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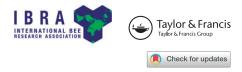
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Testing the efficacy of a thermal camera as a search tool for locating wild bumble bee nests

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Research into how bumble bee colonies respond to the stressors affecting their populations are currently studied in the laboratory using commercially reared *Bombus terrestris* colonies. Understanding how these stressors affect wild bumble bee colonies in the field would be a crucial step forward for the conservation of bumble bee species. Currently, visual cues are used to locate bumble bee nests, using human searchers looking for the worker nest traffic, but the limitations of this method mean that low numbers of nests are found and so a new method that looks to tackle these limitations is needed. Thermal cameras have been considered as a potential nest searching tool because they reduce the visual complexity of the environment by displaying a homogenized thermal landscape to the searcher. In this study, we compare the use of a thermal camera to human searches using two trials: (*i*) using inexperienced volunteers to search along the transect for a known bumble bee nest and (*ii*) using an experienced individual to search across a number of novel locations. We found thermal cameras are not a better nest detection technique than human searches, having low success rates across both trials. We discuss the limitations of thermal cameras as a technique and propose how the technology could be improved for future studies.

Keywords: Bombus; bumble bee; nest detection; thermal camera

Introduction

In order to conserve a species, it is important to understand how they respond to stressors at both the population and individual level. For bumble bees, we know that at the population level they are affected by multiple stressors (Goulson, Nicholls, Botías, & Rotheray, 2015). In eusocial species such as bumble bees, population-level effects are those that impact the number of colonies, as it is the colony as a whole which represents one reproductive unit (Ellis, Knight, Darvill, & Goulson, 2006). Understanding the mechanisms driving these population-level changes must be done at the individual colony level. This is currently not being done in the field due to limitations of current nest detection techniques.

Population-level effects are currently explored using genetic markers (Darvill, Knight, & Goulson, 2004; Herrmann, Westphal, Moritz, & Steffan-Dewenter, 2007). These have provided insight into broad concepts such as colony survival (Carvell et al., 2017; Goulson et al., 2010), foraging distances (Carvell et al., 2012; Knight et al., 2005) and a general outline of large-scale habitat preferences (Chapman, Wang, & Bourke, 2003; Wood, Holland, Hughes, & Goulson, 2015). Studies cannot be conducted at the colony level using these methods as they do not provide exact nest locations. Colony-level studies are currently conducted in the laboratory using commercial *Bombus* colonies (Gegear, Otterstatter, & Thomson, 2006; Gill, Ramos-Rodriguez, & Raine, 2012; Imhoof & Schmid-Hempel, 1999; Stanley et al., 2015). These studies, although useful, are not fully representative of wild bumble bee populations and are also heavily biased toward *B. terrestris*, and *B. impatiens* in the US, and so it is not clear how transferable these findings are for other wild bumble bee species. Field studies of wild bumble bee colonies would provide crucial evidence into how stressors affect colony fitness through impacting queen production, worker production, foraging behavior, and queen mortality.

Locating wild bumble bee nests in order to gather such data is challenging, due to the small nest sizes and the lack of advanced nest detection techniques. Bumble bee colony sizes generally range from 50 to 750 workers depending on the species (Prys-Jones & Corbet, 2011). Consequently, nest traffic, the movement of workers in and out of the nest entrance, is low (Goulson, O'Connor, & Park, 2018). This is a limitation of human searches which rely on these visual nest traffic cues (Lye, Osborne, Park, & Goulson, 2012; O'Connor, Park, & Goulson, 2012; Osborne et al., 2008). Trained sniffer dogs, which rely on olfactory cues to detect nests, were tested as an alternative technique but were no more successful than human searches (O'Connor et al., 2012; Waters, O'Connor, Park, & Goulson, 2011). Another key limitation to the human nest detection method is being unable to visually detect the nest

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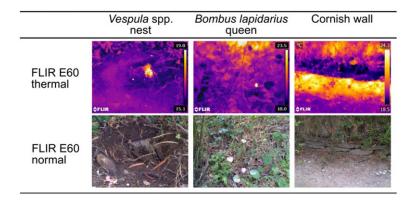


Figure 1. Differences between the thermal images taken using the FLIR E60 and standard human vision images. From left to right the images show: a Vespula spp. nest which has been dug up by a badger (*Meles meles*), a *Bombus lapidarius* queen crawling in the undergrowth, and a Cornish wall; a prominent feature of many of the gardens surveyed during the non-targeted searches.

traffic against a complex background, largely due to the presence of vegetation (Waters et al., 2011). In the current study, we tested a new method that uses heat cues to locate the nest traffic of wild bumble bee nests.

Thermal imaging cameras have been used to study wild animals since 1972 (Graves, Bellis, & Knuth, 1972). The majority of applications for wildlife detection have been for endothermic species, in particular mammals (Cilulko, Janiszewski, Bogdaszewski, & Szczygielska, 2013). Invertebrates are not commonly studied using thermal cameras due to many invertebrates being ectothermic and therefore having a small temperature differential between their body and the background. We feel thermal cameras have the potential to be a good tool for locating wild bumble bee nests as, unlike many invertebrates, social insects such as bumble bees are able to generate and maintain their own body temperatures (Heinrich, 1975; Stabentheiner & Schmaranzer, 1987) often above ambient temperature. Thermal cameras have already been used in some commercial applications to locate invertebrate pests; in the US, it is a key tool for locating termite infestations in domestic and commercial buildings (James & Rice, 2002), and it is also used to locate pest infestations in stored food products and tree plantations (Al-doski, Mansor, & Shafri, 2016; Manickavasagan, Jayas, & White, 2008; Nanje Gowda & Alagusundaram, 2013). Thermal cameras have been considered as a potential nest searching tool because they reduce the visual complexity of the environment by displaying a homogenized thermal landscape (Figure 1). Bee behavior has already been studied using thermal cameras, as tools to monitor the Asian giant honey bees' (Apis dorsata) thermal defenses against an invading wasp (Kastberger & Stachl, 2003), and recording the thoracic temperature of water-drinking honey bees (Apis mellifera) (Kovac & Schmaranzer, 1996). It is also currently being considered as a method for locating the aerial nests of the Asian hornet (Vespa velutina) (Keeling, Franklin, Datta, Brown, & Budge, 2017) in an attempt to control its spread into the UK and across Europe.

The overall aim of our study is to ascertain whether thermal imaging cameras can be used as a new tool for the detection of wild bumble bee nests through enhanced detection of nest traffic or the nest itself in the case of surface nesting species. We had two main objectives: (i) to test the ability of thermal cameras to locate wild bumble bee nests using inexperienced volunteers and (ii) to test the efficacy of thermal cameras compared to human searches using an experienced individual. We discuss the limitations of current thermal camera technology and make recommendations for future research.

Materials and methods

All surveys and experiments for this study took place in south west England (Lat 50°17'N, Long 4°48'W). Experiments were conducted using a FLIR E60 thermal camera (FLIR Systems, E60, 64501-0302). Two types of search method were performed: human-unaided visual surveys and thermal camera surveys (Figure 2). Humanunaided visual searches consisted of the surveyor walking along the transect and using their own visual observations to search for either the nest traffic emanating from a bumble bee nest, or the nest itself in the case of a surface nest. Thermal camera searches consisted of the surveyor walking the transect in the same way as above, but viewing the environment through the viewing screen of the thermal camera. Using this survey method, bumble bees were seen as white objects moving on the viewing screen. Once a white object was detected, visual observations could then be used to determine whether the detected object was a bumble bee. Using the thermal camera, we were most likely to detect the nest traffic rather than the nest itself. Vegetation around the nest may obscure detection of traffic at the entrance, but traffic should still be visible in spite of the vegetation. During both search methods, if a bumble bee was seen it was observed until out of

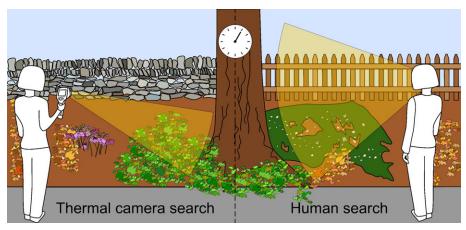


Figure 2. Thermal and human camera search method for the non-targeted searches. The individual is shown surveying an area from the path, which contains examples of different habitat features present during both the targeted and the non-targeted searches: dead leaves, vegetation, Cornish walls, fences, trees, and mossy banks. The individual would walk slowly along the path while continually searching for nests for five minutes. Human and thermal camera searches were performed consecutively. The horizontal field of view for each search method is represented by the yellow beams, 25° for thermal cameras and 60° for human binocular vision. Targeted searches used a similar method, but with a suggested five-minute minimum search time and 10-minute cutoff.

sight to determine whether or not the bumble bee was travelling to a nest.

Targeted searches using non-experts

This trial was conducted on the 26 and 27 July 2016 in Cornwall, UK (Lat $50^{\circ}17'N$, Long $4^{\circ}48'W$). The aim was to test whether thermal cameras are a more effective tool to detect the nest traffic of a bumble bee nest compared to human-unaided visual searches (O'Connor et al., 2012). The 30-m transect used in this trial was located along an established path, where an active *Bombus hortorum* nest had been previously found in a disused rodent hole ~20 m into the transect. The transect was along a bank and encompassed a number of habitat features: leaf litter, exposed soil, natural and planted vegetation and trees.

Members of the public were asked at random to participate in the trial. Participants had no prior training or experience searching for bumble bee nests, and the ages of participants ranged from children to adults, but were not specifically recorded for this study. To ensure all participants had the same basic knowledge level prior to starting their search, they were (i) shown a picture of a bumble bee, (ii) told that bumble bees nest underground (in this case), and (iii) informed they were looking for nest traffic, i.e., worker bumble bees coming in and out of the nest. Providing this information ensured that their search effort was spent actively looking for the nest. Those participants using the thermal camera were given a demonstration of how best to use the thermal camera, i.e., moving it around to view the transect, and were advised that the nest traffic would appear as small, white moving objects on the cameras viewing screen. Once briefed, participants were taken to the starting point and surveyed the right-hand side of the path.

Participants performed targeted "free searches," which ranged from searching from the path, to climbing onto the bank and searching through the vegetation. In total, 25 participants took part, 13 performing thermal camera searches and 12 performing human unaided visual searches. Participants could stop searching at any time, but were advised to search for at least five minutes, and were stopped after 10 minutes if they had not been successful at locating the nest. When a participant found the nest, they were asked to raise their hand for confirmation of success, and at this point, the timer was either stopped or if the participant had incorrectly identified the nest they were given the option to continue searching until the 10-minute period had elapsed. Total search times of both successful and unsuccessful participants, as well as whether they found the nest or not, were all recorded for analysis.

A logistic regression was performed using the statistical program R version 3.4.1 (R Core Team, 2017) with "detection success" as a binary response variable and "search method (camera or human)" as the independent variable, the model was fitted with a binomial family. One thermal camera survey was removed prior to analysis due to the surveyor locating the nest without the thermal camera.

Non-targeted searches using an experienced individual

Between the 25 July and 6 August 2016, searches were performed by a single individual, to test the effectiveness of using a thermal imaging camera as a searching aid when performing nest surveys across various locations. A single search individual was appointed to conduct both the human searches and thermal camera searches. This individual was chosen as she had undertaken multiple bumble bee surveys prior to the study

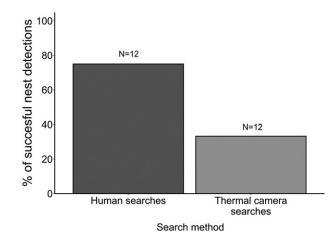


Figure 3. Nest detection success rates for the targeted searches showing the number of times participants successfully located the nest for each search method, presented as a percentage.

Table 1. Coefficients for the best fitting model for the targeted searches. Output generated in the statistical program R version 3.4.1 (R Core Team, 2017) from a logistic regression model fitted with "detection success" as the response variable and fitted with a binomial family.

	/		
Coefficients:	Estimate	Z value	p value
Intercept Search method: Human searches	-0.69±0.61 1.80±0.91	-1.13 1.98	0.258 0.048

and had experience operating thermal cameras. Prior experience is important when testing a novel technique, to reduce the possibilities of "false negatives" which could have occurred if using naïve searchers. Nest surveys were performed across six sites in Cornwall, UK (Lat 50°15'N, Long 5°3'W). Each site was searched for between 92 and 179 minutes. Surveys consisted of fiveminute consecutive searches alternating between human-unaided visual searches and thermal camera searches. For ease, searches were carried out along already established paths. During both human and thermal camera searches, the observer walked along the path at a steady pace, surveying both sides of the path for bumble bee activity. All bumble bees seen were recorded, and their behavior was classified as patrolling, foraging, flying, resting, or entering and exiting a nest. Where possible caste and species were determined. Bumble bees seen flying were observed until they were out of sight to establish if they were flying to a nest. A hood was added to the thermal camera to prevent the observer using their peripheral vision whilst performing thermal camera searches. This was done for the nontargeted searches only, after an individual in the targeted searches located the nest during a thermal camera survey without using the camera. It also reduced glare on the screen to allow for more optimal use of the camera when searching.

To ascertain whether habitat types affected our ability to detect bumble bees and their nests using the thermal camera, all habitats and habitat features present

Table 2. The location of nests found during the non-targeted searches.

Detection method	Number	Habitat
Human	I	Cornish hedge
Human	I	Brick wall
Human	2	Rodent hole
Thermal	I	Rodent hole
	method Human Human Human	method Number Human I Human I Human 2

during the five-minute searches were recorded. These included leaf litter, banks, Cornish hedges, trees, short grassland, flower beds, and long grassland. For analysis, these were simplified to the number of different features in each search area, giving a crude estimate of habitat complexity.

Results

Targeted searches using non-experts

Twenty-five searches were conducted by participants, with 24 being used in the final analysis. Human-unaided visual searches located the nest 75% of the time, whereas individuals using the thermal camera as a search tool were only able to locate the nest 33.3% of the time, and this difference was significant (z_{22} =1.979; p = 0.048; Figure 3). Coefficients for the model output can be seen in Table 1.

Non-targeted searches using an experienced individual

The total search time was 13 hours 48 minutes. Six hours 58 minutes of search time was performed for human-unaided visual searches, and six hours 48 minutes of search time was performed for thermal camera searches. During searches, five nests of four different species were found (Table 2). Only one of these nests was found using the thermal camera, with the other four being found during human-unaided visual searches.

The nest detection rate for human-unaided visual searches was one nest for every one hour 44 minutes of search time, a much faster rate of detection than thermal camera searches which found one nest for every six hours 48 minutes of search time. Due to the small number of nests found, no statistical analysis was performed.

Discussion

We found that the thermal imaging camera did not improve the users' ability to locate bumble bee nests when compared to human searches, and therefore, we recommend that further research into other nest detection methods is needed to enable the successful detection of larger numbers of wild bumble bee nests. In all cases, it was the nest traffic which led to nests being detected; detection of surface nests due to their thermal signal may be possible without the visual cue from

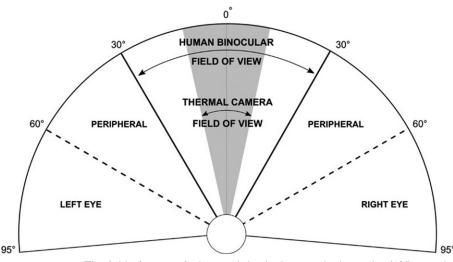


Figure 4. Field of view comparisons. The field of vision of a human (white), showing the binocular (60°), peripheral (120°), and complete (190°) vision range. Compared to the field of view of the FLIR E60 thermal imaging camera (25°) used in this study (grey).

the worker nest traffic, but no such nests were found during our study. The study took place during late-July to early-August at a time when the colonies of some species have or are starting to reach maximum size (Prys-Jones & Corbet, 2011). The majority of colonies in other studies were located within a similar period (Goulson et al., 2018). This may lead to a bias towards detection of larger, successful colonies and therefore reduces the ability to monitor how wild colonies are responding to stressors.

One of the limitations of our study was using nonexpert individuals to survey for nests, which could have led to "false negatives" due to lack of prior nest searching experience. The use of volunteers to collect data is widely used in citizen science and biological recording projects, with around 70,000 individuals in the UK alone annually submitting species observations to recording programs (Pocock, Roy, Preston, & Roy, 2015). There are mixed opinions on the validity of using such "non-experts" for scientific research purposes (Cohn, 2008), but many cite this data collection method as a useful tool (Sauermann & Franzoni, 2015), especially for conservation programs (Johnson et al., 2014). Experience of volunteers was not found to affect their ability to detect wild bumble bee nests when performing human searches (O'Connor et al., 2012). However, when testing a novel nest detection technique, as in our study, the chances of "false negatives" occurring is likely increased due to the combination of the surveyor's lack of prior nest searching experience and their inexperience of using the novel technology. Small differences in detection success between human and thermal camera survey techniques have been shown in other studies (Graves et al., 1972), likely due to discrepancies between surveyors. Therefore, we decided to use a single experienced individual to conduct our non-targeted searches, in an attempt to reduce both variation between surveyors and the occurrence of "false negatives" when performing bumble bee nest surveys across a range of sites. Using a single experienced individual allowed us to more directly compare the efficacy of the two methods for locating nests, rather than the user's ability to successfully operate the thermal camera had we chosen to use multiple inexperienced volunteers.

The second limitation was with the thermal camera itself. The thermal camera lens had a field of view of $25^{\circ} \times 19^{\circ}$, which is much smaller than the human field of view (190°×135°) (Figure 4). This reduced field of view meant that the thermal camera user had to move the camera around in order to survey the same area as a human using their natural field of vision. Due to the small colony sizes of bumble bees (Prys-Jones & Corbet, 2011), nest traffic emanating from the nest entrance is low. Many bumble bee species nest underground, and so will not give off a thermal signal in the way surface nests may do. It is therefore the nest traffic that will enable detection. The need to move the thermal camera around when searching an area due to its small field of view meant the chances of the camera being trained on a nest entrance when a bumble bee was entering or exiting was low. During human-unaided visual searches, bumble bees were often seen in the peripheral and monocular field of vision (Figure 4), giving human searches a wider field of vision with which to survey the environment. To address this, a thermal camera with a wider angle lens could be used, e.g., FLIR thermal imaging cameras with a 45° field of view are currently available. Although larger than that of the thermal camera used in our study, their field of view is still 15° less than a human's binocular vision, and 145° less than a humans binocular and peripheral vision combined (Schneck & Dagnelie, 2011). In addition to a small viewing area, the ability of a thermal camera to detect warm objects within an environment is affected by a number of other factors such as air temperature, distance from the object and the presence of vegetation (Cilulko et al., 2013). These limitations mean that the detection

effectiveness of the thermal camera during the day to detect bumble bee nests was likely reduced. Addressing the current limitations of thermal cameras could improve their use as nest detection tools, for example the use of thermal cameras with wider angled lenses as mentioned above. Further studies would be needed to compare the effectiveness of thermal cameras with a wider field of view to human searches.

The findings from our study further support the argument that human searches are currently the best method at locating bumble bee nests. Our search methods during the non-targeted searches were similar to the "free search" method used by O'Connor et al. (2012) to explore the effectiveness of trained sniffer dogs as a novel bumble bee nest detection method compared to human searches. They recorded a nest detection rate for both humans and sniffer dogs of one nest for every one hour 20 minutes of searching (O'Connor et al., 2012), which is in line with the human search rates found in our own study. Our novel detection approach using the thermal camera as a search tool in comparison performed much worse than both of these methods, finding only one nest during six hours 48 minutes of searching. Our findings corroborate those of O'Connor et al. (2012), showing that human searches are a cost-effective method of locating wild bumble bee nests, as although sniffer dogs performed at the same rate as humans, they are more expensive due to the initial training costs and continued upkeep (Mathews et al., 2013; O'Connor et al., 2012). This is also true for thermal cameras which have large upfront costs. There is still a need for a more effective nest detection method to be developed, which would allow larger quantities of nests to be found more quickly in order to fully study wild colonies in situ.

Conclusion

Due to current lack of efficient detection methods, few field studies on the ecology of natural bumble bee nests exist. Our study was unable to establish a novel nest detection method to replace the currently used human searches. Human searches do not find large numbers of nests, and relatively few studies have used nest detection techniques to actually monitor and study the nests. Without this monitoring of bumble bees at the colony level, we will not be able to understand how stressors that affect them at the population level (Goulson et al., 2015) are actually impacting upon individual colonies, an important area of research due to the continuing decline of bumble bees in the UK (Goulson, Lye, & Darvill, 2008). Studies that have monitored wild nests have given us information on nest predation, survival, disease, and gyne production (Goulson et al., 2018; Osborne et al., 2007) but further and more longer-term monitoring of wild colonies is needed. Future research should focus on better nest detection and monitoring techniques.

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