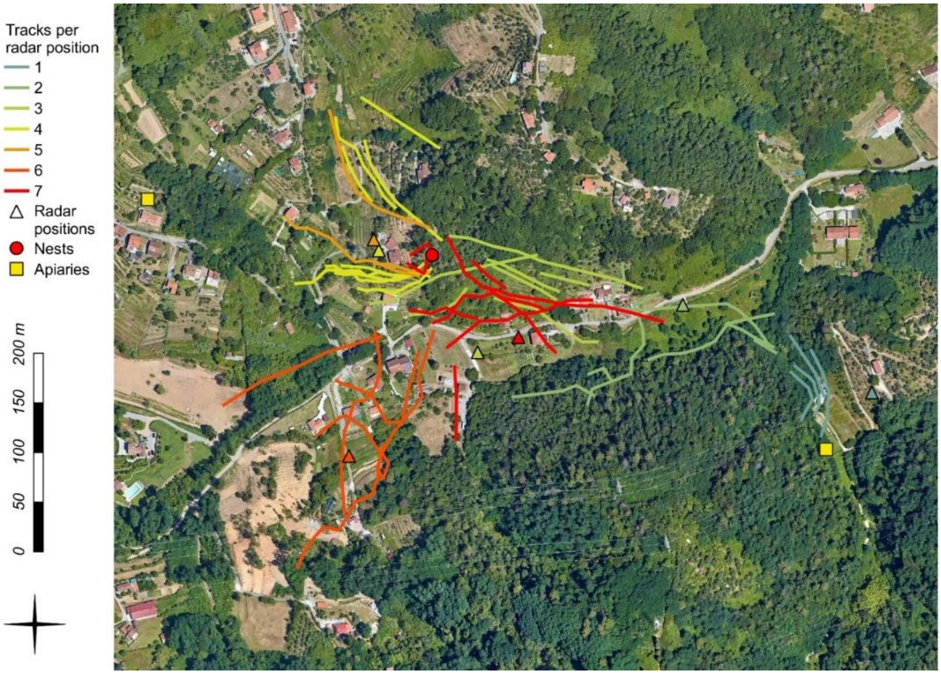

Figure 1. Example of *V. velutina* tracking session with the harmonic radar in Arcola (La Spezia, Italy). *V. velutina* workers were tagged (n = 14) in the apiary close to the first radar position (the easternmost apiary on the map). Subsequently, the harmonic radar was moved accordingly to the flying direction of hornets in other six positions (triangles). Different colours highlight the recorded tracks (n = 46) in relation to each radar position. The red dot indicates the position of the discovered nest of *V. velutina*. Background map by Google Maps (maps.google.com).

Environmental characteristics of the localities are different in terms of land cover, elevation above sea level, slope gradient and road density. Outbreak areas were generally characterised by a prevalence of urbanised or woodland landscapes, while localities inside the colonised range were generally formed by open terrains (Fig. S12 and Table S3). Study areas where the harmonic radar tracking did not allowed to detect the nest position were characterised by: *i)* woodland landscapes with low values in road densities, thus limiting the possibilities of the radar's movement (Fig. S7), *ii)* areas with steep slopes (Fig. S9) and *iii)* highly urbanised areas (Fig. S6).

5.3.2 Length of the tracks

In the nine localities, hornets were tracked by means of the harmonic radar for an overall length of 37 km, by recording 2,580 fixes (positions of the tagged hornets) that allowed the reconstruction of 389 tracks of single hornets (see materials and methods). The mean length per track was 96 ± 62 m with a maximum value of 308 m (Fig. 2). Tracking length among the study areas differ (Kruskal-Wallis test: $H_{(8)} = 30.25$, $P < 0.001$; Figs. S13, S14) but just in one case. The Dunn test with Bonferroni correction indicates a difference only between Dolceacqua (67.7 ± 52.6 m) and the three localities of Arcola (106.4 ± 62.6 m, $P = 0.023$), Finale Ligure (109.5 ± 65.1 m, $P = 0.008$) and Calvo in Ventimiglia (112.5 ± 66.7 m, $P < 0.001$).

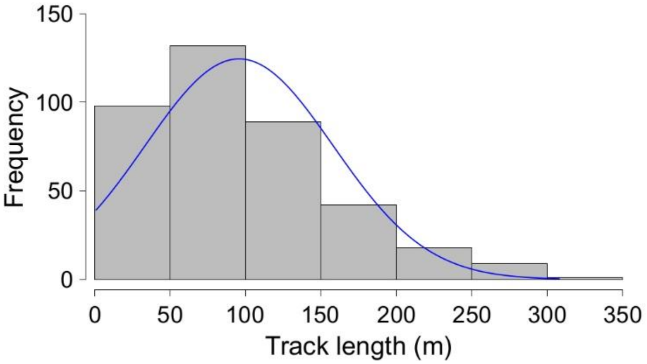

Figure 2. Frequency histogram of the length of the tracks recorded with the harmonic radar. Overall tracks recorded in the nine localities (*n* = 389) and divided by length intervals of 50 m. The line represents the fitting of a normal distribution to the length of the tracks.

A GLMM analysis on environmental characteristics indicates that the slope gradient was the main environmental variable that affected tracking lengths with the harmonic radar (Figs. 3, S15 and Table S4). Despite this negative influence, we were able to track hornets in the worst scenario in terms of slope degree (25.4 ± 7.6° in Latte of Ventimiglia) for a mean distance of 107 ± 73 m per track, with extreme values up to 298 m. On the contrary, land cover does not influence tracking performances negatively since hornets were effectively tracked in open terrains as well as in woodlands or urban areas.

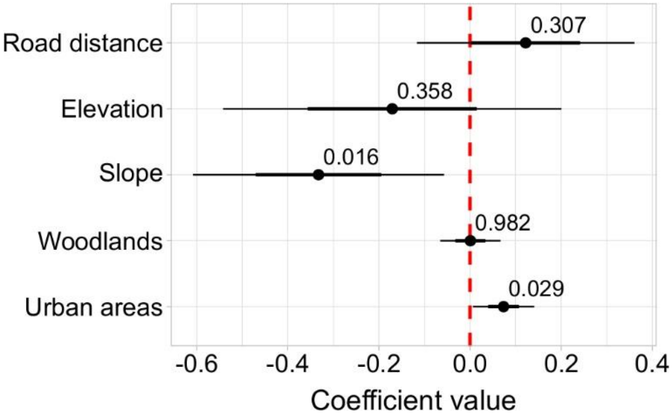

Figure 3. Influence of environmental characteristics on the length of the tracks. The coefficient plot of the GLMM analysis indicates that the slope degree is the only significant variable that affects negatively the length of the tracks with the harmonic radar, while land cover, elevation and road distance have no negative effects. The dots depict the modelled effects (*P* value is reported), inner bars the CI at 50% and outer bars the CI at 95% (see also Table S4 and Fig. S15).

The performances of the harmonic radar tracking, in terms of length of the tracks, are higher than the performance of traditional techniques for following hornets such as visual tracking and triangulation of flying directions (Fig. 4). In the six localities where the two techniques were compared, the harmonic radar allowed to track hornets for a mean length of 98 ± 65 m per track (n_{tracks} = 296), while visual tracking reached a mean length of 32 ± 16 m (n_{tracks} = 66), with a significant difference between the two methods (GLMM: $\beta_{\mathrm{visual\_tracking}} = -1.07$, $SE = 0.09$, $P < 0.001$; null model comparison: $\chi^2 = 98.52$, $df = 1$, $P < 0.001$).

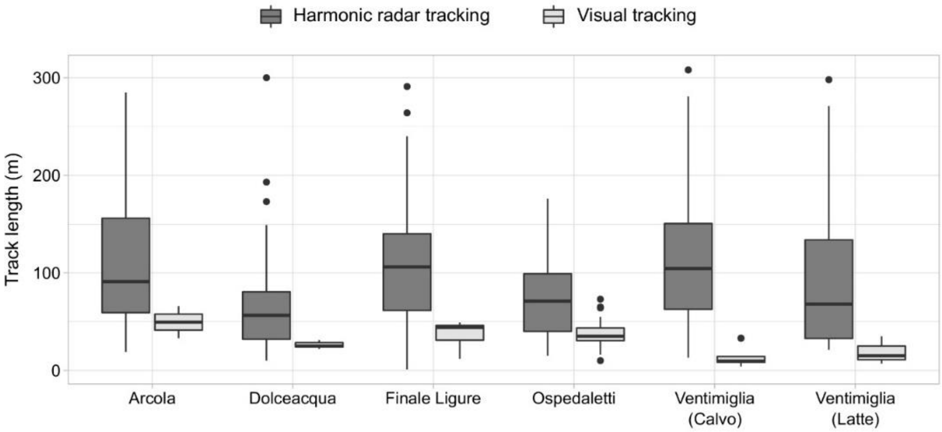

Figure 4. Comparison between tracking techniques. Boxplot of the length of the tracks recorded with the harmonic radar (dark grey) and the length of the tracks recorded with the traditional technique of visual tracking (light grey). Sample size per locality is: Arcola ($n_{radar} = 46$, $n_{visual} = 2$), Dolceacqua ($n_{radar} = 50$, $n_{visual} = 7$), Finale Ligure ($n_{radar} = 43$, $n_{visual} = 25$), Ospedaletti ($n_{radar} = 25$, $n_{visual} = 19$), Calvo of Ventimiglia ($n_{radar} = 92$, $n_{visual} = 10$), Latte of Ventimiglia ($n_{radar} = 40$, $n_{visual} = 3$). Lines represent the median and points depict outlier values.

5.3.3 *Vespa velutina* flying speed

In the three localities of La Spezia district, foraging hornets were flying at a mean ground speed of 6.66 ± 2.31 m s^{-1} ($n = 130$) whereas homing hornets had a mean ground speed of 4.06 ± 1.34 m s^{-1} ($n = 186$; Fig. 5). Flying speed is significantly different between the two groups (Wilcoxon rank-sum test: $Z = 19830$, $P < 0.001$), and values are consistent with previous findings for honey bees (5.2 m s^{-1} and 3.6-5.6 m s^{-1}, Riley et al. 1996, Capaldi et al. 2000), bumble bees (3.0-15.7 m s^{-1}, Osborne et al. 1999) and hornets (5.9 m s^{-1}, Walker 1994). Moreover, good weather conditions were present while tracking hornets in La Spezia, with mean wind speed values ranging between 1.4-2.4 m s^{-1} ($M = 1.8$, $SD = 0.3$). The difference between foraging and homing hornets is probably related to the weight (part of the body of honey bees preyed by the hornets) that homing hornets are carrying to their nests for feeding the brood, but could also be connected to the energy expenditure that hornets encountered during their predation activity. In addition, these findings show that the flying characteristics of *V. velutina* in natural conditions are considerably different from the values recorded in laboratory conditions, e.g. with fly mill experiments (1.6 m s^{-1}, Sauvard et al. 2018), underling the necessities of instruments for directly study flying insects in their natural environments.

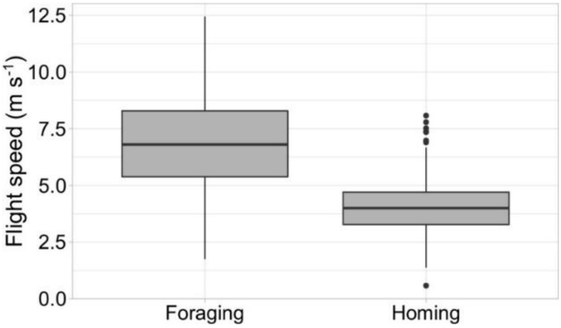

Figure 5. Flight speed of *V. velutina* workers. Foraging (*n* = 130) are hornets flying from the nest to the apiary for hunting honey bees, homing (*n* = 186) are hornets flying back to the nest from the apiary. Lines represent the median and points depict outlier values.

5.4 Conclusions

Our findings highlight the performances of a recent scanning harmonic radar technology that has been developed for tracking flying insects in natural and complex environments (Milanesio et al. 2016, 2017, Maggiora et al. 2019). This technology has been used in the framework of a management strategy developed to contain the spread of an invasive hornet species in Italy (Lioy et al. 2019, Laurino et al. 2020), leading to the detection of *V. velutina* nests either in low-density invasive outbreaks and high-density colonised areas. We were able to operate with the same performances in woodlands, urban areas and open terrains, finding that the only limiting factor is represented by the slope degree, due to the intrinsic characteristics of scanning harmonic radars (beam width). However, also in complex environments characterised by a high degree of slopes (25.4 ± 7.6°), hornets were effectively tracked up to about 300 m of length per single tracks.

These findings highlight the potentialities posed by this recent scanning harmonic radar in tracking and studying the movement of several insect species in the ecosystems. For example, it can be used to extend, in complex natural environments, research works already implemented in flying arenas or flat environments, such as the foraging range of flying insects and their interactions with the ecosystems (Osborne et al. 1999), flying behaviours (Capaldi et al. 2000, Riley et al. 2005), dispersal capabilities (Makinson et al. 2019) or evolutionary aspects (Ovaskainen et al. 2008). At the same time, it could be used for the management of invasive insect species with a similar nesting behaviour, such as several other wasp species (Beggs et al. 2011), in the framework of an early warning and rapid response strategy against biological invasions.

Supplementary material

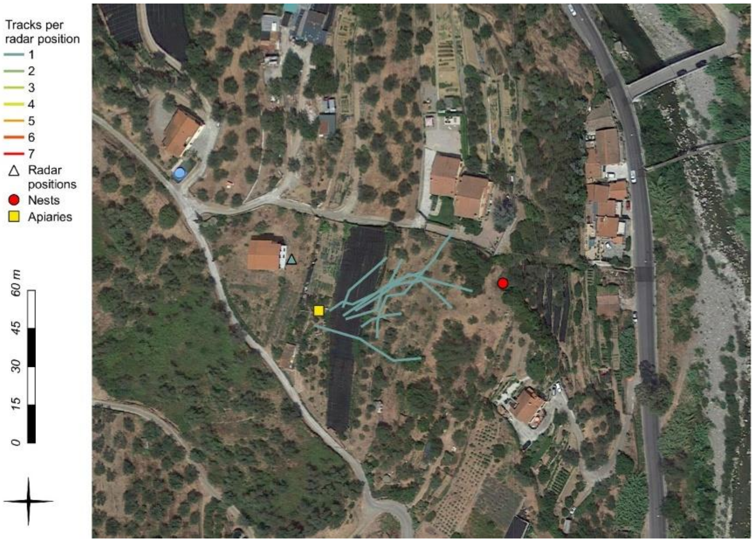

Figure S1. Tracking session in Dolceacqua (31 August - 1 September 2017). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar from one position (triangle) until nest position was discovered (red dot). Background map by Google Maps (maps.google.com).

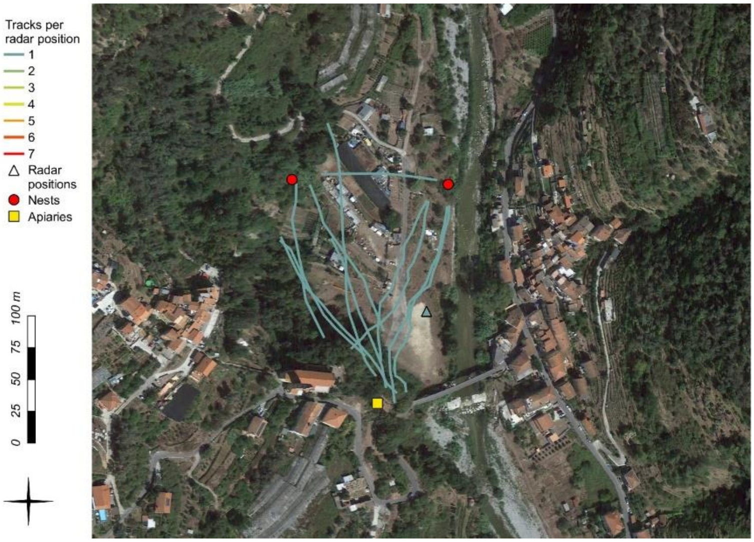

Figure S2. Tracking session in the village of Calvo in Ventimiglia (26-27 October 2017). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar from one position (triangle) until the position of two nests was discovered (red dots). Background map by Google Maps (maps.google.com).

Figure S3. Tracking session in the outbreak of Ameglia (18-19 September 2018). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar from six positions (triangles) until nest position was discovered (red dot). Background map by Google Maps (maps.google.com).

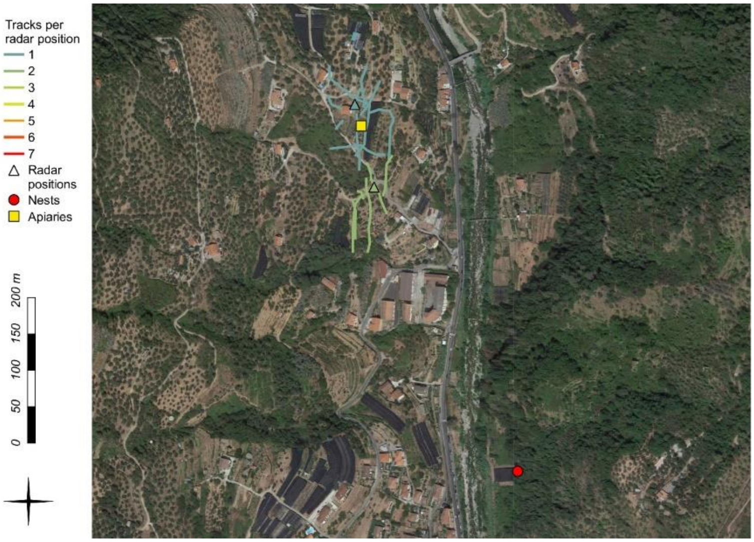

Figure S4. Tracking session in Dolceacqua (7-20 November 2018). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar from two positions (triangles) until nest position was visually discovered with the use of binoculars (red dot). Background map by Google Maps (maps.google.com).

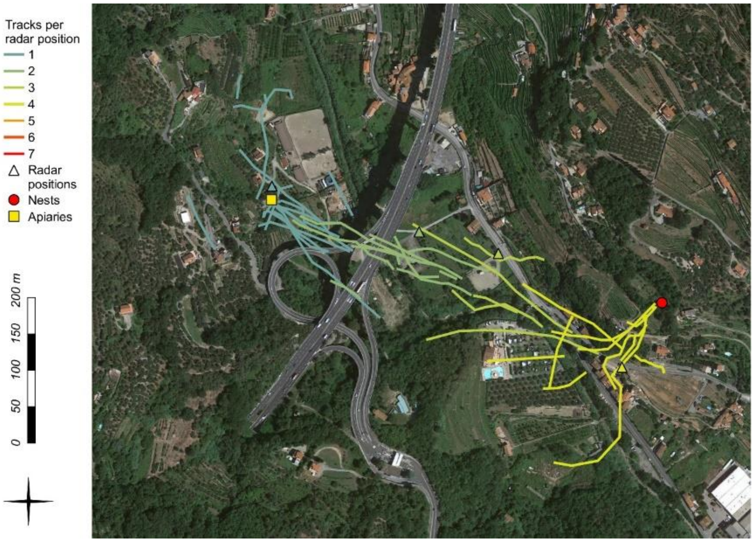

Figure S5. Tracking session in the outbreak of Finale Ligure (4-9 October 2018). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar from four positions (triangles) until nest position was discovered (red dot). Background map by Google Maps (maps.google.com).

Figure S6. Tracking session in Ospedaletti (1-2 August 2018). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar from three positions (triangles) but nest position was not detected in this tracking session. Background map by Google Maps (maps.google.com).

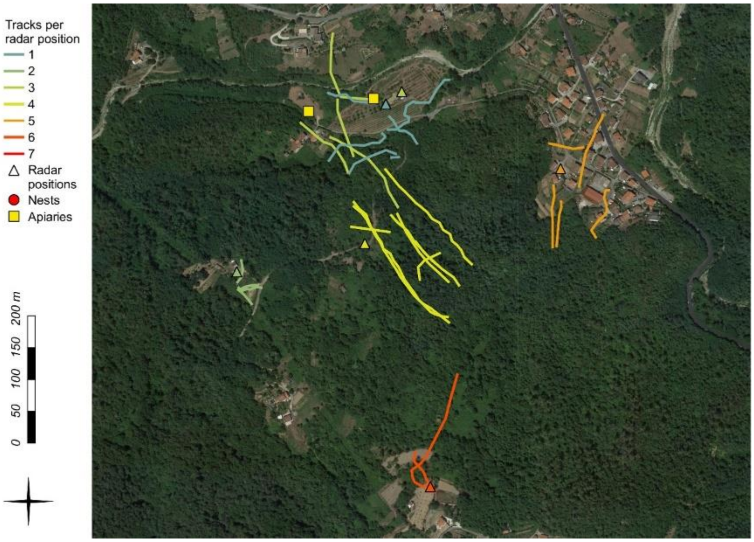

Figure S7. Tracking session in the outbreak of Riccò del Golfo (19 September - 3 October 2018). Hornets were tagged in the apiaries (yellow squares), tracked with the harmonic radar from six positions (triangles) but nest position was not detected in this tracking session. Background map by Google Maps (maps.google.com).

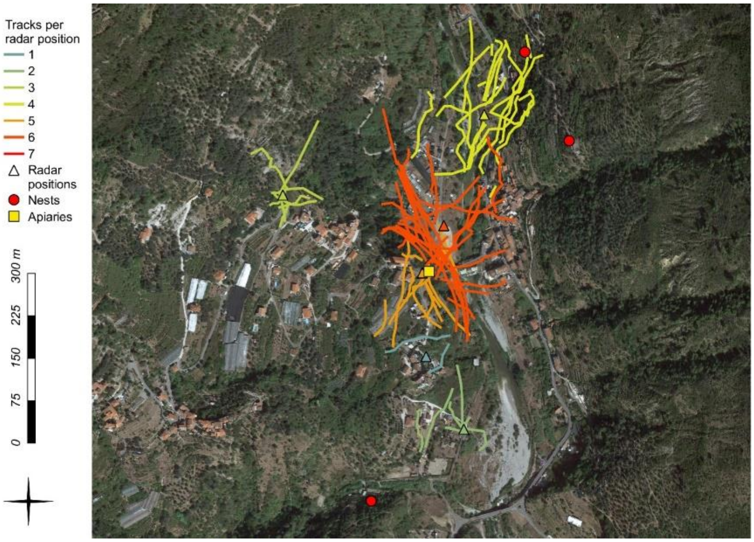

Figure S8. Tracking sessions in the village of Calvo of Ventimiglia in 2018 (24-27 July the first tracking session, 8-9 August the second and 12-17 October the third). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar from two positions for each tracking session (position 1-2 for the first session, 3-4 for the second and 5-6 for the third) until the position of three nests was discovered (red dots) at the end of each tracking session. Background map by Google Maps (maps.google.com).

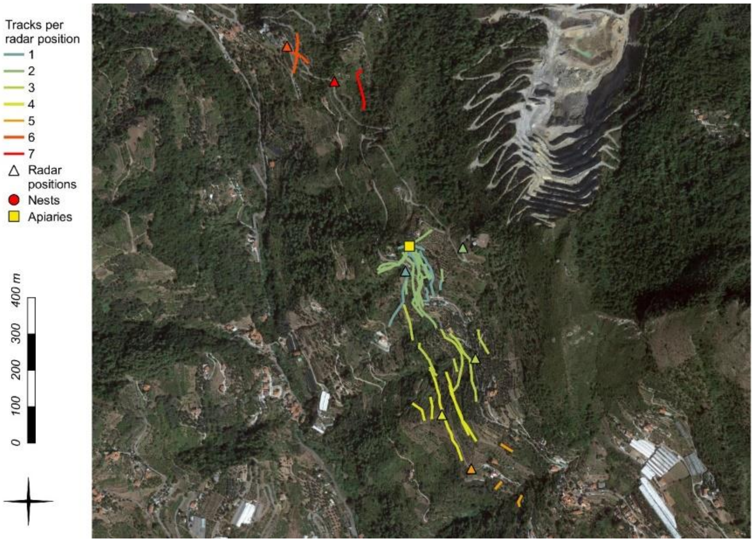

Figure S9. Tracking sessions in the village of Latte of Ventimiglia in 2018 (21-23 August the first tracking session, 5-6 September the second and 19-25 October the third). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar (position 1 for the first session, 2-4 for the second and 5-7 for the third) but nest position was not detected in the area. Background map by Google Maps (maps.google.com).

Figure S10. Tracking session in Camporosso (8-9 July 2019). Hornets were tagged when feeding on floral resources on the east-side of the river Nervia, tracked with the harmonic radar from one position (triangle) until nest position (red dot). Background map by Google Maps (maps.google.com).

Figure S11. Tracking session in Dolceacqua (16-17 July 2019). Hornets were tagged in the apiary (yellow square), tracked with the harmonic radar from three positions (triangles) but nest position was not detected in this tracking session. Background map by Google Maps (maps.google.com).

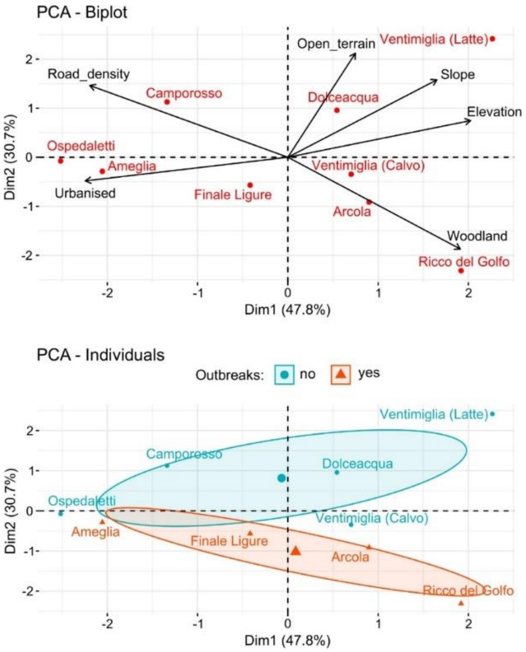

Figure S12. Plot of the PCA analysis on the environmental characteristics of the localities. The biplot highlights the relationship between variables and their correlation with the first and second dimension of the PCA. The individual plot divides the localities in which the harmonic radar operated between new invasive outbreaks (orange) and colonised areas (light blue); the ellipse level is set at 95%.

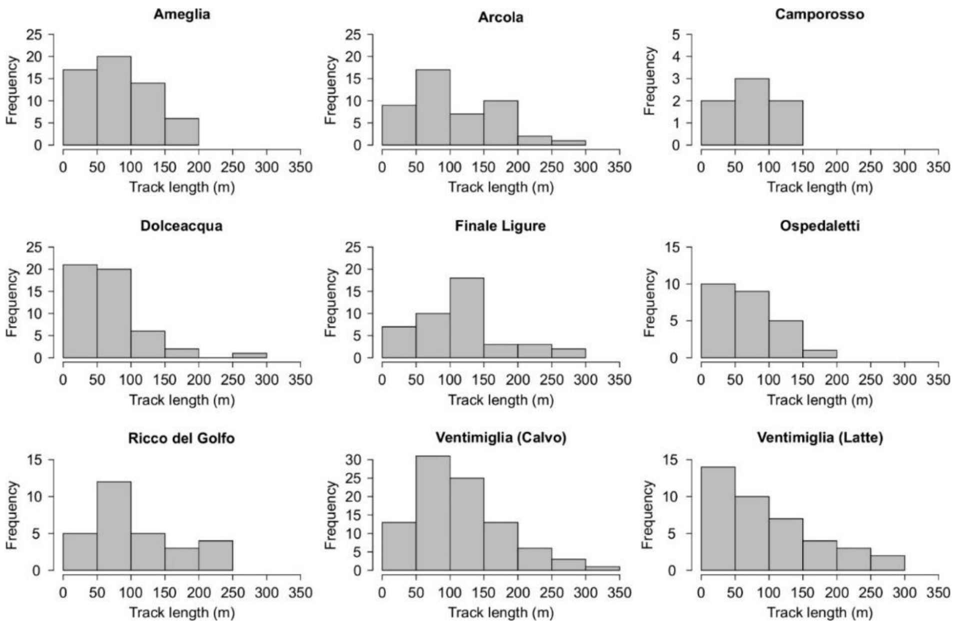

Figure S13. Length of the tracks of the nine localities in which the harmonic radar was operating. Sample size per locality is: Ameglia (n = 57), Arcola (n = 46), Camporosso (n = 7), Dolceacqua (n = 50), Finale Ligure (n = 43), Ospedaletti (n = 25), Riccò del Golfo (n = 29), Calvo of Ventimiglia (n = 92), Latte of Ventimiglia (n = 40).

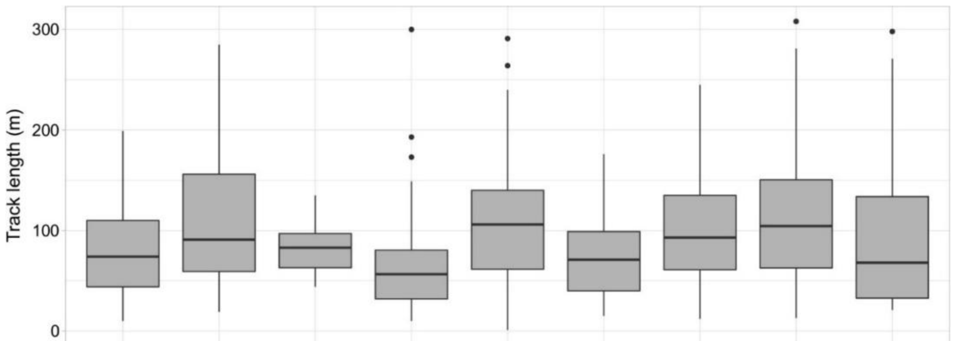

Figure S14. Boxplot of the length of the tracks in which the harmonic radar was operating per localities. Values of the length of the tracks are similar in each locality with the exception of Dolceacqua, where tracks are shorter than the tracks in Arcola, Finale Ligure and Calvo of Ventimiglia. Sample size is the same of Fig. S13. Lines represent the median and points depict outlier values.

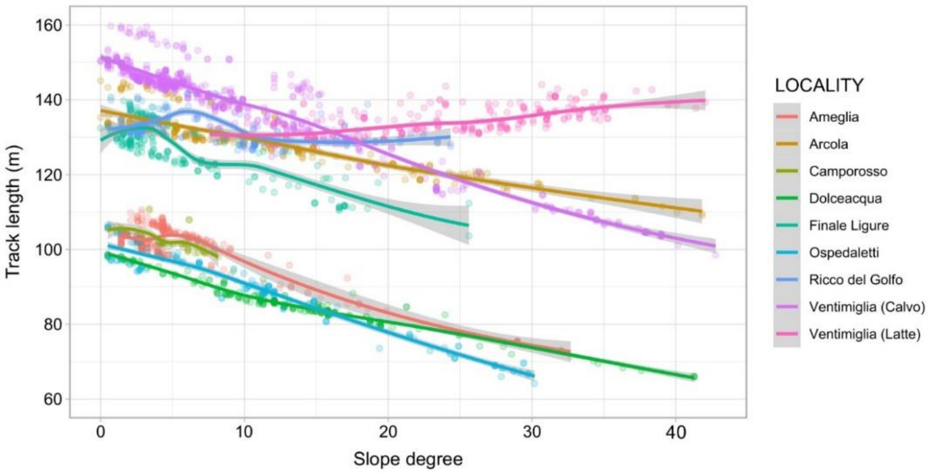

Figure S15. Fitted values of tracking length recorded with the harmonic radar in relation to slope degree and locality. Different colours identify the nine localities where hornets were tracked with the harmonic radar. The smoothed conditional mean was adopted for the fitting; 95% confidence intervals are displayed.

Table S1. Characteristics of the localities in Liguria (Italy) in which hornets have been tracked with the harmonic radar technique. The district of each locality is reported in brackets: La Spezia (SP), Savona (SV), Imperia (IM). Land cover indicates the percentage of open terrains (O), urban areas (U) and woodlands (W). Detected nests are the number of *V. velutina* nests detected with the harmonic radar tracking.

Locality	**Outbreak**	**Lat**	**Long**	**Land cover (%)**			**Elevation (m a.s.l.)**		**Slope (degree)**		**Road Density**	**Detected nests**
				O	**U**	**W**	**Mean**	**SD**	**Mean**	**SD**	**(m ha^{-1})**	**(n)**
Ameglia (SP)	Yes	44.079	9.965	40.9	43.0	16.1	16.2	10.7	4.8	5.1	119.8	1
Arcola (SP)	Yes	44.104	9.913	40.7	10.3	49.0	62.7	20.8	12.9	10.4	79.5	1
Camporosso (IM)	No	43.814	7.632	78.8	17.0	4.3	18.3	9.4	5.8	5.1	114.9	1
Dolceacqua (IM)	No	43.859	7.622	66.7	5.4	27.9	93.0	23.3	14.6	10.4	105.3	2
Finale Ligure (SV)	Yes	44.187	8.303	43.8	27.8	28.4	47.2	16.0	8.8	7.5	89.2	1
Ospedaletti (IM)	No	43.805	7.719	12.5	78.7	8.8	85.0	25.9	11.9	7.6	134.1	0
Riccò del Golfo (SP)	Yes	44.172	9.751	23.9	11.1	65.0	139.6	27.9	8.2	5.4	49.5	0
Calvo of Ventimiglia (IM)	No	43.829	7.555	44.5	16.8	38.7	92.3	33.1	13.6	9.9	85.1	5
Latte of Ventimiglia (IM)	No	43.813	7.563	77.0	1.6	21.4	220.7	50.4	25.4	7.6	94.5	0

Table S2. Results of the harmonic radar tracking activity. For each tracking session we report: the number of tagged hornets, the recorded number of tracks, the cumulative length of the tracks, the number of detected nests and their distance from the apiary where hornets were tagged. Furthermore, an estimate of the radar operation time and the total number of radar positions are reported.

Locality	**Working days**	**Date**		**Tagged hornets (*n*)**	**Tracks recorded (*n*)**	**Cumulative tracks length (m)**	**Detected nests (*n*)**	**Distance nest - apiary (m)**	**Radar operation (h)**	**Radar positions (*n*)**
		from	**to**							
Ameglia	2	18/09/2018	19/09/2018	21	57	4630	1	786	12.0	6
Arcola	3	11/09/2018	13/09/2018	14	46	4893	1	448	23.0	7
Camporosso	2	08/07/2019	09/07/2019	6	7	582	1	136*	10.5	1
Dolceacqua	2	31/08/2017	01/09/2017	42	8	315	1	72	8.0	1
Dolceacqua	5	07/11/2018	20/11/2018	74	15	959	1	520	9.5	2
Dolceacqua	2	16/07/2019	17/07/2019	20	27	2109	0	-	14.0	3
Finale Ligure	3	04/10/2018	09/10/2018	21	43	4710	1	561	10.5	4
Ospedaletti	2	01/08/2018	02/08/2018	57	25	1840	0	-	9.3	3
Riccò del Golfo	4	19/09/2018	03/10/2018	41	29	3035	0	-	12.7	6
Calvo of Ventimiglia	2	26/10/2017	27/10/2017	47	11	1527	2	190; 197	12.5	1
Calvo of Ventimiglia	2	24/07/2018	26/07/2018	43	9	682	1	418	9.3	2
Calvo of Ventimiglia	2	08/08/2018	09/08/2018	33	32	3096	1	423	11.8	2
Calvo of Ventimiglia	2	12/10/2018	17/10/2018	48	40	5049	1	338	7.0	2
Latte of Ventimiglia	3	21/08/2018	23/08/2018	51	12	772	0	-	16.0	1
Latte of Ventimiglia	2	05/09/2018	06/09/2018	61	22	2681	0	-	15.8	3
Latte of Ventimiglia	4	19/10/2018	25/10/2018	78	6	365	0	-	8.5	3

* in the locality of Camporosso, hornets were tagged when feeding on flowerings. In this case, the distance corresponds to the distance between the nest and the feeding point on flowerings (this value was not included when estimating the mean distances of nests from apiaries).

Table S3. PCA analysis on the environmental characteristics of the localities. The first table reports the contribution of the first five dimensions (Dim.1 - Dim.5) of a PCA analysis, the cumulative proportion and the respective standard deviation (*SD*). The second table reports the contribution of the environmental variables to the five dimensions. Biplot and individual plot of the PCA are reported in Fig. S12.

Parameter	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**	**Dim.5**
Proportion of variance	0.4776	0.3070	0.1910	0.0194	0.0050
Cumulative proportion	0.4776	0.7846	0.9756	0.9950	1.0000
Standard deviation	1.6929	1.3572	1.0705	0.3409	0.1731

Variables	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**	**Dim.5**
Open terrain	2.68	33.51	26.47	2.23	0.37
Urbanised	24.10	1.72	23.82	1.53	9.31
Woodlands	17.48	26.09	0.01	10.61	20.06
Elevation	19.63	4.16	27.16	40.58	8.48
Slope	13.06	18.58	20.55	39.16	8.65
Road density	23.06	15.94	1.99	5.88	53.12

Table S4. Results of a GLMM analysis on the relationship between the length of the tracks recorded with harmonic radar and the environmental characteristics. The model with the best fitting is a model that considers all the environmental variables as fixed effects and uncorrelated random intercepts (based on locality) with random slopes (based on slope degree of the study areas). For each variable the following is reported: the estimated coefficient (β), the standard error (*SE*), *Z* and *P* values. The slope degree is the main variable that negatively affects the length of the tracks, while urban areas has a positive influence on the response variable. Woodlands, elevation and road distance do not affect tracking length.

Variables	***β***	***SE***	***Z***	***P***
Urbanised	**0.0736**	**0.0337**	**2.182**	**0.0291**
Woodlands	0.0007	0.0331	0.022	0.9823
Slope	**-0.3325**	**0.1378**	**-2.414**	**0.0158**
Elevation	-0.1707	0.1856	-0.919	0.3579
Road distance	0.1220	0.1194	1.022	0.3068

Selected model: Track_lenght ~ Landcover + Slope + Elevation + Road_distance + (Slope || Locality)
Comparison between the selected model (AIC -1013) and the null model (-981): $\chi^2 = 44.19$, *df* = 6, *P* < 0.001

6.

Effectiveness and selectiveness of traps and baits for catching the invasive hornet *Vespa velutina*

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Abstract: *Vespa velutina* is an invasive hornet that is colonising several countries worldwide, with detrimental effects on multiple components but primarily affecting honey bees and native insect species. Traps for wasps and hornets are commonly used for trapping *V. velutina*, both for monitoring and control purposes. In this study, we compared the performances of two typologies of traps and baits widely used for trapping this invasive hornet, by evaluating their effectiveness and selectiveness in trapping *V. velutina* in two sites during two different periods of the year, spring and autumn. The performance of the traps changed in relation to *i*) the trap's model, *ii*) the bait's typology and *iii*) the period of the year. In spring, traps with common beer as bait were more effective and more selective independently of trap's model than the commercial bait that has been tested. On the contrary, in autumn, just one combination of trap and attractant (the commercial trap and bait) achieved higher effectiveness and selectiveness. Despite the underlined variations among traps and baits, overall catches of *V. velutina* were scanty compared to bycatches of non-target insects, since best performing traps either in term of effectiveness and selectiveness caught 3.65% of the target species in spring and 1.35% in autumn upon the total trapped insects. This highlights the urgent necessity of developing more selective trapping methods for monitoring and particularly for controlling purposes.

Keywords: Asian yellow-legged hornet; *Vespa velutina*; invasive species; monitoring; surveillance; early detection; wasps; trapping; baits

6.1 Introduction

The Asian yellow-legged hornet *Vespa velutina* is an invasive species, introduced in Europe (Monceau et al. 2014a) and in a few other non-native countries of Asia (Choi et al. 2012, Takahashi et al. 2019), where it has established viable and expanding populations (Laurino et al. 2020). As in other hornet and wasp species, the larval stages of *V. velutina* require proteins for their development, which are commonly obtained by preying on other insects such as bees, other wasps or flies, but with a preference for honey bees (Villemant et al. 2011b, Monceau et al. 2014b). In the invaded countries of Europe, the intensive predation of this hornet towards *Apis mellifera* could lead to serious impacts on honey bee colonies, due to the induced foraging paralysis, the homing failure of foraging bees (Requier et al. 2019) and the absence of an effective defensive behaviour against *V. velutina* (Arca et al. 2014). Moreover, its wide predation spectrum might also affect, more in general, insect communities and the ecosystem services they provide, such as pollination (Rojas-Nossa & Calviño-Cancela 2020), although scientific evidence is currently limited. Finally, *V. velutina* represents an economic issue in the invaded countries, both for the impacts associated to the collapse of honey bee colonies (Requier et al. 2019) and the costs related to the implementation of control activities (Barbet-Massin et al. 2020).

Several countries worldwide are monitoring the presence of *V. velutina*, with the aim of detecting new occurrences in areas not yet colonised by the species for the subsequent implementation of control or rapid response strategies, which are based on nest detection and destruction (Budge et al. 2017, Leza et al. 2018, Lioy et al. 2019, Laurino et al. 2020). This surveillance activity is also mandatory for EU countries, since *V. velutina* is listed as an invasive alien species of Union concern (EU Regulation 1141/2016) in the framework of the respective European regulation (EU Regulation 1143/2014).

Monitoring is generally performed by integrating several approaches: *i*) trapping adults, *ii*) spotting nests and *iii*) observing the presence of *V. velutina* on flowers or in apiaries while hunting for honey bees (Demichelis et al. 2014, Porporato et al. 2014, Laurino et al. 2020).

Several models of trap are available for trapping adults, and some of them require attractants (Turchi & Derijard 2018). Sugar-based baits are commonly used for attracting social wasp species (Wegner & Jordan 2005, Sorvari 2013) or trapping *V. velutina* queens in spring (Monceau et al. 2012) or gynes in autumn, while protein-based baits are mainly used in summer for trapping workers around apiaries (Bacandritsos et al. 2006, Turchi & Derijard 2018). Traps with sugar-based baits have been widely used for monitoring the presence of *V. velutina* (Demichelis et al. 2014, Rodríguez-Flores et al. 2019). Furthermore, with an increased density of traps in the environment, this approach has been used as a complementary method for attempting its control by trapping queens (Monceau et al. 2012, Turchi & Derijard 2018), despite its effectiveness appears to be limited (Monceau & Thiéry 2017).

Few studies have compared the performance of traps and baits to understand the effectiveness of trapping *V. velutina* and the consequences on native insect fauna (Monceau et al. 2012, Demichelis et al. 2014, Rojas-Nossa et al. 2018). This is particularly important since the variety of available baits and traps that have been developed (with different shapes, colours and volume capacities) could lead to different attractiveness and then different results on the target species and on native ones (Demichelis et al. 2014, Rojas-Nossa et al. 2018, Turchi & Derijard 2018). Therefore, further investigations are required on this topic, in order to understand which combination of trap and bait could provide the best performances.

In this study, two models of traps and two sugar-based baits (beer and a commercial bait) commonly used for trapping *V. velutina* were combined to compare their effectiveness in trapping *V. velutina* and their effects on non-target insects, accordingly to the monitoring procedures generally adopted by the beekeepers. This allowed to understand which combination of trap and bait, among the tested combinations, is more effective and selective for monitoring the presence of this invasive species. Their performances were analysed in relation to the period of the year in which the sampling was performed (spring and autumn), for evaluating the presence of differences in relation to the seasonality.

6.2 Materials and Methods

6.2.1 Sites, traps and sampling protocol

The sites selected for this experiment were located in Liguria (Italy), inside the area firstly invaded by *V. velutina*: site A, in the village of Sealza (Ventimiglia; N 43.80899, E 7.55111); site B, in the village of Brunetti (Camporosso; N 43.83924, E 7.60659). This area has been colonised by the species since 2013 and the density of *V. velutina* colonies increased similarly in the two sites from 2013 to 2018, ranging between 0.2 to 2.3 nests/km^2 in site A and 0.2 to 1.9 nests/km^2 in site B. Moreover, the two areas were similar in terms of elevation (~300 m a.s.l.) and land cover, with a predominant presence of woodlands (61% in site A and 51% in site B) together with rural and agricultural landscapes (33% in site A and 42% in site B). In addition, the distance between the two sampling sites was more than 5 km, thus no effect of their proximity was expected on the trapping results.

In both sites, two typologies of traps (common PET bottle trap equipped with the TapTrap® yellow cap; VespaCatch® trap made by Véto-pharma) and two baits (common golden ale at 5% of alcohol; VespaCatch® attractant made by Véto-pharma) were combined in a full factorial design and tested for evaluating their effectiveness in trapping *V. velutina* and their impacts towards non-target species

(Fig. 1). The two traps differed in terms of structure, colour and in the number of individual entering points, which was one for the bottle trap with the yellow cap (Fig. 1a) and two for the VespaCatch trap (Fig. 1b). Four trap–bait combinations were tested: TB, bottle trap with beer as bait; TV, bottle trap with VespaCatch attractant as bait; VB, VespaCatch trap with beer as bait; VV, VespaCatch trap with VespaCatch attractant as bait. Traps were filled with the same volume of bait (~250 mL) whether it was beer or the commercial attractant.

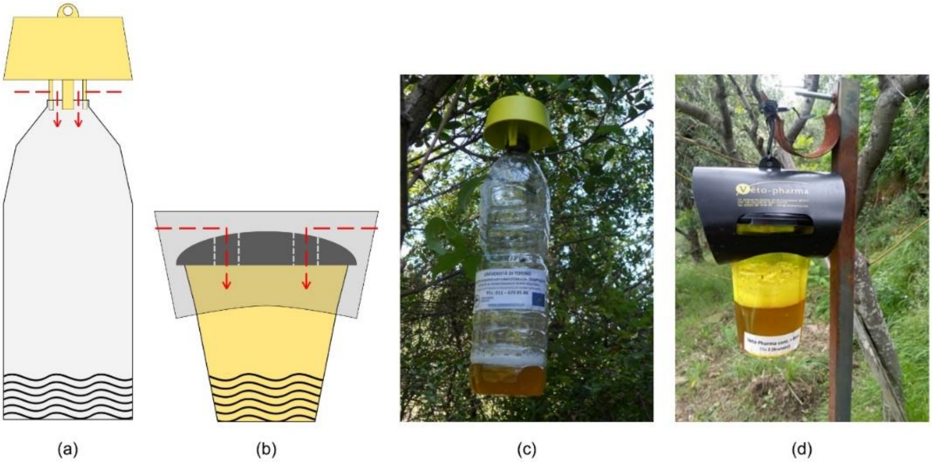


Figure 1. Traps tested in the study: (a) scheme of the bottle trap equipped with the yellow cap (T); (b) scheme of the VespaCatch trap (V); (c) photo of the bottle trap with the yellow cap (T); (d) photo of the VespaCatch trap (V). The red arrows in the two schemes highlight the access route used by the hornets for entering the traps. Both models of traps were filled with beer (B) as bait (trap–bait combination named respectively TB and VB) or VespaCatch (V) attractant (trap–bait combination named respectively TV and VV) for a full factorial experiment.

In each sampling site, 12 monitoring traps (three for each trap–bait combination) were positioned on poles at a height of 1.5 m from the ground, with a distance of 3 m between each trap and clustered per trap–bait combination. To avoid any interference between the surrounding environment and their position, each cluster was moved by one line position during each control (see Fig. S1 for more details on the sampling design in each site).

Monitoring traps were activated during two seasons of 2018: in spring, from the beginning of April to the end of June (82 trapping days in site A and 84 in site B); in autumn, from the beginning of October to the end of December (70 trapping days in site A and 71 in site B). Overall, monitoring traps were checked every 25.6 ± 7.1 days; at the same time, the baits were renewed, and trapped insects collected and preserved in alcohol (70%) for the subsequent taxonomic identification. The sampling interval was higher than previous studies (Demichelis et al. 2014, Porporato et al. 2014, Rojas-Nossa et al. 2018) in terms of number of days between checks, since we were interested in understanding their performance in relation to the procedures adopted by the beekeepers for trapping *V. velutina*, in which checks and change of the attractive baits rarely occur on a daily or weekly basis, but customarily when apiaries are inspected and usually at intervals of 20–30 days.

6.2.2 Data analyses: effectiveness in trapping *Vespa velutina*

The effectiveness of each trap–bait combination in trapping *V. velutina* during the two seasons has been evaluated with a zero-inflated GLMM model with a Poisson distribution (package *glmmTMB*). The trapped number of *V. velutina* was included as response variable, while trap model, bait typology and the season as explanatory variables, taking also into account the interactions among predictors. The sampling sites and the trapping days were included as random effects in the model. After verifying the assumptions (package *DHARMa*), the GLMM model was tested against a null-model and effects of each trap–bait combination extracted for both seasons (packages *emmeans* and *multcomp*).

6.2.3 Data analyses: bycatch of insects and differences between trap-bait combinations

For both seasons, the proportion of trapped insects per taxonomic group was evaluated with a scaled PCA analysis for a first assessment of the differences between each trap–bait combination. Results of the PCA are displayed with a biplot of individuals and variables, with trap–bait combinations as grouping variable (package *factoextra*). Convex hull polygons are used for highlighting individuals from the same group of traps.

Furthermore, for each taxonomic group determined at least at the order level, the difference in the number of trapped insects per trap–bait combination was evaluated with the Fisher's exact test. Differences are displayed with an extended mosaic plot with standardised residuals, where cells representing negative residuals are drawn with broken borders and positive ones are drawn in solid borders, while shades of red and blue indicates different levels of standardised residuals.

6.2.4 Data analyses: selectiveness of the traps

The Fisher's exact test was used to evaluate the selectiveness of each trap–bait combination within a season, by analysing the number of non-target insects against the number of *V. velutina* trapped (2 × 4 contingency tables). A pairwise test of independence with Bonferroni correction has been applied to understand differences between groups within a season (package *rcompanion*). Results of this analysis are displayed in Table 3 as the ratio non-target insects : *V. velutina*. A value lesser than one indicates that a higher number of *V. velutina* is trapped than non-target insects, with a minimum of zero indicating no bycatches towards the non-target group; a value higher than one indicates a lower selectiveness, since a higher number of non-target insects are trapped than *V. velutina*. All data analyses have been performed with the software R 4.0.0 (R Core Team 2019).

6.3 Results

Overall, 213 individuals of *V. velutina* have been trapped in the two sampling seasons (104 in spring and 109 in autumn), accounting for 1.02% of the total trapped insects (Table 1 and Fig. 2). A similar percentage has been recorded for *V. crabro* (261 individuals, 1.25% of the total trapped insects), but with more seasonal variation (192 individuals in spring and 69 in autumn). On the contrary, *Vespula* spp. (0.84%) and *Polistes* spp. (0.10%) were trapped with less frequency than the other wasp species. Diptera and Formicidae were the non-target groups mostly trapped either in spring or in autumn, with variations among seasons and trap–bait combinations. Apoidea (*A. mellifera*, *Bombus* spp. and other Apoidea) were not trapped frequently and, overall, they only accounted for 0.13% of the total trapped insects, while Lepidoptera catches were more frequent than those of Apoidea (382 individuals, 1.84%), but with variations among seasons and trap–bait combinations. Other groups were trapped sporadically and, overall, they accounted for 0.43% of all trapped insects.

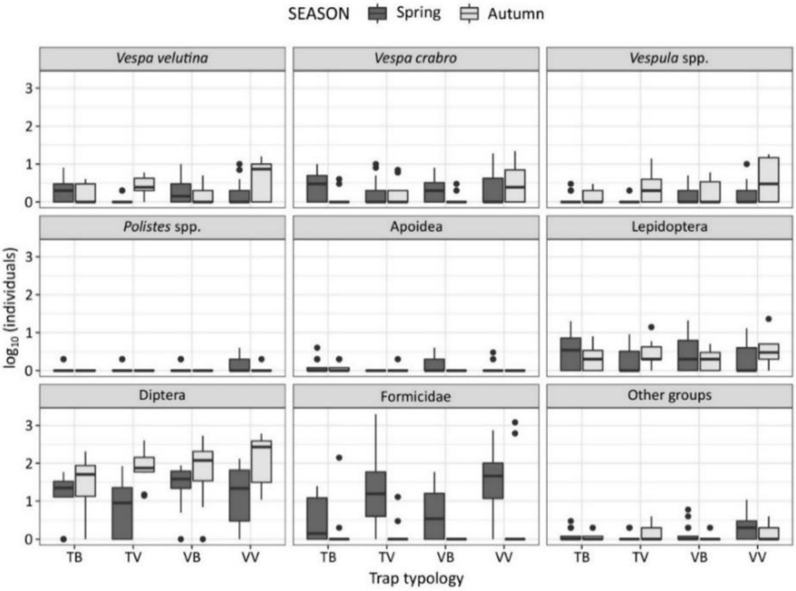


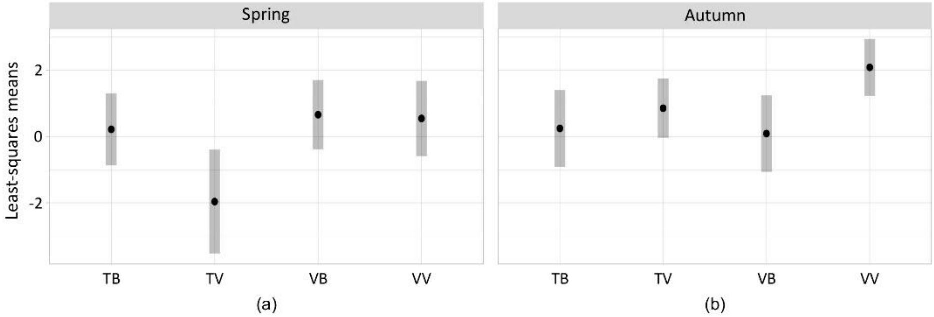
Figure 2. Number of individuals (log$_{10}$ transformed) trapped in spring (dark grey) and autumn (light grey) per species or taxonomic group, and divided among trap–bait combinations. TB: bottle-trap & beer; TV: bottle trap & VespaCatch attractant; VB: VespaCatch trap & beer; VV, VespaCatch trap & VespaCatch attractant.

Table 1. Overall trapping results in the two sampling sites (total column) and results divided between spring and autumn taking into account the four trap–bait combinations. For each period, the mean number of individuals per trap (standard deviation in brackets) and the percentage of trapped individuals for the corresponding species/group out of the total number of trapped insects are reported. TB: bottle-trap & beer; TV: bottle trap & VespaCatch attractant; VB: VespaCatch trap & beer; VV, VespaCatch trap & VespaCatch attractant.

		TOTAL		SPRING								AUTUMN							
				TB		TV		VB		VV		TB		TV		VB		VV	
	N. of Individuals	**Mean per Trap (*SD*)**	**% of Insects**	**Mean per Trap (*SD*)**	**% of Insects**	**Mean per Trap (*SD*)**	**% of Insects**	**Mean per Trap (*SD*)**	**% of Insects**	**Mean per Trap (*SD*)**	**% of Insects**	**Mean per Trap (*SD*)**	**% of Insects**	**Mean per Trap (*SD*)**	**% of Insects**	**Mean per Trap (*SD*)**	**% of Insects**	**Mean per Trap (*SD*)**	**% of Insects**
Hymenoptera Vespoidea																			
Vespa velutina	213	1.48 (2.64)	1.02	1.38 (1.78)	3.65	0.13 (0.33)	0.06	1.63 (2.36)	2.72	1.21 (2.36)	0.80	0.75 (1.09)	1.02	2.08 (1.71)	1.53	0.67 (1.11)	0.39	5.58 (5.22)	1.35
Vespa crabro	261	1.81 (3.16)	1.25	2.54 (2.74)	6.74	1.17 (2.36)	0.52	1.67 (2.25)	2.79	2.63 (4.09)	1.74	0.42 (0.95)	0.57	1.17 (1.99)	0.86	0.25 (0.60)	0.15	3.92 (5.72)	0.95
Vespula spp.	174	1.21 (3.08)	0.84	0.25 (0.52)	0.66	0.04 (0.20)	0.02	0.75 (1.13)	1.26	0.88 (1.92)	0.58	0.42 (0.64)	0.57	2.42 (3.64)	1.78	1.33 (1.84)	0.79	6.50 (7.16)	1.57
Polistes spp.	20	0.14 (0.40)	0.10	0.08 (0.28)	0.22	0.13 (0.33)	0.06	0.21 (0.41)	0.35	0.38 (0.70)	0.25	0.00 (0.00)	0.00	0.00 (0.00)	0.00	0.00 (0.00)	0.00	0.08 (0.28)	0.02
Formicidae	9641	67.0 (258.5)	46.35	5.4 (7.7)	14.26	202.8 (518.8)	90.95	9.9 (14.5)	16.55	101.6 (173.8)	67.50	11.8 (38.7)	15.99	1.2 (3.3)	0.86	0.0 (0.0)	0.00	151.3 (358.7)	36.63
Hymenoptera Apoidea																			
Apis mellifera	18	0.13 (0.45)	0.09	0.29 (0.68)	0.77	0.00 (0.00)	0.00	0.33 (0.75)	0.56	0.08 (0.28)	0.06	0.08 (0.28)	0.11	0.00 (0.00)	0.00	0.00 (0.00)	0.00	0.00 (0.00)	0.00
Bombus spp.	6	0.04 (0.20)	0.03	0.04 (0.20)	0.11	0.00 (0.00)	0.00	0.08 (0.28)	0.14	0.08 (0.28)	0.06	0.00 (0.00)	0.00	0.08 (0.28)	0.06	0.00 (0.00)	0.00	0.00 (0.00)	0.00
Other Apoidea	2	0.01 (0.12)	0.01	0.00 (0.00)	0.00	0.00 (0.00)	0.00	0.00 (0.00)	0.00	0.00 (0.00)	0.00	0.17 (0.37)	0.23	0.00 (0.00)	0.00	0.00 (0.00)	0.00	0.00 (0.00)	0.00
Diptera	9993	69.4 (111.3)	48.04	23.0 (16.5)	60.89	17.1 (23.6)	7.68	41.2 (27.1)	68.99	39.9 (45.3)	26.52	57.8 (56.7)	78.56	125.7 (117.2)	92.52	165.9 (170.0)	97.93	241.1 (200.8)	58.39
Lepidoptera	382	2.65 (3.97)	1.84	4.50 (5.09)	11.93	1.38 (2.29)	0.62	3.46 (4.68)	5.80	1.75 (2.74)	1.16	1.92 (2.36)	2.61	2.75 (3.39)	2.02	1.17 (1.28)	0.69	3.83 (5.62)	0.93
Other groups	90	0.63 (1.49)	0.43	0.29 (0.54)	0.77	0.21 (0.41)	0.09	0.50 (1.15)	0.84	2.00 (2.86)	1.33	0.25 (0.43)	0.34	0.50 (0.87)	0.37	0.08 (0.28)	0.05	0.67 (0.94)	0.16

6.3.1 Effectiveness in trapping *Vespa velutina*

The variable that significantly affected the effectiveness in trapping *V. velutina* was the bait and its interaction with the model of trap and with the season, while no effect was associated to the season on its own (Table 2). In spring, traps equipped with beer as bait were more effective in trapping *V. velutina* independently from the model of trap (Fig. 3; TB: *LSmean* = 0.21, *SE* = 0.55; VB: *LSmean* = 0.66, *SE* = 0.53). On the contrary, VespaCatch attractant became as effective as beer in spring only with its respective trap model (TV: *LSmean* = −1.96, *SE* = 0.79; VV: *LSmean* = 0.54, *SE* = 0.57). In autumn, the effectiveness towards *V. velutina* of traps equipped with beer decreased as well as VespaCatch attractant in bottle traps equipped with TapTrap (TB: *LSmean* = 0.24, *SE* = 0.58; VB: *LSmean* = 0.09, *SE* = 0.58; TV: *LSmean* = 0.85, *SE* = 0.45). High trapping effectiveness in autumn was maintained only by the combination of VespaCatch attractant and its respective trap model (VV: *LSmean* = 2.08, *SE* = 0.43).



(a) (b)

Figure 3. Least-squares means of *V. velutina* catches (log scale) in spring (a) and autumn (b) for each combination of trap and bait, derived from the zero-inflated GLMM of Table 2. TB: bottle-trap & beer; TV: bottle trap & VespaCatch attractant; VB: VespaCatch trap & beer; VV, VespaCatch trap & VespaCatch attractant.

Table 2. Conditional model of the zero-inflated GLMM for evaluating the effectiveness in trapping *V. velutina*. The effect size of the predictors (β), the corresponding standard error (*SE*), *Z* and *p* values are reported. The footnotes describe the structure of the model and the result of the comparison against a null-model.

Variables	β	*SE*	*Z*	*p*
Season (Autumn)	0.03	0.71	0.04	0.968
Bait (VespaCatch)	–2.18	0.63	–3.44	< 0.001
Trap_model (VespaCatch)	0.44	0.26	1.71	0.088
Season (Autumn): Bait (VespaCatch)	2.78	0.79	3.52	< 0.001
Season (Autumn): Trap_model (VespaCatch)	–0.60	0.64	–0.94	0.348
Bait (VespaCatch): Trap typology (VespaCatch)	2.06	0.69	2.97	< 0.01
Season (Autumn): Bait (VespaCatch): Trap typology (VespaCatch)	–0.68	0.95	–0.71	0.475

GLMM Model: *Vespa velutina* ~ Bait typology * Trap typology * Season + (1 | Study area) + (1 | Trapping days). Comparison between the selected model (AIC 436) and the null-model (AIC 521): $\chi^2 = 101$, $df = 8$, $p < 0.001$.

6.3.2 Bycatch of insects and differences between trap-bait combinations

The PCA analysis highlighted variations in the proportion of trapped insects among trap–bait combinations in particular during the spring seasons (Fig. 4). Contribution of the variables to the principal components and loading plots are reported as supplementary material (Table S1 and Fig. S2).

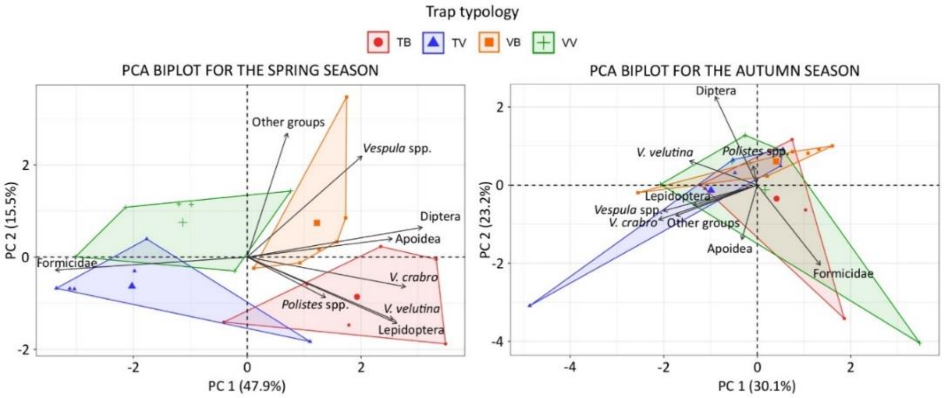


Figure 4. PCA biplot of individuals and variables for the spring (left) and autumn (right) season. Convex hull polygons highlight individuals of each trap–bait combination. TB: bottle-trap & beer; TV: bottle trap & VespaCatch attractant; VB: VespaCatch trap & beer; VV, VespaCatch trap & VespaCatch attractant.

In relation to the season, the Fisher’s exact test highlighted differences between trap–bait combinations in almost all the taxonomic groups considered ($p < 0.001$ in *V. velutina*, *V. crabro*, *Vespula* spp., Lepidoptera, Diptera and Formicidae; $p < 0.05$ in Apoidea), and the only taxon without significant differences is *Polistes* spp. Differences between trap-bait combinations in the Vespidae family and in other taxonomic groups (determined at least at the order level) are displayed respectively in Fig. 5 and Fig. 6.

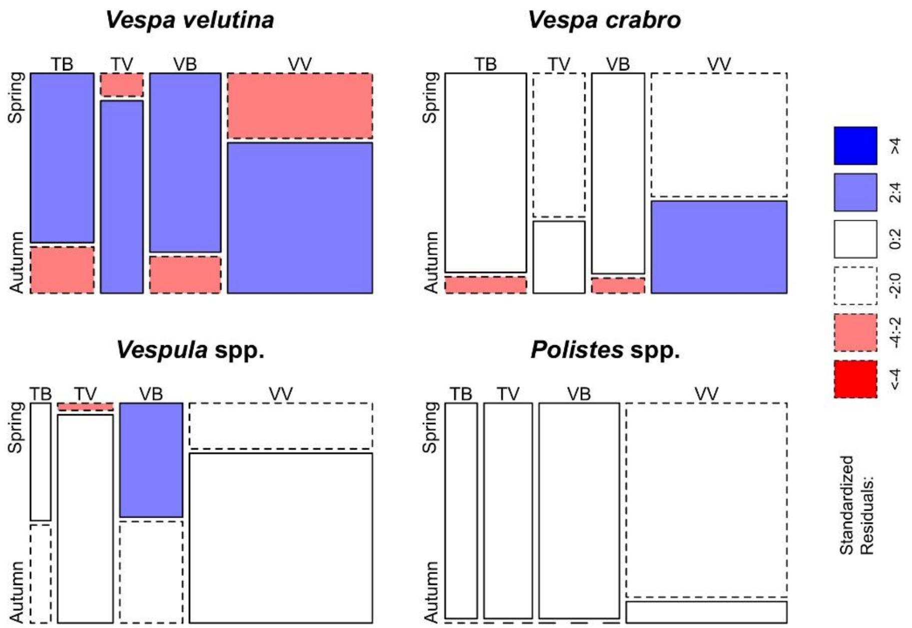


Figure 5. Mosaic plot of the differences between trap–bait combinations per season for the Vespidae family. Cells representing negative residuals are drawn in shades of red and with broken borders while positive ones are drawn in blue with solid borders. TB: bottle-trap & beer; TV: bottle trap & VespaCatch attractant; VB: VespaCatch trap & beer; VV, VespaCatch trap & VespaCatch attractant.

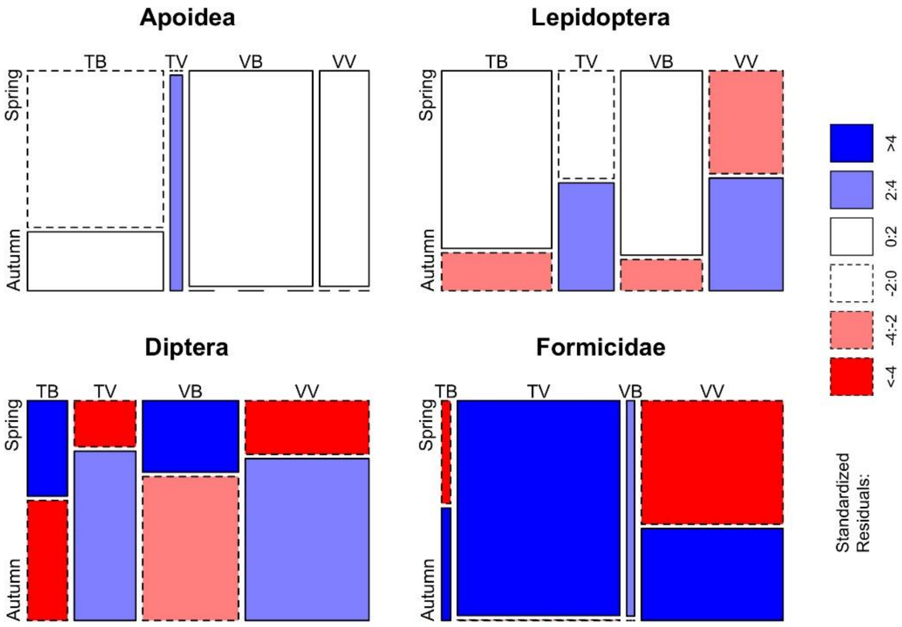


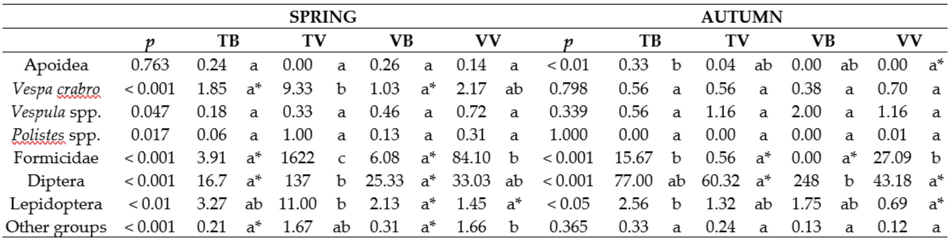
Figure 6. Mosaic plot of the differences between trap–bait combinations per season for Apoidea, Lepidoptera, Diptera and Formicidae. Cells representing negative residuals are drawn in shades of red and with broken borders while positive ones are drawn in blue with solid borders. TB: bottle-trap & beer; TV: bottle trap & VespaCatch attractant; VB: VespaCatch trap & beer; VV, VespaCatch trap & VespaCatch attractant.

6.3.3 Selectiveness of the traps

Overall, traps equipped with beer as bait were significantly more selective in spring than traps with the commercial bait (Table 3). In relation to *V. velutina* catches, TB and VB trapped a significant lesser number of *V. crabro*, Diptera, Formicidae and other taxa than the other trap–bait combinations. Lepidoptera were trapped frequently in spring, however VB and VV traps ensured the best ratio between bycatches of Lepidoptera and *V. velutina* catches. Concerning the other non-target groups, all trap–bait combinations trapped indifferently a small number of Apoidea, *Vespula* spp. and *Polistes* spp.

In autumn, the selectiveness of beer as bait decreases while the one of the commercial bait slightly increases (Table 3), in particular when it is associated with the commercial model of trap. In fact, VV trap allowed to significantly catch a lesser number of Diptera, Lepidoptera and Apoidea in respect to *V. velutina* catches, though Apoidea catches were infrequent with all trap–bait combinations.

Table 3. Results of the Fisher's exact test and of the pairwise test of independence with Bonferroni correction for the analysis of the selectiveness. For each comparison, p-values of the Fisher's exact test are reported. Values for each trap–bait combination represent the ratio non-target insects : *V. velutina*. Letters define similarities among groups in agreement with a pairwise test of independence with Bonferroni correction; letters are ordered alphabetically in relation to their degree of selectiveness (a* = higher degree of selectiveness than the other trap–bait combination).



	SPRING									AUTUMN								
	p	TB		TV		VB		VV		*p*	TB		TV		VB		VV	
Apoidea	0.763	0.24	a	0.00	a	0.26	a	0.14	a	< 0.01	0.33	b	0.04	ab	0.00	ab	0.00	a*
Vespa crabro	< 0.001	1.85	a*	9.33	b	1.03	a*	2.17	ab	0.798	0.56	a	0.56	a	0.38	a	0.70	a
Vespula spp.	0.047	0.18	a	0.33	a	0.46	a	0.72	a	0.339	0.56	a	1.16	a	2.00	a	1.16	a
Polistes spp.	0.017	0.06	a	1.00	a	0.13	a	0.31	a	1.000	0.00	a	0.00	a	0.00	a	0.01	a
Formicidae	< 0.001	3.91	a*	1622	c	6.08	a*	84.10	b	< 0.001	15.67	b	0.56	a*	0.00	a*	27.09	b
Diptera	< 0.001	16.7	a*	137	b	25.33	a*	33.03	ab	< 0.001	77.00	ab	60.32	a*	248	b	43.18	a*
Lepidoptera	< 0.01	3.27	ab	11.00	b	2.13	a*	1.45	a*	< 0.05	2.56	b	1.32	ab	1.75	ab	0.69	a*
Other groups	< 0.001	0.21	a*	1.67	ab	0.31	a*	1.66	b	0.365	0.33	a	0.24	a	0.13	a	0.12	a

* Trap-bait combinations that catch a lower number of non-target insects in proportion to *V. velutina* catches.

6.4 Discussion

With this experiment, we compared the effectiveness and the selectiveness of two typologies of traps and two sugar-based baits that are commonly used for trapping *V. velutina* in several countries worldwide, either for monitoring or control purposes. A difference has been demonstrated among trap–bait combinations in relation to the period of the year. In spring, traps equipped with common beer as bait were trapping a higher number of *V. velutina* independently of the model of trap, while traps with a commercial attractant (VespaCatch) were effective only with its respective trap model, which therefore foresee a higher equipment cost. Conversely, the effectiveness in trapping *V. velutina* of beer-based traps decreased in autumn, and only the VespaCatch trap and bait combination maintained a higher effectiveness towards *V. velutina*. The decrease of the effectiveness of beer-based baits during the autumn might be associated to different environmental temperatures that could modify the olfactive profile of the bait.

Despite the differences associated to traps and baits, the season on its own was not affecting *V. velutina* catches, and this underlines the low performance of these traps for controlling purposes during the autumn. Since the population of *V. velutina* colonies increases along the year, with production peaks of individuals that approximately occurs during the months of October–November (Rome et al. 2015), an increase in the number of hornets in the environment is expected in autumn. However, this increase did not occurred in the number of *V. velutina* trapped during this season, and this casts doubt on the effectiveness of these traps for controlling the species in autumn.

In analogy with previous studies (Monceau et al. 2012, Rodríguez-Flores et al. 2019, Rojas-Nossa et al. 2018), overall *V. velutina* catches represented 1.02% of the total trapped insects, suggesting that all trap–bait combinations will generate an important impact on native insects, especially if used at high densities for controlling purposes. However, an appropriate selection of both trap model and attractant, in relation to the period of the year, could slightly increase the traps performance. This is particularly important in the framework of a monitoring strategy in which a low trap density should still be used as a complementary method for early detecting the presence of *V. velutina* in new areas and thus for the implementation of control/eradication strategies based on early nest detection and destruction (Lioy et al. 2019, Laurino et al. 2020). For example, TB and VB traps in spring were either slightly more effective towards *V. velutina* (respectively 3.65% and 2.72%) and, at the same time, selective for several other groups in relation to *V. velutina* catches; notwithstanding a higher number of Diptera and Lepidoptera are trapped than those caught with traps equipped with the commercial bait, to which is however associated a significant attractiveness towards Formicidae. In autumn, VV traps performed better in terms of effectiveness towards *V. velutina*, however this trap also caught a relatively higher number of Diptera, Lepidoptera, Formicidae and *V. crabro*. Therefore, a local assessment of vulnerable species (e.g., red listed species) before the implementation of monitoring strategies may be useful for evaluating the distribution of traps in the environment, with the aim of minimising the bycatches effect of species already threatened by other external factors. Interestingly, overall Apoidea catches were quite restrained (0.09% for *A. mellifera*, 0.03% for *Bombus* spp. and 0.01% for other Apoidea), indicating a low or negligible impact on this group, which is one of the main taxa responsible for pollination. Therefore, future research for improving trapping performances should be mainly directed to minimise bycatches of Diptera, Lepidoptera, Formicidae and *V. crabro*.

Nevertheless, as previously suggested (Monceau et al. 2012), the rough number of non-target insects is not sufficient for recognising negative effects on the population dynamics. For example, it could be relevant to understand, among the mostly trapped non-target groups (i.e., Diptera and Formicidae) or among a potential vulnerable group (Lepidoptera), which species have been trapped, their conservation status and the proportion of trapped insects compared to the size of the population. One example that highlight this necessity is the fact that many Diptera and Formicidae that were trapped in the two study sites are exotic species, such as the spotted wing drosophila (*Drosophila suzukii*) and the Argentine ant (*Linepithema humile*). In this case, bycatch of exotic species is not a negative result for biodiversity conservation. This may represent a complementary aspect that should be taken into account when planning future experiments on trap performances.

Another factor that could explain the high proportion of Diptera is the length of the sampling interval among checks and bait substitution. This interval has been selected to reflect the approaches generally adopted by the beekeepers for monitoring *V. velutina*, and thus understand the effects of trapping in the framework of the current procedures for monitoring the species. As the days increase, the number of dead insects in the trap increases as well while their conservation status decrease, thus this factor may attract a higher number of

Diptera. However, even with the sampling interval adopted, the mean values per trap of Diptera were considerably smaller than values from other studies (528.67 ± 578.67 individuals) that adopted a shorter sampling interval of 14–15 days and similar traps (Rojas-Nossa et al. 2018). Therefore, even with a longer sampling interval, the tested traps may have performed better in terms of selectiveness towards Diptera, despite this difference could also be related to different environmental and climatic conditions between the study areas (in this case Spain and Italy). This highlights the necessity to test, with a common protocol, the performance of baits and traps in all of the countries where *V. velutina* should be monitored, since results may change due to local characteristics.

In any case, the development of more selective traps and attractive compounds may provide an alternative for monitoring *V. velutina* or controlling the species with a less bycatch effect. An alternative to sugary-based traps is represented by pheromone traps, which have proved to be effective for monitoring and controlling several insect species (Welzel & Choe 2016, Short et al. 2017, Vick et al. 2020). Recently, a pheromone for attracting males of *V. velutina* has been discovered (Wen et al. 2017). This compound could potentially find an application in autumn for monitoring the presence of males or, at a rather higher trap density, also for mating disruption strategies (Monceau et al. 2014a). On the contrary, compounds associated to honey bee colonies (pollen and honey) and the honey bee aggregation pheromone have proved to be attractive for *V. velutina* workers (Couto et al. 2014). These compounds may represent an effective alternative for monitoring or control purposes since the emerging of queens in spring and their performances should be tested against other attractive substances, such as pheromones or the baits tested in this study.

Some of the parameters that should be taken into consideration when planning future studies on trap performances are: trap model, with a focus on design, colour, number and dimension of trap’s entrance, number and dimension of escaping holes for avoiding bycatches of smaller species; bait typology, volume and changes in its composition in relation to the sampling interval; period of the year; sampling intervals between bait's replacement; *V. velutina* density; local characteristics (e.g., climatic conditions, land-use) surrounding the experiment. Testing these parameters in several countries and several areas within each country, with common protocols, and with the possibility to identify trapped insects at the species level, including other taxa rather than Hymenoptera, would contribute to improve trap performances.

6.5 Conclusions

This experiment provides information on the effectiveness of sugary-based traps for catching the invasive hornet *V. velutina* and their potential effects on non-target insects, taking into account the seasons (spring and autumn) in which sugary-based traps are mostly used. Differences between traps and baits were demonstrated: in spring, traps equipped with common beer as bait were both more effective and selective; in autumn, higher performance in terms of both effectiveness and selectiveness were obtained by just one of the four trap–bait combinations. Bycatches of Apoidea were negligible, while other groups were more represented, thus further selective trapping methods should be investigated in the future, particularly when traps are used at a high density for control rather than for monitoring purposes.

Supplementary material

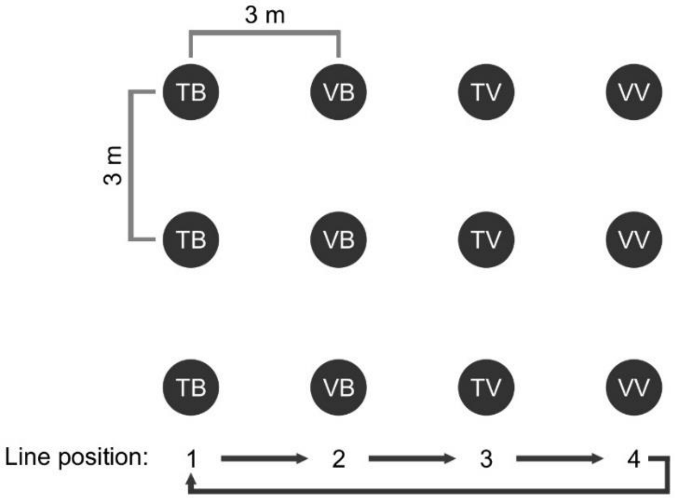


Figure S1. Trapping structure of each sampling site: TB, bottle trap with TapTrap and common beer as bait; VB, VespaCatch trap and common beer as bait; TV, bottle trap with TapTrap and VespaCatch attractant as bait; VV, VespaCatch trap and VespaCatch attractant as bait. At every check, baits were renewed and trap clusters shifted of one line position.

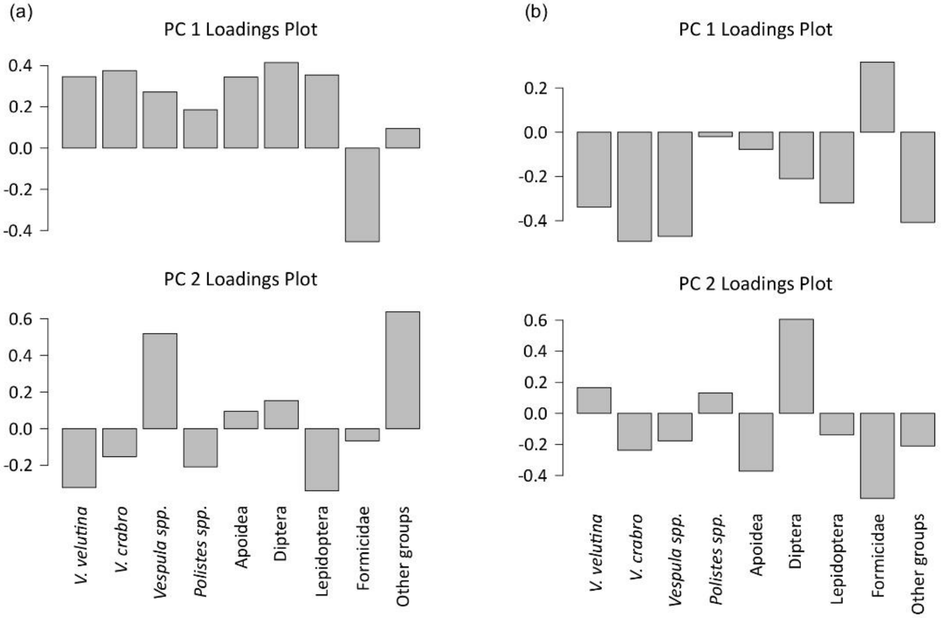


Figure S2. Loading plots of the first and second components of the PCA analysis on spring (a) and autumn data (b).

Table S1. Contribution of the variables to the first, second and third components of the PCA analysis on spring and autumn data.

Season	Species/Group	PC1	PC2	PC3
Spring	*Vespa velutina*	11.99	10.36	0.39
	Vespa crabro	14.08	2.33	7.92
	Vespula spp.	7.39	27.03	0.62
	Polistes spp.	3.45	4.35	79.14
	Apoidea	11.80	0.89	2.30
	Diptera	17.19	2.34	0.88
	Lepidoptera	12.58	11.58	8.58
	Formicidae	20.63	0.45	0.06
	Other groups	0.90	40.68	0.10
Autumn	*Vespa velutina*	11.47	2.74	25.00
	Vespa crabro	24.36	5.63	1.28
	Vespula spp.	22.18	3.14	0.06
	Polistes spp.	0.04	1.69	23.40
	Apoidea	0.60	13.84	15.40
	Diptera	4.43	36.67	6.25
	Lepidoptera	10.25	1.90	18.70
	Formicidae	10.02	29.97	6.30
	Other groups	16.64	4.43	3.61

7.

Introduced *Vespa velutina* does not replace native *Vespa crabro* and *Vespula* species in Italy

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Manuscript submitted for evaluation

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Abstract: Invasive alien species can replace native species with similar ecological requirements, living in their invaded range. For this reason, besides its economic impacts, *Vespa velutina* is raising concerns in Europe, as it can potentially outcompete and replace native wasps. Nevertheless, most evidence for this competition was based on laboratory experiments, and on considerations about the biology and ecology of *V. velutina* and native Vespidae. No field study explored how the abundance of *V. velutina* affected that of native Vespidae, as expected in case of competition. We analysed how the abundance of *V. velutina* influenced that of *Vespa crabro*, four years after the arrival and establishment of *V. velutina* in our study area, in Italy. Moreover, we compared the abundances of three native Vespidae (*V. crabro*, *Vespula vulgaris* and *Vespula germanica*), between our study area and an adjacent uninvaded area with similar environmental conditions. Bayesian Generalized Linear Models revealed that the abundance of *V. velutina* and *V. crabro* was positively associated, where *V. velutina* was scarce. Covariation disappeared only at those trapping sites where *V. velutina* was extremely abundant. The abundances of *V. crabro*, *V. vulgaris* and *V. germanica* were similar between the invaded and the uninvaded area. Overall, our findings indicate that native Vespidae probably avoided or minimised competition with *V. velutina*, at least in its invaded range in Italy. The presence of *V. velutina* did not lead to an evident replacement of *V. crabro* and *Vespula* species.

Keywords: Asian yellow-legged hornet; European hornet; impacts; invasive species; inter-specific competition; niche overlap

7.1 Introduction

Biological invasions are a global driver of change, whose frequency and magnitude are increasing, due to the extended global circulation of people and trades (Simberloff et al. 2013, Seebens et al. 2017). Invasive alien species can affect the population dynamics of native species, in their invaded range, sometimes to the point of their complete replacement (Mckinney & Lockwood 1999, Säterberg et al. 2013), with consequences for communities and ecosystems (Kumschick et al. 2015, Cameron et al. 2016, Carbonell et al. 2017, Stoett et al. 2019). Among alien terrestrial insects, social wasps are particularly successful invaders (Beggs et al. 2011), which were found to outcompete native arthropods and produce large-scale ecological changes on many different occasions (Beggs 2001, Snyder & Evans 2006). This success depends upon the biological traits of social wasps, such as their high reproductive rates, their dispersal abilities, and their flexible habitat and dietary requirements (Moller 1996, Beggs et al. 2011).

The European invasion of the Asian yellow-legged hornet (*Vespa velutina*) is a good example of how social wasps can become successful invaders. Following its introduction to France, in 15 years the species spread and established viable populations across Central and Mediterranean Europe (Arca et al. 2015, Laurino et al. 2020). Such a rapid invasion was due to the capacity of *V. velutina* to use natural and human-mediated dispersal (Robinet et al. 2019). The invasion of *V. velutina* in Europe raised various concern, mostly related to beekeeping (Requier et al. 2019, Laurino et al. 2020) or the economic cost of its management (Barbet-Massin et al. 2020), and in 2016 the species was included in the first list of invasive species of Union concern (EU Regulation n. 1141/2016). However, while available evidence about the socio-economic impacts of *V. velutina* was sufficient to its inclusion in European policymaking, its impacts on native insects other than honey bees remained relatively unexplored.

V. velutina has a semi-specialised diet, centred on honey bees and other insects including social wasps (Villemant et al. 2011b, Monceau et al. 2014a, Islam et al. 2015). Due to its food spectrum, it has been hypothesized that *V. velutina* could well compete with native European Vespidae, at least with those species that have similar ecological requirements (Beggs 2001, Crowder & Snyder 2010, Monceau et al. 2014a), like in other parts of its invaded range (e.g. Japan, Ikegami et al. 2020). In Mediterranean Europe, these could belong to the genus *Vespa*, *Vespula*, *Dolichovespula* or *Polistes*. Furthermore, *V. velutina* is particularly likely to be a successful competitor for the native congener, the European hornet (*Vespa crabro*), due to: *i)* the considerable dietary overlap for protein and sugar resources (Cini et al. 2018); *ii*) smaller levels of boldness, exploration and activity scores for *V. crabro* queens (Monceau et al. 2015a); *iii*) a later seasonal emergence of *V. crabro* compared to that of *V. velutina*, which could then early exploit food resources (Monceau et al. 2015b); *iv*) partial overlap (Bessa et al. 2016, Franklin et al. 2017) and possible competition (Spradbery 1973, Edwards 1980) in nesting site preferences, although *V. crabro* is restricted to cavities or sheltered sites; v) higher reproductive potential of *V. velutina* queens (Poidatz et al. 2018c).

Even by not considering apparent competition, for example mediated by a pathogen (Strauss et al. 2012), *V. velutina* seems to be capable to directly compete with *V. crabro*. Laboratory studies offer the basis for hypothesizing this competition (Cini et al. 2018), but evidences from field-based studies are scarce and limited to conclusions based on overlap in temporal distribution or traits (Monceau et al. 2015b, Kwon & Choi 2020), or derived from the evaluation of habitat requirements and the spatial distribution of the two species (Choi et al. 2012, Bertolino et al. 2016, Monceau & Thiéry 2017, Rojas-Nossa et al. 2018, Rodríguez-Flores et al. 2019). Furthermore, a recent analysis on interspecific hierarchies revealed that *V. crabro* is able to outperform *V. velutina* (Kwon & Choi 2020) in controlled conditions.

In this study, we aim to test if the abundance of *V. velutina* influenced that of *V. crabro*, in an Italian valley where *V. velutina* was well-established at the time of the study. We explicitly hypothesized that *V. velutina* had a causal effect over *V. crabro*, due to niche overlap. Notably, we tested for the following hypothesis: H_1) the abundance of *V. velutina* negatively influenced the abundance of *V. crabro*. To identify this causal effect in an observational setting, like our field study, where species were not manipulated, we accounted for spurious correlation by controlling for relevant environmental confounders. We also evaluated whether the abundances of multiple native Vespidae, *V. crabro, Vespula vulgaris* and *Vespula germanica*, differed between trapping sites in the invaded and the non-invaded area. Due to the pressure exerted by *V. velutina*, we expected that: H_2) the abundances of native wasp species in the invaded area were lower than those in the non-invaded area.

7.2 Materials and Methods

7.2.1 Study area and data collection

The study was carried out in the western Liguria, Italy, in an area that borders with France (Fig. 1). The climate zone is Mediterranean (Cs following Köppen Climate Classification) with dry summer and cold and wet winter and an average annual temperature of about 15 °C. Initially, two study areas were selected, corresponding to two river basins, with a distance between them of about 50 km. The two areas shared similar topographical characteristics and land cover, being covered mostly by young woodlands. The two basins consisted of river valleys with a length of about 20 km, spanning from mountains to the coast, and including an elevation range between 0 and 1300 m a.s.l. At the time of the study, in 2018, one basin had not been invaded by *V. velutina* yet, with few records of individuals and none detected nests, while the other one had been widely colonised by *V.*

velutina at least since 2015. Indeed, in the invaded basin, 103 *V. velutina* nests were detected in the year when the experiment was carried out. These areas have been selected for their wide range of elevations and land covers, for having a good road network and, most of all, for their location with respect to the diffusion of *V. velutina*.

For each river basin, we selected 60 sampling points based on a stratified sampling design that considered the following criteria: *i)* land cover, classified upon the Corine Land Cover classification (woodlands, urban and agricultural areas); *ii)* elevation, with areas divided into three classes of 250 m between 0 and 750 m a.s.l.; *iii)* road network proximity, for experiment feasibility reasons. We considered 750 m a.s.l. as the upper limit for *V. velutina* nesting in Mediterranean areas (Villemant et al. 2011a, Bertolino et al. 2016, Rodríguez-Flores et al. 2019).

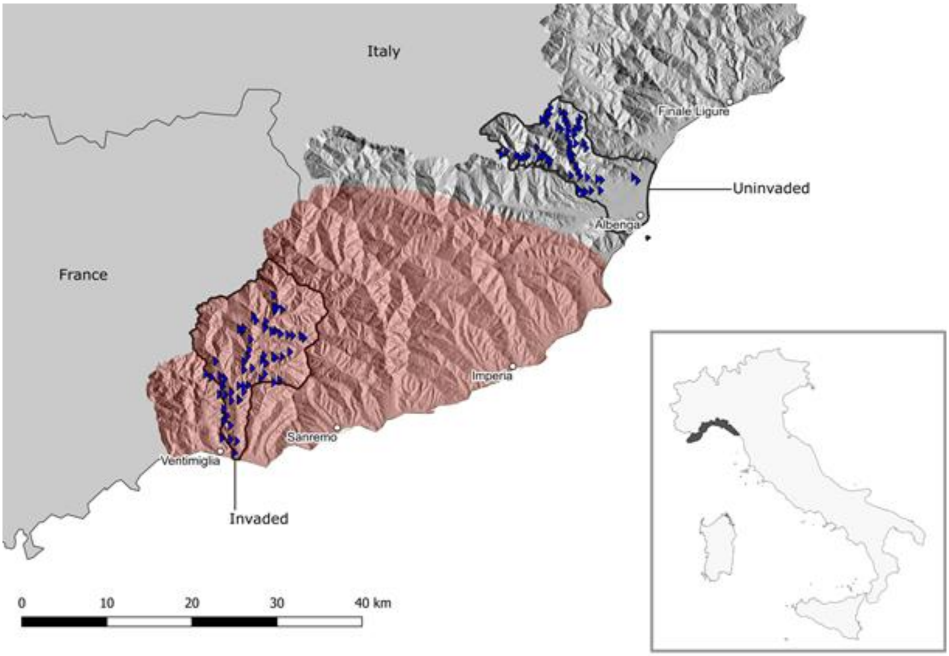

Figure 1. Location of the study region and of the two basins (*V. velutina* invaded/uninvaded) where the sampling was performed. Triangles indicate the position of the sampling traps. The red-brown area is the area colonised by *V. velutina* before the experiment was carried out according to a range analysis of *V. velutina* colonies (see Bertolino et al. 2016 and Lioy et al. 2019 for insights on the methodology).

The study lasted from the end of August until the end of November 2018. Sampling points were visited approximatively every two weeks, in relation to climatic conditions. In both valleys, sampling of Vespidae was carried out with bottle traps commonly used for monitoring social wasp species. These were transparent water bottles in PET rigged with a patented closure, activated with 0.2 l of beer as bait, and they were suspended with an iron wire at about 1.7 m off the ground (Demichelis et al. 2014). Those traps are one of the most widely used tools for hymenopterans trapping (Bacandritsos et al. 2006, Dvořák & Landolt 2006, Sorvari 2013, Lioy et al. 2020b). Sampling lasted 81 days in the invaded valley and 88 days in the uninvaded one. At every sampling visit, we emptied the traps and renewed the bait. Collected Vespidae specimens were recorded, identified to species level by means of a dichotomous key (Buck et al. 2008) and then deposited in the collection of the Department of Agriculture, Forest and Food Science of the University of Turin.

7.2.2 Relationship between *Vespa crabro* and *Vespa velutina*

To better highlight the relationship between *V. crabro* and *V. velutina*, which could have been masked by the absence of the latter in the uninvaded area, we first used data from traps in the invaded area only. We calculated the cumulative abundance of the two species at each trap, by considering only those traps who sampled for more than 70 days (n = 58), to avoid temporal mismatching. Then, we calculated daily abundances for the two species, by dividing trap-specific cumulative abundances per the trapping effort of each trap, in days. Daily abundances were then centred and standardized (Schielzeth 2010).

In this research, we adopted a causal inference framework, to equate the association between *V. velutina* and *V. crabro* to the causal effect of *V. velutina* abundance to that of *V. crabro*. As we already specified in the introduction, *V. velutina* is much more prolific and supposed to outcompete *V. crabro*, then we expected that the association between the two species, in a short timespan like the one of our study, will reflect a directional causal effect. Usually, the coexistence of two species in time, or the facilitating effect of the species A over the species B, is reflected into a positive association between their abundances, in cross-sectional data. On the other hand, when species A outcompetes species B, their abundances are usually negatively associated, or there is a non-linear association, with values of A which at some point stop being positively associated to those of B (Reitz & Trumble 2002, Kumschick et al. 2015).

To identify causal effects in observational settings, where data cannot be manipulated, it is important to control for potential confounders (the "back-door criterion", Pearl 1995, Pearl & MacKenzie 2018), which could affect both the treatment (*V. velutina*) and the outcome variable (*V. crabro*). Based on the available literature, we included the following variables as potential confounders: the median Normalized Difference Vegetation Index (NDVI), the average number of nests of *V. velutina* around the traps between 2016 and 2018, the median slope and aspect values of the terrain around the trap, the elevation of the trap, the Euclidean distance between the trap and the nearest water body, the average density of bee colonies in the municipality where the trap was located, the area covered by olive groves around the trap and the diversity of land cover types around the area. NDVI, the average number of nests, median slope and aspects, olive groves coverage and land cover diversity were calculated over a 500 m radius around each trap. The rationale for covariate inclusion and our causal directed acyclic graph (DAG) is provided in the supplementary material (Appendix S1).

To estimate the causal effect of the abundance of *V. velutina* over the abundance of *V. crabro*, we adopted a Bayesian Generalized Linear Model with a Gamma distribution of the error, a log-link and a moderately informative prior distribution for regression coefficients (Lemoine 2019), standardizing both predictors and the response variable. The model was fitted with four MCMC chains with 5000 iterations and a burn-in of 1000 iterations each. To explore model fitting, we checked for particular patterns in the association between standardized model residuals and fitted values. We also tested for spatial correlation in model residuals, by inspecting the Moran's semivariogram. A complete description of model fitting and diagnostics is available in the supplementary material (Appendix S1).

7.2.3 Differences in the abundance of native Vespidae between the invaded and the uninvaded area

To obtain a more comprehensive picture about the impact of *V. velutina* over native wasps, we compared the abundance of three native species of Vespidae (*V. crabro*, *V. germanica* and *V. vulgaris*) between the invaded and the uninvaded area.

Based on a k-means cluster analysis of environmental covariates surrounding the traps at the two areas, we identified two different clusters of trapping sites, characterized by different environmental conditions. However, the two clusters had a very similar distribution between the two areas, indicating that, overall, environmental conditions between the two areas did not differ markedly (Appendix S1). The environmental similarity between the two areas enabled us to draw conclusions about the effect of the long-term presence of *V. velutina* over the abundance of the three native species. This approach was adopted since the number of traps which did not caught any individual of *V. germanica* (n = 35) and *V. vulgaris* (n = 21) was too high for modelling their association with *V. velutina* in the invaded area, like in the case of *V. crabro*. Moreover, by considering data from the two areas, we also had a secondary source of information about the competition between *V. velutina* and *V. crabro*, which could integrate the findings about the co-occurrence of the two species measured at trapping sites.

As we did not have any baseline knowledge to hypothesize expected differences between areas, nor to calculate statistical power, we did not carry out any statistical test to see whether differences were significant. However, we explored the distribution of catches between the two areas, through boxplots and calculated the overlap of their distributions, through a kernel analysis, to see how the distributions of daily catches for the three species were similar between areas.

7.3 Results

A total of 6632 Vespidae were collected in the two valleys, belonging to five species: *V. crabro* (n = 4721), *V. velutina* (n = 1452), *V. germanica* (n = 317), *V. vulgaris* (n = 141) and *Dolichovespula media* (n = 1). In the invaded area, *V. crabro* was always dominant over *V. velutina* (percentage among Vespidae respectively 62.6% and 33.4%) and the two hornet species were caught in all traps, except for 1 and 2 traps respectively for *V. crabro* and *V. velutina*. Few individuals of *V. velutina* (n = 26) were caught in the uninvaded area. Focusing on the effect of *V. velutina* on *V. crabro*, our best candidate model explained approximately 44.8% of the variability in the abundance of *V. crabro*. We did not detect any pattern when comparing model residuals to fitted values, and the Moran's semivariogram did not indicate the existence of isotropic spatial correlation between the observations (Appendix S1). The model had a quadratic polynomial term linking the abundance of *V. velutina* to the abundance of *V. crabro*. Initially, the relationship between the two species was moderately positive, however, for high values of *V. velutina,* the two species did not covary anymore and the curve reached a plateau (Fig. 2).

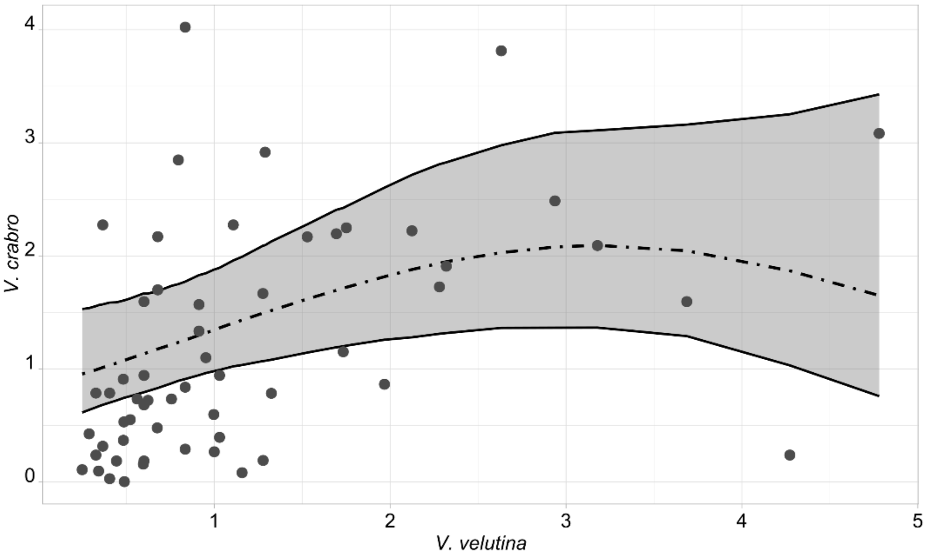

Figure 2. Marginal effects of the daily catches of *V. velutina* over the daily catches of *V. crabro*, in the invaded area.

K-means cluster analysis revealed that the environmental characteristics of trapping points between the invaded and the uninvaded area were relatively similar, and that the two areas could be compared in their distribution of daily catches for the three species. The three species had a similar distribution of daily catches between the two areas, with a substantial overlap (*V. crabro* = 65.35%; *V. germanica* = 40.42%; *V. vulgaris* = 50.39%). Moreover, abundances of *V. crabro* and *V. vulgaris* were higher in the invaded area (mean ± sd, *V. crabro* = 0.53 ± 0.45; *V. vulgaris* = 0.02 ± 0.03) than in the area without *V. velutina* (*V. crabro* = 0.41 ± 0.50; *V. vulgaris* = 0.01 ± 0.01) (Fig. 3).

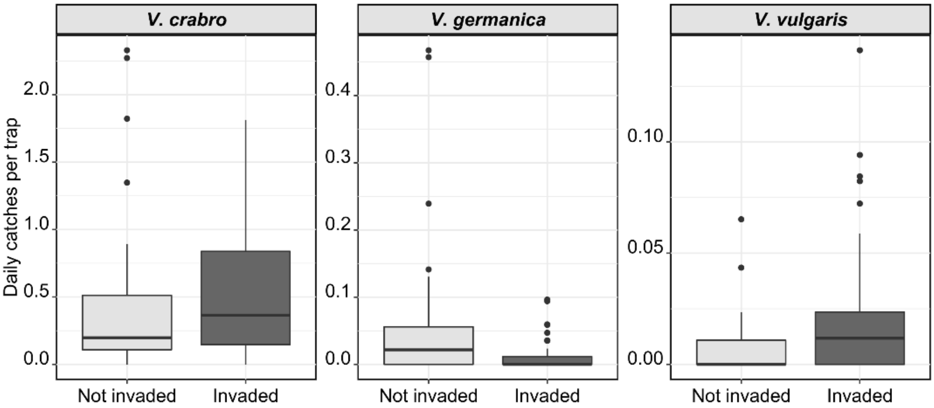

Figure 3. Daily catches of native *V. crabro, V. germanica* and *V. vulgaris* between the not invaded and the invaded areas.

7.4 Discussion

This study constitutes a first attempt, for Mediterranean biotopes, to verify whether invasive alien *V. velutina* and native Vespidae, especially *V. crabro*, negatively covary in their abundances as expected in the case of direct competition. While we expected native *V. crabro* to steadily decline with increasing abundances of *V. velutina*, we found a positive, non-linear, association between the two species, when their numbers were low. Then, at higher abundances, their covariation was weak and characterized by wide credibility intervals. Moreover, when comparing catches between the invaded and the uninvaded areas, we noticed two aspects: *i)* abundances of *V. crabro* were similar between the two areas (and actually higher at the invaded one), and *ii)* abundances of *V. crabro* actually exceeded those of *V. velutina*, contrary to previous studies from Spain and France (Monceau et al. 2013b, Rodríguez-Flores et al. 2019). Taken together, findings from our statistical model and from our comparison of invaded and uninvaded areas, might indicate a lack of competition between the two species, at least at low abundances. This conclusion would align with existing research about direct competition between alien and native species, indicating that competition increases with the number of individuals, due to an increase in the number of inter-specific interactions and a fixed asset of available resources (Ricciardi 2003, Kumschick et al. 2015). Concerning invasive alien social wasps, for example, some studies showed that competition with native species was more pronounced at higher abundances (Beggs 2001). Unfortunately, we observed very few trapping sites characterized by high abundances of *V. velutina*. As a consequence, our model had wide credibility intervals which do not enable us to draw robust conclusion about competition between the two species. Therefore, we do not exclude that the competition between the two species, in contexts where *V. velutina* is very abundant and can fully exploit its phenology and reproductive traits, could be detrimental for the abundances of *V. crabro*.

In this study, we compared also the abundance of Vespidae species between two close areas of NW Italy. The two areas had similar environmental conditions but differed in the presence of *V. velutina*. The comparison between invaded/uninvaded areas is an approach widely adopted to detect the effect of biological invasions (Vilà et al. 2010, Kumschick et al. 2015), also for invasive hymenopteran species (Gotelli & Arnett 2000). The distribution of daily catches of *V. germanica* and *V. vulgaris* showed a considerable overlap between the two areas, as it was noticed for *V. crabro*. These outcomes do not indicate a replacement of native Vespidae by *V. velutina*, at least in the studied area. Although these results may be influenced by the ecological peculiarities of the study area, they highlighted that *V. velutina* impacts are context-dependent, thus its management should not be generalized across all the invaded area but fitted accordingly.

Overall, the results provided in this study bring to the conclusion that, after four years of presence of *V. velutina*, detrimental effects on *V. crabro* are negligible. We advance two non-exclusive hypotheses to explain such lack of competition effects. The first one is that niche overlap between the two species is partial, thus *V. crabro* can escape from competition. Competition usually occurs among close genetically taxa since they share common traits (Reitz & Trumble 2002, Violle et al. 2011), and it leads to the replacement of the less competitive one. In case of niche differentiation, also genetically related species might even display sympatry (Stubbs & Wilson 2004, Aguilera et al. 2013, Bertolino et al. 2013). In analogy with previous studies, we found that the two species used the space similarly, without any clear differentiation in habitat niche (Choi et al. 2012, Rojas-Nossa et al. 2018). However, areas above 600 m. a.s.l have been displayed as more adapted for the colonisation of *V. crabro* (Rodríguez-Flores et al. 2019), while *V. velutina* prefers low altitude areas (Bertolino et al. 2016, Monceau & Thiéry 2017). Such high areas might represent for *V. crabro* both a refuge from competition and a source for new colonisation, especially in areas where it may have been outcompeted. In this study, *V. crabro* might be advantaged since the area is mainly mountainous. The later life cycle of *V. crabro* compared to the one of *V. velutina* has been described either as a mechanism that might favour the alien hornet, due to its earlier access to foraging resources, or as a mechanism that might avoid competition through time partitioning (Monceau et al. 2015b). This last mechanism seems more consistent with the likely lack of competition that we found out. Focusing on competition for food resources, the two hornet species have similar food preferences: both species prey preferentially honey bees (Monceau et al. 2013b, Cini et al. 2018). Nevertheless, an inter-specific competition in predatory activity in front of the hives has not been found (Choi & Kwon 2015, Bonnefond et al. 2020). In addition, the two hornet species are both semi-specialist (Matsuura 1991, Cini et al. 2018), thus a shift in prey target might be a strategy to avoid competition as it was already demonstrated for other arthropods (Wipfli & Merritt 1994). *V. velutina* showed to change food spectrum depending on the nesting site habitat (Villemant et al. 2011b) and a similar trait is predictable for *V. crabro*. Woods and low input agricultural areas, that usually host richer insect communities (Krämer et al. 2012, Medeiros et al. 2019), dominate the study area, so a great variety of potential prey for both hornet species is expected.

The second non-exclusive hypothesis, that we advance, is that there is a competition between the two species, although *V. velutina* is not effectively able to out-compete *V. crabro*. The latter species has proved to have a greater fighting ability, linked to its larger body, which brings *V. velutina* to avoid direct competition with *V. crabro* (Kwon & Choi 2020). *Vespa mandarinia japonica*, which is the biggest Vespidae species as well as the more aggressive in direct fights (Kwon & Choi 2020), is probably acting as an ecological barrier to the spread of *V. velutina* in Japan (Ikegami et al. 2020). A solid population of *V. crabro*, operating as ecological barrier, could be among the reasons that led *V. velutina* to spread in Italy rather slower comparing with the alien hornet expansion in France (Bertolino et al. 2016, Lioy et al. 2019). The nature of interaction between the two species are likely to change depending on their relative abundance, the environment, and the species life-history (Kumschick et al. 2015). *V. velutina* should have constant traits across Europe because of the low genetic variance, the opposite for *V. crabro*, whose reaction to competition should be more variable and population-dependent (Monceau et al. 2015a).

7.5 Implications and perspectives

Our study could be regarded as a field validation of previous experimental studies, exploring the potential competition between *V. velutina* and Vespidae species. This study investigates, for the first time to our knowledge, the effect of the invasion of *V. velutina* over the abundance of native European Vespidae, in a natural environment. *V. velutina* was included in the European list of invasive species of Union concern, since risk assessment acknowledges the impact of *V. velutina* upon honey bees (Marris et al. 2011). Nevertheless, a comprehensive evaluation of risk regarding other species was not possible at that time because of the lack of research addressing this issue. This study provides first field-based knowledge on *V. velutina* impacts on native European wasps. Despite the findings that indicate a lack of negative effects due to *V. velutina*, a long-term monitoring programme of wasp populations should be implemented to detect any potential changes in the interaction with *V. velutina*, and it could provide baseline data for building effective conservation activities. Presently, many European countries have adopted *V. velutina* nest destruction as a primary measure to limit the spread of the species in uninvaded areas and to soften damages to honey bees. This productive sector, which also provides fundamental pollination services, has been identified as in risk, since many diseases, socio-economic and cultural conditions are making such activity less profitable (vanEngelsdorp & Meixner 2010, Jacques et al. 2017). Nevertheless, it has been reported an increase in the number of honey bee colonies in the Mediterranean area, while wild pollinators are steadily declining (Potts et al. 2010, Herrera 2020). On the opposite, the decreasing trend of honey bee colonies, pushed by the further threat of *V. velutina*, might intensify the predation pressure on honey bees and wild insects, especially pollinators, with potential consequences to their conservation and to the pollination ecosystem services. We expect that such changes in predation pressure may exacerbate the interaction between *V. crabro* and *V. velutina*, leading to a possible competition between the invasive and the native species. Therefore, future research should be focused in exploring the mediated effect of honey bee abundance and distribution on the interaction between the two hornet species. At the same time, our study was conducted in a mountain area, where ecological conditions may be favourable to *V. crabro*. We therefore suggest that further investigations are needed to evaluate if species interactions lead to different outcomes in lowland areas.

8.

Conclusions

The results and findings described in the chapters of this PhD thesis provide latest knowledge on the biology, ecology, spread modalities and impacts of the invasive hornet *V. velutina*, as well as new insights on methods, techniques and strategies that could be used for monitoring and controlling the species.

The review on *V. velutina* provides an overview on its diffusion in Europe and its impacts on honey bee colonies, other native insects, and the possible consequences on human health, but it is also delineating the framework for *i)* an early warning and rapid response system for preventing the establishment of the species and *ii)* an integrated pest management strategy for controlling or managing expanding populations.

The multiannual data collection was fundamental to understand the main parameters that influence the spread and distribution of *V. velutina* colonies in the environment, that depends mainly on elevation above sea level and on distance from source sites (colonies of the previous years from which new founder queens originated), besides land cover, or distance from water resources. The main variables explaining *V. velutina* distribution can be used to model and forecast the spread of the species in consecutive years, for identifying the areas with higher probabilities of colonisation for an effective allocation of monitoring and control efforts.

Indeed, areas likely to be colonised should be carefully monitored for early detecting the presence of *V. velutina*, and then establishing effective control measures. Monitoring is typically performed with the collaboration of the beekeepers, that could detect the presence of the Asian yellow-legged hornet during their routinely inspections to the apiaries, and by the use of baited traps for wasps. However, the performance of traps and baits customarily used for this purpose changes in relation to different parameters and, more importantly, none of the tested combinations is assuring a high selectiveness, since overall catches of *V. velutina* were scanty compared to bycatches of non-target insects. This highlights the urgent necessity of developing more selective trapping methods for monitoring or controlling purposes (e.g. in the case of queen’s spring trapping), to avoid a negative side-effect on native insect populations.

The two technologies for locating nests that have been tested (thermal imaging infrared cameras and harmonic radar) are both effective, despite some limitations could occur due to environmental characteristics or operative conditions. The application of these technologies is particularly indicated for the management of small and isolated invasive outbreaks that could occur anywhere in Europe, where environmental and climatic suitability for *V. velutina* exist. Indeed, in these cases, the detection of nests before the reproductive phase of the colonies could change the invasive scenario and potentially lead, at the local level, to the eradication of the invasive species before the establishment of viable populations, and thus allows avoiding the ecological and economic impacts associated to the presence of *V. velutina*. The harmonic radar technology could also be used for other research purposes, leading to new discoveries in the field of insect science. For example, its application in tracking the flight of *V. velutina* brought to discover new information concerning the flying features of the species and its foraging range.

Moreover, even if *V. velutina* is a threat for honey bees and wild bees, it is not confirmed that the species could generate negative effects on wasp communities due to competition or predation, at least in areas recently colonised by the species. This may give hope to the European Vespidae, however long-term samplings are required for confirming this hypothesis.

Finally, the combination of these findings may contribute to improve plans and strategies that all European countries should develop in agreement with the EU Regulation on invasive species, for preventing the establishment of *V. velutina* or for controlling its expanding populations.

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Annex I

List of oral presentations and posters for presenting the results of this PhD research project at national and international conferences.

Oral presentations (speakers in bold and underlined):

[2020] **Lioy S**, Laurino D, Manino A, Porporato M - Life StopVespa, a pilot project for developing a comprehensive management strategy for the Asian yellow-legged hornet *Vespa velutina*. 16th COLOSS eConference, 12-13 October 2020.

[2020] **Lioy S**, Laurino D, Manino A, Porporato M - Management of the invasive hornet *Vespa velutina* in Italy: from surveillance to early warning and control strategies. NEOBIOTA 2020 11th International Conference on Biological Invasions, Vodice, Croatia, 15-18 September 2020.

[2019] **Lioy S**, Laurino D, Manino A, Porporato M - The containment strategy for *Vespa velutina* in Italy: an integrated approach. International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2019] **Bertolino S**, Avagnina A, Lioy S - Does appearance really matter? The perception of Invasive Alien Species shaped by the species attractiveness. International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2019] **Carisio L**, Bianchi E, Lioy S, Porporato M - Does *Vespa velutina* impact on native insects? International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2019] **Romano A**, Capello M, Lioy S, Manino A, Porporato M - Effect of *Vespa velutina* queens trapping on honey bee colonies development. International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2019] **Lioy S** - Proposta di piano di gestione della *Vespa velutina* in Italia. International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2018] **Lioy S**, Bertolino S, Laurino D, Porporato M - *Vespa velutina* in Italy: a modelling approach to predict the new annual colonized area and improve management practices. European PhD Network "Insect Science" - IX Annual Meeting, Firenze, Italy, 14-16 November 2018.

[2018] Carisio L, Lioy S, Porporato M, **Manino A** - Survey of wild bee communities threatened by *Vespa velutina*. 8th Congress of Apidology, Ghent, Belgium, 18-20 September 2018.

[2018] **Lioy S**, Laurino D, Porporato M, Milanesio D, Saccani M, Maggiora R - The harmonic radar to track the invasive hornet *Vespa velutina*: a tool to improve the Early Warning and Rapid Response System for the species. NEOBIOTA 2018 10th International Conference on Biological Invasions, Dun Laoghaire, Dublin, Ireland, 3-7 September 2018.

[2018] **Lioy S**, Laurino D, Manino A, Porporato M - *Vespa velutina* in Italy: an update on the management activities and on the impact of the species on honey bee colonies and biodiversity. Velutina Task Force Meeting (COLOSS), Bilbao, Spain, 28-29 June 2018.

[2018] **Lioy S**, Bertolino S, Manino, Laurino, Porporato - The LIFE STOPVESPA project: establishment of an Early Warning and Rapid Response System and spatial containment of *Vespa velutina*’s populations in Italy. Alien squirrels and other IAS (Invasive Alien Species): impacts and comparison of management experiences, Perugia, Italy, 11-13 April 2018.

[2017] **Lioy S**, Porporato M - Spatial containment of *Vespa velutina* in Italy and establishment of an Early Warning and Rapid Response System. Platform meeting on Invasive Alien Species (IAS), Milan, Italy, 29-30 November 2017.

Posters:

[2019] Laurino D, Carisio L, Lioy S, Manino A, Bianchi E, Porporato M - Impact of *Vespa velutina* on honey bees and other pollinators. 46th Apimondia International Apicultural Congress, Montréal, Quebec, Canada, 8-12 September 2019.

[2019] Porporato M, Laurino D, Lioy S - LIFE STOPVESPA Project: control of *Vespa velutina* in Italy. 46th Apimondia International Apicultural Congress, Montréal, Quebec, Canada, 8-12 September 2019.

[2019] Lioy S, Laurino D, Manino A, Porporato M - An integrated approach for a strategy against *Vespa velutina* in Italy. 15th COLOSS Conference, Montréal, Quebec, Canada, 7-8 September 2019.

[2019] Capello M, Romano A, Lioy S, Manino A, Porporato M - Beer or not beer? Comparison of the attractiveness and selectivity between two types of traps and baits as a control tool for the invasive *Vespa velutina*. International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2019] Carisio L, Bianchi E, Lioy S, Manino A, Porporato M - Wild bee communities across *Vespa velutina* invasion gradient. International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2019] Porporato M, Laurino D, Romano A, Capello M, Avagnina A, Manino A, Lioy S - The experience of LIFE STOPVESPA reporting system up to 2018. International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2019] Viscardi A, Capello M, Romano A, Porporato M, Lioy S, Laurino D, Saccani M, Milanesio D, Maggiora R - Detection of Asian hornet (*Vespa velutina*) colonies using harmonic radar. International Conference *Vespa velutina* and other invasive invertebrates species, Turin, Italy, 22-23 March 2019.

[2018] Lioy S - The LIFE STOPVESPA project: progress and achievements. LIFE Platform meeting on invertebrates, Stirling, Great Britain, 18-19 September 2018.

[2018] Porporato M, Laurino D, Lioy S, Milanesio D, Saccani M, Maggiora R - Harmonic radar tracks *Vespa velutina* flight to nests. XI European Congress of Entomology, Napoli, Italy, 2-6 July 2018.

[2018] Carisio L, Manino A, Lioy S, Sottosanti G, Porporato M - Is *Vespa velutina* a treath to wild bee communities and pollination ecosystem service? XI European Congress of Entomology, Napoli, Italy, 2-6 July 2018.

[2018] Porporato M, Laurino D, Lioy S, Milanesio D, Saccani M, Maggiora R - Harmonic radar tracks *Vespa velutina* flight to nests. Velutina Task Force Meeting (COLOSS), Bilbao, Spain, 28-29 June 2018.

[2018] Carisio L, Lioy S, Capello M, Conti R, Porporato M - Monitoring of the *Vespa velutina* impact on Liguria wild bees communities. XI International Pollination Symposium, Berlin, Germany, 16-20 April 2018.

Annex II

List of the documents prepared for sharing the acquired knowledge with laypersons, stakeholders, and administrations, to promote the transferability of scientific findings for improving the management procedures of the species in Europe.

National Action Plan for the Asian yellow-legged hornet *Vespa velutina*

This document describes the procedures that should be applied in Italy for an effective management of *V. velutina* populations, and in particular the approaches to: *i)* establish surveillance strategies for detecting the presence of *V. velutina*; *ii)* establish early warning and rapid response systems for attempting eradication of new invasive outbreaks; *iii)* establish strategies for the long-term management of *V. velutina* in the invaded areas. The document has been prepared on assignment of the Ministry of the Environment to meet the obligations of the European Regulation on Invasive Alien Species (EU Reg. 1143/2014) and the corresponding national legislation (D.Lgs. 230/2017).

[2020] Lioy S, Bertolino S, Laurino D, Manino A, Porporato M - Piano di gestione nazionale del Calabrone asiatico a zampe gialle *Vespa velutina*. ISPRA - Istituto Superiore per la Protezione e la Ricerca Ambientale. 22 pp.

Effectiveness of rapid eradication attempts reported using NOTSYS notifications in preventing the spread of *Vespa velutina nigrithorax* in Europe

This document describes the environmental suitability of *V. velutina* in Europe based on the occurrences of the species in relation to climatic and anthropic variables, model the spread observed in three invaded countries (Italy, France and Spain) for generating an ensemble model of spread to predict the diffusion in other European countries (Belgium, Netherlands, Germany and UK), taking into account the predicted suitability. The results allow to understand the effectiveness of rapid eradication attempts reported using the notification system for IAS of Union concern, by comparing the actual distribution of the species with the forecasted distribution. The involvement in the preparation of this document is the result of the collaboration established with the UK Centre for Ecology & Hydrology for performing the abroad period foreseen by the Phd research project.

[2020] Hassall R, Purse BV, Barwell L, Booy O, Lioy S, Rorke S, Roy HE - Effectiveness of rapid eradication attempts reported using NOTSYS notifications in preventing the spread of *V. v. nigrithorax* in Europe. Technical note prepared by IUCN for the European Commission. 61 pp. In press.

Information on measures and related costs in relation to species included on the Union list: *Vespa velutina nigrithorax*

This document describes the measures and related costs that member states of Europe should apply for an effective prevention, eradication, control, and management of *V. velutina* populations.

[2019] Lioy S, Manino A, Porporato M - Information on measures and related costs in relation to species included on the Union list: *Vespa velutina nigrithorax*. Technical note prepared by IUCN for the European Commission. 33 pp.

The management strategy for *Vespa velutina* in Italy: an integrated approach

This document has been prepared for the beekeepers, to describe the diffusion of *V. velutina* in Italy, the management strategy that has been developed for limiting the spread of the species and the activities developed by the University of Turin in the framework of the Life StopVespa project.

[2020] Lioy S, Laurino D, Manino A, Porporato M - La strategia di controllo di *Vespa velutina* in Italia: un approccio integrato. L’Apicoltore Italiano, 4: 11-14.