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ORIGINAL ARTICLE

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.

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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.

Key words alien species; Asian yellow-legged hornet; monitoring; nest detection; remote sensing; thermography

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*., 2014).

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*., 2013; Monceau *et al*., 2014; 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 give 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*., 2014; 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.

Materials and methods

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 800–1400 nm wavelengths with a spatial resolution of 320 × 240 px. All thermal images were processed using the GORATEC Thermography Studio software.

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).

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.

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

The correlation between *TDI* and the qualitative visibility score values estimated in 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.

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 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.

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). 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).

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. These experiments 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*., 2014; 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*., 2018); 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.

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Disclosure

The authors have no conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject of this manuscript.

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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 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*

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

Figure legends

Fig. 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.

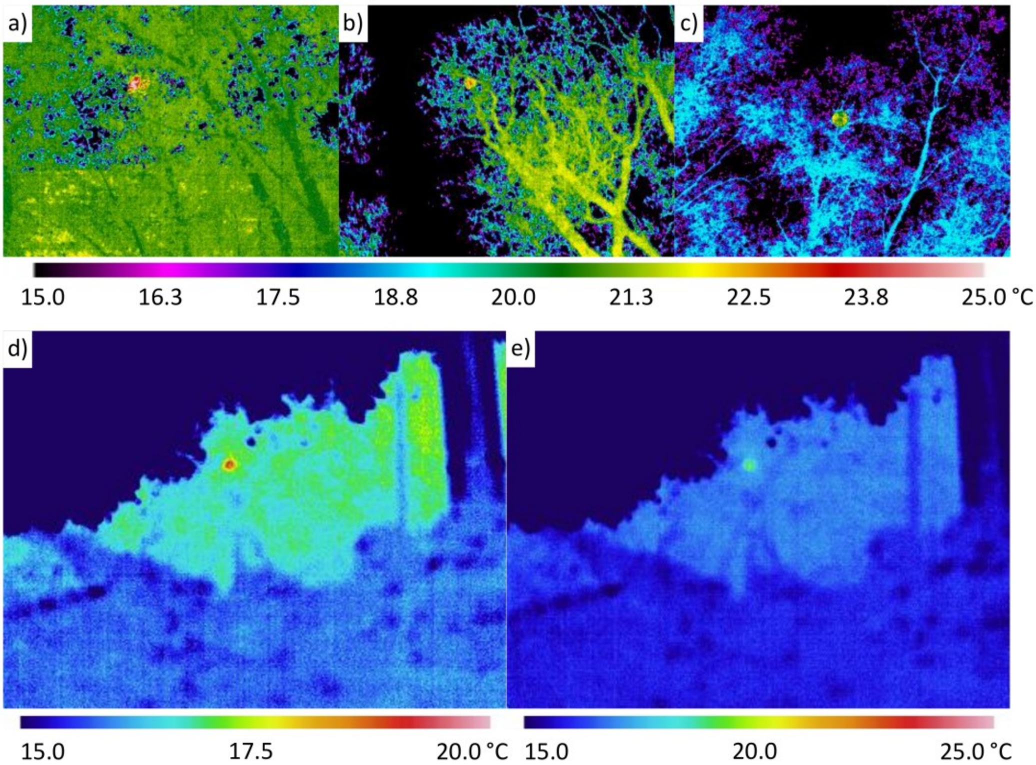

Fig. 2 Boxplot of the Thermal Detectability Index (*TDI*) in relation to the following variables: a) qualitative score of nest visibility (1 = not visible; 2 = poorly visible; 3 = clearly visible); b) *V. velutina* nests; c) time of the day when the sampling was performed (morning/evening); d) presence/absence 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.

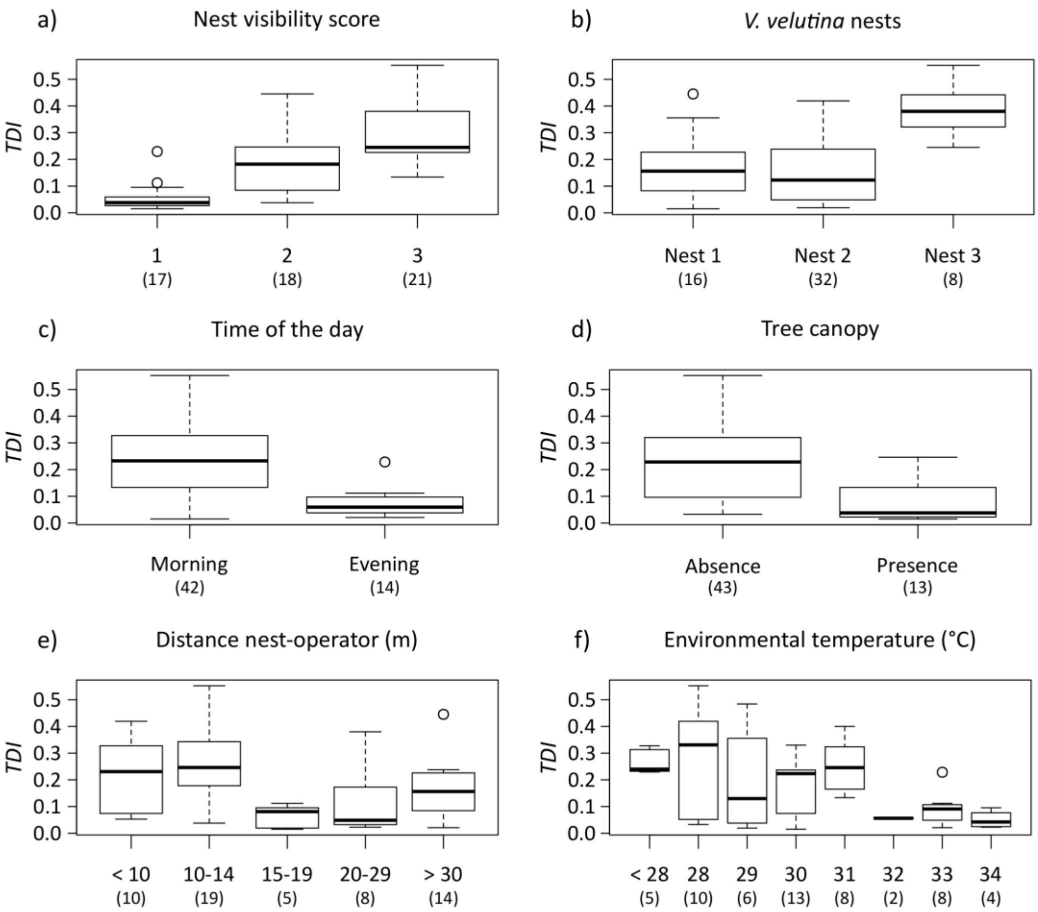

Fig. 3 Predicted values of the Thermal Detectability Index (*TDI*) in relation to the three variables of the LMM analysis: the time of the day (morning on the left and evening on the right); the distance between the nest and the operator in metres (on the *x*-axis); the presence (blue) or absence (red)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