The impact and spread of the invasive garden ant: an alien invasive species in the UK

Phillip Buckham-Bonnett

PhD Thesis

University of York

Biology

December 2019

Abstract

Alongside climate change and habitat loss, invasive non-native species are a major threat to the natural world. Ants are amongst the most widespread and damaging invasive species. The invasive garden ant, *Lasius neglectus*, has only recently been detected in the United Kingdom and is the country's first invasive ant species. This thesis aims to assess the impact and spread of this species in the UK. In this thesis I carry out a UK-wide risk assessment for the species and develop a protocol for experimentally assessing its potential impact on an economically important crop plant. I investigate behaviours that may contribute to its success as an invasive species. I evaluate the feasibility of commonly used pesticides for the control of *Lasius neglectus*, and conclude that granular products, while convenient for large-scale application, are not suitable for this species due to low palatability; this may hinder future control attempts. I carry out a large-scale survey to assess the distribution of this easily-overlooked species, and conclude that while it is not as widely distributed in botanic gardens as expected, the number of urban sites where it occurs is increasing rapidly. In conclusion *Lasius neglectus* is difficult to detect and hard to eradicate once established, so efforts should be made to reduce its spread from the areas in which it currently occurs.

Contents

Acknowledgements

This thesis has been the supported by the contributions of a great many people for which I am very grateful.

First and foremost, I would like to thank my supervisor, Elva Robinson. Without her help, input, inspiration, guidance, encouragement and even some video analysis, the project would not have been as rewarding and I would not have finished this thesis. I am also very grateful to Paul Lee as a supervisor, to Dan Franks as a TAP member and supervisor, and Peter Mayhew as a TAP member for all their contributions and support.

I am very grateful to NERC and Hymettus for funding my PhD and to the Non-Native Species Secretariat for providing additional funds for research assistants. I also would like to thank the NNSS and the National Trust for providing and dispatching the traps and trapping materiel used in the botanic garden survey. I am grateful to Bayer, Bábolna Bio and DuPont for supplying the pesticides used in the experiments described in chapter 4.

The placements with Hymettus have been one of the highlights of my PhD and I would like to thank all those involved, especially Paul Lee, Murdo Macdonald, Mike Edwards, Rowan Edwards, Scotty Dodd, Paddy Saunders, Brian Banks, Stephen Carroll, Nikki Gammans, Stewart Taylor, Athayde Tonhasca and Hayley Wiswell.

My fieldwork would not have been possible without the support of the National Trust and the staff at Hidcote Manor, especially Chris Charman, Ann Davies and Glynn Jones. I am also grateful to John Righton for allowing me to run part of my experiment in his sheep field and for advice on how to stop the sheep eating it. My field work was also supported by the Cambridge University Botanic Gardens and I would like to thank all their staff especially Sally Petitt, Beverly Glover and Peter Sheld. My Cambridge field work was also very generously supported by Mary Vickers who provided me with accommodation. I am grateful to the staff at the sites I surveyed, for the permission to look for ants and also for the occasional cups of tea!

I would like to thank past and present members of the Robinson Lab, for all their help, support, interesting discussions, red pens of justice and camaraderie. I would especially like to thank Duncan Proctor, Sam Ellis, Yi-Huei Chen, Eleanor Drinkwater, Dom Burns, Josie Monaghan, Meg Holgate, Valentin Lecheval and Joshua Sammy. I would also like to thank members of the Biology Department, YCCSA, GReBE, and the Theoretical Ecology and Whole Organism groups, especially Jon Pitchford, Jamie Woods, Leda Cai, Tom Hartley, Emma Rand, Melanie Smee, Stuart Nattrass, Ellie

Heyworth, Richard Law, Sarah Collings, Andy Suggitt and Gail Stride. I am grateful to Monica Bandeira and Sarah Christmas for their administrative support, Phil Roberts for permission to use his ant photographs and Mark Bentley for his fantastic workshop creations.

I would like to thank Clive Boase for all his contributions and advice (even once answering the phone whilst at the top of a ladder!) on the pesticide and survey work and for first showing me around the Hidcote site. I am grateful to Alba Aguion for all her work on the first pesticide experiment and enthusiasm for learning survival analysis. I am grateful to Mike Fox for his ant identification assistance. I would like to thank Sebastian Mellor for discussions about and help with statistical analysis and coding. I am grateful to Tommy Czaczkes, Stefan Popp and Sophie Evison for being so willing to collaborate on tactile communication and all their work. I would like to thank Niall Moore, David Bullock and Simon Toomer for all their contributions and input through the *Lasius neglectus* task group.

I am very grateful to all the volunteers who helped with the video analysis for chapter 4: Alice Bedois, Leda Cai, Laura Dunsby, Maria Guaita, Kate Hutchinson, Robyn Kinnersley, Alexander Kyte, Anna Middlemiss, Sarah Pickering, Charlotte Sauer and Lakesha Smith. I would like to thank all the staff at sites which took part in the survey for their help deploying and returning the traps for the botanic garden survey. I would like to thank Sarah Nicholson, Sarah Pickering and Tom Rudd, for processing the samples; and Sarah Pickering and Tom Rudd for their help with sample analysis for the botanic garden survey. I also would like to thank the staff in stores for tolerating all the dubious looking parcels labelled “ants” that I have received over the years.

I would like to thank Helen Roy and Peter Mayhew for an enjoyable and stimulating viva and their very useful comments and suggestions for improving this thesis.

Finally, I would like to thank my parents for all their support during my PhD.

Declaration

I declare that this thesis is a presentation of original work and my contribution is detailed below. This material has not previously been presented for an award at this, or any other, university. All sources are acknowledged as references.

Author contribution statements

Chapter 1 (General introduction)

Written by Phillip Buckham-Bonnett (PBB) with input from Elva Robinson (EJHR) and Paul Lee (PL)

Chapter 2 (Risk Assessment)

Written by PBB with input from EJHR and PL. This is published on the Non-Native Species Secretariat website at the following links: http://www.nonnativespecies.org/index.cfm?pageid=143

https://secure.fera.defra.gov.uk/nonnativespecies/downloadDocument.cfm?id=2065

Chapter 3 (Tactile Communication)

This chapter represents a collaborative project to which PBB made significant contributions. More detail on the origin and contributions to this project is provided in the preface to Chapter 3, but in summary: experimental design involved Sophie Evison (SEFE), Tomer Czaczkes (TC), Stefan Popp (SP), EJHR and PBB. Data collection was performed by SP and PBB. Data analysis was performed by TC. The paper and appendix B were written by TC with contributions from PBB, SEFE, EJHR and SP. Material additional to the paper (Appendix A) was authored by PBB. This work is published as detailed below (Popp et al., 2018).

Popp, S., Buckham-Bonnett, P., Evison, S.E.F., Robinson, E.J.H., Czaczkes, T.J., 2018. No evidence for tactile communication of direction in foraging Lasius ants. Insectes Soc. 65, 37–46.

Chapter 4 (Pesticide efficacy)

Experimental design was by PBB, Alba Aguion (AA), Clive Boase (CB), PL and EJHR. Data collection for Experiment 1 was performed by PBB and AA; data collection for Experiment 2 and Appendix E was performed by PBB. Data analysis was performed by PBB. The chapter and Appendices C, D and E were written by PBB with input from PL, CB, AA and EJHR.

Chapter 5 (Distribution)

Part 5a (Wide-scale survey) was designed by PBB, EJHR, CB, PL and Niall Moore (NM) with input from Mike Fox (MF), David Bullock (DB) and Simon Toomer (ST). Data collection was carried out by site managers under the direction of PBB, CB, ST & DB. Sample processing was carried out by PBB. Data analysis was performed by PBB. This section and Appendix F was written by PBB with input from EJHR.

Part 5b was performed and written by PBB with input from EJHR and PL.

Part 5c was written by PBB.

Chapter 6 (General Discussion)

Written by PBB with input from EJHR.

Appendix A

(See Chapter 3 above)

Appendix B

(See Chapter 3 above)

Appendix C

(See Chapter 4 above)

Appendix D

(See Chapter 4 above)

Appendix E

(See Chapter 4 above)

Appendix F

(See Chapter 5 above)

Appendix G

This data collection instruction sheet used in Chapter 5 was written by CB, and is included here to document the complete methods used.

Appendix H

Experiment was designed by PBB, EJHR and PL. Data collection was performed by PBB. Sample processing and data analysis were performed by PBB. The section was written by PBB with input from EJHR and PL.

Appendix I

This appendix comprises 3 parts. The video was produced by PBB. The 2 information sheets were written by PBB with input from EJHR, PL and MF. The magazine article was written by PBB with input from EJHR.

Chapter 1: General Introduction

Invasive species

Non-native species are those which have actively or passively been introduced by humans to an area beyond their natural range. Invasive species are non-native species that become established (form self-sustaining populations) where they are introduced, and cause environmental, economic or societal harm. The rate of the establishment of non-native species globally has been increasing since around 1800 as a correlate of the growth in human transport networks (Hulme, 2009) and the number of recorded invasive species has doubled in the last fifty years (IPBES, 2019). Whilst a very small proportion of the non-native species arriving become established, those that do can have serious impacts. The estimated annual economic cost of non-native species in the European Union is in the region of 12.5 – 20 billion Euros, of which at least 1.5 billion can be attributed to terrestrial invertebrates (Keller et al., 2011). Responding to biological invasions is more expensive than responding to natural disasters (Ricciardi et al., 2011) and tackling invasions is made difficult by various political as well as biological factors (Crowley et al., 2017; Keller et al., 2011; Prior et al., 2018). The economic impacts of invasive species are often measured at a national, regional or global scale but the impacts such as a loss of ecosystem services or impact on health and livelihoods are experienced at a local or individual scale (EEA, 2012; Shackleton et al., 2019; Yongo and Outa, 2015), and can be particularly severe in developing countries (Paini et al., 2016). As well as economic impacts, invasive species can have major evolutionary and ecological impacts on native species and environments (Gurevitch and Padilla, 2004; Linders et al., 2019; Mooney and Cleland, 2001; Strayer et al., 2006). The rate of biological invasions has been found to correlate positively with temperature so is likely to be an increasing problem with global warming (Huang et al., 2011). There is no evidence that the number of invasive species worldwide is approaching saturation and indeed, the rate at which new invasions are identified is increasing for many taxa, including insects (Seebens et al., 2017).

Invasive Species in the UK

The UK Biodiversity Indicators 2019 report from the Joint Nature Conservation Committee states that 3,208 non-native species have been identified in Great Britain of which 193 are thought to have a negative impact on native species. The number of invasive species in the terrestrial, freshwater and marine environments has been increasing since the 1960-69 assessment period (although the number of terrestrial invasive species did not increase between the 2000-09 and 2010-18 periods). Also of concern is the increase in the proportion of invasive species that are established in more than 10% of the territory (Department of Environment Food and Rural Affairs, 2018). New invasive species continue to become established and those already here are expanding their ranges. Two ant species were included in the list; the Argentine ant *Linepithema humile* and the invasive garden ant *Lasius neglectus* (in Harrower et al., 2019).

Horizon scanning is used as a systematic tool to try to identify and assess future threats in order to allow the most efficient prioritisation of resources to combat invasive species (Roy et al., 2014a). Horizon scanning should be repeated at frequent intervals to increase the probability that novel invasive species are detected and can have a narrow or broad taxonomic focus and cover a narrow or wide geographic area. Known invasive species in other territories should not be the limit of horizon scanning activities as the ever-increasing global connectivity provides access to novel sources of potentially invasive species (Seebens et al., 2018). The UK also uses a risk assessment scheme to collate and evaluate information about invasive species that are anticipated or have already arrived which can be used to inform policy decisions (see chapter 2). European Union policy on invasive species prioritises action to combat 36 plant and 30 animal species of Union concern (as of August 2019). Members are required to act to reduce the likelihood of introduction, implement protocols for detecting and eradicating new arrivals and manage those species which are already established (UE, 2014). This was then adopted into UK law in 2019 with a focus 14 species that are widespread in England and Wales.

As an island nation, Great Britain is in an advantageous position in terms of preventing new arrivals in terrestrial and freshwater habitats. The seas act as a physical barrier to many invasive species that have been introduced to continental Europe thus helping to prevent spread from existing invasive populations. Accidental introductions resulting from the movement of goods and people remains a risk, however, but leaving the European Union also presents us with an opportunity to review invasive species policy. Other island nations such as New Zealand have much stricter biosecurity protocols at and before the border (Hulme, 2020) but these have associated costs both in terms of loss of trade and biosecurity enforcement. The much lower endemism in the UK might make these costs harder to justify politically but the staggering economic cost of some invasive species should also be taken into account. For example, a single plant pathogen *Hymenoscyphus frazineus* (responsible for ash dieback) is expected to cost the UK economy £7.6 billion over the next ten years. This far outweighs the value of the entire live horticultural trade which in 2017 was worth only £300 million per year (Hill et al., 2019).

Ants as invasive species

Introduced non-native ant species can be found in all of the biogeographic realms excluding Antarctica, with the Oceania and Nearctic regions hosting the most species, whilst the Neotropical and Indomalaya regions are the largest sources (McGlynn, 1999). Over 200 taxonomically diverse ant species have been introduced outside their natural range (McGlynn, 1999; Suarez et al., 2010) with several of these being particularly damaging invasive species.

Characteristics that increase the probability of an ant species being transported to new sites include: a small body size (meaning they are easily overlooked), opportunistic nesting and small nests (increasing the probability of being stow-away or overlooked) and anthropophilic tendencies increasing the likelihood of contact with humans. Once at a new site, polydomy and unicoloniality help to reduce intraspecific competition; mass recruitment foraging strategies and broad diets allow exploitation of local resources; and polygyny, intranidal mating and dependent colony foundation can aid growth and expansion (Rabitsch, 2011). Not all of these traits are shared by all invasive ant species, however so predicting invasive capacity can be challenging. Analysing the nutritional dimensions of ant species is a novel approach which can potentially help to predict its capacity for invasion. Species with a broad region of nutritional space (the ability to survive and reproduce on a diet within a wide range of nutrient proportions as opposed to being constrained to a narrow spectrum) are more likely to have a higher invasive potential (Shik and Dussutour, 2020).

Ants can be particularly expensive invasive species. *Solenopsis invicta*, for example, is estimated to result in losses and damages worth $600million and require $400million in control measures in the US annually (Pimentel et al., 2005). Invasive ants often found vast polydomous colonies. For example *Linepithema humile* colonies in California can extend over an area greater than 600m^2 in the summer and are estimated to contain over 5 million individuals (Heller et al., 2008). They can also have profound ecological impacts through a wide variety of mechanisms such as: altering the structure of native communities; affecting seed dispersal; impacting ecosystem function; interfering with obligate mutualisms; reducing diversity; predation; causing extinctions; and interfering with pollination (Holway et al., 2002; Lach, 2008; Lessard et al., 2009; Ness and Bronstein, 2004; Wittman, 2014). An extreme example of the negative impact of an invasive ant species is the yellow crazy ant, *Anoplolepis gracilipes*, where interactions between the ants, scale insects which they tend and crabs on which the ants predate, cause 'invasional meltdown' (Abbott, 2006; Abbott and Green, 2007; Green et al., 2011).

Historical research effort (number of publications) has previously focussed heavily on just two invasive ant species *Solenopsis invicta* and *Linepithema humile* while the others are underrepresented (Bertelsmeier et al., 2016).

The invasive garden ant, *Lasius neglectus*

The Physiology, Genetics, and Life-History Characteristics of *Lasius neglectus*

Lasius neglectus is likely to have evolved from, or shared a last common ancestor with *Lasius turcicus* and mating strategy, male morphology and differences in genitalia represent barriers to mating between the two species (Cremer et al., 2008; Seifert, 2000). The most recent assessment suggests that the species' native range includes large parts of Uzbekistan (Stukalyuk, 2020). *Lasius neglectus* is morphologically very similar to *Lasius alienus* but this is due to convergence rather than relatedness (Van Loon et al., 1990). Such morphological similarities often mean that the ant is not correctly identified.

Lasius neglectus exhibits a suite of characteristics typical of invasive ant species. These include: intranidal mating (Boomsma et al., 1990; Espadaler and Rey, 2001; Seifert, 2000); absence of worker oviposition (Espadaler and Rey, 2001); dependent colony founding (Espadaler and Rey, 2001); polygyny (Boomsma et al., 1990; Cremer et al., 2008); weak intraspecific competition and "supercolony" formation (Boomsma et al., 1990; Cremer et al., 2008; Espadaler et al., 2004; Van Loon et al., 1990); and small, hyper-abundant workers (Van Loon et al., 1990). For a review of how these characteristics can contribute to ant invasions see Holway et al. (2002).

Lasius neglectus has lower brood development times and a greater initial production of workers by newly mated queens than *Lasius niger* (although this evaluation did not account for possible effects of temperature) (Espadaler and Rey, 2001). In Spain, temperature does not appear to affect egg laying rate (Espadaler et al., 2004) and a mean air temperature of around -5°c in the coldest month at two sites where the ant is present in Asia suggests cold tolerance (Seifert, 2000). High fecundity, rapid worker development and thermal tolerance are likely contributors to the success of this species in Europe.

The Ecology and Behaviour of *Lasius neglectus*

Lasius neglectus has a very large range in Eurasia and as of 2000 (its known range has since expanded) was found between 1°E-75°E and 36°N-52°N; between sea level and 1750m altitude; and is likely to have originated from an Asian Steppe habitat (Seifert, 2000).

Queen number and brood abundance has been found to be fairly even throughout a single colony and it is estimated that there are 800 workers per square metre and in the region of 35500 to 360000 queens over 14ha at one site in Spain (Espadaler et al., 2004). High ant densities have also been reported in Hungary where *Lasius neglectus* can be eight times more abundant than *Lasius niger* (Tartally, 2000). See figure 1.1 for an example of high *Lasius neglectus* worker densities in the UK.

Figure 1.1 – *Lasius neglectus* workers foraging on silver birch. Cambridge, UK. 2016

Lasius neglectus forms strong mutualisms with aphids. In Spain *L. neglectus* collect (approximately 2.5 times) more honeydew per tree than *L. grandis* and also exhibit a higher aphid tending frequency than the native species. This is partly due to aphids tended by the invasive ant producing honeydew at a higher rate and partly due to the greater abundance of the invasive ant. Conversely, *L. grandis* workers ware significantly more likely to be found carrying prey items than *L. neglectus* (Paris and Espadaler, 2009). *Lasius neglectus* workers remain active 24 hours per day in Spain (Rey and Espadaler, 2004).

Figure 1.2 – Lasius neglectus worker collecting aphid honeydew. Cambridge, UK. 2016.

Not only does *Lasius neglectus* monopolise nest space and food resources but workers immediately capture queens of other species that land in invaded areas post mating flight (Paris and Espadaler, 2012). This aggressive tendency can also be seen in worker-worker interactions. *Lasius neglectus* is dominant in one on one interactions with three native species of *Lasius* in Spain. This was most strongly demonstrated by biting where *neglectus* was significantly more likely to perform biting than any one of the three native species. A (non-significant) trend was also found with *neglectus* workers from the edge of the colony being more likely to be aggressive than workers from the centre (Cremer et al., 2006).

Models show that currently 40% of Europe is climatically suitable for *Lasius neglectus* but this could rise to around 50% with climate change. The argentine ant *Linepithema humile* is the next most potentially problematic ant in Europe with around 20% of the land climatically suitable. The whole of UK (except some highland areas in Scotland) is currently suitable (Bertelsmeier et al., 2015a). The range expansion of *Lasius neglectus* locally is only between a few and 90 metres per year at existing sites but new distant sites are also colonised; behaviour typical of a tramp species relying on human mediated dispersal (Espadaler et al., 2007). Aggression tests, genetic analysis, and cuticular hydrocarbon (CHC) analysis agree that relatedness does not correlate with distance between European populations of *Lasius neglectus* further supporting a human mediated transport hypothesis (Ugelvig et al., 2008). Whilst *Lasius neglectus* is mainly found in highly disturbed habitats (Seifert, 2000) there have been isolated reports of the ant invading more natural habitats (Paris and Espadaler, 2012).

Planning the response to an established non-native invasive species

In order to plan the response to a non-native invasive species, it is necessary to collect data about the species' current distribution/abundance, the impact of the species, its biological characteristics, the feasibility of any mitigation approaches, and also to take into consideration moral and ethical considerations (Figure 1.3). I discuss these five areas in the context of *Lasius neglectus* below.

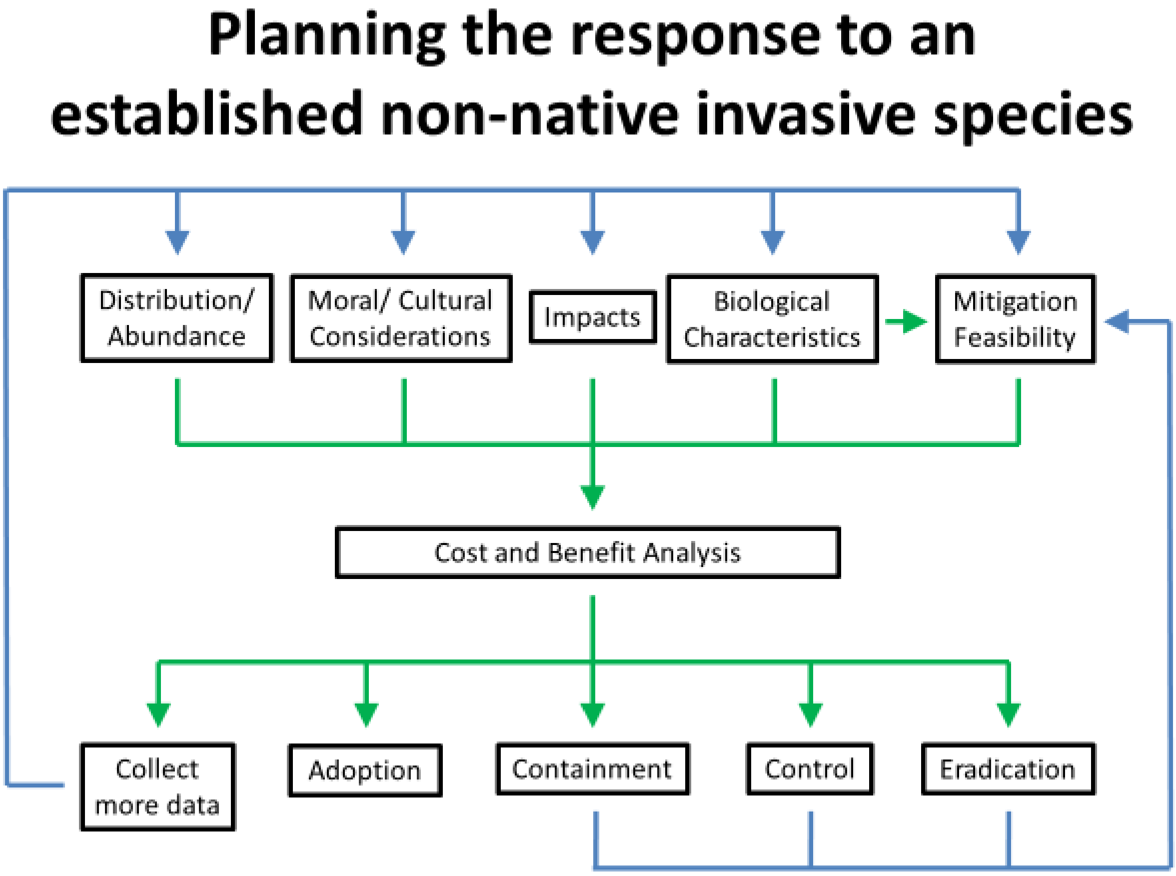


Figure 1.3 - A schematic of the stages and processes involved in planning and implementing the response to an invasive species.

Distribution/Abundance

Unfortunately, assessing the UK distribution of *Lasius neglectus* is not easy. The morphological similarities between *L. neglectus* and native ant species mean that the invasive ant's presence is easily overlooked. Amongst the uniformly brown *Lasius* species found in the UK, *L. neglectus* can be separated from *Lasius niger* and *Lasius platythorax* by the absence of erect hairs on its antennal scapes and hind tibiae. Separating *L. neglectus* from *Lasius alienus* and *Lasius psammophilus* requires an assessment of the mandibular dentition. *Lasius neglectus* usually has seven teeth whereas *L. alienus* and *L. psammophilus* usually have eight (Fox, 2010). However, the dentition in *L. neglectus* can be quite variable (see figure 1.4) meaning several individuals are required to confirm the identification. Ecological characteristics such as the high abundance of ants in established *L. neglectus* populations, combined with the lack of hairs on the antennal scapes and hind tibiae, can also be to used aid identification. However, as the progression from establishment to "superabundance" in *L. neglectus* has not been described, the absence of an unusually high number of ants does not necessarily equate to the absence of *L. neglectus*.

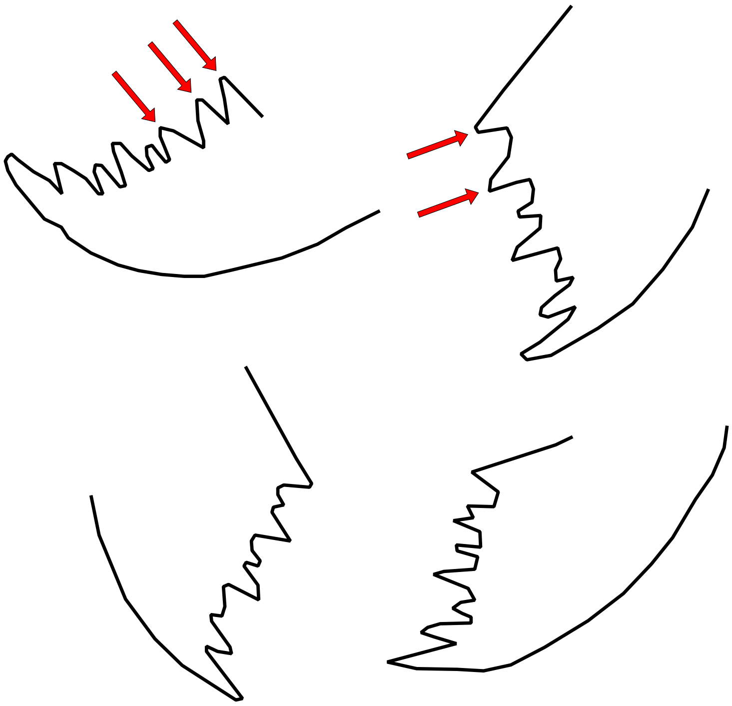


Figure 1.4 – Tracings of dentition patterns from photographs of four *Lasius* mandibles. Clockwise from top left: Typical *L. alienus* (eight teeth, three of which are basal – denoted with arrows); typical *L. neglectus* (seven teeth, two of which are basal – denoted with arrows); atypical *L. neglectus*; atypical *L. neglectus* (the right hand mandible of the same ant shown in the top right trace highlighting the amount of variation even within individuals.

Requiring a specialist skill set for identifying *Lasius neglectus*, combined with the need for a microscope means that assessing the distribution of this species is not suitable for “citizen science” recording. As a result, requests for recording effort are most usefully targeted at the select audience (such as naturalists) who are likely to have required ID skills. Visiting sites that are likely to have a high propagule pressure may also be useful. “Propagule pressure” (the combined effect of the number of individuals arriving and number of arrival events) has been proposed as a potentially important measure for predicting the likelihood of a non-native species becoming established at a particular site (Lockwood et al., 2005). Whilst this does not seem to hold true for some taxa, for example trees (Nuñez et al., 2011), it has been shown to be important in some ant invasions (Rice and Silverman, 2013). Sites that are likely to have a high propagule pressure for *Lasius neglectus* are those receiving potted plants, such as botanic gardens. Chapter 2 includes data on the distribution of *Lasius neglectus* at the start of this PhD project; Chapter 5 comprises a large-scale survey, focussed on botanic and formal gardens, which provides a more comprehensive and up to date assessment of its distribution.

Characteristics

A characteristic key to the success of many ant species (in particular invasive ant species) is their ability to efficiently exploit food resources in a spatially and temporally heterogeneous environment (Hölldobler and Wilson, 1990). Many ant species with larger colony sizes use mass recruitment (a communication system based on the deposition and gradual decay of pheromones that stimulate positive chemotaxis) to regulate their foraging effort (Beckers et al., 1989). However, there are disadvantages to such an approach as it can result in run-away positive feedback, and the chemical trails reflect the historical but not necessarily the current abundance of food. In some invasive species such as *Monomorium pharaonis,* additional signals have been shown to aid in the regulation of foraging in mass recruiting species (Robinson et al., 2008, 2005). There is some evidence to suggest that tactile interactions between workers during foraging have a communication role in mass recruiting species (Reznikova and Ryabko, 2001). As *Lasius neglectus* forms foraging trails with a greater density of workers than native species, the rate at which between-worker encounters occur is also likely to be higher. If these encounters were being used to transfer information about the presence of food resources, they are likely to be of proportionally higher importance to the success of the invasive species. Chapter 3 addresses whether tactile communication in *Lasius neglectus* could contribute to its invasion success.

Mitigation Feasibility

There are two main aspects of mitigation for *Lasius neglectus*. Firstly, stopping the spread, either locally within an infested site, or at a broader scale preventing introduction to new sites. For ants, the measures needed to stop this depend strongly on the dispersal mechanism (i.e. colony foundation via flight versus budding, whether queens found alone etc); this is discussed in the risk assessment in Chapter 2. Secondly, existing populations can be targeted to reduce numbers or eliminate completely. The main tool for this is insecticides and there is a whole suite of modes of action and methods of delivery. The specific problem with targeting ants is the need to kill the queens, the reproductive individuals, rather than just the foragers, who are the ones likely to encounter an insecticide bait or spray application. This means that contact pesticides alone are unlikely to be effective; instead it is necessary to use a pesticide that can be ingested by workers and taken back to the nest, and passed by trophallaxis to other colony members. The active ingredient needs to be slow-acting, to allow this propagation through the colony. The challenge is therefore to find a sufficiently toxic but slow acting active-ingredient that is not repellent to the ant species and a bait matrix for this ingredient that is sufficiently attractive. The solution to this challenge differs between ant species (Hoffmann et al., 2016). As *Lasius neglectus* is a relatively recent invasive species, there has been limited work on species-specific control measures. Approaches developed for the UK-native species *Lasius niger* may not be appropriate; for example *L. niger* control typically focusses on targeting the nest; this is less effective for ants such as *Lasius neglectus* which are polydomous with nests spread out over large areas. Baiting approaches are more targeted and discriminating than large-scale pesticide application, but are more affected by the species' ecology and behaviour. In Chapter 4 we evaluate the suitability of several widely-used ant baits for the control of *Lasius neglectus* and the role that the ant's foraging behaviour has on their effectiveness.

Impacts

Lasius neglectus is associated with: a reduction in native ant (particularly congeneric) species richness in Spain (Paris and Espadaler, 2012); and a reduction in the species richness of Coleoptera, Formicidae and Isopoda but an increase in richness of Hemiptera in Hungary (Nagy et al., 2009). In Hungary, the ant was also "superdominant" with a far greater abundance than all other arthropod species combined. In both the UK (Boase, 2014) and Spain (Espadaler and Rey, 2001), Lasius neglectus enters human dwellings and other buildings and is considered a pest.

Lasius neglectus is reported to form very strong mutualisms with aphids (Paris and Espadaler, 2009; Van Loon et al., 1990) and is also able to forage at extra-floral nectaries (Rey and Espadaler, 2004). In extreme examples, the aphid load resulting from the presence of *Lasius neglectus* can kill host trees (Espadaler and Rey, 2001). Pathogen and parasite release are also likely to contribute to its success as an invader (Cremer et al., 2008; Rey and Espadaler, 2004).

Appendix H asks what impact *Lasius neglectus* could have on plant growth within the UK, and how this might be mediated by its interactions with aphids.

Ethics/Moral and Cultural Considerations

Perceptions of the public

Science and conservation take place within (and contribute to) the ethical framework of society. It is therefore worth considering the ethics of responding to invasive species to ensure their treatment is morally justifiable but also to avoid damaging the relationship between scientists or conservation practitioners and the general populous, especially where the science or conservation is publicly funded.

People often have an affiliation for “charismatic” species. This is utilized in conservation fundraising where species such as pandas, polar bears or whales that people are unlikely to ever see in the wild are used as mascots to encourage people to donate. Characteristics such as “charisma” are worth taking into account when planning the response to an invasive species. We might, for example, expect a stronger backlash against attempts to cull invasive mammals such as grey squirrels or muntjac deer than we might control measures targeting invasive invertebrates or plants.
It is also advisable to consider any specific cultural value, particularly to indigenous peoples (Todd et al., 2015), possessed by an invasive species itself or the organisms it impacts. We must ensure sufficient information is available to the public about the justification for any control.

It is also worth considering the language used when discussing non-native species. Epithets such as “invasive”, “alien” and “colonising” for example often have pejorative connotations so could bias opinions about a particular species for non-specialists (Colautti and MacIsaac, 2004).

Open Data

Open access to data is very important for invasive species research and management (Groom et al., 2015). Access is essential for ensuring the accuracy of “horizon scanning” and risk assessment initiatives and helps to facilitate a rapid response when invasive species reach a new area. Data can have expected future uses e.g. species distribution data from a single year could be combined with datasets from subsequent years to assess the spread of a particular organism, but can also have unanticipated future uses. Publishing information details of control attempts and their effectiveness helps to avoid reuse of ineffective techniques and therefore reduce the potential suffering inflicted on target and non-target organisms. Collating information about non-native species into a centralised repository, for example the Great Britain Non-Native Species Information Portal (Roy et al., 2014b), is also useful.

Our responsibilities

Most people would agree that species have the right to exist. There are some exceptions, for example organisms which cause diseases with the most severe pathology. The ethics of vertebrate (and to some extent invertebrate) research is centred on the policy of the “three Rs” - Replacement, Reduction and Refinement (Lindsjö et al., 2016). There is a growing awareness and concern about the use and ethical treatment of animals in science (Drinkwater et al., 2019). Applying the same principles to dealing with non-native invasive species will help to ensure their ethical treatment. The best approach (practically as well as ethically) is to prevent invasive species arriving and becoming established in a novel location as this reduces the potential need to harm them. Whilst populations of invasive species may seem undesirable, they could have unique attributes such as their population structure, phenotypes, genetics, behavioural syndromes or even cultures. These are often perceived as units to conserve in native populations (Daniels et al., 2001; Sato et al., 2009; Whitehead et al., 2004). Ultimately humans are responsible for invasive species because it is humans, for the most part, who transport them to new areas.

Response and Next Steps

The evaluation process in response to an invasive species needs to weigh up the seriousness of the impacts and the scale at which they occur, and the potential for this to change for the worse in the future. The evaluation process should also consider the likelihood of any mitigation measures being successful, given the species’ biological characteristics, and what the value of a partial mitigation would be. The financial resources available must also be taken into consideration, along with the moral and ethical angles and the uncertainties in the data, which could lead to unintended impacts of a response. All of this taken together can enable an informed cost-benefit analysis, leading to the choice of one of the response outcomes listed in Figure 1.3. This cost-benefit analysis should be a process which is repeated as new evidence comes to light, and whatever the chosen response outcome, more information will always be valuable to improve the decision process. This thesis contains a risk assessment for *Lasius neglectus* in the UK (Chapter 2), and also acts on the recommendations of that risk assessment to collect more data across a range of areas (Chapters 3-5) that enable an informed cost-benefit analysis for *Lasius neglectus*.

References

Abbott, K.L., 2006. Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. Divers. Distrib. 12, 101–110.

Abbott, K.L., Green, P.T., 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. Oikos 116, 1238–1246.

Beckers, R., Goss, S., Deneubourg, J.L., Pasteels, J.M., 1989. Colony size, communication, and ant foraging strategy. Psyche (Stuttg). 96, 239–256.

Bertelsmeier, C., Blight, O., Courchamp, F., 2016. Invasions of ants (Hymenoptera : Formicidae) in light of global climate change. Myrmecological News 22, 25–42.

Bertelsmeier, C., Luque, G.M., Hoffmann, B.D., Courchamp, F., 2015. Worldwide ant invasions under climate change. Biodivers. Conserv. 24, 117–128.

Boase, C., 2014. *Lasius neglectus* (Hymenoptera : Formicidae) in the UK : status, impact and management. In: Proceedings of the Eighth International Conference on Urban Pests. pp. 223–228.

Boomsma, J.J., Brouwer, A.H., Van Loon, A.J., 1990. A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe. Insectes Soc. 37, 363–375.

Colautti, R.I., MacIsaac, H.J., 2004. A neutral terminology to define ‘invasive’ species. Divers. Distrib. 10, 135–141.

Cremer, S., Ugelvig, L. V, Drijfhout, F.P., Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Hughes, D.P., Schulz, A., Petersen, K.S., Konrad, H., Stauffer, C., Kiran, K., Espadaler, X., d’Ettorre, P., Aktaç, N., Eilenberg, J., Jones, G.R., Nash, D.R., Pedersen, J.S., Boomsma, J.J., 2008. The evolution of invasiveness in garden ants. PLoS One 3, e3838.

Cremer, S., Ugelvig, L. V, Lommen, S.T.E., Petersen, K.S., Pedersen, J.S., 2006. Attack of the invasive garden ant: aggression behaviour of *Lasius neglectus* (Hymenoptera: Formicidae) against native *Lasius* species in Spain. Myrmec. Nachr 9, 13–19.

Crowley, S.L., Hinchliffe, S., McDonald, R.A., 2017. Conflict in invasive species management. Front. Ecol. Environ. 15, 133–141.

Daniels, M.J., Beaumont, M.A., Johnson, P.J., Balharry, D., Macdonald, D.W., Barratt, E., 2001. Ecology and genetics of wild-living cats in the north-east of Scotland and the implicaitons for the conservation of the wildcat. J. Appl. Ecol. 38, 146–161.

Department of Environment Food and Rural Affairs, 2018. Biodiversity 2020: A strategy for England's wildlife and ecosystem services Indicators.

Drinkwater, E., Robinson, E.J.H., Hart, A.G., 2019. Keeping invertebrate research ethical in a landscape of shifting public opinion. Methods Ecol. Evol. 2041–210X.13208.

EEA, 2012. The impacts of invasive alien species in Europe. EEA Technical report, No 16/2012.

Espadaler, X., Rey, S., 2001. Biological constraints and colony founding in the polygynous invasive ant *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 48, 159–164.

Espadaler, X., Rey, S., Bernal, V., 2004. Queen number in a supercolony of the invasive garden ant, *Lasius neglectus*. Insectes Soc. 51, 232–238.

Espadaler, X., Tartally, A., Schultz, R., Seifert, B., Nagy, C., 2007. Regional trends and preliminary results on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 54, 293–301.

Fox, M.G., 2010. First incursion of Lasius neglectus (Hymenoptera: Formicidae), an invasive polygynous ant in Britian. Br. J. Ent. Nat. Hist. 23, 259–261.

Green, P.T., O'Dowd, D.J., Abbott, K.L., Jeffery, M., Retallick, K., Mac Nally, R., 2011. Invasional meltdown: Invader-invader mutualism facilitates a secondary invasion. Ecology 92, 1758–1768.

Groom, Q., Desmet, P., Vanderhoeven, S., Adriaens, T., 2015. The importance of open data for invasive alien species research, policy and management. Manag. Biol. Invasions 6, 119–125.

Gurevitch, J., Padilla, D.K., 2004. Are invasive species a major cause of extinctions? Trends Ecol. Evol. 19, 470–4.

Harrower, C.A., Rorke, S.L., Roy, H.E., 2019. England Biodiversity Indicators -Technical background document - 20. Pressure from invasive species.

Heller, N.E., Ingram, K.K., Gordon, D.M., 2008. Nest connectivity and colony structure in unicolonial Argentine ants. Insectes Soc. 55, 397–403.

Hill, L., Jones, G., Atkinson, N., Hector, A., Hemery, G., Brown, N., 2019. The £15 billion cost of ash dieback in Britain. Curr. Biol. 29, R315–R316.

Hoffmann, B.D., Luque, G.M., Bellard, C., Holmes, N.D., Donlan, C.J., 2016. Improving invasive ant eradication as a conservation tool: A review. Biol. Conserv. 198, 37–49.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Springer-Verlag Berlin Heidelberg.

Holway, D., Lach, L., Suarez, A., 2002. The causes and consequences of ant invasions. Annu. Rev. Ecol. Syst. 33, 181–233.

Huang, D., Haack, R. a, Zhang, R., 2011. Does global warming increase establishment rates of invasive alien species? A centurial time series analysis. PLoS One 6, e24733.

Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46, 10–18.

Hulme, P.E., 2020. Plant invasions in New Zealand: global lessons in prevention, eradication and control. Biol. Invasions 22, 1539–1562.

IPBES, 2019. Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.

Keller, R.P., Geist, J., Jeschke, J.M., Kühn, I., 2011. Invasive species in Europe: ecology, status, and policy. Environ. Sci. Eur. 23, 23.

Lach, L., 2008. Floral visitation patterns of two invasive ant species and their effects on other hymenopteran visitors. Ecol. Entomol. 33, 155–160.

Lessard, J., Fordyce, J., Gotelli, N., Sanders, N., 2009. Invasive ants alter the phylogenetic structure of ant communities. Ecology 90, 2664–2669.

Linders, T.E.W., Schaffner, U., Eschen, R., Abebe, A., Choge, S.K., Nigatu, L., Mbaabu, P.R., Shiferaw, H., Allan, E., 2019. Direct and indirect effects of invasive species: Biodiversity loss is a major mechanism by which an invasive tree affects ecosystem functioning. J. Ecol. 107, 2660–2672.

Lindsjö, J., Fahlman, Å., Törnqvist, E., 2016. Animal Welfare From Mouse To Moose—Implementing the Principles of the 3Rs in Wildlife Research. J. Wildl. Dis. 52, S65–S77.

Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20, 223–228.

McGlynn, T.P., 1999. The worldwide transfer of ants: Geographical distribution and ecological invasions. J. Biogeogr. 26, 535–548.

Mooney, H. a, Cleland, E.E., 2001. The evolutionary impact of invasive species. Proc. Natl. Acad. Sci. U. S. A. 98, 5446–51.

Nagy, C., Tartally, A., Vilisics, F., Merkl, O., Szita, É., Szél, G., Podlussány, A., Rédei, D., Csösz, S., Pozsgai, G., Orosz, A., Szövényi, G., Markó, V., 2009. Effects of the invasive garden ant, *Lasius neglectus* VAN LOON, BOOMSMA & ANDRÁS-FALVY, 1990 (Hymenoptera: Formicidae), on arthropod assemblages: pattern analyses in the type supercolony. Myrmecological News 12, 171–181.

Ness, J., Bronstein, J., 2004. The effects of invasive ants on prospective ant mutualists. Biol. Invasions 6, 445–461.

Nuñez, M.A., Moretti, A., Simberloff, D., 2011. Propagule pressure hypothesis not supported by an 80-year experiment on woody species invasion. Oikos 120, 1311–1316.

Paini, D.R., Sheppard, A.W., Cook, D.C., De Barro, P.J., Worner, S.P., Thomas, M.B., 2016. Global threat to agriculture from invasive species. Proc. Natl. Acad. Sci. U. S. A. 113, 7575–7579.

Paris, C.I., Espadaler, X., 2009. Honeydew collection by the invasive garden ant *Lasius neglectus* versus the native ant *L. grandis*. Arthropod. Plant. Interact. 3, 75–85.

Paris, C.I., Espadaler, X., 2012. Foraging Activity of Native Ants on Trees in Forest Fragments Colonized by the Invasive Ant *Lasius neglectus*. Psyche A J. Entomol. 2012, 1–9.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288.

Prior, K.M., Adams, D.C., Klepzig, K.D., Hulcr, J., 2018. When does invasive species removal lead to ecological recovery? Implications for management success. Biol. Invasions 20, 267–283.

Rabitsch, W., 2011. The hitchhiker's guide to alien ant invasions. BioControl 56, 551–572.

Rey, S., Espadaler, X., 2004. Area-wide management of the invasive garden ant *Lasius neglectus* (Hymenoptera: Formicidae) in Northeast Spain. J. Agric. Urban Entomol. 21, 99–112.

Reznikova, Z.I., Ryabko, B., 2001. A study of ants' numerical competence. Comput. Inf. Sci. 5, 111–126.

Ricciardi, A., Palmer, M.E., Yan, N.D., 2011. Should Biological Invasions Be Managed as Natural Disasters? Bioscience 61, 312–317.

Rice, E.S., Silverman, J., 2013. Propagule pressure and climate contribute to the displacement of *Linepithema humile* by *Pachycondyla chinensis*. PLoS One 8, e56281.

Robinson, E.J.H., Green, K.E., Jenner, E.A., Holcombe, M., Ratnieks, F.L.W., 2008. Decay rates of attractive and repellent pheromones in an ant foraging trail network. Insectes Soc. 55, 246–251.

Robinson, E.J.H., Jackson, D.E., Holcombe, M., Ratnieks, F.L.W., 2005. Insect communication: "no entry" signal in ant foraging. Nature 438, 442.

Roy, H.E., Peyton, J., Aldridge, D.C., Bantock, T., Blackburn, T.M., Britton, R., Clark, P., Cook, E., Dehnen-Schmutz, K., Dines, T., Dobson, M., Edwards, F., Harrower, C., Harvey, M.C., Minchin, D., Noble, D.G., Parrott, D., Pocock, M.J.O., Preston, C.D., Roy, S., Salisbury, A., Schönrogge, K., Sewell, J., Shaw, R.H., Stebbing, P., Stewart, A.J. a, Walker, K.J., 2014a. Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. Glob. Chang. Biol. 20, 3859–71.

Roy, H.E., Preston, C.D., Harrower, C.A., Rorke, S.L., Noble, D., Sewell, J., Walker, K., Marchant, J., Seeley, B., Bishop, J., Jukes, A., Musgrove, A., Pearman, D., Booy, O., 2014b. GB Non-native Species Information Portal: documenting the arrival of non-native species in Britain. Biol. Invasions 16, 2495–2505.

Sato, J.J., Yasuda, S.P., Hosoda, T., 2009. Genetic Diversity of the Japanese Marten (*Martes melampus*) and Its Implications for the Conservation Unit. Zoolog. Sci. 26, 457–466.

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Van Kleunen, M., Winter, M., Ansong, M., Arianoutsou, M., Bacher, S., Blasius, B., Brockerhoff, E.G., Brundu, G., Capinha, C., Causton, C.E., Celesti-Grapow, L., Dawson, W., Dullinger, S., Economo, E.P., Fuentes, N., Guénard, B., Jäger, H., Kartesz, J., Kenis, M., Kühn, I., Lenzner, B., Liebhold, A.M., Mosena, A., Moser, D., Nentwig, W., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H.E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., Walker, K., Ward, D.F., Yamanaka, T., Essl, F., 2018. Global rise in emerging alien species results from increased accessibility of new source pools. Proc. Natl. Acad. Sci. U. S. A. 115, E2264–E2273.

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., Kartesz, J., Kenis, M., Kreft, H., Kühn, I., Lenzner, B., Liebhold, A., Mosena, A., Moser, D., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H.E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., Van Kleunen, M., Walker, K., Weigelt, P., Yamanaka, T., Essl, F., 2017. No saturation in the accumulation of alien species worldwide. Nat. Commun. 8, 1–9.

Seifert, B., 2000. Rapid range expansion in *Lasius neglectus* (Hymenoptera, Formicidae) – an Asian invader swamps Europe. Mitt. Mus. Nat. kd. Berl., Dtsch. entomol. Z. 47, 173–179.

Shackleton, R.T., Shackleton, C.M., Kull, C.A., 2019. The role of invasive alien species in shaping local livelihoods and human well-being: A review. J. Environ. Manage. 229, 145–157.

Shik, J.Z., Dussutour, A., 2020. Nutritional Dimensions of Invasive Success. Trends Ecol. Evol. 35, 691–703.

Strayer, D.L., Eviner, V.T., Jeschke, J.M., Pace, M.L., 2006. Understanding the long-term effects of species invasions. Trends Ecol. Evol. 21, 645–51.

Stukalyuk, S. V., 2020. Uzbekistan — the alleged native range of the invasive ant *Lasius neglectus* (Hymenoptera, Formicidae): geographical, ecological and biological evidences. Zoodiversity 54, 111–122.

Suarez, A. V., McGlynn, T.P., Tsutsui, N.D., 2010. Biogeographic and Taxonomic Patterns of Introduced Ants. In: Ant Ecology. p. 233.

Tartally, A., 2000. Notes on the coexistence of the supercolonial *Lasius neglectus* Van Loon, Boomsma et Andrásfalvy 1990 (Hymenoptera: Formicidae) with other ant species. Tiscia 1990, 43–46.

Todd, J.H., Barratt, B.I.P., Tooman, L., Beggs, J.R., Malone, L.A., 2015. Selecting non-target species for risk assessment of entomophagous biological control agents : Evaluation of the PRONTI decision-support tool 80, 77–88.

UE, 2014. Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species, Official Journal of the European Union.

Ugelvig, L. V, Drijfhout, F.P., Kronauer, D.J.C., Boomsma, J.J., Pedersen, J.S., Cremer, S., 2008. The introduction history of invasive garden ants in Europe: integrating genetic, chemical and behavioural approaches. BMC Biol. 6.

Van Loon, A.J., Boomsma, J.J., Andrasfalvy, A., 1990. A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe description and general Biology. Insectes Soc. 37, 348–362.

Whitehead, H., Rendell, L., Osborne, R.W., Würsig, B., 2004. Culture and conservation of non-humans with reference to whales and dolphins: Review and new directions. Biol. Conserv. 120, 427–437.

Wittman, S., 2014. Impacts of invasive ants on native ant communities (Hymenoptera: Formicidae). Myrmecological News 19, 111–123.

Yongo, E., Outa, N.., 2015. Effects of Water Hyacinth (Eichhornia Crassipes Solm) Infestation on Water Quality , Fish Diversity and Abundance in the. Lakes, Reserv. ponds 9, 67–74.

Chapter 2: GB Non-native Species Rapid Risk Assessment (NRRA) for *Lasius neglectus*

Introduction to the role and scope of GB Non-native Species Risk Assessments

In Great Britain, Non-native Species Risk Assessments are a standardised tool for collating and appraising relevant current information about a specific non-native invasive species. The assessment evaluates the probability and means of introduction and spread, the species' likely impacts, and possible management options. The degree of uncertainty in the available information is also qualified (Baker et al., 2008; Mumford et al., 2010). 'Great Britain Non-native Species Risk Assessments' take the form of standard questions covering the entry, establishment, spread, impacts and management options for a target species. A similar process can be used for examining threats to particular ecosystems or to evaluate specific invasion pathways.

Once completed, GB Non-native Risk Assessments undergo peer review to help to reduce any potential assessor bias. An alternative approach to an individual assessment and peer-review process could be a collective review performed by a panel of experts (Vanderhoeven et al., 2017). Publishing risk assessments and allowing time for public or stakeholder consultation can also help to reduce bias in the assessment of the evidence (Vanderhoeven et al., 2017). The Great Britain Non-native Risk Assessments are published on the GB Non Native Species Secretariat website for three months after peer review to allow public comment of the evidence presented (NNSS, 2020).

The application of the same risk assessment protocol to multiple species helps to provide comparable information to aid in prioritising action at the policy decision stage (Mumford et al., 2010). These Risk Assessments provide the scientific evidence about invasive species and potential management options and are used as evidence upon which to carry out a policy decision making process which must also consider things like the practicality and cost of management options. The steps in the risk assessment process are outlined in figure 2.1.

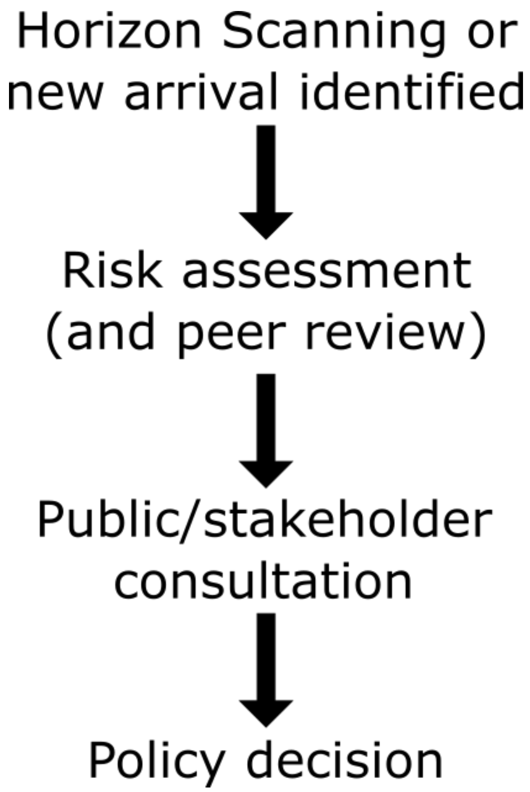


Figure 2.1 – a simplified diagram showing the path from the commissioning of a risk assessment to it feeding into a policy decision making process.

GB Non-native Species Rapid Risk Assessment (NRRA)

Rapid Risk Assessment of: *Lasius neglectus* (Invasive Garden Ant)
Author: Phillip Buckham-Bonnett, Elva J H Robinson

Version: Draft 1 (*06/05/2016*), Peer Review (*04/11/2016)*, NNRAP 1st review (*Nov 2016*), Draft 2 (*11/01/2017*), etc.
Signed off by NNRAP: TBC
Approved by Programme Board: [sent September 2015]
Placed on NNSS website: TBC

Introduction:

The rapid risk assessment is used to assess invasive non-native species more rapidly than the larger GB Non-native Risk Assessment. The principles remain the same, relying on scientific knowledge of the species, expert judgement and peer review. For some species the rapid assessment alone will be sufficient, others may go on to be assessed under the larger scheme if requested by the Non-native Species Programme Board.

Guidance notes:

- We recommend that you read all of the questions in this document before starting to complete the assessment.
- Short answers, including one word answers, are acceptable for the first 10 questions. More detail should be provided under the subsequent questions on entry, establishment, spread, impacts and climate change.
- References to scientific literature, grey literature and personal observations are required where possible throughout.

1 - What is the principal reason for performing the Risk Assessment? (Include any other reasons as comments)

Response: *To rapidly assess the risk associated with this species in Great Britain*

2 - What is the Risk Assessment Area?

Response: *Great Britain*

3 - What is the name of the organism (scientific and accepted common; include common synonyms and notes on taxonomic complexity if relevant)?

Response:

Lasius neglectus Van Loon, Boomsma & Andrásfalvy, 1990 (Hymenoptera, Formicidae)

Common names: Invasive Garden Ant (preferred), Asian Super-Ant

The species has sometimes been incorrectly synonymised with *Lasius turcicus* due to morphological similarities (Seifert, 2000). In records predating its description in 1990, the species is often thought to be *Lasius alienus*.

4 - Is the organism known to be invasive anywhere in the world?

Response:

Yes. *Lasius neglectus* is a widespread invasive pest in Europe and Asia Minor and has been recorded at over two hundred sites across twenty countries (see Figure 2.2).

Global distribution information can be found at:

www.creaf.uab.es/xeg/Lasius/Ingles/distribution.htm

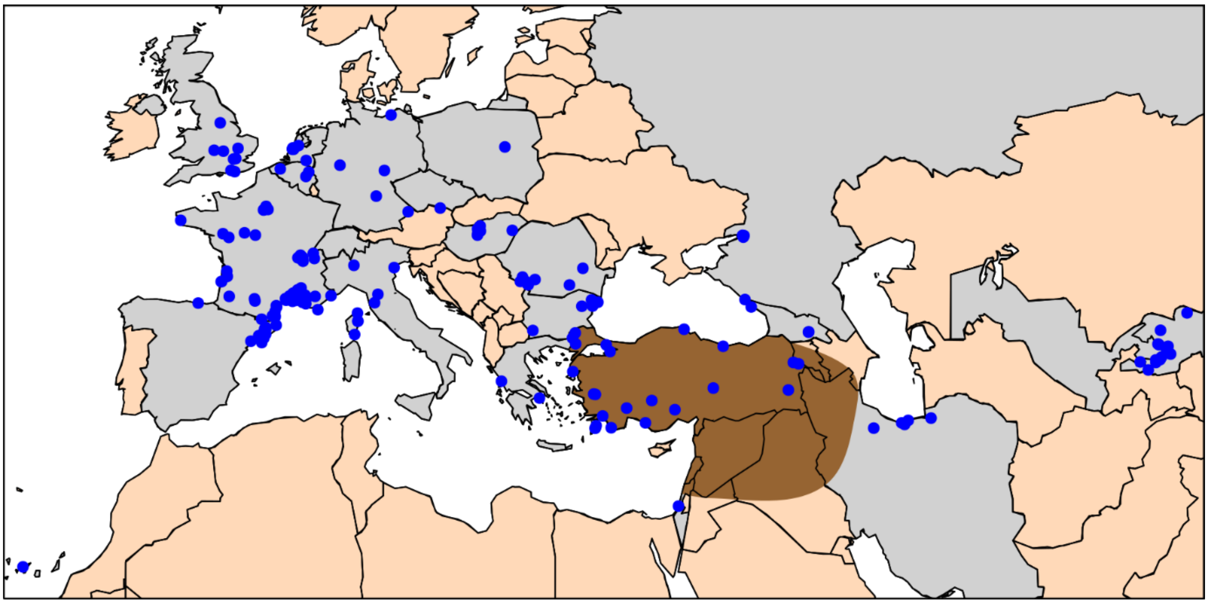


Figure 2.2 – Map showing the location of all known *Lasius neglectus* colonies. Blue dots represent locations where the ant has been found, countries with colonies are indicates in grey, and the likely home range of the ant [adapted from Cremer et al. (2008)**]** is indicated in brown**.** Data from (Boase, *pers. comm.*; Espadaler and Bernal, 2015; Gippet et al., 2016; Le Parisiene, 2015; *pers. obs.* [PBB])**.**

5 - What is the current distribution status of the organism with respect to the Risk Assessment Area?

Response:

As of the NRRA Draft 2 date, *Lasius neglectus* is established at seven locations in Great Britain and has been successfully eliminated from one location (see Figure 2.3).

It was first found at Hidcote Manor, Gloucestershire in 2009 where it occupies approximately 14ha (Boase, 2014; Fox, 2010)**.** A small satellite colony has formed in a quarry approximately 800m to the East of the main colony most likely as a result of transport from Hidcote (Boase, *pers. comm.*).

Lasius neglectus was found in low numbers at Stowe, Buckinghamshire in 2010 on building materials imported from Italy. An immediate eradication response appears to have prevented the species becoming established at this site (Boase, *pers. comm.*).

Lasius neglectus was found in the Cambridge University Botanic Gardens in 2010 (*pers. obs.* [PBB]) where it is now well established covering an area of approximately five hectares (Boase, *pers.comm*, *pers. obs.* [PBB]).

In 2014 *Lasius neglectus* was found in Hendon, North London. An initial inspection found the species occupies at least one hectare of residential and commercial properties (Boase, *pers. comm.*).

In 2016 *Lasius neglectus* was found in the village of Kirk Smeaton in North Yorkshire. It extends approximately 500 metres along a road and currently affects in the region of sixty residential properties (*pers. obs.* [PBB]).

In 2016 *Lasius neglectus* was also found in the grounds and buildings of a farm and school near Rodmell in East Sussex where it occupies at least two to three hectares (Boase, *pers.comm.*). Control attempts so far have had little success.

In 2016 *Lasius neglectus* was also detected in Eastbourne, East Sussex where it occupies an area of at least 7ha. The species is found in residential properties, gardens, college buildings and pavements and has reached pest status (Boase, *pers. comm.*).

In 2016 a *Lasius neglectus* colony was detected in a luxury apartment block in the vicinity of Holland Park, London (W8). The extent of this infestation is unknown (Boase, *pers. comm.*).
Moreover, *Lasius neglectus* is taxonomically cryptic (i.e. superficially similar to some British ant species), therefore hard to detect. This means *L. neglectus* usually goes unnoticed until it reaches pest status and is therefore likely to be present at more sites than currently known. It is important to note that the size of a colony is not necessarily and indicator of establishment date as colonies can grow and shrink at different rates (Tartally et al., 2016).

Lasius neglectus is often found in and around buildings. This is likely a result of human mediated transport and factors such as the availability of suitable nest sites. Warmth for overwintering may also be a benefit but *Lasius neglectus* is able to overwinter in much colder areas that Great Britain, for example sites where the mean air temperature in the coldest month of -4.4°C (Seifert, 2000).

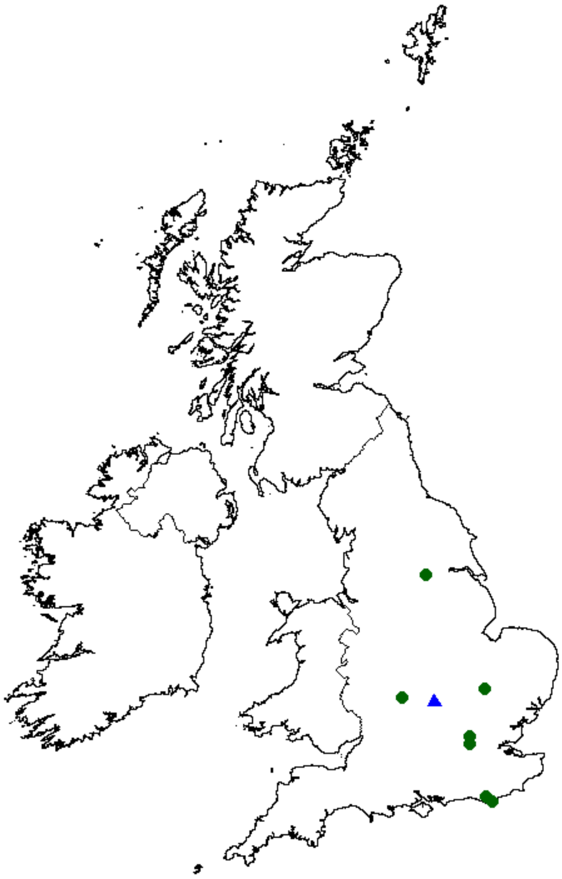


Figure 2.3 – The location of known *Lasius neglectus* colonies (green circles), sites where the species has been eradicated (blue triangles). Information correct as of NRRA review date.

6 - Are there conditions present in the Risk Assessment Area that would enable the organism to survive and reproduce? Comment on any special conditions required by the species?

Response:

The whole of Great Britain (with the exception of some mountainous regions) is thought to be currently climatically suitable for *Lasius neglectus*, with the potential range increasing under climate change forecasts (Bertelsmeier et al., 2015a). *Lasius neglectus* can survive in a wide range of habitats. Whilst it is usually found in highly disturbed areas such as gardens, parks, urban areas and pasture (Boase, 2014; Czechowska and Czechowski, 2003; Espadaler, 1999; Espadaler and Bernal, 2003), it can also invade natural sites (Paris and Espadaler, 2012).

Lasius neglectus exhibits very flexible foraging behaviour, exploiting a wide range of food sources. It forages on both floral and extra-floral plant nectaries (Espadaler et al., 2007; *pers. obs.* [PBB]) and forms mutualisms with a diverse group of honeydew producing insects, including some of which are non-native (*pers. obs.* [PBB]). This means that the species is highly likely to be able to find food sources at new sites. Once established, *L. neglectus* causes an increase in the abundance of taxa such as aphids which it utilises as a food resource (Paris and Espadaler, 2009) promoting its continued success.

Lasius neglectus forms supercolonies comprising multiple non-antagonistic nests that can each contain multiple queens (Boomsma et al., 1990; Espadaler et al., 2004). This, combined with the species' aggressive behaviour towards other ant species (Bertelsmeier et al., 2015b; Cremer et al., 2006; Santarlasci et al., 2014) means that native ant communities are unable to resist the spread of the invasive ant. The low parasite prevalence in *L. neglectus* and low levels of intraspecific aggression (Cremer et al., 2008) also contribute to its ability to readily establish, survive, and thrive in novel locations.

7 - Does the known geographical distribution of the organism include ecoclimatic zones comparable with those of the Risk Assessment Area or sufficiently similar for the organism to survive and thrive?

Response: Yes – see response to questions 4 and 6.

8 - Has the organism established viable (reproducing) populations anywhere outside of its native range (answer N/A if you have answered 'yes' to question 4)?

Response: N/A

9 - Can the organism spread rapidly by natural means or by human assistance?

Response:

Natural spread

Unlike most species in the genus *Lasius*, *Lasius neglectus* queens do not usually fly during or post mating. However, queen morphology suggests that they should be capable of flight (Espadaler and Rey, 2001) and on one occasion a queen was found suspended in a spider's web in a location that would have been difficult to access without flight (Schultz and Seifert, 2005). The rarity of dispersal by flight means that the spread of the invasive ant locally is relatively slow and the species does not tend to arrive at novel sites via this means. Instead, colonies bud off new nests from existing colonies (dependent colony foundation). Budding involves a queen (or queens) moving to a new nest site with part of the population of adult workers. The area occupied by colonies has been recorded as expanding by an average of 13m per year in all directions with new buds forming up to 30m away

(Tartally, 2006). Elsewhere, figures ranging between an increasing radius of 2.75m and 10.6m per year have been reported (Espadaler and Bernal, 2007).

The rate of natural spread is dependent upon the habitat available in the location of the colony. *Lasius neglectus* is less likely to spread into areas dominated by coniferous plants than warmer, more open areas (Tartally, 2006), and dense, overgrown vegetation is unfavourable (Schultz and Seifert, 2005). Roads and pavements provide a conduit for the rapid spread of *L. neglectus* (Tartally, 2006; *pers. obs.* [PBB]) by providing warm and robust nest sites. The dependence of some colonies on aphids in large trees has been suggested as a factor limiting their spread (Boomsma et al., 1990) but the species also shows a preference for isolated trees over core woodland areas (Paris and Espadaler, 2012). This suggests a trade off between thermal requirements and food availability. *Lasius neglectus* appears to be thermophilic in Great Britain, and most abundant in open habitats with exposed soil or stones with nearby food sources (*pers. obs.* [PBB]).

The maximum possible area occupied by a *Lasius neglectus* colony is unknown but the largest reported area is 20ha (Le Parisiene, 2015). This is considerably larger than any of the known Great Britain colonies.

Human mediated dispersal

Human mediated dispersal in the soil of potted plants is the most likely mechanism for the spread of *Lasius neglectus* over distances greater than 100m (Boomsma et al., 1990; Espadaler et al., 2007; Schultz and Busch, 2009). *Lasius neglectus* is frequently found in locations that are associated with botanical exchange (see question 5). Other possible mechanisms for accidental human mediated dispersal include the disposal of construction or green waste (Boase, 2014). The movement of soil and building materials are likely to be important for human mediated dispersal at local and regional scales (i.e. hundreds of metres to hundreds of kilometres). For example the movement of soil to build embankments is linked to the establishment of sixty nine *L. neglectus* supercolonies in the vicinity of Lyon, France (Gippet et al., 2017). The behavioural, chemical and genetic similarities between and within populations in Europe (Ugelvig et al., 2008) support the hypothesis of natural dispersal over short distances and human mediated dispersal over large distances.

10 - Could the organism itself, or acting as a vector, cause economic, environmental or social harm in the Risk Assessment Area?

Response:

Environmental Harm

Lasius neglectus saturates the habitat it invades and reaches very high abundances (Boomsma et al., 1990; Espadaler et al., 2004). *Lasius neglectus* is highly aggressive towards other ants (Cremer et al., 2006) and its numerical advantage allows it to outcompete larger native ant species (Santarlasci et al., 2014). The effects on native ant species can be dramatic with their being excluded from the core area of *Lasius neglectus* colonies (Tartally, 2000). In addition to its impact on native ant communities, the presence of *L. neglectus* also reduces the overall species diversity in an area and in particular reduces the richness of isopods (Nagy et al., 2009). In contrast, the presence of the invasive ant tends to lead to an increase in the abundance of aphids (Paris and Espadaler, 2009), and other non-aphid Hemiptera (Nagy et al., 2009). *Lasius neglectus* feeds on honeydew excreted by Hemiptera and protects them from predators and parasitoids (Espadaler, 1999). In Spain *Lasius neglectus* is estimated to collect more than twice as much honeydew per tree as native ants. This removal of phloem sap is likely to have a not insignificant impact on the health of the trees (Paris and Espadaler, 2009). There have even been anecdotal reports that the aphid load resulting from the presence of *Lasius neglectus* can kill the host trees (Espadaler, 1999). Impacts are highly localised around each colony.

Economic Harm

The biggest potential for economic harm from *Lasius neglectus* is probably via its interaction with aphids. If *Lasius neglectus* were to spread into agricultural areas, particularly those using organic farming methods, it could have a significant impact on yield. The deleterious effects of native ant species (via their interaction with aphids) on crop plants have already been reported (Banks and Macaulay, 1967).

Lasius neglectus has been blamed for damaging electrical equipment in a variety of locations where is it a pest species (Espadaler, 1999; Jolivet, 1986; Rey and Espadaler, 2004). Whilst the precise monetary value of this damage is rarely assessed, a colony at Saint-Desirat, France is estimated to have caused €5000 worth of damage over 4 years (Le Parisiene, 2015). The cost of reducing ant numbers within homes both in terms of materials used and expertise required is likely to be significant over time. The presence of *L. neglectus* could also have a negative impact on the value of property in an area, if the infestation were publicised. Measures taken to prevent the spread of ants from existing locations (e.g. restrictions on the movements of plants and soil) also result in a cost both in terms of inconvenience and money (Boase, 2014).

Social Harm

Lasius neglectus workers do not possess a sting, do not spray formic acid, and are too small to break the skin when biting humans. However, the species readily invades homes, causing distress to residents, and can interfere with activities such as gardening (*pers. obs.* [PBB]). There are no known human allergies to *L. neglectus*. The social harm caused by *L. neglectus* is through annoyance rather than danger.

Vector

The nests of *Lasius neglectus* are home to various myrmecophiles (ant-partnered symbionts), including species such as the cricket *Myrmecophilus fuscus* which are not native to Great Britain (Stalling et al., 2015). Some of these, for example the woodlouse *Platyarthrus schoblii* have been co-introduced outside of their native range alongside the invasive ant (Tartally et al., 2004). Whilst these are not known to cause economic or social harm, their impact on native myrmecophiles (e.g. the woodlouse *Platyarthrus hoffmannsteggii*) is unknown. Some *L. neglectus* colonies in Europe are infected with the fungal pathogen *Laboulbenia formicarum* (Tragust et al., 2015). This ant-specific pathogen is originally from North America and if introduced to Great Britain could potentially spread to native ant species as it has on Madeira (Espadaler and Santamaria, 2003). In North America, the pathogen is known to infect 17 species in the ant subfamily Formicinae (references in: Espadaler et al., 2011).

Table 2.1 – Summary of harm caused by *Lasius neglectus* with locations of examples

Harm	Location	References
Host/ mutualist to other invertebrates not native to Great Britain	Great Britain [Hidcote], Hungary, Spain	(Hornung et al., 2005; Stalling et al., 2015; Tartally et al., 2004; *pers. obs.* [PBB])
Host for ant pathogens not native to Great Britain	France, Spain	(Konrad et al., 2015; Tragust et al., 2015)
Alters native invertebrate community structure	Hungary	(Nagy et al., 2009)
Increases aphid abundance locally	Great Britain [Cambridge, Hidcote, Kirk Smeaton], Hungary, Spain	(Nagy et al., 2009; Paris and Espadaler, 2009; *pers. obs.* [PBB]; Sheld, *pers. comm.*)
Damages plants (through interaction with aphids)	Spain	(Espadaler, 1999)
Excludes native ant species	Great Britain	(Boase, 2014; *pers. obs.* [PBB])
Damage to electrical equipment	Great Britain [Kirk Smeaton], France, Spain	(Jolivet, 1986; Le Parisiene, 2015; Sheld, *pers. comm.*)
Annoyance and distress to residents in affected areas	Great Britain [Cambridge, Hidcote, Kirk Smeaton, Rodmell], Netherlands	(Mabelis et al., 2010; *pers. obs.* [PBB])
Economic losses due to control measures etc	Great Britain [Hidcote]	(Boase, 2014)

Entry Summary

Estimate the overall likelihood of entry into the Risk Assessment Area for this organism (comment on key issues that lead to this conclusion).

Response: *very likely*

Confidence: *very high*

Comments (include list of entry pathways in your comments):

Lasius neglectus has already arrived in Great Britain and is known to be established at seven locations (see Figure 2.3). It is not known whether these populations represent independent introductions from outside Great Britain. There is a risk of further introductions, both from Europe and from existing Great Britain populations.

The most likely pathway for the entry of *L. neglectus* into Great Britain is in the soil associated with potted plants. Therefore, the species is most likely to arrive at areas associated with horticultural exchange as these will have a higher propagule pressure. This includes sites involved in the horticultural trade (e.g. garden centres) but also those involved with plant curation, display or research (e.g. botanic gardens and gardens open to the public). Shipping ports and airports tend to have a high propagule pressure for non-native ant species (Ward et al., 2006). The ability for *L. neglectus* to survive in highly disturbed habitats means that Great Britain points of entry linked to horticultural exchange with Europe are also high risk locations.

Accidental transport with building materials from Europe (as seen with the arrival at Stowe in 2010) is also possible.

Further pathways relating to the spread of *Lasius neglectus* within the Great Britain are detailed in the "Spread Summary" section.

Establishment Summary

Estimate the overall likelihood of establishment (comment on key issues that lead to this conclusion).

Response: *likely*
Confidence: *very high*
Comments (state where in Great Britain this species could establish in your comments):
A mathematical model suggests that the climate of the whole of the Great Britain (with the exception of some mountainous regions) is currently within the climatic range of existing *Lasius neglectus* populations (both native and invasive). The suitable area is expected to increase under climate change (Bertelsmeier et al., 2015a). (See Bertelsmeier et al. (2015a) Figure 3 for a map.) Whilst this approach does not account for the possible effects of anthropogenic microclimate at invasion sites, *L. neglectus* is reported from one site in its native range that has a mean air temperature in the coldest month of -4.4°C (Seifert, 2000). This indicates that the species should be able to survive British winters.

Lasius neglectus is very flexible in both its foraging behaviour (Espadaler et al., 2007; Schultz and Busch, 2009, *pers. obs* [PBB]) and the range of habitats it can occupy (Paris and Espadaler, 2012; Seifert, 2000). In addition, *L. neglectus* is highly aggressive towards other ant species (Cremer et al., 2006). These factors mean the species is well suited to establishment in novel areas (see Question 6).

There are, however, several examples of *Lasius neglectus* colonies ceasing to expand, shrinking, or even disappearing after having become established (Tartally et al., 2016, 2004). The factors contributing to these changes are unclear but could include: climatic variables; a reduction in resource availability (e.g. food or nest sites); adaptation of local pathogens or competitors; and genetic impediments resulting from inbreeding or isolation (Tartally et al., 2016).

Spread Summary

Estimate overall potential for spread (comment on key issues that lead to this conclusion).

Overall response: *intermediate*

Confidence: *high*

Sub scores:

Natural spread only:

Response: *slow*

Confidence: *high*

Human facilitated spread only:

Response: *rapid*

Confidence: *high*

Comments (in your comments list the spread pathways and discuss how much of the total habitat that the species could occupy has already been occupied):

Lasius neglectus currently only occupies a very small fraction of the total habitat that it could potentially occupy, in part due to its low rate of natural dispersal.

Natural spread

Once established at a site, a *Lasius neglectus* colony will expand the area that it occupies if there is suitable habitat for it to expand into. Highly disturbed habitats and urban areas where sufficient food resources are available are likely to allow a higher rate of spread than areas such as grassland or dense woodland. The rate of spread is likely to be low and natural dispersal to new sites unlikely.

Human facilitated spread

The risk of human facilitated spread is high. *Lasius neglectus* is cryptic (i.e. sufficiently similar to native ant species to not be noticed) and is a small species so there is a high likelihood of it not being noticed upon import to Great Britain. Not all known Great Britain populations have measures in place to prevent the spread to new locations so there is also a high risk of accidental transport.

Moreover, it is highly likely that further unknown populations exist and the risk of spread from these is also high. With the exception of sites liked with horticultural exchange, it is difficult to predict where the species might be transported. Two Great Britain populations (Hendon and Kirk Smeaton) do not have any apparent strong links with the horticulture so it is unclear how *Lasius neglectus* arrived at these sites.

Potential pathways for spread include:

Transport in soil with potted plants
Transport on building materials or waste
Transport in soil (bulk quantities)
Transport in garden waste
Transport in agricultural materials e.g. hay.

Impact Summary

Estimate overall severity of impact (comment on key issues that lead to this conclusion)

Overall response: *moderate*

Confidence: *medium*

Sub-scores

Environmental impacts:

Response: *major*

Confidence**:** *medium*

Economic impacts:

Response: *moderate*

Confidence: *medium*

Social impacts:

Response: *minor*

Confidence: *medium*

Comments (include list of impacts in your comments):

Environmental impacts

The environmental impact of *Lasius neglectus* can be severe but tend to be highly localised around colonies. Impacts include: reducing the abundance of native ants; reducing the richness of native isopods; acting as a vector for other non-native species and diseases; increasing the abundance of Hemiptera (particularly aphids); and potentially impacting plant health via the increase in Hemiptera numbers.

Economic Impacts

Economic impacts resulting from *Lasius neglectus* include: costs relating to the replacement of damaged electrical equipment; the cost of control/ eradication measures; loss of income as a result of control/ eradication measures; a potential impact on property value; and the potential for negative impacts on agricultural production.

Social impacts

The main social impact of *L. neglectus* is the distress and inconvenience caused when it invades homes, properties and gardens.

Uncertainty

The medium confidence in these response levels is in part due to: a lack of data on the impacts of *Lasius neglectus* in Great Britain; the fact that it has only emerged as a pest in the last 30 years so long-term effects are unknown; and the difficulty is estimating the true abundance of this ant in the Great Britain.

Climate Change

What is the likelihood that the risk posed by this species will increase as a result of climate change?

Response: *high*

Confidence: *high*

Comments (include aspects of species biology likely to be effected by climate change (e.g. ability to establish, key impacts that might change and timescale over which significant change may occur):

Lasius neglectus is probably near to the northern edge of its potential range in Great Britain. Whilst the potential Great Britain range will only slightly increase under climate change (Bertelsmeier et al., 2015a), an increase in average temperatures would most likely increase the favourability of Great Britain to the establishment of this species. In Great Britain *Lasius neglectus* appears to be thermophilic in its habitat preference at the local scale (*pers. obs.* [PBB]). Thermophily has also been reported in the most northerly known populations of *L, neglectus* on the European mainland (Schultz and Busch, 2009).

A change in climate that leads to less severe winters or overall warming in Great Britain is likely to be conducive to the spread and persistence of *Lasius* neglectus. A change in Great Britain's climate may also lead to an increase in the demand for and importing of plants native to infected countries such as France and Spain. This could increase the rate at which *Lasius neglectus* arrives in Great Britain.

Conclusion

Estimate the overall risk (comment on the key issues that lead to this conclusion).

Response: *high*

Confidence: *medium*

Comments:

Whilst the impacts of *Lasius neglectus* can be severe, both economically and environmentally, they tend to be very localised around colonies. The whole of Great Britain represents a potential habitat for this species but the natural spread of colonies is slow and they are unlikely to reach new sites via natural means. However, the species is cryptic meaning that novel introductions could easily be overlooked. In addition, *Lasius neglectus* populations tend to be identified only once the ant reaches pest status. It is therefore likely that the species is more abundant than we are currently aware and the risk of accidental human-mediated spread from unknown populations is high. The rate at which new populations are discovered globally is increasing exponentially (Espadaler et al., 2007).

A lack of data on the effects of *Lasius neglectus* in Great Britain and the long term stability and survival of *Lasius neglectus* colonies generally adds uncertainty to this conclusion.

Management options (brief summary):

1 - Has the species been managed elsewhere? If so, how effective has management been?

Response:

Multiple studies have reported that control attempts have been unsuccessful but have not indicated what measures were employed (Espadaler, 1999; Schultz and Busch, 2009)**.**

A field-based trial in Spain used a fourfold approach to attempt to reduce *Lasius neglectus* numbers (Rey and Espadaler, 2004). Trees were fogged with insecticides to kill aphid, tree trunks were painted with a contact insecticide, soil in and around the colony was injected with insecticides and granular bait stations were deployed in houses (see Table 2.2 for details). This approach had some success in reducing ant numbers. However, the effects were reduced by rainfall in the second year, the contribution of each component is unclear and phoxim (the pesticide used in two of the approaches) is no longer approved for use in the European Union (European Commission 2007/393/EC, 2007).

A field-based trail in Great Britain used a single approach to control *Lasius neglectus* numbers (Boase, 2014). A gel-based insecticide bait (Maxforce® Quantum gel) was used applied in and around houses (see Table 2.3 for details). This approach achieved a 91% reduction in ant number over one week. However, application of gel-based pesticides is highly labour intensive and unsuitable for scaling up to large areas. A laboratory-based trial of the efficacy of four granular pesticides has been unable to identify a commercially available granular insecticide bait that performs as well as Maxforce® Quantum gel (Buckham-Bonnett et al., *in prep*).

One successful eradication has occurred in Great Britain. The *Lasius neglectus* arriving at Stowe on stone from Italy were immediately identified allowing the whole shipment to be fumigated with phosphine (Boase, 2014). Subsequent surveys for the ant at Stowe have found no evidence of its presence.

Table 2.2 – Insecticide products and use in Rey and Espadaler (2004)

Product	**Ingredient**	**Concentration of active ingredient**	**Concentration after dilution**	**Use**	**Approximate application**
Fendona®	α-cypermethrin	6%	0.04%	Tree trunk spray	0.6 L /tree
Baythion®	phoxim (foxim)	50%	0.05%	Soil injection	5 L /injection (100 L/house)
Efitax®	α-cypermethrin	4%	0.00%	Tree canopy fogging	4 L /tree
Confidor®	imidacloprid	20%	0.02%	Tree canopy fogging	4 L /tree
Blattanex®	phoxim (foxim)	0.08%	NA (granular)	Bait stations in houses	5 - 10 per house

Table 2.3 – Insecticide products and use in Boase (2014)

Product	**Ingredient**	**Concentration of active ingredient**	**Concentration after dilution**	**Use**	**Approximate application**
Maxforce® Quantum	imidacloprid	0.03%	NA (gel)	Injected into bait stations/ natural cracks and crevices	0.2 g/m^2

2 - List the available control / eradication options for this organism and indicate their efficacy.

Response:

If a *Lasius neglectus* colony is identified when it is small (i.e. within approximately two years of establishment), intensive treatment with a variety of measures (see Table 2.4) followed by monitoring to ensure the treatment's effectiveness should result in its eradication.

There have been no successful attempts at eradicating large colonies but their size can be limited using the methods outlined in Table 2.4. Granular baits appear to have a low palatability for *Lasius neglectus* (Buckham-Bonnett et al, *in prep*) whereas gel-based insecticides are effective but highly labour intensive to apply. Water storing crystals such as those used against the Argentine ant (Boser et al., 2014) are likely to be the best toxicant delivery method for large areas, but research into their use with *Lasius neglectus* is required. This should include an evaluation of the effectiveness of various different active ingredients for the species (Hoffmann et al., 2016).

Table 2.4 – control/eradication options

Measure	**Disadvantages**	**Effectiveness**
Gel ant baits	Highly labour intensive application	High
Granular ant baits	Low palatability for *Lasius neglectus*	Medium - Low
Fumigation	Works best in an enclosed area	High
Contact pesticides e.g. painted on trees.	Highly labour intensive application Effects reduced by rain	Medium
Water storing crystals (laced with insecticide)	Untested with *Lasius neglectus*	Likely high

3 - List the available pathway management options (to reduce spread) for this organism and indicate their efficacy.

Response:

Spread from outside Great Britain

Preventing the import of *Lasius neglectus* into Great Britain is likely to be difficult due to its wide geographical distribution. However, monitoring sites with a high propagule pressure e.g. botanic gardens, garden centres etc. would help to increase the probability that the ant was caught soon enough after arrival for eradication to take place.

Spread from within Great Britain

A list of pathway management practices in place at Hidcote to prevent the spread of *Lasius neglectus* to other locations is provided in Table 2.1 of Boase (2015). In summary, these measures prohibit the removal from site of materials which could also contain queens/ brood of *L. neglectus*. The measures include preventing the transport of plants off site (unless from an ant free area), prohibiting the disposal of garden waste off site, and preventing building waste/ soil being removed from the site. The application of management strategies such as these are particularly important at sites which distribute plants to other locations.

Garden waste/ rubbish bins collected by councils for infested areas also present a potential (although less likely) pathway. This could lead to the ants being transferred to waste processing sites and then on to other new locations. If a colony were located on farmland, the movement of materials such as soil could facilitate the spread of the ant.

It is likely that new potential pathways will emerge that are specific to the area new colonies inhabit. Assessment of new cases is required to ensure that these pathways are identified.

4 - How quickly would management need to be implemented in order to work?

Response:

As the number of new cases discovered globally is increasing exponentially (Espadaler et al., 2007), the sooner pathway management practices are implemented, the more effective they are likely to be. Preventing the spread of *Lasius neglectus* to new locations is the most important measure, followed by steps to eradicate new colonies as soon after establishment as possible.

However, it is likely to be very difficult and expensive to eradicate large established colonies so a rapid response here is not important. The cost of potential control measures alongside other long term economic impacts of the ant should be considered before a decision not to eradicate large colonies is made.

References

Baker, R.H.A., Black, R., Copp, G.H., Haysom4, K.A., Hulme, P.E., Thomas, M.B., Brown, A., Brown, M., Cannon, R.J.C., Ellis, J., Ellis, M., Ferris, R., Glaves, P., Gozlan, R.E., Holt, J., Howe, L., Knight, J.D., MacLeod, A., Moore, N.P., Mumford, J.D., Murphy, S.T., Parrott, D., Sansford, C.E., Smith, G.C., St-Hilaire, S., Ward, N.L., 2008. The UK risk assessment scheme for all non-native species. Biol. Invasions– from Ecol. to Conserv. 7, 46–57.

Banks, C.J., Macaulay, E.D.M., 1967. Effects of *Aphis fabae* Scop, and of its attendant ants and insect predators on yields of field beans (*Vicia faba* L.). Ann. Appl. Biol. 60, 445–453.

Bertelsmeier, C., Luque, G.M., Hoffmann, B.D., Courchamp, F., 2015a. Worldwide ant invasions under climate change. Biodivers. Conserv. 24, 117–128.

Bertelsmeier, C., Ollier, S., Avril, A., Blight, O., Jourdan, H., Courchamp, F., 2015b. Colony-colony interactions between highly invasive ants. Basic Appl. Ecol. 17, 106–114.

Boase, C., 2014. *Lasius neglectus* (Hymenoptera : Formicidae) in the UK : status, impact and management. In: Proceedings of the Eighth International Conference on Urban Pests. pp. 223–228.

Boomsma, J.J., Brouwer, A.H., Van Loon, A.J., 1990. A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe. Insectes Soc. 37, 363–375.

Boser, C.L., Hanna, C., Faulkner, K.R., Cory, C., Randall, J.M., Morrison, S.A., 2014. Argentine Ant Management in Conservation Areas: Results of a Pilot Study. Monogr. West. North Am. Nat. 7, 518–530.

Buckham-Bonnett, P., Aguión, A., Boase, C., Lee, P., Robinson, E.J.H., n.d. Evaluating the palatability and toxicity of commercially available pesticides for large scale use on the Invasive Garden ant (*Lasius neglectus*).

Cremer, S., Ugelvig, L. V, Drijfhout, F.P., Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Hughes, D.P., Schulz, A., Petersen, K.S., Konrad, H., Stauffer, C., Kiran, K., Espadaler, X., d'Ettorre, P., Aktaç, N., Eilenberg, J., Jones, G.R., Nash, D.R., Pedersen, J.S., Boomsma, J.J., 2008. The evolution of invasiveness in garden ants. PLoS One 3, e3838.

Cremer, S., Ugelvig, L. V, Lommen, S.T.E., Petersen, K.S., Pedersen, J.S., 2006. Attack of the invasive garden ant: aggression behaviour of *Lasius neglectus* (Hymenoptera: Formicidae) against native *Lasius* species in Spain. Myrmec. Nachr 9, 13–19.

Czechowska, W., Czechowski, W., 2003. Further record of *Lasius neglectus* Van Loon, Boomsma et Andrasflavy (Hymenoptera:Formicidae) for Warsaw, with a key to the Polish species of the subgenus Lasius s.str. Fragm. faun. 46, 195–202.

Espadaler, X., 1999. *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy, 1990 (Hymenoptera, Formicidae), a potential pest ant in Spain. Orsis 14, 43–46.

Espadaler, X., Bernal, V., 2003. Exotic ants in the Canary Islands (Hymenoptera, Formicidae). Vieraea 31, 1–7.

Espadaler, X., Bernal, V., 2007. *Lasius neglectus*. Control Trials. [WWW Document]. URL http://www.creaf.uab.es/xeg/Lasius/Ingles/control.htm (accessed 2.18.16).

Espadaler, X., Bernal, V., 2015. *Lasius neglectus* a polygynous, sometimes invasive, ant: Distribution [WWW Document]. URL http://www.creaf.uab.es/xeg/lasius/Ingles/distribution.htm (accessed 2.2.16).

Espadaler, X., Lebas, C., Wagenknecht, J., Tragust, S., 2011. *Laboulbenia formicarum* (Ascomycota, Laboulbeniales), an exotic parasitic fungus, on an exotic ant in France. Vie Milieu 61, 41–44.

Espadaler, X., Rey, S., 2001. Biological constraints and colony founding in the polygynous invasive ant *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 48, 159–164.

Espadaler, X., Rey, S., Bernal, V., 2004. Queen number in a supercolony of the invasive garden ant, *Lasius neglectus*. Insectes Soc. 51, 232–238.

Espadaler, X., Santamaria, S., 2003. *Laboulbenia formicarum* Thaxt. (Ascomycota, Laboulbeniales) crosses the Atlantic. Orsis (Org. Sist.) 18, 97–101.

Espadaler, X., Tartally, A., Schultz, R., Seifert, B., Nagy, C., 2007. Regional trends and preliminary results on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 54, 293–301.

European Commission 2007/393/EC, 2007. Commission decision of 6 June 2007 concerning the non-inclusion of diazinon in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance.

Fox, M.G., 2010. First incursion of Lasius neglectus (Hymenoptera: Formicidae), an invasive polygynous ant in Britian. Br. J. Ent. Nat. Hist. 23, 259–261.

Gippet, J.M.W., Mondy, N., Diallo-Dudek, J., Bellec, A., Dumet, A., Mistler, L., Kaufmann, B., 2017. I'm not like everybody else: urbanization factors shaping spatial distribution of native and invasive ants are species-specific. Urban Ecosyst. 20, 157–169.

Hoffmann, B.D., Luque, G.M., Bellard, C., Holmes, N.D., Donlan, C.J., 2016. Improving invasive ant eradication as a conservation tool: A review. Biol. Conserv. 198, 37–49.

Hornung, E., Vilisics, F., Tartally, A., 2005. Occurrence of *Platyarthrus schoblii* (Isopoda, Oniscidea) and its ant hosts in Hungary. Eur. J. Soil Biol. 41, 129–133.

Jolivet, P., 1986. Les fourmis et la télévision. L' Entomol. 42, 321–323.

Konrad, M., Grasse, A. V, Tragust, S., Cremer, S., 2015. Anti-pathogen protection versus survival costs mediated by an ectosymbiont in an ant host. Proc. Biol. Sci. 282, 20141976.

Le Parisiene, 2015. Ardèche : un village envahi par les fourmis. Le Paris.

Mabelis, A.A., van Loon, A.J., Dekoninck, W., 2010. Verovert de plaagmier Nederland? Entomol. Ber. 70(2), 30–36.

Mumford, J.D., Booy, O., BAKer, R.H.A., Rees, M., Copp, G.H., Black, K., Holt, J., Leach, A.W., Hartley, M., 2010. Invasive non-native species risk assessment in Great Britain. Asp. Appl. Biol. 104, 49–54.

Nagy, C., Tartally, A., Vilisics, F., Merkl, O., Szita, É., Szél, G., Podlussány, A., Rédei, D., Csösz, S., Pozsgai, G., Orosz, A., Szövényi, G., Markó, V., 2009. Effects of the invasive garden ant, *Lasius neglectus* VAN LOON, BOOMSMA & ANDRÁS-FALVY, 1990 (Hymenoptera: Formicidae), on arthropod assemblages: pattern analyses in the type supercolony. Myrmecological News 12, 171–181.

NNSS, 2020. GB Non-native Species Secretariat - Risk Assessment [WWW Document]. URL http://www.nonnativespecies.org/index.cfm?pageid=143

Paris, C.I., Espadaler, X., 2009. Honeydew collection by the invasive garden ant *Lasius neglectus* versus the native ant *L. grandis*. Arthropod. Plant. Interact. 3, 75–85.

Paris, C.I., Espadaler, X., 2012. Foraging Activity of Native Ants on Trees in Forest Fragments Colonized by the Invasive Ant *Lasius neglectus*. Psyche A J. Entomol. 2012, 1–9.

Rey, S., Espadaler, X., 2004. Area-wide management of the invasive garden ant *Lasius neglectus* (Hymenoptera: Formicidae) in Northeast Spain. J. Agric. Urban Entomol. 21, 99–112.

Santarlasci, A., Martelloni, G., Frizzi, F., Santini, G., Bagnoli, F., 2014. Modeling warfare in social animals: a “chemical” approach. PLoS One 9, e111310.

Schultz, R., Busch, T., 2009. The northernmost record of the invasive garden ant, *Lasius neglectus* (Hymenoptera: Formicidae). Myrmecological News 12, 183–186.

Schultz, R., Seifert, B., 2005. *Lasius neglectus* (Hymenoptera: Formicidae)–a widely distributed tramp species in Central Asia. Myrmecol News 7, 47–50.

Seifert, B., 2000. Rapid range expansion in *Lasius neglectus* (Hymenoptera, Formicidae) – an Asian invader swamps Europe. Mitt. Mus. Nat. kd. Berl., Dtsch. entomol. Z. 47, 173–179.

Stalling, T., Espadaler, X., Carrón, N.O.Z., 2015. First record of the ant-loving cricket *Myrmecophilus fuscus* Stalling, 2013 (Orthoptera: Myrmecophilidae) in mainland Spain. Sociobiology 62, 481–483.

Tartally, A., 2000. Notes on the coexistence of the supercolonial *Lasius neglectus* Van Loon, Boomsma et Andrásfalvy 1990 (Hymenoptera: Formicidae) with other ant species. Tiscia 1990, 43–46.

Tartally, A., 2006. Long term expansion of a supercolony of the invasive garden ant, *Lasius neglectus* (Hymenoptera: Formicidae). Myrmecologische Nachrichten 9, 21–25.

Tartally, A., Antonova, V., Espadaler, X., Csősz, S., Czechowski, W., 2016. Collapse of the invasive garden ant, *Lasius neglectus*, populations in four European countries. Biol. Invasions 18, 3127–3131.

Tartally, A., Hornung, E., Espadaler, X., 2004. The joint introduction of *Platyarthrus schoblii* (Isopoda: Oniscidea) and *Lasius neglectus* (Hymenoptera : Formicidae) into Hungary. Myrmecologische Nachrichten 6, 61–66.

Tragust, S., Feldhaar, H., Espadaler, X., Pedersen, J.S., 2015. Rapid increase of the parasitic fungus *Laboulbenia formicarum* in supercolonies of the invasive garden ant *Lasius neglectus*. Biol. Invasions 17, 2795–2801.

Ugelvig, L. V, Drijfhout, F.P., Kronauer, D.J.C., Boomsma, J.J., Pedersen, J.S., Cremer, S., 2008. The introduction history of invasive garden ants in Europe: integrating genetic, chemical and behavioural approaches. BMC Biol. 6.

Vanderhoeven, S., Branquart, E., Casaer, J., D'hondt, B., Hulme, P.E., Shwartz, A., Strubbe, D., Turbé, A., Verreycken, H., Adriaens, T., 2017. Beyond protocols: improving the reliability of expert-based risk analysis underpinning invasive species policies. Biol. Invasions 19, 2507–2517.

Ward, D.F., Beggs, J.R., Clout, M.N., Harris, R.J., O'Connor, S., 2006. The diversity and origin of exotic ants arriving in New Zealand via human-mediated dispersal. Divers. Distrib. 12, 601–609.

Chapter 3: Tactile communication in *Lasius* ants

Tactile communication in *Lasius* ants

Science is a collaborative endeavour and the following chapter is the result of work carried out by multiple research groups sharing resources and ideas. I here explain the background context to the collaborative work presented in Chapter 3.

Previous work on tactile communication in *Lasius niger*

The first indication that *Lasius niger* might use tactile communication was from a T-maze experiment performed by S.E.F. Evison but these results were not published in a journal.

An apparent communication effect of ant-ant interactions in *Lasius niger* during foraging was also observed during an experiment examining the role of “footprint” hydrocarbons in foraging (Buckham-Bonnett 2013 - Master’s thesis).

In 2014 P. Buckham-Bonnett and E.J.H. Robinson, in collaboration with S.E.F. Evison, performed an experiment which appeared to demonstrate the use of tactile communication in *Lasius niger* in a T-maze set up. The aim of this had been to use video analysis try to identify the proximate mechanism for communication but none could be detected (Buckham-Bonnett, Robinson & Evison, unpublished data).

Preliminary work on tactile communication in *Lasius neglectus*

P. Buckham-Bonnett and E.J.H. Robinson designed and performed a proof of concept experiment which appeared to indicate the use of directional communication during foraging in *Lasius neglectus* - see appendix A

Parallel work on tactile communication in *Lasius niger*

At the same time, S. Popp and T. Czaczkes began experiments to replicate and explain S.E.F. Evison’s results in *Lasius niger.* Initially the two labs were addressing the problem independently, but when this was realised, the collaboration published in Chapter 3 was undertaken.

Notice of contributions to “No evidence for tactile communication of direction in foraging *Lasiu*s ants”

Experimental Design

The design of the *Lasius neglectus* experiment was by P. Buckham-Bonnett and E.J.H. Robinson (with the idea to use two platforms adapted from the methods by S. Popp). The experiment’s design was chosen to match that of the “proof of concept” experiment performed with *Lasius neglectus* as closely as possible. The design of the *Lasius niger* experiments was by S. Popp and T. Czaczkes.

Data Collection

Approximately 74% of the *Lasius neglectus* data collection was by P. Buckham-Bonnett, 26% by S. Popp. The *Lasius niger* data collection was by S. Popp.

Data Analysis and Writing

Data analysis was performed by T. Czaczkes. The paper was primarily written by T. Czaczkes but P. Buckham-Bonnett (and other authors) made contributions to early drafts and had the opportunity to comment on the version submitted for publication.

No evidence for tactile communication of direction in foraging *Lasius* ants

Stefan Popp[1,2], Phillip Buckham-Bonnett[3], Sophie E. F. Evison[4, *], Elva J. H. Robinson[3, *], and Tomer J. Czaczkes[1,*]

[1] Institute of Zoology, Universität Regensburg, Universitätsstraße 31, D-93053 Regensburg, Germany

[2] Department of Behavioral Physiology and Sociobiology, Biocenter, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany

[3] Department of Biology and York Centre for Complex Systems Analysis, Heslington, York, YO10 5DD, UK

[4] Animal and Plant Sciences, University of Sheffield, Alfred Denny Building, Western Bank, Sheffield, S10 2TN, UK

[*] Corresponding authors

Abstract

The idea that ants communicate when meeting on a trail is beguiling, but evidence for this is scarce. Physical communication in ants has been demonstrated to play a role as a modulator of behaviours such as alarm and recruitment. Honeybees can communicate the location of a resource using an advanced motor display – the waggle dance. However, no equivalent of the waggle dance has been described for any ant species, and it is widely believed that ants cannot communicate the location of resources using motor displays. One group of researchers report several demonstrations of such communication in *Formica* ants; however, these results have been largely ignored. More recently some evidence arose that *Lasius niger* foragers returning from a food source can communicate to outgoing foragers the direction that should be taken at the next bifurcation by means of physical contact on the trail. Here, we make a concerted effort to replicate these results. Although initial results seemed to indicate physical communication, once stringent controls to eliminate pheromone cues were put in place, no evidence for physical communication of food location could be found. This null result was replicated independently by a different research group on a closely related species, *L. neglectus*. We conclude that neither *L. niger* nor *L. neglectus* foragers communicate resource location using physical contact. Our results increase the burden of proof required for other claims of physical communication of direction in ants, but do not completely rule out this possibility.

Key words

Motor displays – tactile communication – distance homing – *Lasius niger* – *Lasius neglectus* - antennation

Introduction

> *"The story that ants talk by touching antennae is probably the most deeply rooted idea most people have about ants. It is also a story of considerable age. Yet the evidence that ants do have an antennal language is extremely thin".* Sudd (1967) *– An Introduction to The Behaviour of Ants*

An observation made by almost anybody who has ever watched ants forage is that ants encountering nestmates on a trail will often pause and make antennal contact. As observers, we cannot help but imagine that some form of communication is taking place. There is strong evidence that several ant species use a series of motor displays to modulate their recruitment behaviour (Hölldobler, 1971; Hölldobler and Wilson, 1990, 1978), such as priming nestmates to follow pheromone trails, or signalling that a pheromone trail leads to a food source or a nest site (Hölldobler, 1971). As ant trails often form a branching network of paths, and much ant foraging occurs on plants (which again constitute a ramifying system), it seems plausible that some sort of directional signalling of food location would lead to more efficient foraging. This hypothesis was indeed suggested over two centuries ago (Huber, 1810) and found support from the eminent myrmecologist Erich Wasmann (1905). In light of Karl von Frisch's remarkable discovery of the honey bee waggle dance (Von Frisch, 1967, 1923), such a supposition seemed a lot more reasonable. Undoubtedly, ants meeting on a trail ascertain each other's colony identity (Akino et al., 2004; Mamiko et al., 2005). Odour cues from successful ants returning to the nest are also likely to be gathered by the outgoing ant, which can inform the foragers as to what type of food is available (Le Breton and Fourcassie, 2004; Roces, 1994, 1990). It is likely that odour cues on returning foragers can trigger previously learned associations between food odours and foraging locations (Czaczkes et al., 2014), in a manner similar to odour cue transfer via trophallaxis in honey bees (Balbuena et al., 2012; Farina et al., 2005; Grüter et al., 2008). Despite the temptation to assume that more than simple cue-sensing is occurring during ant-ant interactions, there remains very little support for anything more complex, such as signal exchange (Sudd, 1967). In their landmark book, Hölldobler and Wilson (1990) state that "ants antennate nestmates in order to smell them, not to inform them".

There is, however, one notable exception to the lack of support for tactile directional information transfer in ants: the findings of Reznikova and colleagues (reviewed in Reznikova, 2017, 2008), and the related work of Novgorodova (2006). Reznikova and Ryabako (1994) describe a series of experiments in which scouts from two *Formica* species (*F. polyctena* and *F. sanguinea*) were able to communicate complex directional information to other foragers via physical contact. Forager groups that could physically interact with an informed scout were able to find the location of a food source at the end of a multiply-bifurcating maze much more accurately and rapidly than groups that were not allowed to interact with an informed scout. These results implied that the informed scout could communicate a series of turns to naïve foragers. In a second experiment reported in the same paper, and replicated in Reznikova and Ryabko (2001), scout ants were allowed to find a food source on one branch of a comb-like maze consisting of 25 or more branches, all emerging from a single main stem in one direction. Groups of foragers subsequently contacted by the informed scout then achieved remarkable accuracy in finding the food source: in one experiment (Reznikova and Ryabko, 2001) ant groups made zero mistakes in 117 of 152 trials. The authors stressed that in every experiment steps were taken to ensure that no information apart from direct physical contact from the informed scout was available to the otherwise naïve foragers. Using variations of these experimental paradigms, and by measuring the time scout ants spent communicating with their team of naïve foragers, Reznikova and Ryabko (1994, 2001) describe further impressive information-processing feats by these ants. These include simple arithmetic operations such as addition and subtraction, and information-compression abilities. Novgorodova (2006) replicated some of the findings of Reznikova and Ryabko (1994) in a related species; *Formica pratensis*. The results appeared to corroborate the previous findings, and showed that otherwise naïve foragers which had contacted an informed scout spent significantly less time searching for a feeder at the end of a maze than foragers that had no contact with informed scouts. However, as decision accuracy was not provided, the results could equally well be explained by faster searching by the contacted naïve ants.

The findings of Reznikova and colleagues are startling, but they have had little impact on the scientific community, perhaps as the results seem unlikely. However, the uncovering of many seemingly unlikely facts have been the cornerstone of scientific progress for centuries. Moreover, in light of the honey bee waggle dance and the complex motor displays performed by other ants (Hölldobler, 1976, 1971; Hölldobler and Wilson, 1978), such claims are perhaps not quite so far-fetched. Indeed, one experiment suggests that honey bees can also count, albeit to a limit of five items (Dacke and Srinivasan, 2008). More concrete doubts on these findings are cast by analyses of antennation during trophallaxis (Bonavita-Cougourdan and Morel, 1984; Lenoir, 1982), in which no conclusive patterns could be found. Lenoir (1982) concludes that the Shannon information density of antennal contact in *Myrmica rubra* is too low to support complex directional communication. Rather, it is argued, such communication would be more suited to modulation, for example of trophallaxis time or rate. Indeed, McCabe et al. (2006) support this claim by showing that antennation patterns during trophallaxis correlate with food quality and colony hunger levels in the ant *Camponotus mus*. However, the communication periods observed by Reznikova et al. included more than just trophallaxis, and Reznikova and Ryabko (1994, 2001) argue that numerical information is transmitted by the duration of antennation, not the pattern of antennal strikes, as assumed by Lenoir. Indeed, Reznikova et al. explicitly tested for, and found no evidence of, tactile communication of direction in *M. rubra* (Reznikova and Ryabko, 1994). Lastly, a major reason for the lack of acceptance of antennation as a directional communication method is that, unlike the honey bee waggle dance, the underlying mechanism has not been elucidated, and thus this putative communication system remains a 'black box' (Reznikova, 2007).

From a theoretical standpoint, the additional benefit of such a communication system is not wholly clear. Chemical recruitment systems are already available to these ants, although their reliance on pheromonal recruitment may vary (e.g. Aron et al., 1993; Thienen et al., 2014). Antennation may add another source of information to the large array of information sources which ants are known to use when making directional decisions (Czaczkes et al., 2015b). It may also be that an additional physical system could help prevent ant colonies becoming 'trapped' by outdated pheromone trails or memories, by acting to counter such information (Beckers et al., 1990; Czaczkes et al., 2016; Goss et al., 1989).

The phenomenon of transfer of directional information via physical contact was investigated in a different species of ant, *Lasius niger* in the doctoral thesis of Evison (2008). This study appeared to suggest that ant-ant communication could convey directional information in this species, but in a far more modest manner (e.g. 'go left', or 'go left then left', but not 'go left then right'), and with more modest accuracy: 66-69% accuracy on a single bifurcation. This accuracy was somewhat lower than the accuracy of foragers that had other information cues, such as visual memory and trail pheromone (Evison, 2008; Evison et al., 2008), even after having made only one previous visit to a food location (Czaczkes et al., 2011; Czaczkes and Heinze, 2015), and lower than the trail following accuracy of *L. niger* for moderately strong trails (Czaczkes et al., 2017; Evison et al., 2008; Thienen et al., 2014). Again, the results of Evison (2008) were critically received, and were published only in thesis form. Here, we make a collaborative effort between three laboratory groups to add weight to the findings of Reznikova et al., in an attempt to clarify this enigmatic phenomenon. Stringent control experiments suggest that the effect initially found by three of the groups may have been confounded. This study is therefore an important addition to the curious case of directional information transfer via physical contact in ants.

Methods

Three experiments were run in total: an initial experiment which was later found to be flawed (experiment 0, see appendix B for details), an experiment in which all factors were adequately controlled (experiment 1), and a confirmatory experiment run in a different laboratory to experiment 1 (experiment 2). Full details of experiment 1 will be presented below, followed by a more concise description of experiment 2. Full details of experiment 0 are presented in appendix B

Study species and animal maintenance for experiment 1

We used 10 queenless colony fragments of the black garden ant, *Lasius niger* (Linnaeus), collected in 2014 from eight different colonies on the University of Regensburg campus. Each colony was housed in a plastic box (40×30×20cm) with a layer of plaster on the bottom. Each box contained a circular plaster nest (14cm diameter, 2cm high). Colonies contained c. 1000 workers and small amounts of brood. The ants were fed three times per week with Bhatkar diet, a mixture of egg, agar, honey and vitamins (Bhatkar and Whitcomb, 1970). Colonies were deprived of food for four days prior to each trial to give high and consistent motivation for foraging and pheromone deposition. Water was provided *ad libitum.*

Experimental procedure

Overview

In all experiments ants that knew the location of a food source at the end of a T-maze (henceforth "informed ants") were allowed to make contact with ants that did not know the food location henceforth "contacted naïve ants". The contacted naïve ants were then tested for their arm choice on the T-maze. If information acquired by the informed ants is transferred to the contacted naïve ants, we expect these ants to choose the correct arm significantly more often than chance (an even split between the two branches). In this experiment, as a control, the arm choice of uncontacted naïve ants (which were not allowed to make contact with an informed ant) was tested.

Food location learning in *L. niger* is rapid but not instantaneous. On average, foragers require 2-3 visits to a food source on one arm of a T-maze to make over 95% correct decisions (Czaczkes and Heinze, 2015; Grüter et al., 2011). Thus, to ensure that informed ants were indeed informed, we required them to make at least 3 visits to the food source before information transfer was tested. Lastly, *L. niger* workers make extensive use of pheromone trails to guide nestmates to food sources (Beckers et al., 1993; Evison et al., 2008). So as to test only for ant-ant physical communication, contamination by trail pheromone must be entirely eliminated. Our first attempt to do this failed (see appendix B). Thus, in this experiment separate T-mazes were used for informed and naïve ants.

Detailed description of methods – experiment 1

The experiment was carried out in a laboratory space with many high contrast objects which could act as landmarks. The experimenter always sat at the head end of the apparatus. A colony was connected to the testing apparatus via a paper covered drawbridge. The apparatus was constructed out of Perspex, and consisted of two 80mm long, 5mm wide paths (the 'communication section'), an additional 80mm long path (the 'buffer section') and a T-maze (see figure 3.1). The stem of the T-maze was 150mm long and 5mm wide, and the head was 220mm long and 20mm wide. The entire apparatus was raised on stilts over water moats, to prevent ants from escaping. Two identical T-mazes were constructed arranged next to each other on a board. This allowed the T-mazes to be rapidly exchanged by sliding the board back and forth. One of the T-mazes was used exclusively for the informed ants, and the other exclusively for the naïve ants. The entire apparatus was covered with disposable paper overlays. The stem overlays had been kept in the nest for at least 24 hours prior to use, to ensure that they were marked with colony-specific home range markings and encourage direct walking and reduce U-turning (Devigne and Detrain, 2006; Lenoir et al., 2009). A drop of 1M sucrose solution on a 20x20mm acetate sheet was placed at the end of one arm of the T-maze and acted as a sugar feeder.

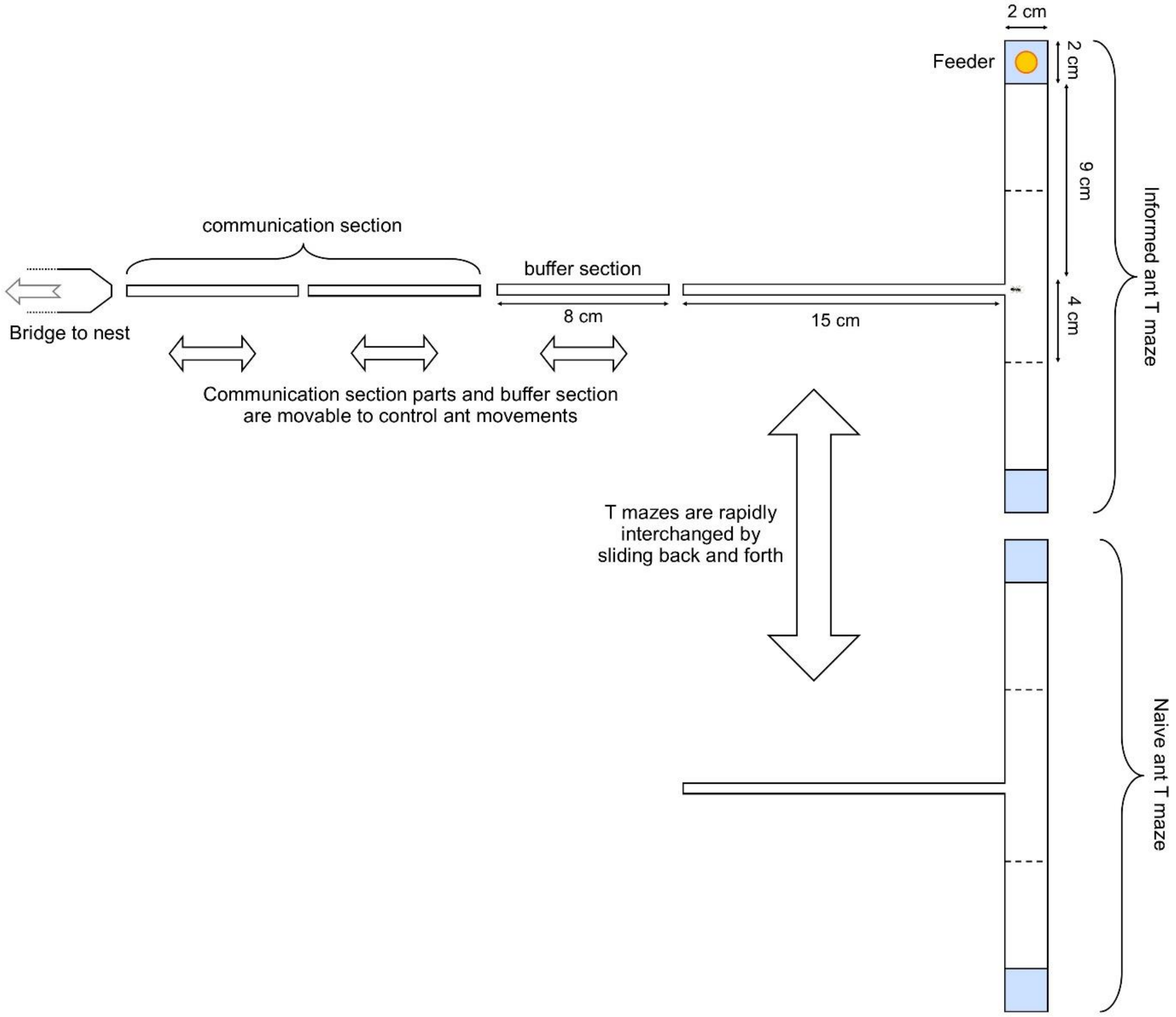


Figure 3.1 – Experimental setup for experiment 1. Two marked (=informed) ants with knowledge of the feeder location are allowed to make repeated return visits to the feeder. On their return visits they may be allowed to encounter naïve ants on the communication section, by allowing a naïve ant onto the first section and the informed ant onto the second section, then joining the two sections. The T-mazes are slid along so as to replace the maze the informed ant walked on with a maze unmarked by pheromone. The contacted naïve ant is then allowed, via the buffer section, onto the maze, and its arm choice decision is noted. The figure, including ant entering the T-maze head, is to scale.

Several ants were allowed onto the apparatus, and the first two to find the feeder were marked individually on the abdomen with acrylic paints. These ants would become the informed ants. All other ants were removed from the apparatus. The marked ants were allowed to feed, return to the nest, unload the sucrose, and make three more return visits to the feeder. During this initial training phase, no other ants were allowed onto the apparatus. The paper overlays on the T-maze head, but not the stem, were replaced with unmarked paper every time the ants walked over them. This was done so as to ensure that the informed ants had to rely on their memories for navigation, rather than their previously deposited pheromone trail. The maze was cleaned with ethanol after every 5 return visits of the informed ants to remove any traces of pheromone which may have reached the plastic.

After the informed ant had fed for the fourth time and was about to return to the nest, several naïve ants were allowed onto the bridge and one of them was further allowed onto the first platform of the meeting section. As soon as the informed ant stepped onto the second platform, the segments were connected to allow physical contact between the two ants. Ants could thus make contact at any point on the communication sections, or occasionally on the buffer section. Data were collected from contacted naïve ants only if they were contacted by the informed ant with both antennae on the head or antennae. The interactions between informed ant and contacted naïve ant lasted no longer than c. 1 second in the majority of the cases, and consisted of a stereotypical movement sequence- As soon as the ants touched each other with their antennae, they stopped running and occasionally even recoiled slightly. They then turned their heads toward each other and stroked the head of the opposite ant a few times with their antennae, after which both ants proceeded on their way. The contacted naïve forager sometimes turned its head after the returning ant, but quickly moved on in the direction of the food source. A few informed ants seemed to consistently avoid stopping for the interaction and ran past the outbound ants with very little interaction. No data were collected from these interactions; data was only collected from ants when they were contacted by the informed ant with both antenna on the head or the antenna.

After contact had been made, the informed ant was allowed to proceed back to the nest, and the outbound naïve ant was immediately allowed onto the buffer section. The T-maze the informed ant had walked on was then replaced by the naïve ant T-maze, and the naïve ant was allowed from the buffer section onto the T-maze. We recorded the initial decision of the naïve ant using decision lines located 4cm away from the middle line. We also recorded which end of the T-maze the informed ant reached first (henceforth the final decision). An ant was considered as having made a decision when both of its antennae crossed the decision line or the end of the T-maze head respectively. Additionally, we also recorded the delay from ant-ant contact to reaching the T-head and end of the maze. If an ant did not make a decision within 90 seconds after contacting the informed ant, it was considered not motivated and rejected for data collection. 15 out of 500 (=3%) ants were rejected for this reason. After the ant reached the end of the maze it was removed from the experiment and not reintroduced back into the colony, to prevent pseudoreplication.

The position of the feeder, and whether a control or an ant-ant contact trial was run, was varied between trials, and arranged in such a way that all colonies were tested with all side and control permutations equally, but with all permutations spaced equally over the course of the experiment. We aimed to test 20 ants per trial. In total 460 ants over 24 trials were tested with ant-ant contact, and 438 ants over 23 trials were tested in the control treatment (no contact).

Experiment 0

A similar experiment was carried out prior to experiment 1, which differed in some key methodological details, and thus failed to adequately control for trail pheromone contamination. For a detailed description of the methodological differences between these experiments, see appendix B.

Confirmatory experiment on *Lasius neglectus* - Experiment 2

Concurrent to experiment 1 being run at the University of Regensburg by SP & TJC, PBB & EJHR were carrying out very similar experiments at the University of York. Initial pilot results seemed to suggest an effect of ant-ant communication on direction choice accuracy, but similar issues to those described for experiment 0 (see appendix B) likely played a role. To confirm the lack of effect we describe in experiment 1, a confirmatory experiment was carried out in the University of York by SP, PBB & EJHR. The methods used differed slightly due to differences in working style between the two labs. However, the key method of using different, sliding T-mazes for the informed and naïve ants was maintained. Rather than describe the methods in full, we will only describe the differences in experimental design between this experiment and experiment 1.

Study species and animal maintenance

Four queenless *Lasius neglectus* colonies, collected in 2015 at Hidcote, Gloucestershire were used in the experiment. Colonies contained between 500 and 2000 workers and small amounts of brood. Colonies were fed 3 times per week on a 50% honey solution and a chopped mealworm. Colonies were deprived of food for 3 - 5 days prior to testing.

Experimental procedure

All experiments were carried out at the University of York. C. 25% of the data was collected by SP, who collected the data for the other two experiments described. The remainder were collected by PBB.

Rather than having separate test and control trials, in this experiment naïve ants were simply brought onto the apparatus as the informed ants were returning. No attempt to force contact between the naïve and informed ant was made. Naïve ants which made contact with the informed ants were considered contacted naïve ants, and ants which by chance did not contact the informed ant were considered controls (uncontacted naïve ants). As such, no communication section was used in the experimental setup (see figure B4). Deliberate control trials, in which uncontacted naïve ants were tested after the informed ant had been removed, were also carried out. Decision lines were drawn 25mm from the centre of the T-maze. The T-maze stem did not have a constriction. Paper overlays were not used on the apparatus but the T-maze was cleaned with 80% ethanol between replicates.

In this experiment, rather than using two highly informed ants, which make many return visits to the feeder, each informed ant only made one visit to the feeder. Thus, an ant was allowed onto the experimental setup, allowed to find the sucrose and drink, and as it returned a naïve ant was brought onto the experimental setup and allowed to contact the informed ant on the stem of the T maze. The informed ant was then removed just before it left the T-maze, and prevented from returning to the nest. This method has the benefit of having a much larger range of informed ants, making each data-point more independent. However, this method has the drawback of low information certainty in the informed ant: *Lasius niger* can reliably learn the location of a feeder at the end of a T-maze in between 1 and 3 visits: After one visit foragers show between 75% and 80% accuracy (Czaczkes et al., 2015a; Grüter et al., 2011). Thus, we can assume that between 20% and 25% of ants considered 'informed' did not possess accurate information. Indeed, this might be even higher, even uninformed ants choose the correct side half the time, by chance. However, even disregarding this, and assuming 100% accurate and effective ant-ant physical communication, the maximum accuracy we could expect in this experiment is 75-80%.

Lastly, rather than using a movable bridge to bring ants onto the apparatus, ants were allowed to climb onto a toothpick in their nest, and then allowed to climb off onto the apparatus.
The number of ants tested per trial was variable, ranging from one to 22.

Statistical analysis

Statistical analyses were carried out in R 3.1.0 (R Core Team, 2012) using Generalised Linear Mixed Models (GLMMs) in the LME4 package (Bates et al., 2014). Following Forstmeier & Schielzeth (2011) we included in the tested models only factors and interactions for which we had a-priori reasons for including. As multiple ants were tested per trial, we added the trial identity as a random effect. The decisions of the ants (correct/incorrect) were modelled using a binomial distribution and logit link function.

To test whether treatment affected the accuracy of the ants, we used the following model formula:

Decision = treatment * ant order + (trialID as a random effect)

Ant order is the order in which the naïve ants were tested. We added this factor to test for possible pheromone contamination (see appendix B3), as if pheromone contamination was occurring, it would result in higher accuracy for ants tested later.

The same model formula was used to examine both the initial and final decisions of the ants. All results reported were corrected for multiple testing using the Benjamini-Hochberg (1995) method. Exact binomial tests were carried out in R using the binom.test function. All binomial tests were two-tailed.

Results

Experiment 1

We found no evidence for tactile communication of direction between foraging ants. The initial choice made by the contacted naïve ants which came into contact with informed ants did not differ from random (exact binomial test, 248/460 correct decisions, probability of success 0.54, P = 0.10, see figure 3.2A). Whether naïve ants contacted an informed ant or not did not significantly predict decision accuracy (GLMM, Z = 0.49, P = 0.95). The order an ant was tested in, and the interaction between order and treatment, were also not significant predictors of choice accuracy (order, Z = 0.304, P = 0.95, interaction, Z = -0.103, P = 0.95).

If the final choices made by the ants is considered, the results remain qualitatively identical. Naïve ants which came into contact with informed ants did not differ from random (exact binomial test, 223/460 correct decisions, probability of success 0.48, P = 0.54, see figure 3.2A). The treatment naïve ants underwent (contacting an informed ant or not) was not a predictor of decision accuracy (GLMM, Z = -0.84, P = 0.79). The order an ant was tested in, and the interaction between order and treatment, were also not significant predictors of choice accuracy (order, Z = 0.29, P = 0.79, interaction, Z = 0.90, P = 0.79).

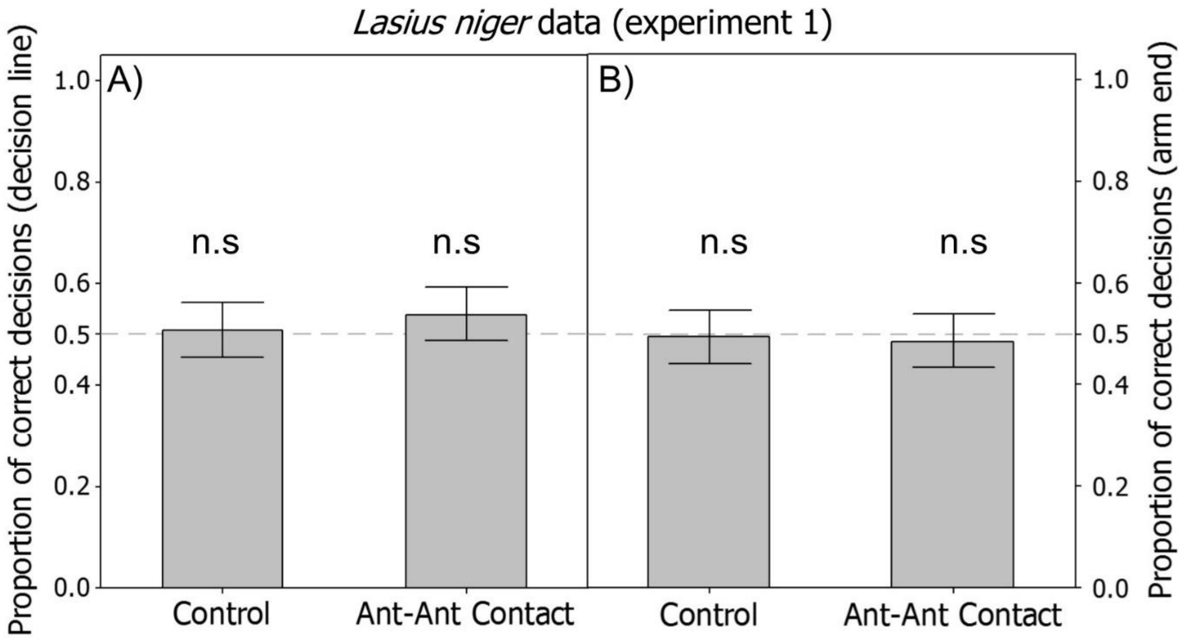


Figure 3.2 – No evidence of tactile communication of direction in *Lasius niger* ants. Naïve ants heading towards a food source which had made antennal contact with well-informed ants returning from a food source were no more likely to choose the correct route than naïve ants that had not made antennal contact with an informed ant. This is true both when considering the initial decision (A, 248 / 460 contacted ants and 223 / 438 control ants chose the correct arm) and the final decision (B, 223/460 contacted ants and 216 / 438 control ants chose the correct arm). Error bars show 95% confidence intervals for the mean.

Experiment 2

The proportion of correct decisions ants made on control and ant-ant contact trials was not different (GLMM, Z = 0.26, P = 0.795, see figure 3.3). The choices of both control and test ants did not differ from random (exact binomial test, control: 106 / 205 correct decisions, probability of success = 0.52, P = 0.675, test: 106 / 200 correct decisions, probability of success = 0.53, P = 0.437).

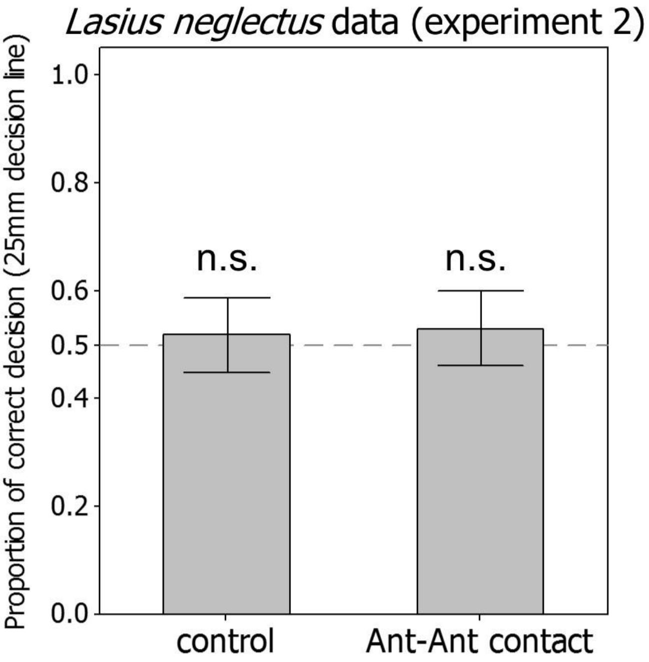


Figure 3.3 – Decision accuracy of *Lasius neglectus* ants in the confirmatory experiment. The arm choice of both the control and test (ant-ant contact) ants did not differ from an even split between the two branches. Error bars show 95% confidence intervals for the mean.

Experiment 0 – initial experiment with flawed experimental design.

The initial choice of naïve ants which had made contact with informed ants was correct significantly more than half the time (exact binomial exact test, 206 / 299 correct decisions, probability of success 0.69, P < 0.0001, see figure 3.4A). This effect almost disappears, however, if the final decision is considered (165 / 299 correct decisions, probability of success 0.55, P = 0.08, see figure 3.4B). Control ants do not choose differently from an even split between the two branches either in terms of the initial decision (exact binomial exact test, 77 / 160 correct decisions, probability of success 0.48, P = 0.69) or the final decision (74/160 correct decision, probability of success 0.46, P = 0.384). Decision accuracy increases over the course of the experiment (Z = 2.59, P = 0.0095, see figure B3), suggesting contamination by pheromones over the course of each trial (see S1 for details).

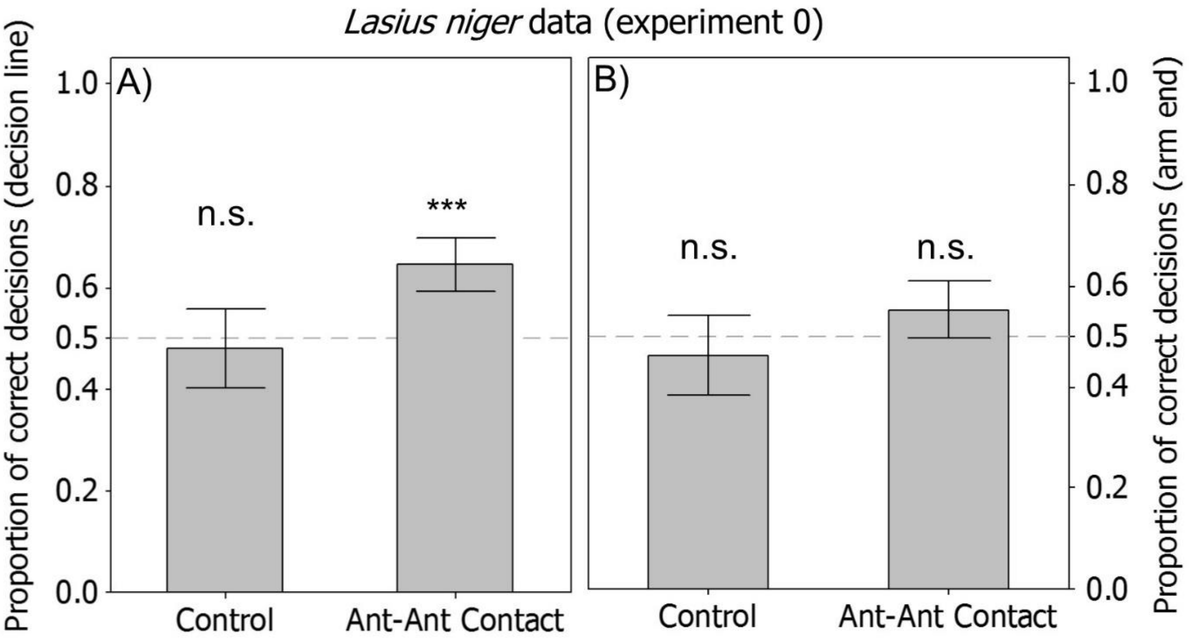


Figure 3.4 – Decision accuracy of *Lasius niger* ants in the initial, flawed trial. The initial choice of contacted naïve ants (A, measured by crossing a decision line 4 cm from the centre of the T-maze stem) were correct significantly more often than expected by chance. The initial choices of uncontacted naïve ants (controls), and the final choice of both groups (B), were not different from random. Error bars show 95% confidence intervals for the mean.

Discussion

Our experiments failed to find support for the hypothesis that ants can communicate food locations by physical interaction. This null result was confirmed in both *L. niger* and in a second, independently performed experiment using *L. neglectus*. We therefore add to the body of evidence that ants cannot communicate direction via physical contact during foraging. We also believe that the combined effort among our three groups is an important highlight to this almost decade long research. Each group believed the initial positive results were sound; only the collaborative effort highlighted the methodological flaw that led to these misleading findings.

While an initial experiment (experiment 0, see appendix B) seemed to find evidence for such communication, a careful analysis of the data revealed that these results were due to a flawed methodology. Specifically, it is likely that pheromonal contamination on the stem of the T-maze resulted in the higher accuracy of the contacted naïve ants. We conclude this from three lines of evidence. Firstly, the accuracy of naïve ants increases over the course of the experiment, suggesting pheromone accumulation. Secondly, the increase in accuracy is only evident when the initial decision of the ants, as defined by crossing a decision line close to the junction, is considered. When the final decision of the naïve ants is considered, as defined by the end of the T-maze reached first, the pattern disappears. This indicates local pheromone contamination around the T-maze junction. Lastly, when completely separate T-mazes are used for informed and naïve ants (as in the main experiment and in the confirmatory experiment), contacted ants do no better than uncontacted ants.

We included a detailed analysis of the flawed experiment 0 (see appendix B), as we feel that important lessons can be learned from it. It is worth noting that pilot experiments by PBB and EJHR (unpublished data) found similar results to the flawed experiment reported in S1, but that again once the stringent control for pheromone contamination was implemented these effects also disappeared (Experiment 2). That both groups initially failed to control the experiments properly demonstrates how difficult it can be to exclude all biases in the data. It is likely that the results reported by Evison (2008) are similarly flawed. In these experiments, the choice zone was replaced between each trial, but the zone leading up to this was never replaced and would have been contaminated with pheromones that may have biased decisions leading up to the branch point. The use of disposable paper overlays to remove pheromones deposited during an experiment is a widespread technique, as it is rapid, simple, and does not involve using cleaning solvents that might disturb the ants. However, the results of experiment 0 suggest that this method is not sufficient to ensure the complete removal of pheromone trails, especially in experiments involving many ant passages.

Do our results also cast doubt on those of Reznikova and Raybako (2008; 1994), and Novgorodova (2006)? Parallels must be drawn with caution. Firstly, Reznikova and Raybako (1994) mention in passing that two species of ants tested, *Myrmica rubra* and *Formica cunicularia*, showed no evidence for tactile communication of food location. Reznikova (2008) argues that tactile communication of food location will only arise in ants which form very large and complex colonies, and forage over very large areas, and will only be used in complex environmental situations (i.e. multiple bifurcations). *L. niger* form moderately sized colonies of several thousand workers or more – a comparable size to that of *F. sanguinea* (Seifert, 2007)*,* in which such communication was reported. Nonetheless, their territory size is smaller than that of the three *Formica* species in which physical communication was found. Furthermore, while the *Formica* species and *Lasius* species all rely heavily on honeydew, and must solve broadly similar problems to forage successfully, their foraging organisation is no doubt different. Indeed, foraging teams consisting of specialised workers performing specific roles (such as trophobiont guarding, honeydew harvesting, and honeydew transporting) have been described for *F. polyctena*. *Lasius niger*, on the other hand, are reported to show much less specialisation during foraging, with no stable task partitioning via 'foraging teams' (Novgorodova, 2015). The physical communication described by Reznikova and Ryabko (1994) relied on the presence of these stable foraging teams consisting of one scout and 5-8 recruits, and scouts would communicate food location only with their team mates. It is not clear why such specialisation is beneficial, although it may allow long-term specialisation of different teams in different foraging locations(Czaczkes et al., 2015a; Salo and Rosengren, 2001). While there seems no *a priori* reason to expect physical communication of food location only when robust foraging teams are present, this is a possibility. Lastly, the character of the ant-ant contacts in the two studies was very different. In the work of Reznikova and Ryabko (1994, 2001) and Novgorodova (2006), information transfer contacts occurred mainly in the nest, and required many tens of seconds. The exact definition of 'contact duration' in these studies is somewhat unclear. The contacts used in Evison (2008) and in the present study occurred on the foraging platform, and lasted only a few seconds. Thus, the two different groups of studies may have been studying different types of contacts.

In spite of the large differences between the current study and the work of Reznikova et al., our results do increase the burden of proof required to fully accept physical communication of food location by ants. Our study demonstrates how easy it is to miss critical experimental flaws, resulting in overlooked chemical directional information being available to the ants. While we could detect no major flaws in the methodology of Reznikova and Rybako (1994) or that of Nogorodova (2006), it is notoriously difficult to fully describe an experimental design in prose. With such extraordinary claims, extraordinarily robust evidence must be brought forward. This may take the form of repeated video documentation of these effects, or better yet, a replication of these results by an unaffiliated research group. While direct replication of experiments may be unappealing to most researchers, similar research in a different group of ants might be more attractive. *Oecophylla longinoda* forms large, dominant colonies with complex organisation, and has been demonstrated to make extensive use of motor displays (Hölldobler and Wilson, 1978). If physical communication of food location is to be searched for in an ant group unrelated to the previous demonstrations, we feel *O. longinoda* would be a good place to start.

In this study we set out to test whether brief contacts on a foraging trail between an informed and uninformed *Lasius niger* worker transfer directional information. Our results demonstrate that they do not. The difficulties we had in performing a fair experiment, despite three experienced groups leading their own trajectory, highlight the importance of very stringent controls for such experiments. Multi-group efforts have brought many challenging fields of research in diverse topics forward. Such successful multi-group efforts may be competitive, such as in the question of metacognition in animals (Smith et al., 2008) or cognitive maps in insects (Collett et al., 2013; Wehner and Menzel, 1990), or collaborative, for example in understanding the evolution of (eu)sociality (Kennedy et al., 2017). Our results also raise the burden of proof for claims of physical communication of food location in ants. However, our results do not rule out that such communication may happen in other situations and in other species. Reliable, independent, well-documented replication of any such findings will be necessary for claims of physical communication of location by ants to be broadly accepted by the scientific community.

Acknowledgements

Many thanks to two anonymous reviewers for comments on an earlier version of this manuscript. TJC was supported by a DFG Emmy Noether group leader grant (grant number CZ 237/1-1). PBB was supported by a NERC Industrial CASE studentship with Hymettus awarded to EJHR (NE/L008904/1).

References

Akino, T., Yamamura, K., Wakamura, S., Yamaoka, R., 2004. Direct behavioral evidence for hydrocarbons as nestmate recognition cues in *Formica japonica* (Hymenoptera: Formicidae). Appl. Entomol. Zool. 39, 381–387.

Aron, S., Beckers, R., Deneubourg, J.L., Pasteels, J.M., 1993. Memory and chemical communication in the orientation of two mass-recruiting ant species. Insectes Soc. 40, 369–380.

Balbuena, M.S., Molinas, J., Farina, W.M., 2012. Honeybee recruitment to scented food sources: Correlations between in-hive social interactions and foraging decisions. Behav. Ecol. Sociobiol. 66, 445–452.

Bates, D., Maechler, M., Bolker, B., Al, E., 2014. lme4: Linear mixed-effects models using Eigen and S4.

Beckers, R., Deneubourg, J.L., Goss, S., 1993. Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. J. Insect Behav. 6, 751–759.

Beckers, R., Deneubourg, J.L., Goss, S., Pasteels, J.M., 1990. Collective decision making through food recruitment. Insectes Soc. 37, 258–267.

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate : A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57, 289–300.

Bhatkar, A., Whitcomb, W.H., 1970. Artificial Diet for Rearing Various Species of Ants. Florida Entomol. 53, 229.

Bonavita-Cougourdan, A., Morel, L., 1984. Les activités antennaires au cours des contacts trophallactiques chez la fourmi *Camponotus vagus* Scop. Ont-elles valeur de signal? Insectes Soc. 31, 113–131.

Collett, M., Chittka, L., Collett, T.S., 2013. Spatial memory in insect navigation. Curr. Biol.

Czaczkes, T.J., Castorena, M., Schürch, R., Heinze, J., 2017. Pheromone trail following in the ant *Lasius niger*: high accuracy and variability but no effect of task state. Physiol. Entomol. 42, 91–97.

Czaczkes, T.J., Czaczkes, B., Iglhaut, C., Heinze, J., 2015a. Composite collective decision-making. Proc. R. Soc. B Biol. Sci. 282.

Czaczkes, T.J., Grüter, C., Jones, S.M., Ratnieks, F.L.W., 2011. Synergy between social and private information increases foraging efficiency in ants. Biol. Lett. 7, 521–4.

Czaczkes, T.J., Grüter, C., Ratnieks, F.L.W., 2015b. Trail Pheromones: An Integrative View of Their Role in Social Insect Colony Organization. Annu. Rev. Entomol. 60, 581–599.

Czaczkes, T.J., Heinze, J., 2015. Ants adjust their pheromone deposition to a changing environment and their probability of making errors. Proc. R. Soc. B Biol. Sci. 282, 20150679-.

Czaczkes, T.J., Salmane, A.K., Klampfleuthner, F.A.M., Heinze, J., 2016. Private information alone can trigger trapping of ant colonies in local feeding optima. J. Exp. Biol. 219, 744–751.

Czaczkes, T.J., Schlosser, L., Heinze, J., Witte, V., 2014. Ants use directionless odour cues to recall odour-associated locations. Behav. Ecol. Sociobiol. 68, 981–988.

Dacke, M., Srinivasan, M. V., 2008. Evidence for counting in insects. Anim. Cogn. 11, 683–689.

Devigne, C., Detrain, C., 2006. How does food distance influence foraging in the ant *Lasius niger*: the importance of home-range marking. Insectes Soc. 53, 46–55.

Evison, S.E.F., 2008. Foraging Organisation in Ants. University of Sheffield.

Evison, S.E.F., Petchey, O.L., Beckerman, A.P., Ratnieks, F.L.W., 2008. Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. Behav. Ecol. Sociobiol. 63, 261–267.

Farina, W.M., Grüater, C., Díaz, P.C., 2005. Social learning of floral odours inside the honeybee hive. Proc. R. Soc. B Biol. Sci. 272, 1923–1928.

Forstmeier, W., Schielzeth, H., 2011. Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. Behav. Ecol. Sociobiol. 65, 47–55.

Goss, S., Aron, S., Deneubourg, J.L., Pasteels, J.M., 1989. Self-organized shortcuts in the Argentine ant. Naturwissenschaften 76, 579–581.

Grüter, C., Balbuena, M.S., Farina, W.M., 2008. Informational conflicts created by the waggle dance. Proc. Biol. Sci. 275, 1321–7.

Grüter, C., Czaczkes, T.J., Ratnieks, F.L.W., 2011. Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav. Ecol. Sociobiol. 65, 141–148.

Hölldobler, B., 1971. Recruitment behavior in *Camponotus socius* (Hym. Formicidae). Z. Vgl. Physiol. 75, 123–142.

Hölldobler, B., 1976. Recruitment behavior, home range orientation and territoriality in harvester ants, *Pogonomyrmex*. Behav. Ecol. Sociobiol. 1, 3–44.

Hölldobler, B., Wilson, E.O., 1978. The multiple recruitment systems of the african weaver ant *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 3, 19–60.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Springer-Verlag Berlin Heidelberg.

Huber, P., 1810. Recherchez sur les Meœrs des Fourmis lndigenes. JJ Paschoud, Paris.

Kennedy, P., Baron, G., Qiu, B., Freitak, D., Helanterä, H., Hunt, E.R., Manfredini, F., O'Shea-Wheller, T., Patalano, S., Pull, C.D., Sasaki, T., Taylor, D., Wyatt, C.D.R., Sumner, S., 2017. Deconstructing Superorganisms and Societies to Address Big Questions in Biology. Trends Ecol. Evol.

Le Breton, J., Fourcassie, V., 2004. Information transfer during recruitment in the ant *Lasius niger* L. (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 55, 242–250.

Lenoir, A., 1982. An informational analysis of antennal communication during trophallaxis in the ant *Myrmica Rubra* L. Behav. Processes 7, 27–35.

Lenoir, A., Depickère, S., Devers, S., Christidès, J.P., Detrain, C., 2009. Hydrocarbons in the ant *Lasius niger*: from the cuticle to the nest and home range marking. J. Chem. Ecol. 35, 913–21.

Mamiko, O., Ayako, W.-K., Kazuyo, F., Masayuki, I., Fumio, Y., Yuji, S., Tomoyosi, N., Ryohei, Y., 2005. Ant Nestmate and Non-Nestmate Discrimination by a Chemosensory Sensillum. Science (80-.). 309, 311–314.

McCabe, S., Farina, W.M., Josens, R.B., 2006. Antennation of nectar-receivers encodes colony needs and food-source profitability in the ant *Camponotus mus*. Insectes Soc. 53, 356–361.

Novgorodova, T. a., 2015. Organization of honeydew collection by foragers of different species of ants (Hymenoptera: Formicidae): Effect of colony size and species specificity. Eur. J. Entomol. 112, 688–697.

Novgorodova, T.A., 2006. Experimental investigation of information transmission in *Formica pratensis* (Hymenoptera, Formicidae) using "binary tree" maze. Entomol. Rev. 86, 287–293.

R Core Team, 2012. R: A Language and Environment for Statistical Computing.

Reznikova, Z., 2007. Dialog with black box: Using information theory to study animal language behaviour. Acta Ethol.

Reznikova, Z., 2008. Experimental paradigms for studying cognition and communication in ants (Hymenoptera: Formicidae). Myrmecol. News 11, 201–214.

Reznikova, Z., 2017. Studying animal languages without translation: An insight from ants, Studying Animal Languages without Translation: An Insight from Ants.

Reznikova, Z., Ryabko, B., 1994. Experimental study of the ants' communication system, with the application of the information theory approach. Memorab. Zool. 48, 219–236.

Reznikova, Z.I., Ryabko, B., 2001. A study of ants' numerical competence. Comput. Inf. Sci. 5, 111–126.

Roces, F., 1990. Olfactory conditioning during the recruitment process in a leaf-cutting ant. Oecologia 83, 261–262.

Roces, F., 1994. Odour learning and decision-making during food collection in the leaf-cutting ant *Acromyrmex lundi*. Insectes Soc. 41, 235–239.

Salo, O., Rosengren, R., 2001. Memory of location and site recognition in the ant *Formica uralensis* (Hymenoptera: Formicidae). Ethology 107, 737–752.

Seifert, B., 2007. Die Ameisen Mittel-und Nordeuropas. lutra.

Smith, J.D., Beran, M.J., Couchman, J.J., Coutinho, M.V.C., 2008. The comparative study of metacognition: Sharper paradigms, safer inferences. Psychon. Bull. Rev. 15, 679–691.

Sudd, J.H., 1967. An introduction to the behaviour of ants. Edward Arnold, London.

Thienen, W. Von, Metzler, D., Choe, D.H., Witte, V., 2014. Pheromone communication in ants: A detailed analysis of concentration-dependentdecisions in three species. Behav. Ecol. Sociobiol. 68, 1611–1627.

Von Frisch, K., 1923. Über die" Sprache" der Bienen. Zool Jb Physiol 40, 1–186.

Von Frisch, K., 1967. The dance language and orientation of bees. Belknap Press, Cambridge.

Wasmann, E., 1905. Comparative studies in the psychology of ants and of higher animals, 2nd ed, Comparative studies in the psychology of ants and of higher animals / by Eric Wasmann. B Herder, London.

Wehner, R., Menzel, R., 1990. Do Insects Have Cognitive Maps. Annu. Rev. Neurosci. 13, 403–414.

Chapter 4: Integrating foraging ecology informs approaches to invasive ant eradication

Title: Integrating foraging ecology informs approaches to invasive ant eradication

Authors: Phillip Buckham-Bonnett[1], Alba Aguion[2], Clive Boase[3], Paul Lee[4], Elva JH Robinson[1]

Author affiliations:

1 Department of Biology and York Centre for Complex Systems Analysis, University of York, York, UK

2 Ecology and Animal Biology Department, Universidade de Vigo, Vigo, Spain

3 The Pest Management Consultancy, UK

4 Hymettus Ltd, UK

Abstract

Invasive alien species are a global conservation problem. Invasive ants can be particularly damaging, but due to their discrete populations, eradication is an achievable and quantifiable conservation target. The invasive garden ant (*Lasius neglectus*) is a recently emerged and rapidly spreading invasive species in Europe. We aimed to identify a suitable insecticide for large-scale eradication of colonies of *L. neglectus*. We assessed the suitability of four granular insecticidal ant baits for use on *L. neglectus* in controlled laboratory trials under a range of conditions. Mortality in three of the granular treatments was not significantly higher than a pesticide-free control. The fourth, Imidacloprid, resulted in 7.8 times higher mortality than the control, but less than half the mortality of a gel-based pesticide that is effective for small-scale control of *L. neglectus* in the field. The mortality rate of two of the granular insecticide treatments increased with elevated humidity but despite this, all four are effectively unsuitable for use in eradication attempts. In behavioural tests, the ants showed a strong preference for aqueous rather than granular food, indicating that low palatability rather than toxicity was the likely cause of the low mortality. Indeed, it seems probable that granular insecticide baits in general are not suitable for use with this species. Our methods provide a template for identifying the most effective control methods for other invasive ant species. We suggest that further research into the control of *L. neglectus* should focus on hydrogel delivery mechanisms which are more suited to the foraging ecology of this species.

Keywords:
Invasive alien species
Eradication
Invasive ant
Pest management
Foraging ecology
Pesticide

Key message:

The invasive garden ant *Lasius neglectus* is spreading as a pest species. Gel-based insecticides are effective, but inefficient to apply.
We tested alternative pesticide application methods to assess mortality and behavioural responses.
Mortality in lab colonies treated with granular insecticides is lower than for a gel-based insecticide.
Ants fed more readily on aqueous food resources than dry ones; suggesting granular insecticides are unpalatable.
Polyacrylamide water-storing crystals are a promising pesticide delivery mechanism for *L. neglectus*, while granular substrates are not.

Author Contribution Statement:

All authors conceived and designed the research. AA and PBB conducted experiment 1. PBB and EJHR conducted experiment 2. PBB conducted experiment 3. PBB analysed the data. PBB and EJHR wrote the manuscript. All authors read and approved the manuscript.

Introduction

Invasive alien species are a significant conservation problem. They can change the composition of local species communities, contribute to species extinctions and alter ecosystem function. They also cause problems for humans, including the social and financial costs of control and eradication, the loss of crops, the transmission of disease, damage to infrastructure, and disruption to recreation and tourism activities (Pejchar and Mooney, 2009; Pimentel et al., 2005). Among invasive species, insects are one of the most financially costly taxa for humans with global expense conservatively estimated to be in the region of $76.9 billion annually (Bradshaw et al., 2016) and as the number of introductions of non-native species is increasing, costs are likely to rise. Social insect species are heavily represented among the invasive insects: 7 out of 17 terrestrial invertebrates from the IUCN top 100 worst invasive species are social insects (Lowe et al., 2000). Many have a worldwide distribution and these numerically dominant invaders can have major impacts (Evans et al., 2013; Holway et al., 2002; Lach et al., 2010; McGlynn, 1999; Sanders et al., 2003). For example, the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island has been implicated in 'invasional meltdown': the ant depletes native crabs while promoting growth of native and non-native scale bugs leading to dramatic changes in understorey composition and tree canopy dieback (Abbott and Green, 2007). Social insects pose particular problems for control and eradication due to their social structure i.e. workers, the cast most likely to encounter pesticides, are not reproductive units (Gentz, 2009; Hoffmann et al., 2016). However, colonies of invasive ants are often concentrated populations with discrete borders which makes their eradication achievable, with the right tools. This makes the eradication of populations of invasive ants at a global scale a quantifiable conservation target.

The ant *Lasius neglectus* has emerged relatively recently as an invasive species, and has been studied throughout its invasion history since it was identified in Budapest, Hungary (Van Loon et al., 1990). It originated in Asia Minor (Cremer et al., 2006; Seifert, 2000) and is able to survive low winter temperatures in its native range. Most invasive ant species are tropical in origin, which limits their ability to survive in cooler climates, confining them to human-associated habitats, e.g. homes and greenhouses, at higher latitudes. In contrast, *L. neglectus* can overwinter in natural habitats in northern Europe. Climate modelling indicates that large areas in Europe and North America could support the species (Bertelsmeier et al., 2015a), making it a more significant conservation problem in these regions than previous invasive ant species. The potential range of *L. neglectus* is expected to increase with climate change (Bertelsmeier et al., 2015a).

Lasius neglectus has characteristics that contribute to its invasive success. Like all major invasive ant species, *L. neglectus* forms polydomous and polygynous colonies; i.e. colonies are spread between many socially connected nests, and have multiple reproductively active queens. The resulting potential for rapid growth and limited intraspecific competition allows them to reach very high densities across large areas, and together with their aggressive behaviour, gives them an advantage in interspecific competition (Bertelsmeier et al., 2015a; Cremer et al., 2006; Espadaler et al., 2004; Santarlasci et al., 2014). Native ants can be entirely excluded from the core areas of *L. neglectus* territories and can dramatically change biodiversity, increasing aphid populations due to mutualism, and decreasing the population sizes of other taxa including beetles and woodlice (Nagy et al., 2009). Their impacts span trophic levels, through effects on other ant species, Hemiptera and plants, and they can act as hosts for non-native myrmecophiles (Tartally et al., 2004). *Lasius neglectus* is a flexible forager, opportunistically making use of novel food resources when it invades. Colonies collect honeydew from various Hemiptera (both native and non-native), visit floral and extrafloral nectaries, and exploit anthropogenic food sources (Buckham-Bonnett and Robinson, 2017), and to a lesser extent predate or scavenge on insects (Paris and Espadaler, 2009). This increases the chances that a colony arriving at a new site will find a suitable food source. While the ants are not directly dangerous to humans, in addition to foraging in human habitation, they can damage domestic electrical equipment, and the high density at which they occur can distress the residents of affected houses. Pesticide treatments can be costly, and for businesses, indirect measures put in place to prevent their spread can also be expensive (Boase, 2014).

The natural spread of *L. neglectus* is limited by its mode of colony foundation. New colonies are established through colony budding following intranidal (within the nest) mating rather than nuptial flights (Espadaler et al., 2007). This means that the natural spread at a site is by workers and queens walking to a new nest site; this is slow and makes natural dispersal to new sites unlikely. Instead, the species is spread through human-mediated dispersal, via the plant trade, in building materials or movement of soil (Gippet et al., 2017; Van Loon et al., 1990). As of November 2018, *L. neglectus* had been found at over 200 sites across 21 countries (Espadaler and Bernal, 2018) most of which are outside the species' native range (Cremer et al., 2008; Seifert, 2000).

It is best practice to prevent the introduction of invasive ants as controlling established populations is challenging (Hoffmann et al., 2010). Attempts to control invasive *L. neglectus* have met with variable levels of success. At one site in Buckinghamshire, UK *L. neglectus* were identified on stone that had been recently imported from Italy. The stone was treated with phosphine gas which eradicated the ants (Boase, 2014). This type of approach would not be suitable for established colonies. Near Barcelona, Spain, an established population extending 14 hectares was treated with a range of techniques, including multiple pesticides and fogging of food-aphids (Rey and Espadaler, 2004). This multi-threaded approach was somewhat successful at reducing numbers, although its success in the second year was reduced by rain. Whilst multi-threaded approached to ant eradication can be highly successful (Hoffmann et al., 2016), they make it difficult to isolate the more effective techniques from the suite used. Another control attempt was made in Zurich, Switzerland, again using multiple active insecticidal ingredients and application techniques. The efficacy of the treatment measures was not quantified (Landau et al., 2017). At a smaller-scale site in Gloucestershire, UK, a gel-based pesticide was used. This was highly effective at controlling ant numbers locally to bait stations in the field, but successful application requires regular manual replenishing of bait stations (Boase, 2014). This means that a gel-based approach is too labour intensive to scale up to a larger area.

A much less labour-intensive approach to ant eradication is the use of a bait matrix (e.g. a granular formulation) that can be applied by hand or, for large scale invasions, even from a helicopter (Hoffmann et al., 2016) and remain available to the ants for longer than a gel-based medium, which will rapidly dry out. A successful bait is characterised by a non-repellent toxicant in combination with an attractive food source. Toxicants with a delayed action are preferred in order to allow transmission to non-foraging ants e.g. queens (Hoffmann et al., 2010). Manual application of pesticide granules has been used successfully in the eradication of populations of invasive ant species including *Pheidole megacephala* and *Wasmannia auropunctata* (Hoffmann et al., 2016) but it is currently unclear whether it would be effective on *L. neglectus* and if so, which of the commercially available pesticides would be most suitable for eradicating *L. neglectus* over large areas. The aim of this study is to provide pest-control practitioners, landowners, pesticide manufacturers and policy makers with information about whether suitable tools are available for eradicating this invasive species. We do so by carrying out controlled laboratory experiments on the efficacy of four granular pesticides as applied to the invasive garden ant, *L. neglectus.*

Methods

Experiments were performed using the invasive garden ant (*L. neglectus*). The ants used in Experiment 1 were collected from Hidcote (Gloucestershire, United Kingdom) in November 2014 and the ants used in Experiment 2 were collected from Hidcote in September 2015. They were then maintained under standard laboratory conditions in Fluon® coated boxes at 22 ± 1.5°C under a 12h:12h light dark cycle. A 50% honey solution and chopped mealworm larvae (*Tenebrio molitor*) were provided three times per week and water was available *ad libitum*. The ants collected in 2014 were divided into four stock colonies and the ants collected in 2015 were divided into ten stock colonies.

The efficacy of one gel based and four granular ant baits (see table 4.1) was examined in this study. These are all commercially available products designed for use with a variety of ant species. The Imidacloprid gel performs well when used to manage *L. neglectus* in the field (Boase, 2014) so was included in this study as a positive control.

Table 4.1 – The five ant baits investigated in this study with details of their active ingredients and other components, further details in Appendix C, table C1**.**

Pesticide (Manufacturer)	Insecticidal Ingredients	Matrix Type	Matrix Attractants	Start Mass in Experiment (g)
PROTECT® COMBI Ant Bait (Bábolna Bio Ltd)	0.1% Acetamiprid 0.25% S-methoprene	Granules	Protein, sugar	1
Maxforce® Quantum (Bayer)	0.03% Imidacloprid	Gel	Sugar	0.3
Maxforce® Complete (Bayer)	1% Hydramethylnon	Granules	Protein, sugar, fats	1
Baythion® Ant Bait (Bayer)	0.05% Imidacloprid	Granules	Sugar	1
Advion® Fire Ant Bait (DuPont)	0.045% Indoxacarb	Granules	Defatted corn grits, soy bean oil	1

Experiment 1 – Pesticide mortality effects

The aim of this experiment was to determine the effect on mortality of the five pesticides under controlled laboratory conditions. Data collection took place between January and May 2015. *Lasius neglectus* sub-colonies for testing were created from four stock colonies. Each sub-colony contained approximately 2500 ants. Each test sub-colony was created by taking ten extra-nest ants, thirty intra-nest ants and a small number of larvae (5 ± 2 individuals) from the parent stock colony. Foragers and other workers may behave differently (Czaczkes et al., 2014), so colony composition was standardised at 1:3 ratios based upon observed ratios of extra- and intra-nest workers in the stock colonies. Brood presence is important for natural foraging behaviour (Herbers and Choiniere, 1996; Portha et al., 2004). At the start of the experiment, test sub-colonies (hereafter 'test colonies') contained 40 ± 1 workers (Appendix C, table C2).

Six test colonies were formed from each of the stock colonies, one test colony for each of the pesticide treatments (table 4.1) and one as a negative control that would not receive a pesticide treatment. Each test colony was housed in a 185 x 145 mm box with sides coated in Fluon®. A nest was provided, constructed from a tube coated in translucent red acetate (transmission < 20% for $\lambda <$ 600nm) with water and cotton wool at the closed end. Within each box, water was available *ad* *libitum* and three plastic bait stations were present, placed equidistant from the nest (figure 4.1). One bait station contained cotton wool saturated with 1.5ml honey solution. This solution was replaced weekly and had 1ml water added to it daily to keep it moist. A second bait station had half a meal worm added to it three times per week. These food sources were present to simulate the food that an invasive population of *L. neglectus* may already have available during the application of a pesticide treatment. Ants were allowed to explore the box and the first two bait stations for one day, and then the third bait station was added. This contained either a known amount of pesticide (table 4.1) or was empty (the negative control treatment, allowing baseline mortality to be measured).

As a control for changes in pesticide mass in response to environmental conditions, e.g. through the loss or gain of atmospheric moisture, five bait stations, each containing the treatment dose (table 4.1) of one of the five pesticides, were also maintained in identical conditions, but in isolation from ants, for the duration of the mortality experiment.

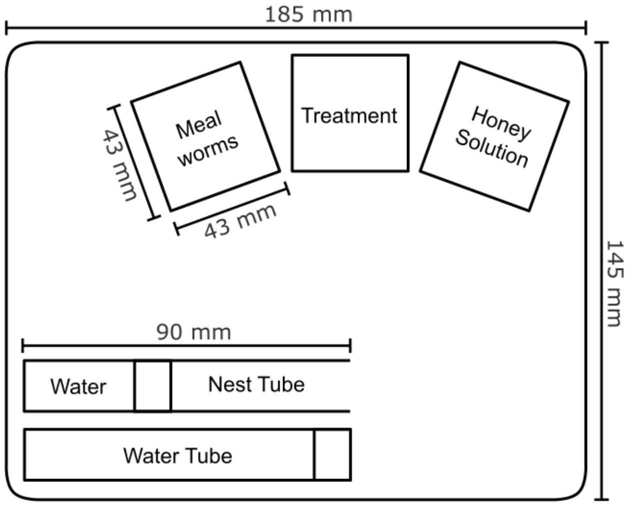


Figure 4.1 - Experimental arena, showing the position of the nest tube relative to the three bait stations and the water tube in each test colony.

Daily, for each test colony: any dead ants were counted and removed from the box; the total number of ants outside the nest was counted; and the numbers of ants visiting each of the three bait stations were recorded. The mass of the pesticide remaining in each test colony was measured three times per week. In addition, the mass of the pesticide in each no-ant control was recorded at the same intervals (see Appendix D, section D7).

Following data collection on day 42, a transparent acetate lid and a small dish containing damp gypsum plaster were added to each test box. One millilitre of water was then added to the gypsum plaster daily and data collection proceeded as before. The same process was applied to the no-ant control boxes. The aim of these steps was to increase the humidity in the vicinity of the test colonies. The effect of this treatment was measured in one test box: the humidity was raised from an average of 26.9% RH to an asymptote at 53% RH. Box temperature was unaffected. See Appendix C for details.

Following data collection on day 68, the bait stations containing the sucrose and the meal worms were removed from each colony and then data collection proceeded as before (unless all workers in the sub-colony were deceased). This step aimed to increase the hunger levels in the test colonies to see whether this increased the effectiveness of the pesticide baits (as determined by mortality).

Experiment 2 – the behavioural responses of individual ants to the pesticide treatments

This experiment was designed to test whether the mortality results from experiment 1 were the result of differences in toxicity or palatability between the treatments.

The behavioural response of individual *L. neglectus* workers was investigated in response to ten bait treatments. These were: the five pesticides (table 4.1), water, a sucrose solution, granular sucrose, and mealworm fragments. For each trial, a sample of one of the baits was placed in a bait station in the centre of a clean circular arena (100mm diameter) with sides coated in Fluon®. A single *L. neglectus* worker was then released at the edge of the arena. The arena was filmed from above, from release of the ant until ten minutes after the ant had discovered the food source. For each bait, ten ants were tested, one from each of the ten stock colonies. A new bait and bait-station was used each time. No ants were used more than once during testing. One video for the granular sucrose treatment was lost between capture and analysis reducing the sample size for this treatment to nine.

Videos were analysed to record ants both interacting with and feeding on baits. Recording interactions took the form of recording the total number of seconds in which the ant was in contact with the bait during the 10 minutes following its discovery. An ant was deemed to be in contact with a bait if any part of the ant crossed a virtual decision line at the edge of the bait station. Each video was analysed by a minimum of two independent observers and where there was not a consensus on the status of a particular second, an additional observer adjudicated. All video analysis was performed by observers blind to the identity of the substrate in the bait station.

Recording whether the ant was feeding was deemed too subjective to be assessed by observers directly from the videos. Instead, in order for a second to be deemed a “feeding second” the ant’s head had to be over the feeder in both the current frame and the previous extracted frame and not have moved by more than the diameter of an ant’s head (to allow for the potential in a small error in the coordinates of the centre of the ant’s head). To record these data, for each video, one frame for each of the interaction seconds (determined in the previous phase) was extracted from the video using FFmpeg (2016). These frames were then analysed to produce coordinates for the centre of the ant’s head and the position of the bait station. Analysis was carried out in R (R Core Team, 2018) using the packages EBImage (Pau et al., 2010). The frames were analysed in a random order and blind to the identity of the frame.

Experiment 3 – hydrogel delivery proof of concept

We performed a “proof of concept” experiment to determine whether *L. neglectus* would feed on sucrose solution delivered via saturated polyacrylamide crystals. Details of this experiment are presented in Appendix E.

Statistical analysis

Analyses were carried out using the R statistical environment (R Core Team, 2018). Following Forstmeier and Schielzeth (2011), only terms and interactions that we *a priori* expected to explain the results were included in the statistical models, and these models were not simplified by removing non-significant terms. Survival data were analysed using Cox Proportional Hazards models or Cox Frailty (mixed effects) models. The proportion of ants inside and outside of the nest in each treatment box daily was analysed using a generalised linear mixed effects model with a binomial structure and logistic link function. The amount of time that individual ants spent interacting with or feeding on substrates in the palatability experiment was not suitable for Analysis of Variance technique due to non-normality of residuals (feeding and interacting) and non-equality of variances (interacting). Instead, Kruskal-Wallis tests were performed. Further details can be found in Appendix C.

Results

Experiment 1 – Pesticide mortality effects

Across all treatments, 961 ants were observed, and 332 deaths were recorded in the first 42 days. Pesticide impact on ant survival was tested using a Cox frailty model. Both pesticide treatment (Cox model fixed effect X^2 = 311.44, df = 5, p <0.001) and ant colony (Cox model frailty term X^2 = 52.98, df = 2.931, p <0.001) significantly affected ant survival (figure 4.2).

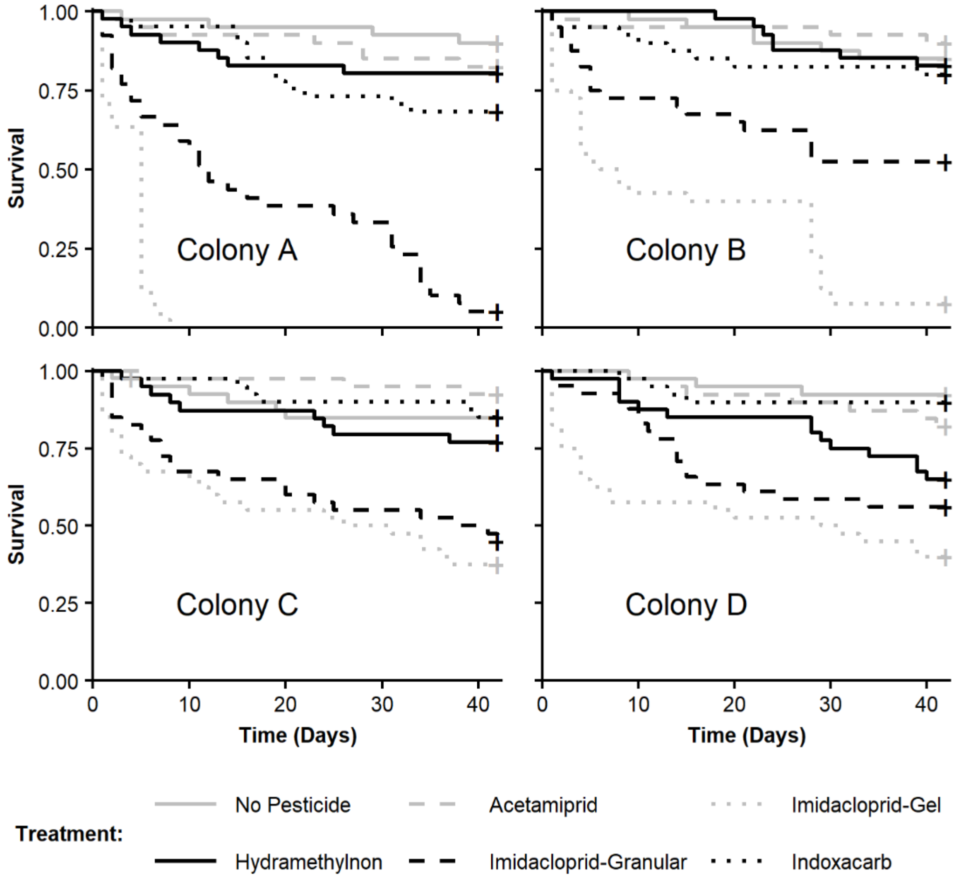
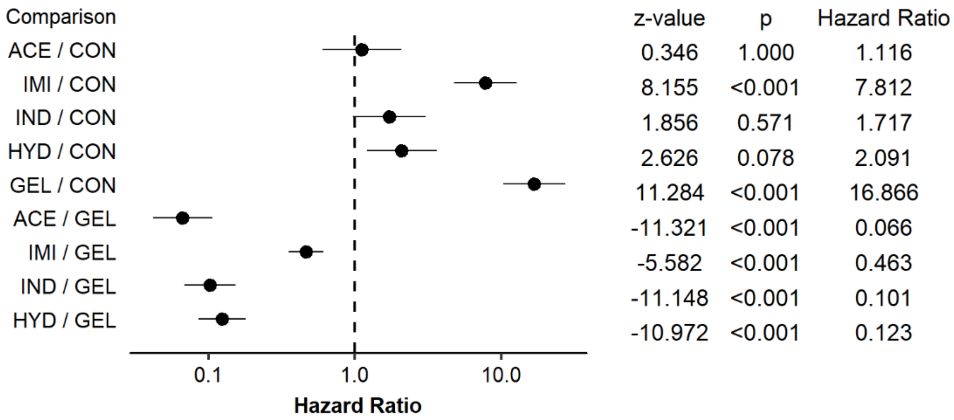


Figure 4.2 - Kaplan-Meier estimators of survival under each treatment, separated by colony, during the first 42 days of the experiment. Censored results indicated with a cross.

Imidacloprid was the only granular pesticide treatment with a mortality rate significantly higher than the control (pesticide free) treatment (Figure 4.2). Ants in the granular Imidacloprid treatment were 7.8 times as likely to die as ants in the pesticide free group at any time point (z = 8.16, df = 1, p <0.001). The Imidacloprid gel treatment also had an ant mortality rate significantly higher than the pesticide free treatment. Ants in the Imidacloprid gel treatment were 16.9 times as likely to die as ants in the pesticide free group at any time point (z = 11.28, df = 1, p <0.001). Whilst the granular

Imidacloprid treatment led to a higher mortality rate than the pesticide-free control treatment, mortality was still significantly lower than in the gel treatment. Ants in the granular Imidacloprid treatment are only 0.46 times as likely to die as ants in the Imidacloprid gel group at any time point (z = -5.58, df = 1, p <0.001). The mortality rate was also significantly higher in the Imidacloprid gel than in all the other granular pesticide treatments (figure 4.3).



Comparison	z-value	p	Hazard Ratio
ACE / CON	0.346	1.000	1.116
IMI / CON	8.155	<0.001	7.812
IND / CON	1.856	0.571	1.717
HYD / CON	2.626	0.078	2.091
GEL / CON	11.284	<0.001	16.866
ACE / GEL	-11.321	<0.001	0.066
IMI / GEL	-5.582	<0.001	0.463
IND / GEL	-11.148	<0.001	0.101
HYD / GEL	-10.972	<0.001	0.123

Figure 4.3 - Summary of pairwise treatment comparisons and associated hazard ratios (p-values adjusted with a Bonferroni correction). CON = Control treatment (no pesticide present); ACE = granular Acetamiprid treatment; IMI = granular Imidacloprid treatment; IND = granular Indoxacarb treatment; HYD = granular Hydramethylnon treatment; GEL = Imidacloprid gel treatment (positive control). A hazard ratio greater than 1 indicates that the probability of death at any time point is higher in the focal group than in the reference group (listed as focal/reference).

The effects of humidity on pesticide efficacy

Here we ask whether increased humidity affects mortality differently in the pesticide and control treatments. We therefore use the interaction term (pesticide-treatment* humidity-condition) to assess whether low humidity contributes to the initially low mortality observed in some pesticide treatments during the first 42 days of the experiment.

Four states were possible, combining low and high humidity, with pesticide presence/absence. Data for the low-humidity condition were collected during days 1-42 of the experiment, with the high-humidity condition being applied sequentially and data being collected during days 43-68 of the experiment.

The efficacy of each of the four granular pesticides treatments was affected slightly differently by the increase in humidity. Overall, combining both low and high humidity states whilst food was available, ants in the Acetamiprid treatment were no more likely to die than those in the control group (Cox PH model, z = 0.278, d.f. = 1, p = 0.781). However, the application of the high-humidity-condition resulted in a significantly greater increase in mortality risk to ants in the Acetamiprid treatment than to ants in the control treatment (Cox PH model, z = 2.41, d.f. = 1, p = 0.016, figure 4.4A).

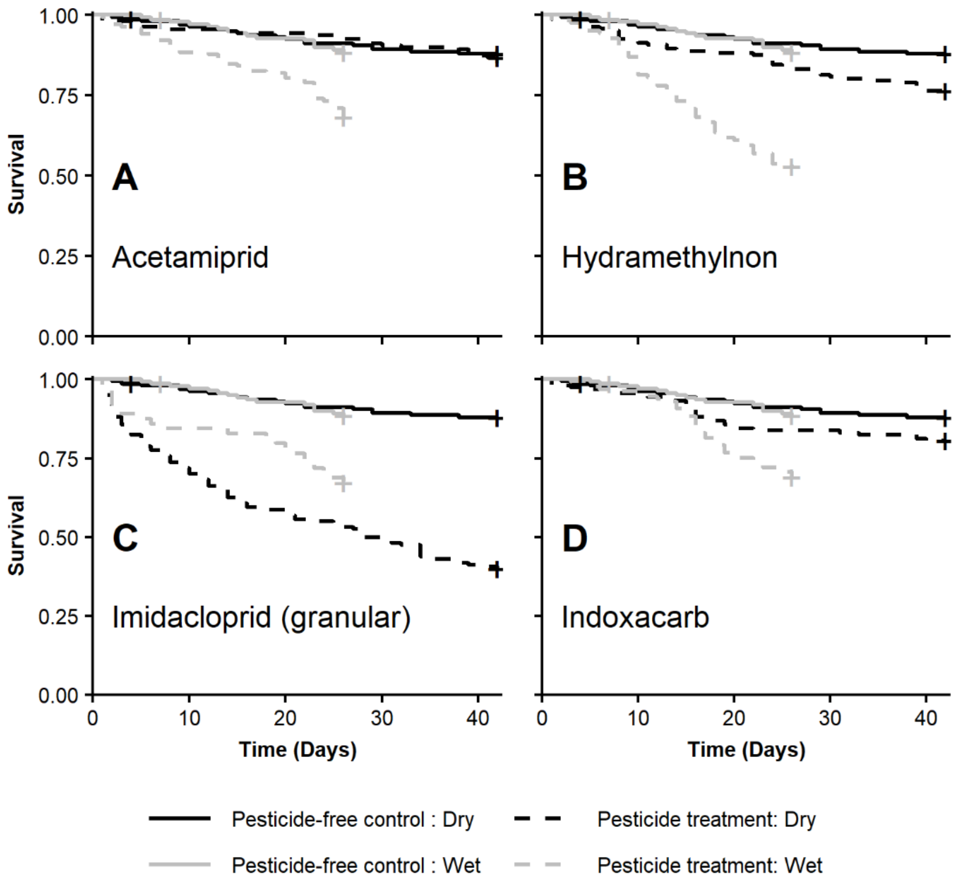


Figure 4.4 - Kaplan-Meier estimators of survival under high-humidity (Wet) and low-humidity (Dry) conditions, separated by pesticide treatment, pooled across all colonies. For each colony the Dry condition was applied immediately before the Wet condition, hence starting sample sizes differed between conditions (see Appendix D, section D3). The key comparison is whether increasing the humidity affects the pesticide treated colonies differently from the controls. Censored results indicated with a cross.

In contrast, over the same period ants in the Hydramethylnon treatment were more likely to die than those in the control group (Cox PH model, z = 2.633, d.f. = 1, p = 0.008). This contrasts with the result generated when just the low humidity phase is modelled and is driven by the increased humidity resulting in a significantly greater increase in mortality risk to ants in the Hydramethylnon treatment than to ants in the control treatment (Cox PH model, z = 2.266, d.f. = 1, p = 0.023, figure 4.4B). For both Acetamiprid and Hydramethylnon, these results suggest that the low mortality in the treatment during the low-humidity, food available phase of the experiment may have been due to the low humidity.

Across the low and high humidity states, ants in the granular Imidacloprid treatment were more likely to die than those in the control group (Cox PH model, z = 7.908, d.f. = 1, p < 0.001). Surprisingly, the application of the high-humidity-condition resulted in a borderline-significantly greater *decrease* in mortality risk to ants in the granular Imidacloprid treatment than to ants in the control treatment (Cox PH model, z = -1.956, d.f. = 1, p = 0.051 figure 4.4C).

Finally, ants in the Indoxacarb treatment were no more likely to die than those in the control group (Cox PH model, z = 1.818, d.f. = 1, p = 0.069). The application of the high-humidity-condition did not result in a significantly greater increase in mortality risk to ants in the Indoxacarb treatment compared to the control (Cox PH model, z = 1.33, d.f. = 1, p = 0.185, figure 4.4D), so there is no evidence to suggest that the low mortality in the Indoxacarb treatment during the first phase of the experiment was due to the low humidity.

The effects of starvation on pesticide efficacy

Here we ask whether removing the alternative food source affects mortality differently in the pesticide and control treatments. We therefore use the interaction term (pesticide-treatment* food-presence) to assess whether the availability of an alternative, and possibly higher quality, food resource might contribute to the initially low mortality observed in some treatments. The low food treatment was applied sequentially after the high humidity treatment, so only ants that survived the first 68 days were considered for this analysis.

Four states (all with high humidity) combining food available and unavailable, with pesticide presence/absence were possible: pesticide and food present; pesticide and food absent; pesticide-present but food absent; pesticide absent but food present. Data for the food-present status was collected during days 43-68 of the experiment with the food-absent status being applied sequentially and data being collected during days 69-96 of the experiment.

The efficacy of each of the four granular pesticides treatments was affected in a similar manner by the removal of the alternative food source under high humidity. Overall, combining both the food available and food unavailable high humidity states, ants in each of the pesticide treatments were more likely to die than those in the control group (Acetamiprid Cox PH model, $z = 3.851$, d.f. = 1, $p < 0.001$; Hydramethylnon Cox PH model, $z = 5.855$, d.f. = 1, $p < 0.001$; Imidacloprid granular Cox PH model, $z = 3.611$, d.f. = 1, $p < 0.001$; Indoxacarb Cox PH model, $z = 3.654$, d.f. = 1, $p < 0.001$).

However, removing the alternative food source had a significantly greater effect on the mortality rate in the control treatment than any of the pesticide treatments (Acetamiprid Cox PH model, $z = -3.71$, d.f. = 1, $p < 0.001$, figure 4.5A; Hydramethylnon Cox PH model, $z = -5.756$, d.f. = 1, $p < 0.001$, figure 4.5B; Imidacloprid granular Cox PH model, $z = -3.149$, d.f. = 1, $p = 0.002$, figure 4.5C; Hydramethylnon Cox PH model, $z = -4.095$, d.f. = 1, $p < 0.001$, figure 4.5D.) Therefore, we found no evidence to support the hypothesis that availability of an alternative food source causes the low mortality rate observed in the granular pesticide treatments in the low-humidity, food available phase of the experiment.

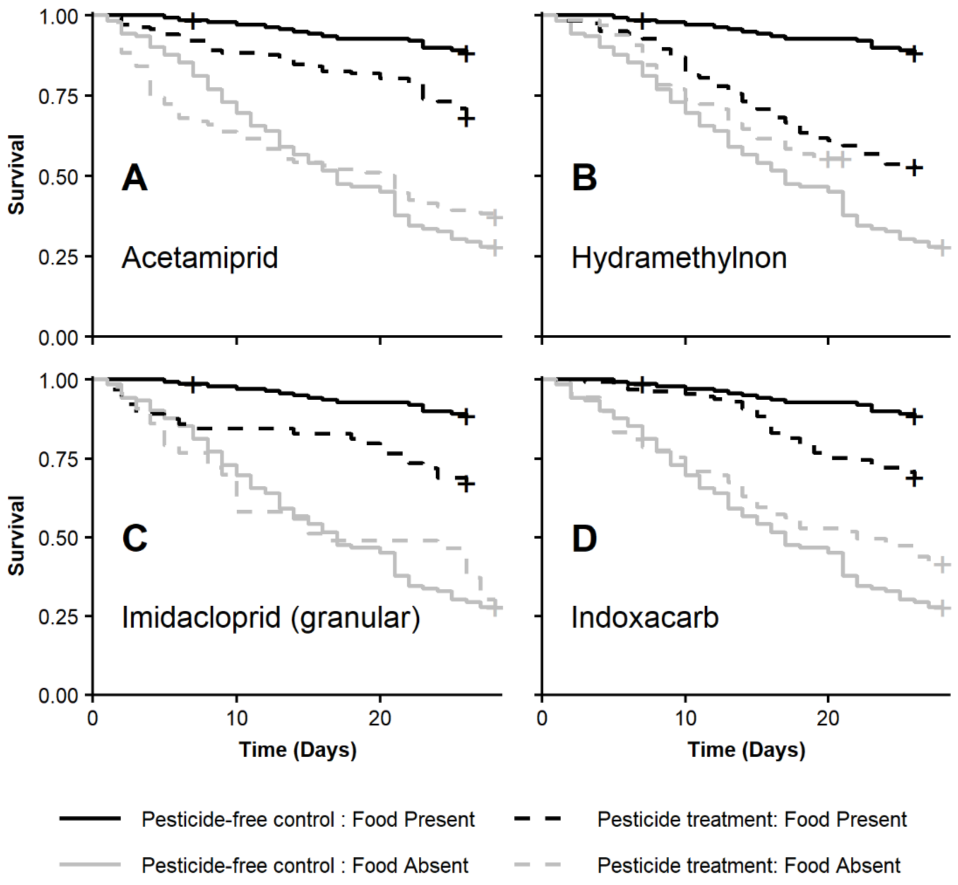


Figure 4.5 - Kaplan-Meier estimators of survival with food (sucrose solution and mealworms) present or absent, separated by pesticide treatment, pooled across all colonies. For each colony the food present treatment was applied immediately before the food absent treatment, hence starting sample sizes differed between treatments (see Appendix D, section D4). Analysis is applied only to ants surviving to the high humidity treatment. The key comparison is whether removing food affects the pesticide treated colonies differently from the controls. Censored results indicated with a cross.

Table 4.2 - Summary of findings from mortality investigations

Pesticide treatment	**Mortality risk relative to pesticide-free control in first 42 days (low humidity + food available)**	**Does low humidity condition explain the results from the first 42 days?**	**Does availability of food source explain the results from the first 42 days?**
Imidacloprid Gel	Very high	**NA** - not tested due to low surviving sample size	**NA** - not tested due to low surviving sample size
Imidacloprid granular	High	**No** - mortality risk during 0 - 68 days significantly higher than control	**No** - mortality risk during 69 - 96 days significantly higher than control
Acetamiprid	No significant difference	**Yes** but mortality risk during 0 - 68 days not significantly different from control	**No** but mortality risk during 69 - 96 days significantly higher relative to pesticide-free control
Hydramethylnon	No significant difference	**Yes** and mortality risk during 0 - 68 days significantly higher relative to pesticide-free control	**No** but mortality risk during 69 - 96 days significantly higher relative to pesticide-free control
Indoxacarb	No significant difference	**No** and mortality risk during 0 - 68 days not significantly different relative to pesticide-free control	**No** but mortality risk during 69 - 96 days significantly higher relative to pesticide-free control

Sub-lethal effects

Here we ask whether pesticide treatment had a sublethal effect on the behaviour of the ants during the first 42 days of the experiment. We therefore use the interaction term (treatment* day) to assess the proportion of live ants observed outside the nest changed during the experiment. The pesticide treatments did not result in a change in the absolute proportion of ants outside the nest (the intercept, i.e. the predicted proportion of ants outside the nest at time zero, was not significantly different in any of the pesticide treatments when compared to the control group; see Appendix D, section D5 for pairwise comparisons). The pesticide treatments also did not alter the rate at which the proportion of ants outside the nest changed, with the exception that the proportion of ants outside the nest decreased significantly quicker in the Imidacloprid-gel treatment than in the control (Wald test z= -4.860, df=1 p<0.001; see figure 4.6 and Appendix D, section D5).

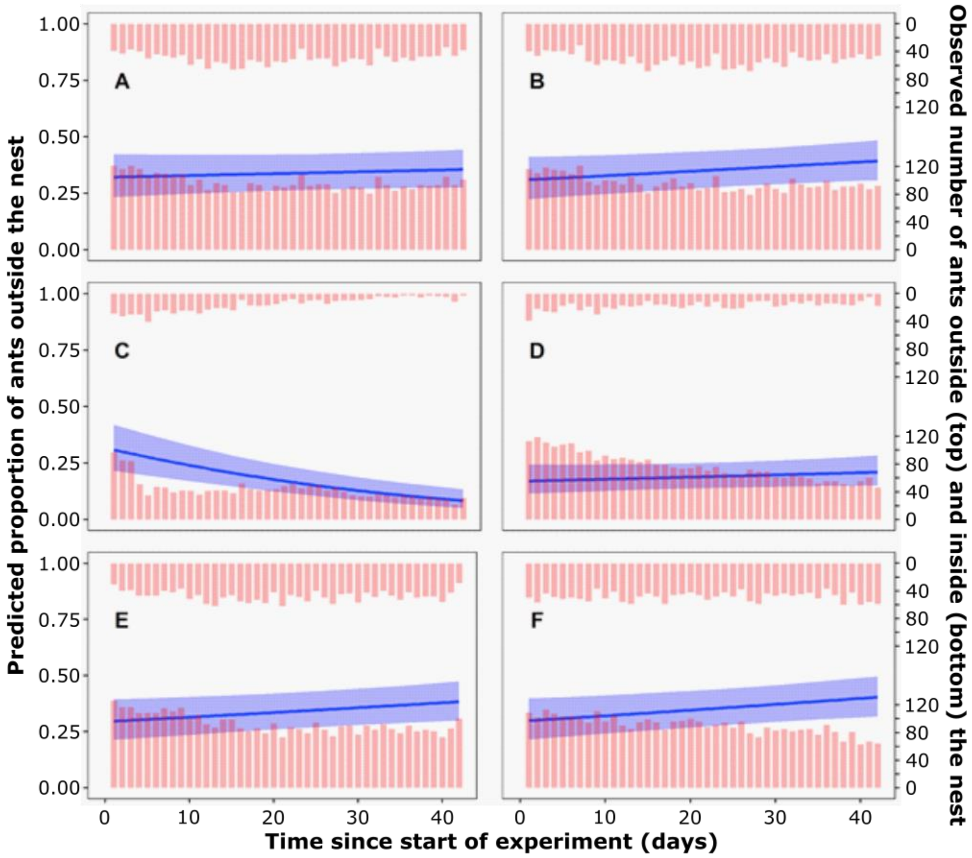


Figure 4.6 - Marginal fixed effects showing the predicted proportion of ants outside the nest each day and 95% confidence interval. Predictions separated by treatment and averaged over the random effects. Panels show: A - Pesticide-free control treatment, B - Acetamiprid, C - Imidacloprid Gel, D - granular Imidacloprid, E - Indoxacarb, and F - Hydramethylnon. The bars represent the number of ants inside the nest (bottom) and the number of ants outside the nest (top) averaged across colony.

Experiment 2 - the behavioural responses of individual ants to the pesticide treatments

Here we examine the behavioural response of individual ants to either one of the four granular pesticides, the gel-based pesticide, granular sucrose, sucrose solution, chopped mealworms or water. One datapoint from an Imidacloprid gel treatment (where the ant became stuck in the gel) was a significant outlier so was excluded from the analysis. Removing it had very little impact on the overall results (see Appendix D, section D56). There was very little difference in the amount of time that ants spent interacting with the different treatments (figure 4.7 panel A). There were, however, quite considerable differences in the amount of time the ants spent feeding at the different resources. Ants spent longer feeding on the wet resources (the sucrose solution, the imidacloprid gel and the water) than the dry resources (including the granular pesticides, mealworms and granular sucrose control). See figure 4.7 panel B and Appendix D, section D6.

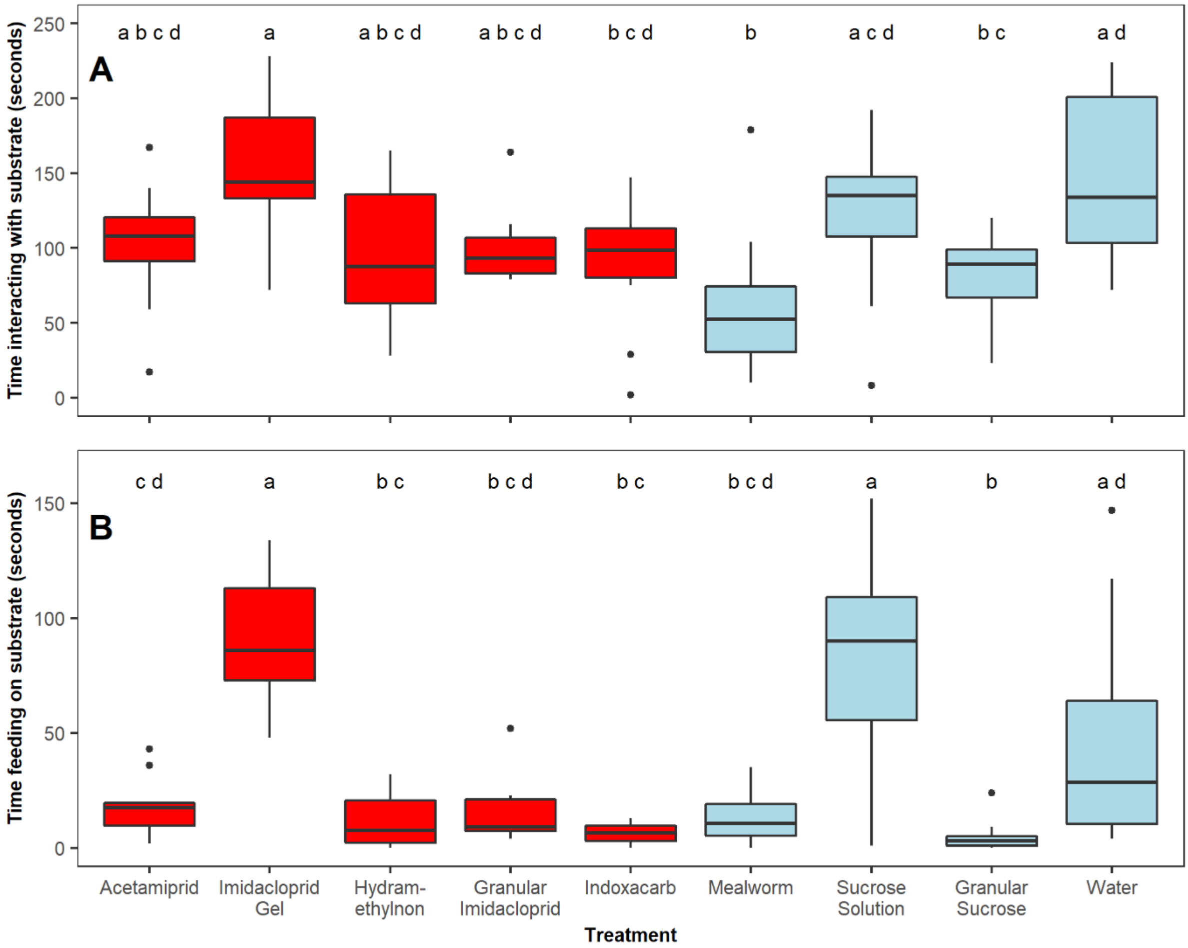


Figure 4.7 - Time spent interacting with (panel A) or feeding on (panel B) the contents of a bait station separated by treatment. Red boxes are pesticide treatments and blue boxes are non-pesticide resource comparisons. Matching letters indicate no significant difference in population

distributions (Kruskal-Wallis with Dunn's post hoc test using Benjamini-Hochberg false discovery rate adjustment to control for multiple testing - see Appendix D, section D6 for details).

Discussion

Our results demonstrate that, despite being formulated for use against other ant species, the four tested granular ant baits are likely to be ineffective for the control or eradication of *L. neglectus*. The granular Imidacloprid was the only one of the four to result in mortality greater than the control under dry conditions. Compared to the Imidacloprid gel treatment however, its performance is poor. The high mortality rate under the Imidacloprid gel treatment helps to validate our experimental design by acting as a positive control, as this gel treatment is effective at reducing the size of *L. neglectus* populations in the field (Boase, 2014). Our palatability tests show that dry food sources (e.g. granular sucrose and the four granular ant baits) are less palatable to *L. neglectus* than aqueous food resources (e.g. sucrose solution and the gel pesticide). A strong preference for aqueous (and sugary) food resources is also shown by the Argentine ant *Iridomyrmex humilis* (Baker et al., 1985). This indicates that the low efficacy of the four granular baits tested against *L. neglectus* is most likely due to low palatability rather than low toxicity, and suggests that granular baits in general may not be suitable for use with this species at least under dry conditions.

Field conditions are likely to be on average wetter than standard lab conditions, so to simulate this in the laboratory we also assessed mortality under increased humidity. This resulted in a significantly greater increase in mortality in both the Acetamiprid and the Hydramethylnon treatments relative to the pesticide free control group, although overall mortality was still relatively low in these treatments. Hydramethylnon is effective only through ingestion (i.e. no contact toxicity) so the increased mortality under high humidity is most likely due to an increase in the palatability of the bait under wetter conditions. These data suggest that the efficacy of some granular treatments may be improved under damp conditions, but when considering use in the field, this must be offset by the possibility of weathering reducing the attractiveness of baits (Hara et al., 2014). The unexpected result that the mortality in the Imidacloprid granular treatment was lower under high humidity than low humidity conditions, in contrast to the other granular pesticide treatments, might have been a result of the fewer ants surviving until the high humidity condition in the granular Imidacloprid treatment than the other granular treatments. The sequential nature of the humidity and starvation treatments also means we are unable to eliminate the possibility that an increase in mortality under later conditions was due the conditions themselves and not a slow response to the pesticide treatment. However, the inclusion of the pesticide-free control treatment demonstrates that the observed effects were not simply due to an increase in background mortality over time. Future work should randomise the order of treatment conditions and also assess bait palatability over a range of field-realistic humidity conditions.

In most field contexts, invasive ants have established alternative food sources, so we initially provided food to our test colonies as well as the pesticide baits. Some control attempts target the ants' food sources as well as the ants themselves (Rey and Espadaler, 2004), so we also investigated the effects of removing the alternative food source. This additional change did not increase the mortality in any of the granular pesticide treatments relative to the pesticide-free control group. This could be a further indication of the low palatability of the baits trialled, even though the ants were starving, the mortality data suggests that they did not switch to eating the pesticides. It is worth bearing in mind that our experiment provided two extremes of food availability i.e. shifting from a surplus to famine. Colonies in the field are likely to exist somewhere between these extremes. Moreover, a colony's nutritional requirements can vary seasonally and this results in changes to behavioural responses to food resources (Cook et al., 2011). As our colonies were maintained under 12:12 light:dark cycle it is not possible to determine their potential seasonal state. Simultaneously targeting a colony's food resource during an eradication campaign is unlikely to be detrimental to the overall outcome and may indeed help if the ants have a highly palatable bait as an alternative food source.

Pesticides can have sublethal effects: behavioural or physiological changes that can cause considerable disruption and eventually result in colony failure. For example, numerous studies have demonstrated sublethal effects of neonicotinoid pesticides on pollinators (Alkassab and Kirchner, 2017; Godfray et al., 2014) and Imidacloprid has sub-lethal effects on the foraging behaviour of *Solenopsis invicta* (Wang et al., 2015). Sub-lethal doses of Imidacloprid reduce the number of extra-nest foraging workers in *Lasius niger* (Thiel and Köhler, 2016). We also used the number of extra-nest ants as a measure of colony foraging activity in case sub-lethal behavioural impacts of the pesticides could be detected. At the start of the experiment, overall around a third of the ants were active outside the nest. This is close to the ratio that we used in constructing the colonies (25% extranest workers) suggesting our initial colony composition was appropriate. The proportion of ants outside the nest over time did not vary with treatment, with the exception of the Imidacloprid gel treatment where fewer ants left the nest as the experiment progressed. It is not clear from our results whether the decrease in the number of ants outside the nest is a behavioural response of the colony to exposure to the Imidacloprid gel treatment or (given the high mortality in this treatment) whether the change is due to foraging ants having a disproportionately high mortality rate. Repeating the experiment using a lower concentration of Imidacloprid might help to elucidate this dichotomy.

Queens are the reproductive units of the colony so eliminating them is crucial for a successful eradication. Like all invasive ant species, *L. neglectus* is polygynous and queen numbers can be very high (Espadaler et al., 2004). Our experiment did not examine the effect of the pesticides on *L. neglectus* queens (no queens were included in the test sub-colonies). However, ant queens are dependent on foraging workers for food, and the rate of colony reproductive output, growth and spread will be negatively impacted if worker population is dramatically reduced (Hee et al., 2000; Warner et al., 2018). While queenless nests are common in polydomous ant colonies, brood presence is required for normal functioning of ant sub-colonies, so we included brood in our test colonies; however, the fate of the brood included at the start is unclear. Some of the brood may have developed into workers, but this cannot have been a major factor because the final total number of ants (live and dead) never exceeded 41; alternatively, the brood could have died or been eaten. Another note of caution that should be raised is that all the stock colonies came from the same population of *L. neglectus* and therefore may not represent the full range of variation within the species. Interestingly, despite the fact that all the ant colonies used in the mortality experiments came from the same population, there was significant variation in mortality between colonies. This was unexpected and may have related to differences in queen health or colony nutritional state prior to the experiment; it warrants further investigation.

Our results suggest that granular pesticide delivery is unlikely to be useful in control of *L. neglectus*; conversely, while the gel-based method is effective, it is not suitable for large-scale application. The most promising pesticide delivery mechanism for the control of *L. neglectus* is likely to be the use of polyacrylamide (Buczkowski et al., 2014a) or alginate (Tay et al., 2017) hydrogel crystals. When saturated with a sucrose solution and soluble toxicant, these materials present a liquid food resource for ants that can be hand scattered over large areas and is much less susceptible to desiccation than formulated gel insecticides. Both polyacrylamide and alginate hydrogel baits in combination with an aqueous insecticide are effective tools for the control of the Argentine ant (*Linepithema humile*) in the field (Buczkowski et al., 2014b; Tay et al., 2017). Preliminary work (Appendix E) demonstrates that *L. neglectus* will readily feed from polyacrylamide crystals saturated with a sucrose solution, providing a proof-of-concept for this approach to control for the invasive garden ant.

We propose that further work should be carried out to identify a suitable aqueous insecticide or combination of insecticides to use in combination with hydrogel crystals for the control of *L. neglectus*. An ideal active ingredient should be non-repellent to *L. neglectus* and lead to high mortality but also be slow acting enough to ensure that it is distributed to non-foraging workers, larvae, and queens. In this experiment, we tested the insecticides individually in order to try to isolate their effects, but the use of multiple active ingredients may have additive or synergistic effects which warrant investigation (Zhu et al., 2017). If possible, the longer term effects of exposure and the development of possible avoidance behaviour (Wada-Katsumata et al., 2013) should also be considered. Ultimately, field-based trials will be required to assess the optimal application effort in order to achieve eradication of the desired level of population reduction. The seasonal timing of treatment should also be considered to maximise effectiveness in relation to the ants' seasonal nutritional requirements (Cook et al., 2011).

We believe that with a small amount of work to identify the most suitable insecticidal active ingredients to use in conjunction with hydrogel crystals, the effective large-scale control or even eradication of populations of *L. neglectus* should be achievable. Our approach demonstrates the importance of integrating the target species' ecology into planned control measures. It also highlights the value of determining the species-specific effectiveness of pesticide delivery mechanisms in a controlled environment before use in the field where the causes of failure can be harder to identify. We recommend the use of a similar protocol for developing control measures for other invasive social insects.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Acknowledgements:

PBB acknowledges funding from the Natural Environment Research Council (NERC) and Hymettus Ltd. We are grateful to Bayer, Bábolna Bio and DuPont for providing the granular pesticide samples used in these experiments. We would like to thank AMH Bedois, LL Cai, L Dunsby, M Guaita, KL Hutchinson, RF Kinnersley, A Kyte, AK Middlemiss, S Pickering, C Sauer and LHN Smith for assisting with the video analysis. We would also like to thank D Burns, E Drinkwater, DW Franks, M Holgate, V Lecheval, and P Mayhew for comments on earlier drafts of this manuscript.

References

Abbott, K.L., Green, P.T., 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. Oikos 116, 1238–1246.

Alkassab, A.T., Kirchner, W.H., 2017. Sublethal exposure to neonicotinoids and related side effects on insect pollinators: honeybees, bumblebees, and solitary bees. J. Plant Dis. Prot. 124, 1–30.

Baker, T.C., Van Vorhis Key, S.E., Gaston, L.K., 1985. Bait-preference Tests for the Argentine Ant (Hymenoptera: Formicidae). J. Econ. Entomol. 78, 1083–1088.

Bertelsmeier, C., Luque, G.M., Hoffmann, B.D., Courchamp, F., 2015. Worldwide ant invasions under climate change. Biodivers. Conserv. 24, 117–128.

Boase, C., 2014. *Lasius neglectus* (Hymenoptera : Formicidae) in the UK : status, impact and management. In: Proceedings of the Eighth International Conference on Urban Pests. pp. 223–228.

Bradshaw, C.J.A., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., Barbet-Massin, M., Salles, J.M., Simard, F., Courchamp, F., 2016. Massive yet grossly underestimated global costs of invasive insects. Nat. Commun. 7, 1–8.

Buckham-Bonnett, P., Robinson, E.J.H., 2017. GB Non-native Species Rapid Risk Assessment : Rapid Risk Assessment of: *Lasius neglectus* (Invasive Garden Ant).

Buczkowski, G., Roper, E., Chin, D., 2014a. Polyacrylamide Hydrogels: An Effective Tool for Delivering Liquid Baits to Pest Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 107, 748–757.

Buczkowski, G., Roper, E., Chin, D., Mothapo, N., Wossler, T., 2014b. Hydrogel baits with low-dose thiamethoxam for sustainable Argentine ant management in commercial orchards. Entomol. Exp. Appl. 153, 183–190.

Cook, S.C., Eubanks, M.D., Gold, R.E., Behmer, S.T., 2011. Seasonality directs contrasting food collection behavior and nutrient regulation strategies in ants. PLoS One 6, e25407.

Cremer, S., Ugelvig, L. V, Drijfhout, F.P., Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Hughes, D.P., Schulz, A., Petersen, K.S., Konrad, H., Stauffer, C., Kiran, K., Espadaler, X., d'Ettorre, P., Aktaç, N., Eilenberg, J., Jones, G.R., Nash, D.R., Pedersen, J.S., Boomsma, J.J., 2008. The evolution of invasiveness in garden ants. PLoS One 3, e3838.

Cremer, S., Ugelvig, L. V, Lommen, S.T.E., Petersen, K.S., Pedersen, J.S., 2006. Attack of the invasive garden ant: aggression behaviour of *Lasius neglectus* (Hymenoptera: Formicidae) against native *Lasius* species in Spain. Myrmec. Nachr 9, 13–19.

Czaczkes, T.J., Schlosser, L., Heinze, J., Witte, V., 2014. Ants use directionless odour cues to recall odour-associated locations. Behav. Ecol. Sociobiol. 68, 981–988.

Espadaler, X., Bernal, V., 2018. *Lasius neglectus* Distribution [WWW Document]. URL http://www.creaf.uab.es/xeg/Lasius/Ingles/distribution.htm (accessed 11.20.18).

Espadaler, X., Rey, S., Bernal, V., 2004. Queen number in a supercolony of the invasive garden ant, *Lasius neglectus*. Insectes Soc. 51, 232–238.

Espadaler, X., Tartally, A., Schultz, R., Seifert, B., Nagy, C., 2007. Regional trends and preliminary results on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 54, 293–301.

Evans, T.A., Forschler, B.T., Grace, J.K., 2013. Biology of Invasive Termites: A Worldwide Review. Annu. Rev. Entomol. 58, 455–474.

Forstmeier, W., Schielzeth, H., 2011. Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. Behav. Ecol. Sociobiol. 65, 47–55.

Gentz, M.C., 2009. A review of chemical control options for invasive social insects in island ecosystems. J. Appl. Entomol. 133, 329–335.

Gippet, J.M.W., Mondy, N., Diallo-Dudek, J., Bellec, A., Dumet, A., Mistler, L., Kaufmann, B., 2017. I'm not like everybody else: urbanization factors shaping spatial distribution of native and invasive ants are species-specific. Urban Ecosyst. 20, 157–169.

Godfray, H.C.J., Blacquière, T., Field, L.M., Hails, R.S., Petrokofsky, G., Potts, S.G., Raine, N.E., Vanbergen, A.J., McLean, A.R., 2014. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc. R. Soc. B Biol. Sci. 281.

Hara, A.H., Aoki, K.L., Cabral, S.K., Niino-DuPonte, R., 2014. Attractiveness of gel, granular, paste, and solid formulations of ant bait insecticides to the little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae). Proc. Hawaii. Entomol. Soc. 46, 45–54.

Hee, J.J., Holway, D.A., Suarez, A. V., Case, T.J., 2000. Role of propagule size in the success of incipient colonies of the invasive Argentine ant. Conserv. Biol. 14, 559–563.

Herbers, J.M., Choiniere, E., 1996. Foraging behaviour and colony structure in ants. Anim. Behav. 51, 141–153.

Hoffmann, B.D., Abbott, K.L., Davis, P.D., 2010. Invasive Ant Management. In: Lach, L., Parr, C.L., Abbott, K.L. (Eds.), Ant Ecology. Oxford University Press.

Hoffmann, B.D., Luque, G.M., Bellard, C., Holmes, N.D., Donlan, C.J., 2016. Improving invasive ant eradication as a conservation tool: A review. Biol. Conserv. 198, 37–49.

Holway, D., Lach, L., Suarez, A., 2002. The causes and consequences of ant invasions. Annu. Rev. Ecol. Syst. 33, 181–233.

Lach, L., Parr, C., Abbott, K., 2010. Ant ecology. Oxford University Press.

Landau, I., Mueller, G., Schmidt, M., 2017. First occurrence of *Lasius neglectus* (Hymenoptera: Formicidae) in Zurich, Switzerland: distribution and control measures. In: Proceedings of the Ninth International Conference on Urban Pests. pp. 111–116.

Lowe, S., Brownw, M., Boudjelas, S., De Poorter, M., 2000. 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. Invasive Species Spec. Gr. a Spec. Gr. Species Surviv. Comm. World Conserv. Union 12, 12.

McGlynn, T.P., 1999. The worldwide transfer of ants: Geographical distribution and ecological invasions. J. Biogeogr. 26, 535–548.

Nagy, C., Tartally, A., Vilisics, F., Merkl, O., Szita, É., Szél, G., Podlussány, A., Rédei, D., Csösz, S., Pozsgai, G., Orosz, A., Szövényi, G., Markó, V., 2009. Effects of the invasive garden ant, *Lasius neglectus* VAN LOON, BOOMSMA & ANDRÁS-FALVY, 1990 (Hymenoptera: Formicidae), on arthropod assemblages: pattern analyses in the type supercolony. Myrmecological News 12, 171–181.

Paris, C.I., Espadaler, X., 2009. Honeydew collection by the invasive garden ant *Lasius neglectus* versus the native ant *L. grandis*. Arthropod. Plant. Interact. 3, 75–85.

Pau, G., Fuchs, F., Sklyar, O., Boutros, M., Huber, W., 2010. EBImage---an R package for image processing with applications to cellular phenotypes. Bioinformatics 26, 979–981.

Pejchar, L., Mooney, H.A., 2009. Invasive species, ecosystem services and human well-being. Trends Ecol. Evol. 24, 497–504.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288.

Portha, S., Deneubourg, J.-L., Detrain, C., 2004. How food type and brood influence foraging decisions of *Lasius niger* scouts. Anim. Behav. 68, 115–122.

R Core Team, 2018. R: A Language and Environment for Statistical Computing.

Rey, S., Espadaler, X., 2004. Area-wide management of the invasive garden ant *Lasius neglectus* (Hymenoptera: Formicidae) in Northeast Spain. J. Agric. Urban Entomol. 21, 99–112.

Sanders, N.J., Gotelli, N.J., Heller, N.E., Gordon, D.M., 2003. Community disassembly by an invasive species. Proc. Natl. Acad. Sci. 100, 2474–2477.

Santarlasci, A., Martelloni, G., Frizzi, F., Santini, G., Bagnoli, F., 2014. Modeling warfare in social animals: a “chemical” approach. PLoS One 9, e111310.

Seifert, B., 2000. Rapid range expansion in *Lasius neglectus* (Hymenoptera, Formicidae) – an Asian invader swamps Europe. Mitt. Mus. Nat. kd. Berl., Dtsch. entomol. Z. 47, 173–179.

Tartally, A., Hornung, E., Espadaler, X., 2004. The joint introduction of *Platyarthrus schoblii* (Isopoda: Oniscidea) and *Lasius neglectus* (Hymenoptera : Formicidae) into Hungary. Myrmecologische Nachrichten 6, 61–66.

Tay, J.W., Hoddle, M.S., Mulchandani, A., Choe, D.H., 2017. Development of an alginate hydrogel to deliver aqueous bait for pest ant management. Pest Manag. Sci. 73, 2028–2038.

Team, F., 2016. FFmpeg.

Thiel, S., Köhler, H.-R., 2016. A sublethal imidacloprid concentration alters foraging and competition behaviour of ants. Ecotoxicology.

Van Loon, A.J., Boomsma, J.J., Andrasfalvy, A., 1990. A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe description and general Biology. Insectes Soc. 37, 348–362.

Wada-Katsumata, A., Silverman, J., Schal, C., 2013. Changes in taste neurons support the emergence of an adaptive behavior in cockroaches. Science (80-.). 340, 972–975.

Wang, L., Zeng, L., Chen, J., 2015. Sublethal Effect of Imidacloprid on *Solenopsis invicta* (Hymenoptera: Formicidae) Feeding, Digging, and Foraging Behavior. Environ. Entomol. 44, 1544–1552.

Warner, M.R., Lipponen, J., Linksvayer, T.A., 2018. Pharaoh ant colonies dynamically regulate reproductive allocation based on colony demography. Behav. Ecol. Sociobiol. 72, 1–13.

Zhu, Y.C., Yao, J., Adamczyk, J., Luttrell, R., 2017. Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (*Apis mellifera*). PLoS One 12, 1–16.

Chapter 5: Assessing the UK distribution of *Lasius neglectus*

This chapter comprises 3 parts. 5a is a report on a large-scale survey across the United Kingdom; 5b is a report on a new discovery of a *Lasius neglectus* population, the first in the north of the UK; 5c is a collation of data from multiple sources to give an up-to-date overview of the current distribution of the species.

5a - Large scale survey for a cryptic invasive insect

Abstract

1. Accurate information about the distribution of invasive species is vital for conservation policy and management. However, assessing the distribution of cryptic invasive species is challenging.
2. *Lasius neglectus* Van Loon, Boomsma et Andrásfalvy, 1990 (Hymenoptera: Formicidae) is a cryptic invasive ant species with an extensive distribution in Europe that has negative impacts on native invertebrates. The lack of a nuptial flight limits natural dispersal in this species but accidental transport to new sites by humans occurs.
3. Botanic gardens and other sites with extensive plant collection have a high propagule pressure (introduction effort) for non-native insects accidentally transported with potted plants.
4. We used adhesive traps to survey botanic and other gardens open to the public across the UK for the presence of *L. neglectus*.
5. Although traps were returned from 100 sites across the UK, no new sites with *L. neglectus* were identified.
6. Most sites returned traps with native ants: this increases our confidence in the negative results for *L. neglectus* as it often excludes native ant species.
7. Our results indicate that *L. neglectus* is not prevalent as a cryptic invasive species in botanic gardens and suggest that such sites may be less important for this species than predicted.

Introduction

Invasive alien species cause major conservation and economic problems (Kenis et al., 2009; Pejchar and Mooney, 2009; Pimentel et al., 2005; Strayer et al., 2006). Invasive insects alone are estimated to result in an annual global cost in excess of US$76 billion (Bradshaw et al., 2016). Through competition and predation, and by acting as vectors for pathogens, invasive insect species can have significant negative impacts on native invertebrates. For example, the conservation impacts of invasive species such as the harlequin ladybird (*Harmonia axyridis*), the common wasp (*Vespula vulgaris*) and the big headed ant (*Pheidole megacephala*) are dramatic, leading to reduced survival, population declines and, in some cases, local extinctions of native insect species (Beggs, 2001; Comont et al., 2014; Hoffmann et al., 1999; Roy et al., 2012). High quality data on the abundance and distribution of invasive species is important for managing their populations, minimising their spread and assessing their potential conservation impacts (Groom et al., 2015), especially where the species is cryptic (difficult to detect) in its non-native range (Jarić et al., 2019).

In western Europe, the invasive garden ant *Lasius neglectus* Van Loon, Boomsma et Andrásfalvy, 1990 (Hymenoptera: Formicidae) is a cryptic and harmful invasive species. At invasion sites, *L. neglectus* can achieve very high population densities (Espadaler et al., 2004) and also occupy large areas (Paris and Espadaler, 2009; Tartally, 2006). This, combined with the species' aggressiveness towards individuals of other ant taxa (Cremer et al., 2006) has several negative ecological consequences. Invertebrate community composition can be impacted, with reductions in the abundance of native ant species, beetles and woodlice (Nagy et al., 2009). In contrast, populations of Hemiptera (in particular aphids), which the ants farm for honeydew, can increase dramatically (Paris and Espadaler, 2009) meaning the ants may also have an indirect negative impact on plant performance (Espadaler, 1999). In addition to its ecological impacts, *L. neglectus* is a pest, entering buildings in large numbers where it is a nuisance and sometimes damages electrical equipment (Rey and Espadaler, 2004).

Lasius neglectus has an extensive distribution in Europe (Espadaler and Bernal, 2018) and shows a similar mode of dispersal to many invasive ant species. The spread of ants at existing sites is slow (tens of metres per year) but new colonies are frequently discovered many kilometres from the nearest known one (Espadaler et al., 2007). The low spread rate at existing sites is due to the lack of a nuptial flight: colonies grow by budding, with groups moving to new areas on foot. In contrast, movement over longer distances is due to human-mediated dispersal, for example accidental transport in potted plants (Pospischil, 2011; Seifert, 2010; Tartally et al., 2004; Van Loon et al., 1990). This pattern of spread means that unlike many invasive species whose impacts are often diffusely spread across landscapes, the impact of *L. neglectus* populations is highly concentrated in a few areas. The spread of invasive species is exacerbated by both global human activities and by climate change, which can increase their potential range (Hulme, 2017), especially allowing hot-climate species to move into temperate regions which may previously have been unsuitable. Being ectotherms, invasive insects are particularly likely to benefit from climate change, both from increases to their potential ranges, but also by release from dependence on human-associated habitats e.g. hothouses. The potential range of *L. neglectus* currently includes most of the UK, with the exception of some mountainous regions, and this is predicted to increase as a result of climate change (Bertelsmeier et al., 2015a).

In total, *L. neglectus* has been identified from ten sites in the UK (see table 5.1) and eliminated from one (Boase, 2014) but the total number of sites where the species is present is likely to be higher. *Lasius neglectus* is morphologically and ecologically very similar to several congenerics found in the UK. This makes confirming the identification of the species challenging without training, practice and suitable equipment. In particular, ecological and morphological similarities to the highly abundant, synanthropic species *Lasius niger* mean that populations of *L. neglectus* are frequently overlooked. This has occurred at several sites in the UK including Hidcote Manor Gardens (Hidcote), Gloucestershire where residents were aware of an “ant problem” for decades before *L. neglectus* was identified there in 2009 (Boase, 2014; Fox, 2010). Similarly, residents of Kirk Smeaton, North Yorkshire, affected by the UK’s most northerly known population of *L. neglectus*, were aware of the unusual abundance of ants for at least six years prior to their identification in 2016 (Sheldon *pers. comm.*).

Table 5.1 - Sites of confirmed populations of *L. neglectus*.

Location	**Year identified**	**Further information**
Hidcote Manor Gardens (Gloucestershire)	2009	Formal Garden
Stowe (Buckinghamshire)	2010	Formal Garden/Park - eradicated
Cambridge (Cambridgeshire)	2010	Botanic Garden
Hendon (Greater London)	2014	Urban/Residential
Kirk Smeaton (North Yorkshire)	2016	Rural/Residential
Rodmell (East Sussex)	2016	Rural/Residential
Eastbourne (East Sussex)	2016	Urban
Holland Park (Greater London)	2016	Residential?
Branscombe (Devon)	2017	Rural/Residential?
Cheshunt (Hertfordshire)	2018	Residential?

Lasius neglectus was first identified from a population whose distribution included the gardens of the Company for the Development of Fruit and Ornamental Production in Budapest, Hungary (Van Loon et al., 1990) and since then, *L. neglectus* has been found in several other botanic gardens including: Debrecen, Hungary (Tartally et al., 2004); Rostoc, Germany, (Schultz and Busch, 2009); Yalta, Ukraine (Stukalyuk and Radchenko, 2018); and Cambridge, UK (Buckham-Bonnett and Robinson, 2017). Accidental transport in potted plants is likely to be responsible for some of the instances of the transport of *L. neglectus* to new sites (Tartally et al., 2004; Van Loon et al., 1990). Sites with plant collections often exchange materials with similar sites; this can lead to an increase in propagule pressure i.e. a high probability of non-native species arriving which, in turn, can drive a higher rate of establishment (Lockwood et al., 2005). Additionally, sites with large plant collections or plant displays often have characteristics which can facilitate the establishment of *L. neglectus* upon arrival. Mature trees hosting large aphid populations are likely to play an important role in allowing establishment as the aphids provide a food resource for the ants (Espadaler et al., 2007; Van Loon et al., 1990). Whilst *L. neglectus* is able to survive at locations with colder winters than the UK, for example a mean temperature of -5.5°C in the coldest month (Seifert, 2000), structures such as glasshouses frequently found at sites with plant collections may also play a role in the establishment of the species. Many gardens open to the public also sell potted plants, so if *L. neglectus* is present at these sites, the risk of it spreading to new sites is potentially higher than it would be in a purely residential environment. Preliminary data (see supplementary information) suggested that that non-native ants are likely to be found in botanic gardens in the UK.

Given that botanic gardens are high risk sites for *L. neglectus* and the ants are easily overlooked, a systematic survey of botanic and formal gardens across the UK is important because it has the potential to prevent further unintentional introductions. Various methods are employed to survey ants, each with its own benefits and limitations. One of the more effective methods for surveying for *L. neglectus* is for someone familiar with the species' ecology and appearance to manually search a target area. This approach has drawbacks however: at low population sizes the ants could be overlooked; warm, dry weather is required for optimal search conditions; the method is slow; travel to target sites is expensive; and the number of people with the training to perform the search is limited. Over large geographical scales, for example at a national level, passive trapping collection methods are a potentially less labour-intensive approach. Traps can be sent to sites of interest, be deployed locally before being returned to a central location for specimen identification. Adhesive traps are particularly useful for invertebrate sampling as they can be deployed by someone without prior experience using them and do not contain any chemical harmful to humans. They can then be sealed in bags to encase the catch and frozen to humanely kill any invertebrates that remain alive (Drinkwater et al., 2019). Trapping approaches have the potential to generate data that is comparable to the results of expert surveying (Sumner et al., 2019).

The aim of this study is to determine whether *L. neglectus* is prevalent in botanic gardens, formal gardens and other sites with extensive plant collections in the UK. This research will help to inform the management of a potential invasion pathway i.e. from formal gardens within the UK to other sites. By helping to assess the distribution of *L. neglectus* in the UK, this research will also provide information towards the feasibility of a potential national eradication campaign.

Methods

Sites targeted

Traps were sent to 191 gardens and sites open to the public in summer 2016, covering a wide geographical range within the UK (see figure 5.1). Sites were either owned or managed by the National Trust or listed as members of the Plant Network (https://plantnetwork.org/). The sites included formal, display and botanic gardens as well as some parks and country properties open to the public. Two sites where the presence of *L. neglectus* had already been confirmed were included as a positive control.

Sampling methods

Four Killgerm AF® hinged plastic insect monitors (traps) were sent to each site. On site, an adhesive card was placed inside each trap and approximately 5g of honey was poured onto the adhesive face at one edge and allowed to run onto the plastic case. The honey was used to attract ants which then became trapped on the adhesive card. Site staff were instructed to deploy the traps in four separate locations for between two and five days. They were asked to select sites that would have conditions that were favourable for ants (see supplementary Appendix G for details). After retrieval from the field, the traps were placed in a sealed plastic bag and stored in a freezer for a minimum of 12 hours before being returned to the University of York for analysis.

Analysis of samples

All invertebrates were removed from the adhesive cards. Ants were stored in 80% ethanol and non-ants were stored in 60% ethanol. Ants were then identified to species level using the key in Skinner and Allen (2013). Any ants that keyed out as *L. alienus* in this resource were then checked against a *L. neglectus* identification sheet (Buckham-Bonnett et al., 2016). An online resource from the Bees, Ants and Wasps Recording Society was also used to aid identification of ants in the *Myrmica scabrinodis* group (Attewell, 2011).

Data Visualisation and Statistical Analysis

Maps were produced in R (R Core Team, 2018) using the “sp” package (Pebesma, E.J., 2005) and GADM shapefiles (GADM, 2017). Dunn’s tests were performed to examine the impact of physical factors on the number of species caught using the “dunn.test” package (Dinno, 2017) and “ggplot2” (Wickham, 2016) was used to plot the data.

Results

From the 191 sites targeted, 103 sets of traps were returned from 100 sites. These sites had a broad geographic distribution in the UK (figure 5.1) and included botanic gardens, parks, arboreta, the gardens of historic country properties and other sites with extensive plant collections (see supplementary information for the full list).

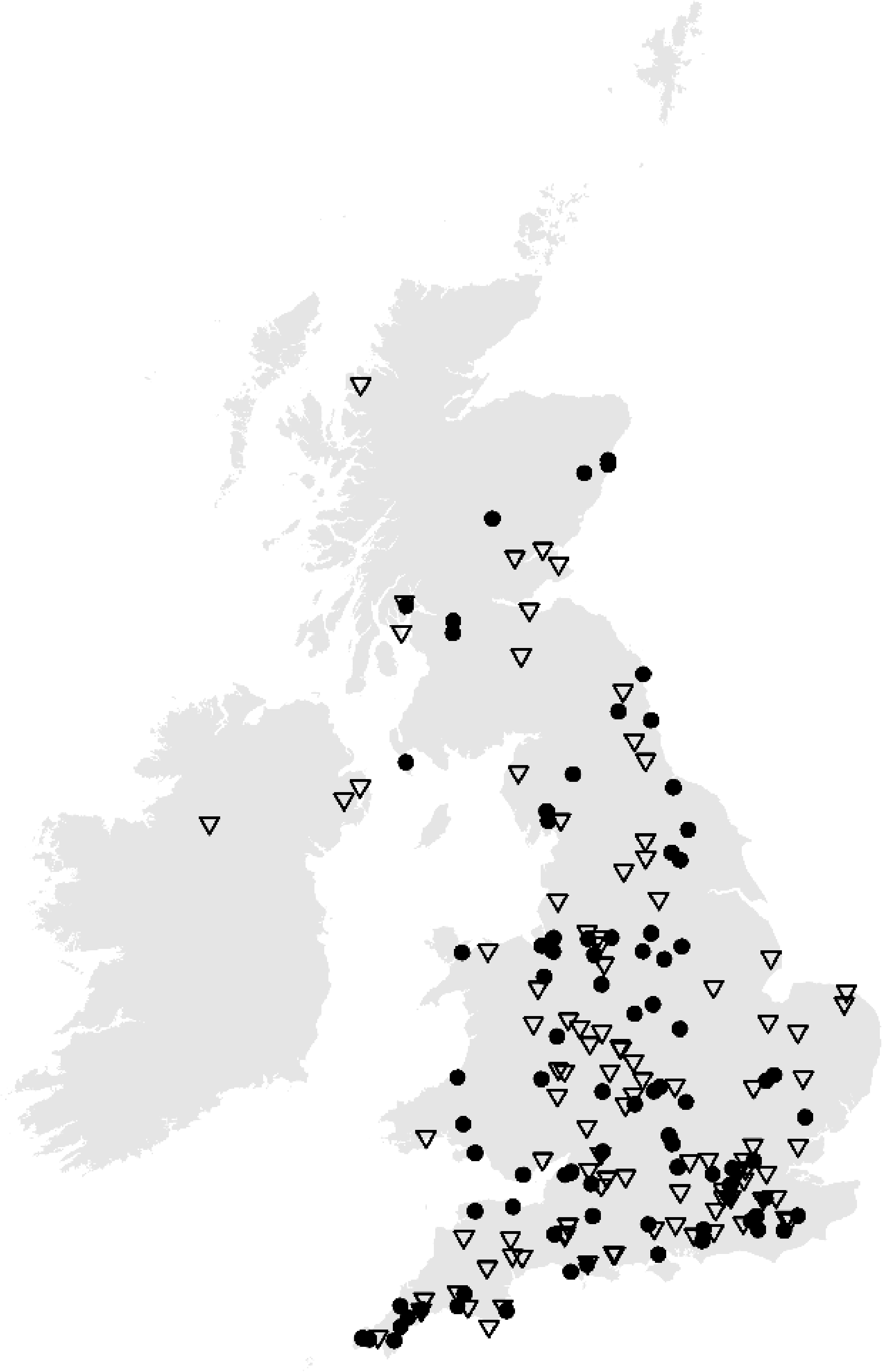


Figure 5.1 - Locations of sites targeted by survey. Sites which returned traps are shown as solid circles (n=100), those which did not are shown as inverted triangles (n=91). (Three of the 100 sites returned two sets of traps, see table F1)

Of the 103 sets of traps returned, 91 sets included traps with non-ant invertebrates and 74 sets included traps with ants, mostly native species (figure 5.2). *Lasius neglectus* excludes native ants species (Nagy et al., 2009), so the presence of native ants increases the confidence associated with results indicating the absence of *L. neglectus*. This is particularly true for the most ecologically similar species such as *L. niger*. Fifty-one sites returned traps with native *Lasius* species (figure 5.3) and no new sites with *L. neglectus* were identified.

Two sets of traps were returned from Hidcote Manor in Gloucestershire where the presence of *L. neglectus* has previously been confirmed (figure 5.3). All eight of the traps from this site contained *L. neglectus*. Indeed, the entire adhesive surfaces of all eight glue pads were covered with *L. neglectus* workers. No traps sent to other sites achieved this level of ant cover. The other potential positive control site did not return any traps. Across all traps, all the ants were workers with the exception of three queens (one *Lasius* and two *Tetramorium bicarinatum*). A summary of ant species found is presented in table 5.2.

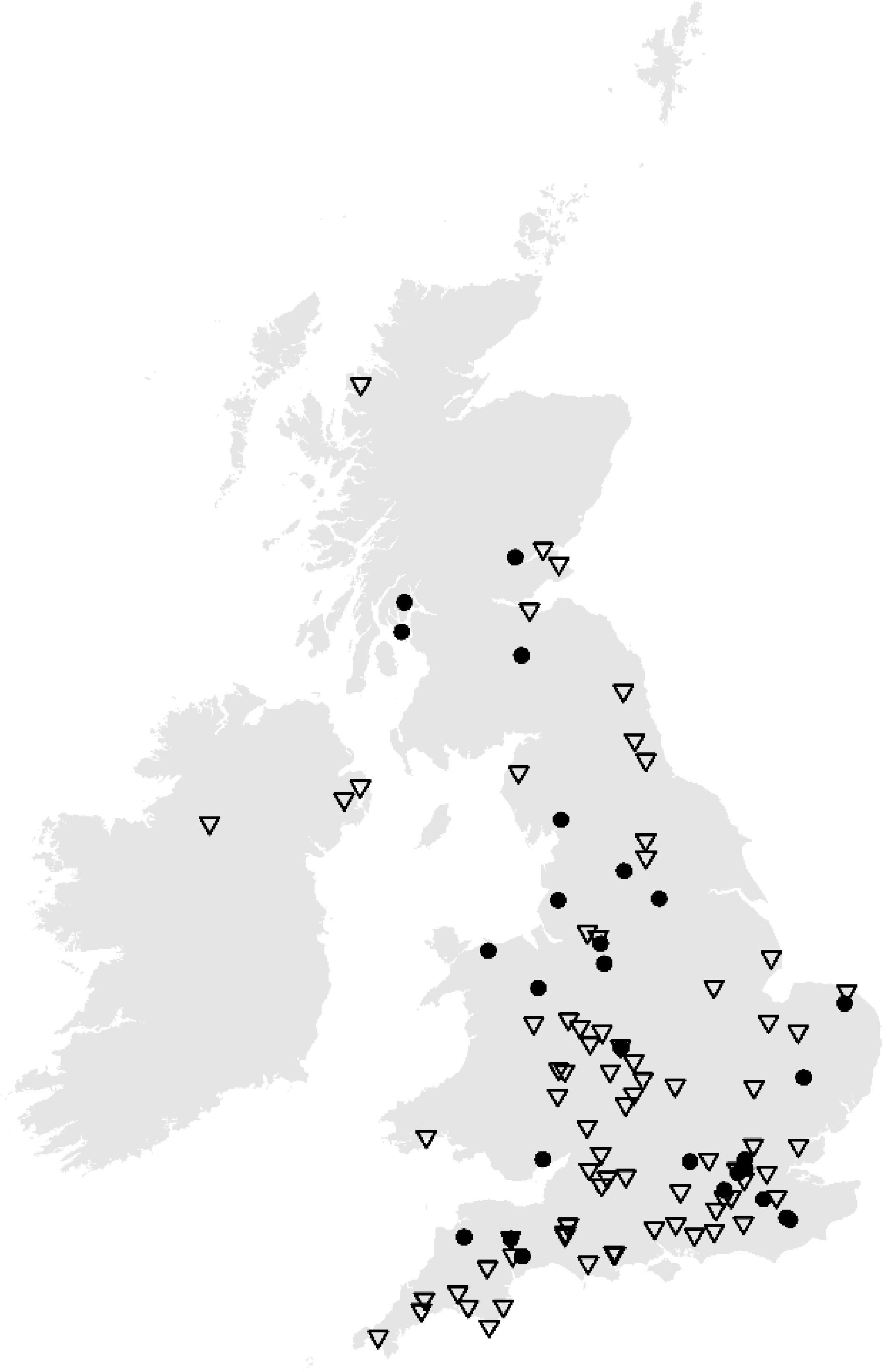


Figure 5.2 - Of the 103 sets returned, 74 sets contained traps with ants (indicated by triangles) and 29 sets contained only traps without ants (circles).

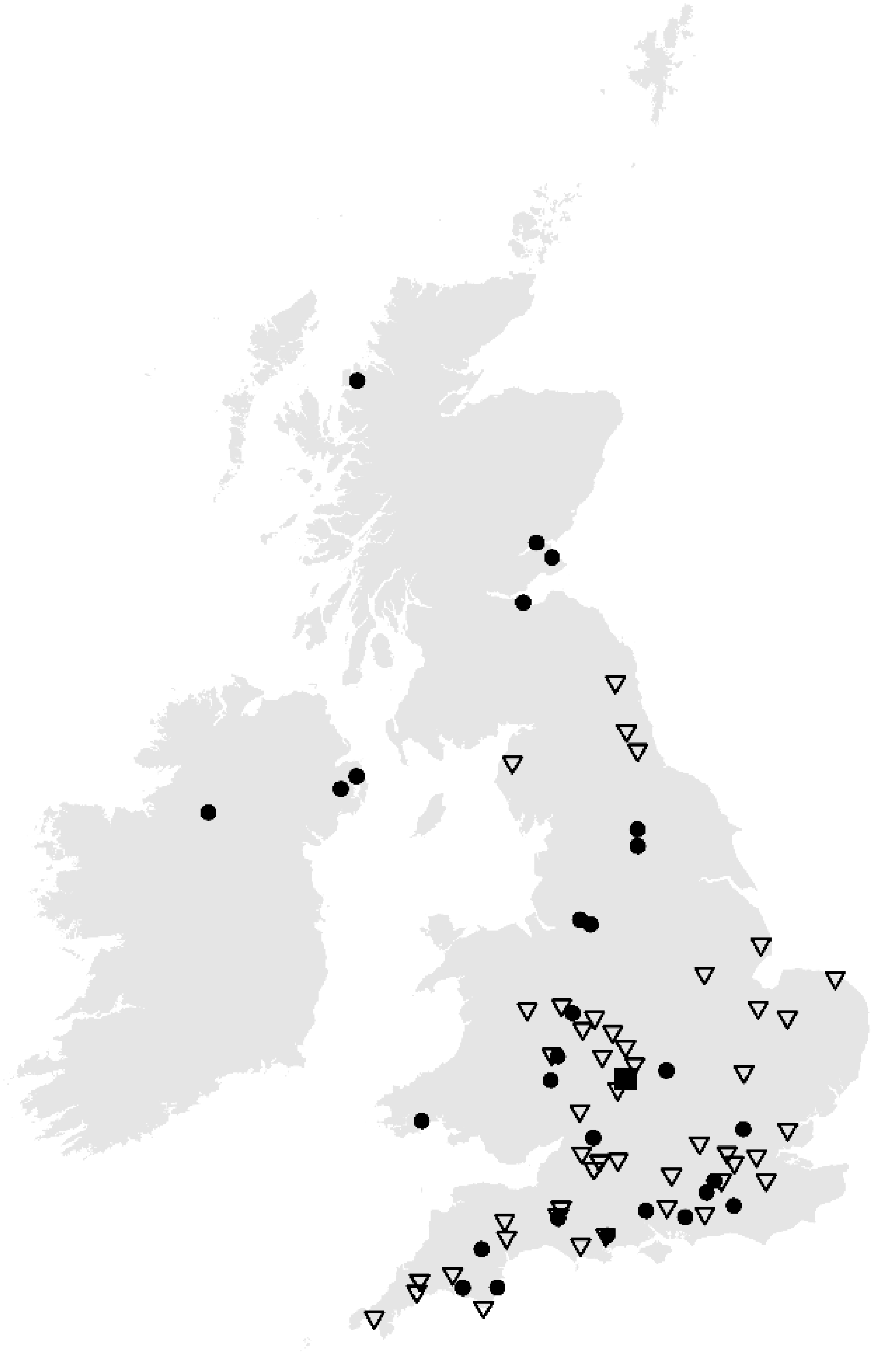


Figure 5.3 - Of the 73 sites returning traps with ants, those marked with triangles returned traps with native *Lasius* species (n=51); Hidcote (black square), returned traps with *L. neglectus*; sites marked by circles returned traps only with ants from other genera (n=21).

Table 5.2 - summary of ant species detected in the survey: "s.l." (sensu lato) denotes instances where identification beyond species group was not possible.

Taxa	**Number of sites**	**Number of traps**
Formica fusca	1	1
Formica lemani	1	1
Lasius neglectus	1 (Hidcote)	8 (2 sets)
Lasius niger	49	97
Lasius flavus	4	4
Myrmecina graminicola	6	6
Myrmica rubra	17	19
Myrmica ruginodis	14	17
Myrmica scabrinodis	7	7
Tapinoma sp. (most likely *T. erraticum*)	1	4
Tetramorium bicarinatum (non-native)	1	3
Tetramorium caespitum	1	1
Myrmicinae (not identified beyond sub-family level)	11	13
Lasius flavus s.l.	2	2
Lasius sabularum s.l.	1	1

Several physical factors could affect the number of ant species caught. Date deployed, deployment duration, longitude and latitude did not have a significant impact on the number of ant species caught after adjusting for multiple comparisons (figure 5.4 and table 5.3).

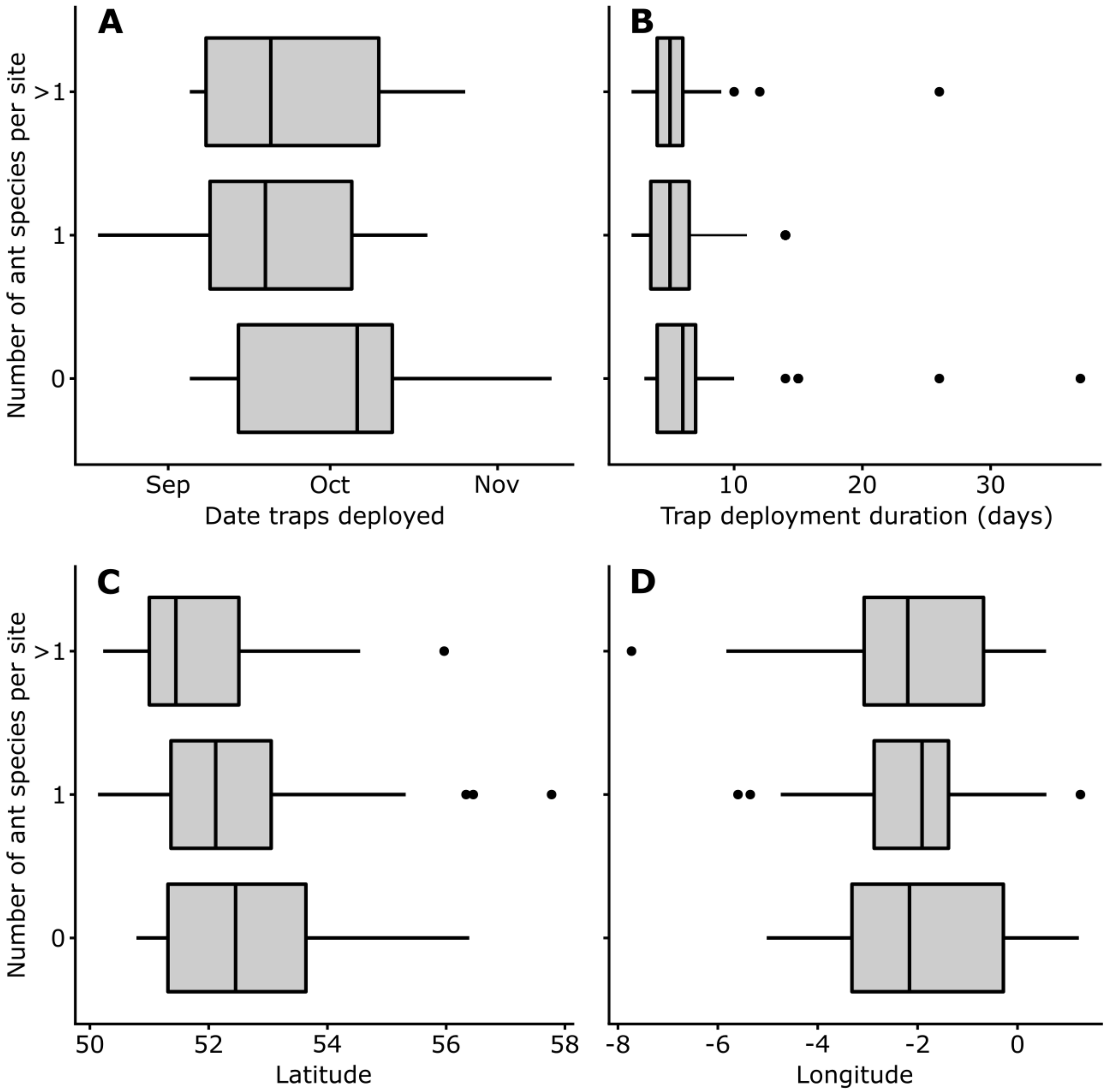


Figure 5.4 - Physical factors and their impact on the number of ant species caught. Panels show the impact of: (A) the date the traps were deployed (n = 92); (B) duration of trap deployment (n= 87); (C) site latitude (n = 103); (D) site longitude (n = 103). Box extends between 25th and 75th percentile (=IQR), vertical line shows position of median value. Whiskers extend to 1.5*IQR from the nearest hinge or to the most extreme value if it deviates less than 1.5*IQR from the nearest hinge. Points are values that deviate more than 1.5*IQR from the nearest hinge.

Table 5.3 - Impact of physical factors on the number of species caught. Z-statistics for Dunn's test between groups within each physical factor. In all cases the reported p-value is larger than the corresponding alpha level, indicating that the effect was not significant at the 0.05 level after adjusting for multiple comparisons.

Physical Factor	**Between group comparison (number of species caught)**	**z-statistic**	**p-value**	**α level (to adjust for multiple comparisons)**
Date deployed	>1 vs 0	-1.399	0.081	0.007
	>1 vs 1	0.515	0.303	0.010
	0 vs 1	1.974	0.024	0.005
Deployment Duration	>1 vs 0	-1.660	0.048	0.005
	>1 vs 1	-0.140	0.444	0.025
	0 vs 1	1.623	0.052	0.006
Latitude	>1 vs 0	-2.017	0.022	0.004
	>1 vs 1	-1.605	0.054	0.006
	0 vs 1	0.556	0.289	0.008
Longitude	>1 vs 0	-0.276	0.391	0.013
	>1 vs 1	-0.126	0.450	0.050
	0 vs 1	0.165	0.434	0.017

Discussion

Despite the broad geographical coverage of our survey, no new sites with *L. neglectus* were identified and the total number of known UK sites remains at ten; however, other ant species, mostly native, were detected at over half the sites that returned traps. When traps do not contain *L. neglectus*, the presence of native ant species increases confidence in the negative result, because established colonies of *L. neglectus* generally exclude native ants from the core regions of their territories (Nagy et al., 2009). The complete lack of detection of any new *L. neglectus* sites was unexpected as we had hypothesized that they would have a high probability of spreading to sites with extensive plant collections. Our results strongly suggest that this is not the case. Our absence data will provide an important baseline for the sites surveyed if *L. neglectus* is detected there in the future. Having a “known absence” timepoint will limit the timespan when the species could have been introduced and therefore increase the probability of identifying the introduction pathway.

Adhesive traps proved to be a useful tool for surveying a cryptic ant species. They made sampling a large number of sites across a wide geographical area relatively easy to administer and the approach was lower cost than visiting all sites to survey them manually. They were also effective: there was significant overlap between the results of the trap survey and those of a preliminary manual search survey where sites were covered by both (see supplementary info). In addition, *L. neglectus* workers (but no native ant species) were found on all traps returned from Hidcote, Gloucestershire. This is a site where the presence of *L. neglectus* was already known, so the reliable detection of the species at this site helps to confirm that our trap based survey methodology was suitable. The unusual abundance of ants at sites where *L. neglectus* is established stands out. Our instructions for deploying the traps included prioritising “anywhere where numbers of small, active, dark-coloured ants have been seen” (see appendix G). We can therefore take the negative results (i.e. absence of *L. neglectus*) as a good indication that the species is neither established nor abundant at the sites targeted. We cannot, however, be certain of detecting *L. neglectus* if it were confined to a very small area or present in low numbers, for example following a very recent introduction. Resampling a subset of sites (particularly to account for variation in site area) with more traps, and/or performing a manual search at a subset of sites, could be used help validate the adequacy of our sampling approach and confidence in the negative result.

Whilst the use of adhesive traps to survey botanic gardens was suitable for generating presence/absence point data for the target and also native ant species, there are limitations to this method. The data do not give a full picture of ant abundance or species richness, and all sites received the same number of traps per set, but not all sites are of equal area. This makes comparisons of the native ant species caught between sites unreliable; such comparisons were not an aim of our study. Additionally, there was variation in the date and duration of deployment that would need to be standardised if future surveys aimed to compare ant communities at the different sites, although we detected no impact of this variation on our data. Another drawback of using glue traps to sample for invertebrates was that some of the samples were damaged (e.g. legs and antennae lost) when they were removed from the adhesive pads and in some cases, glue remained attached to the specimens. This meant that it was not possible to identify some samples to species level, particularly where only one or two individuals were caught. These damaged specimens were always either Myrmicine or yellow *Lasius* species so the damage did not reduce our ability to assess whether *L. neglectus*, a dark *Lasius* (Formicine) species, had been caught.

Large parts of the UK, such as residential areas, without a local contact to deploy the traps would be much more challenging to survey with this methodology. For areas such as these, raising awareness amongst the public and professional pest controllers is likely to be a better monitoring tool. Other sites such as ports, through which non-native species are likely to transit, may require more continuous monitoring. Trained scent detection dogs are a possible solution. These have been successfully used to check for the presence of a variety of species including plants such as the spotted knapweed, *Centaurea stoebe* (in Goodwin et al. 2010) and rodents such as Franklin's ground squirrel *Poliocitellus franklinii* (in Duggan et al. 2011). When used in the context of invasive ants, detection dogs can identify the presence of a target species with very high accuracy at ecologically relevant numbers of ants, even when other ant species are present (Lin et al., 2011). This approach also has limitations however: false positives can be a problem; training the dogs is expensive; and travel to target sites is also expensive.

It is important to ensure that invertebrates collected for scientific studies are used as ethically as possible (Drinkwater et al., 2019). In order to efficiently survey sites over such a large geographical area it was necessary to identify the samples at a central location, which meant it was necessary to kill what was caught. Several steps were taken to minimise the impact of the methodology on non-target species. The adhesive pads were deployed in rigid plastic boxes that would prevent larger animals entering. The traps were deployed in locations favourable for *L. neglectus;* this will have helped reduce unnecessary bycatch. In order to make best use of the invertebrates that were caught, we identified all the ants to species level where possible and these data will be openly available via the Bees, Wasps and Ants Recording Society database. The loss of a small number of workers from an ant colony will have a low ecological impact. The timing of our survey (September to October) resulted in only three queens being caught, further reducing the ecological impact of the survey. In addition, we will also make the non-ant invertebrate samples available for use elsewhere. It is also worth noting that a trapping methodology may not be appropriate if any non-target organisms of conservation concern are known to be present at a site.

It appears that *L. neglectus* is not as prevalent at botanic gardens and other sites with significant plant collections in the UK as predicted. This is good news because sites like these often exchange plants with each other or sell them to the public, so would have been at risk of spreading the ants even further. However, the probability that *L. neglectus* is established at other sites in the UK of which we are currently unaware remains high. Seven of the locations in the UK where *L. neglectus* has been found have been urban or residential sites which are much more difficult to survey. Increasing awareness amongst the public about *L. neglectus* and implementing measures to stop the ants spreading from sites where it is already established are likely to be necessary for monitoring and limiting the spread of this species.

Acknowledgements

We thank the staff at sites which took part in the survey for their help deploying and returning the traps. We are grateful for the input of C Boase, O Booy, D Bullock, M Fox, N Moore and S Toomer during the design and implementation of this study. We would like to thank S Nicholson, S Pickering and T Rudd, for processing the samples; and S Pickering and T Rudd for performing the ant identification work. We would like to thank E Drinkwater, J Eames, V Lecheval, and J Sammy for comments on earlier drafts of this manuscript. We are grateful for the support of the National Trust and the Non-Native Species Secretariat (NNSS) who provided the trapping materials and helped with the logistics of trap deployment. We are also grateful to the NNSS for providing a stipend for the two students who helped with the sample identification work. PBB was funded by the Natural Environment Research Council (grant number NE/L008904/1) and Hymettus Ltd.

References

Attewell, 2011. *Myrmica* Scapes [WWW Document]. www.bwars.com. URL http://www.bwars.com/sites/www.bwars.com/files/diary_downloads/myrmica-scabrinodis-group-scapes-images.pdf

Beggs, J., 2001. The ecological consequences of social wasps (*Vespula* spp.) invading an ecosystem that has an abundant carbohydrate resource. Biol. Conserv. 99, 17–28.

Bertelsmeier, C., Luque, G.M., Hoffmann, B.D., Courchamp, F., 2015. Worldwide ant invasions under climate change. Biodivers. Conserv. 24, 117–128.

Boase, C., 2014. *Lasius neglectus* (Hymenoptera : Formicidae) in the UK : status, impact and management. In: Proceedings of the Eighth International Conference on Urban Pests. pp. 223–228.

Bradshaw, C.J.A., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., Barbet-Massin, M., Salles, J.M., Simard, F., Courchamp, F., 2016. Massive yet grossly underestimated global costs of invasive insects. Nat. Commun. 7, 1–8.

Buckham-Bonnett, P., Lee, P., Robinson, E.J.H., 2016. Identifying the invasive garden ant (*Lasius neglectus*) [WWW Document]. URL http://bwars.com/sites/www.bwars.com/files/info_sheets/L.neglectus_ID_sheet_v2.pdf

Buckham-Bonnett, P., Robinson, E.J.H., 2017. GB Non-native Species Rapid Risk Assessment : Rapid Risk Assessment of: *Lasius neglectus* (Invasive Garden Ant).

Comont, R.F., Roy, H.E., Harrington, R., Shortall, C.R., Purse, B. V., 2014. Ecological correlates of local extinction and colonisation in the British ladybird beetles (Coleoptera: Coccinellidae). Biol. Invasions 16, 1805–1817.

Cremer, S., Ugelvig, L. V, Lommen, S.T.E., Petersen, K.S., Pedersen, J.S., 2006. Attack of the invasive garden ant: aggression behaviour of *Lasius neglectus* (Hymenoptera: Formicidae) against native *Lasius* species in Spain. Myrmec. Nachr 9, 13–19.

Dinno, A., 2017. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums.

Drinkwater, E., Robinson, E.J.H., Hart, A.G., 2019. Keeping invertebrate research ethical in a landscape of shifting public opinion. Methods Ecol. Evol. 2041–210X.13208.

Duggan, J.M., Heske, E.J., Schooley, R.L., Hurt, A., Whitelaw, A., 2011. Comparing detection dog and livetrapping surveys for a cryptic rodent. J. Wildl. Manage. 75, 1209–1217.

Espadaler, X., 1999. *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy, 1990 (Hymenoptera, Formicidae), a potential pest ant in Spain. Orsis 14, 43–46.

Espadaler, X., Bernal, V., 2018. *Lasius neglectus* Distribution [WWW Document]. URL http://www.creaf.uab.es/xeg/Lasius/Ingles/distribution.htm (accessed 11.20.18).

Espadaler, X., Rey, S., Bernal, V., 2004. Queen number in a supercolony of the invasive garden ant, *Lasius neglectus*. Insectes Soc. 51, 232–238.

Espadaler, X., Tartally, A., Schultz, R., Seifert, B., Nagy, C., 2007. Regional trends and preliminary results on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 54, 293–301.

Fox, M.G., 2010. First incursion of Lasius neglectus (Hymenoptera: Formicidae), an invasive polygynous ant in Britian. Br. J. Ent. Nat. Hist. 23, 259–261.

GADM, 2017. Database of Global Administrative Areas [WWW Document]. URL https://gadm.org/data.html

Goodwin, K.M., Engel, R.E., Weaver, D.K., 2010. Trained Dogs Outperform Human Surveyors in the Detection of Rare Spotted Knapweed (*Centaurea stoebe*). Invasive Plant Sci. Manag. 3, 113–121.

Groom, Q., Desmet, P., Vanderhoeven, S., Adriaens, T., 2015. The importance of open data for invasive alien species research, policy and management. Manag. Biol. Invasions 6, 119–125.

Hoffmann, B.D., Andersen, A.N., Hill, G.J.E., 1999. Impact of an introduced ant on native rain forest invertebrates: *Pheidole megacephala* in monsoonal Australia. Oecologia 120, 595–604.

Hulme, P.E., 2017. Climate change and biological invasions: evidence, expectations, and response options. Biol. Rev. 92, 1297–1313.

Jarić, I., Heger, T., Castro Monzon, F., Jeschke, J.M., Kowarik, I., McConkey, K.R., Pyšek, P., Sagouis, A., Essl, F., 2019. Crypticity in Biological Invasions. Trends Ecol. Evol. 34, 291–302.

Kenis, M., Auger-Rozenberg, M.A., Roques, A., Timms, L., Péré, C., Cock, M.J.W., Settele, J., Augustin, S., Lopez-Vaamonde, C., 2009. Ecological effects of invasive alien insects. In: Ecological Impacts of Non-Native Invertebrates and Fungi on Terrestrial Ecosystems. pp. 21–45.

Lin, H., Chi, W., Lin, C., Tseng, Y., Chen, W., 2011. Fire Ant-Detecting Canines : A Complementary Method in Detecting Red Imported Fire Ants. Househ. Struct. Insects 104, 225–231.

Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20, 223–228.

Nagy, C., Tartally, A., Vilisics, F., Merkl, O., Szita, É., Szél, G., Podlussány, A., Rédei, D., Csösz, S., Pozsgai, G., Orosz, A., Szövényi, G., Markó, V., 2009. Effects of the invasive garden ant, *Lasius* *neglectus* VAN LOON, BOOMSMA & ANDRÁS-FALVY, 1990 (Hymenoptera: Formicidae), on arthropod assemblages: pattern analyses in the type supercolony. Myrmecological News 12, 171–181.

Paris, C.I., Espadaler, X., 2009. Honeydew collection by the invasive garden ant *Lasius neglectus* versus the native ant *L. grandis*. Arthropod. Plant. Interact. 3, 75–85.

Pebesma, E.J., R.S.B., 2005. Classes and methods for spatial data in R. R news 5.

Pejchar, L., Mooney, H.A., 2009. Invasive species, ecosystem services and human well-being. Trends Ecol. Evol. 24, 497–504.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288.

Pospischil, R., 2011. Role of tropical greenhouses for introduction and establishment of foreign ant species (Hymenoptera: Formicidae) in Central Europe. In: Proceedings of the Seventh International Conference on Urban Pests. pp. 59–66.

R Core Team, 2018. R: A Language and Environment for Statistical Computing.

Rey, S., Espadaler, X., 2004. Area-wide management of the invasive garden ant *Lasius neglectus* (Hymenoptera: Formicidae) in Northeast Spain. J. Agric. Urban Entomol. 21, 99–112.

Roy, H.E., Adriaens, T., Isaac, N.J.B., Kenis, M., Onkelinx, T., Martin, G.S., Brown, P.M.J., Hautier, L., Poland, R., Roy, D.B., Comont, R., Eschen, R., Frost, R., Zindel, R., Van Vlaenderen, J., Nedvěd, O., Ravn, H.P., Grégoire, J.-C., de Biseau, J.-C., Maes, D., 2012. Invasive alien predator causes rapid declines of native European ladybirds. Divers. Distrib. 18, 717–725.

Schultz, R., Busch, T., 2009. The northernmost record of the invasive garden ant, *Lasius neglectus* (Hymenoptera: Formicidae). Myrmecological News 12, 183–186.

Seifert, B., 2000. Rapid range expansion in *Lasius neglectus* (Hymenoptera, Formicidae) – an Asian invader swamps Europe. Mitt. Mus. Nat. kd. Berl., Dtsch. entomol. Z. 47, 173–179.

Seifert, B., 2010. Intranidal mating, gyne polymorphism, polygyny, and supercoloniality as factors for sympatric and parapatric speciation in ants. Ecol. Entomol. 35, 33–40.

Skinner, G.J., Allen, G.W., 2013. Ants (Naturalists' Handbooks). Pelagic Publishing.

Strayer, D.L., Eviner, V.T., Jeschke, J.M., Pace, M.L., 2006. Understanding the long-term effects of species invasions. Trends Ecol. Evol. 21, 645–51.

Stukalyuk, S. V., Radchenko, A.G., 2018. *Lasius neglectus* Van Loon et al. (Hymenoptera, Formicidae), an Invasive Ant Species in Crimea. Entomol. Rev. 98, 690–701.

Sumner, S., Bevan, P., Hart, A.G., Isaac, N.J.B., 2019. Mapping species distributions in 2 weeks using citizen science. Insect Conserv. Divers.

Tartally, A., 2006. Long term expansion of a supercolony of the invasive garden ant, *Lasius neglectus* (Hymenoptera: Formicidae). Myrmecologische Nachrichten 9, 21–25.

Tartally, A., Hornung, E., Espadaler, X., 2004. The joint introduction of *Platyarthrus schoblii* (Isopoda: Oniscidea) and *Lasius neglectus* (Hymenoptera : Formicidae) into Hungary. Myrmecologische Nachrichten 6, 61–66.

Van Loon, A.J., Boomsma, J.J., Andrasfalvy, A., 1990. A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe description and general Biology. Insectes Soc. 37, 348–362.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

5b The Extent of Lasius neglectus in Kirk Smeaton (April 2016)

Methods

Following the receipt of ants from Dave Williamson on 29/03/2016 which I identified as the invasive garden ant (*Lasius neglectus*), I visited Kirk Smeaton on 10/04/2016 to determine the extent of the colony. The weather was sunny with an air temperature of 13°C. Public areas (streets, footpaths, parks etc) were checked for the presence of ants via visual inspection. Worker size is a characteristic that can be used to identify the invasive ant once its presence in an area has been confirmed using other morphological characteristics. Some voucher specimens were taken at the edge of the colony or if the species identity based on size was unclear.

Distribution

The results of this sampling process are shown on the map in figure 5.5. The *Lasius neglectus* colony extends for approximately 500m along one road (Water Lane) and along both branches of a circular street (Springfield Crescent). It is highly likely that the ants are also present in the approximately 60 houses and an area of allotments adjoining these roads. The pasture and arable fields adjoining sections of Water Lane represent a less suitable habitat for the ant but it is possible that it has also spread into these areas to some extent. The number of records does not necessarily reflect the abundance of ants as a greater sampling effort was used at the edges of the colony to help correctly identify the boundary.

Impact

Residents report that the ant has been a pest for a number of years (at least five), invading homes.

Origin of the ant in the village

The invasion point of *Lasius neglectus* in Kirk Smeaton is likely to have been near the junction between Water Lane and Springfield Crescent. This area is close to the centre of the colony and residents of Springfield Crescent report that the ants were initially a problem in this area before spreading South to the rest of the street. I have checked the nearest garden centre for the presence of the invasive ant but did not find it.

Spread of the ant

The *Lasius negelctus* colony has nest entrances in the cracks between the curb stones. This means that the road network in the village will help the ants to spread (by providing suitable nest sites) and makes their presence easy to observe. It is likely that the high abundance of ants of native species (particularly *Lasius niger*) in Kirk Smeaton will reduce the speed at which the invasive ant can spread but dispersal into the rest of the village is inevitable without intervention. The ant is unlikely to rapidly spread out into the agricultural land around the village but hedges and field margins could provide a suitable invasion corridor. Farms in and around the village could also become accidentally responsible for the spread of the ant if they become infected and then move material (straw etc) to other areas.

Two bridges (a footbridge to the West and a road bridge to the East) connect Kirk Smeaton to the village of Little Smeaton. The *Lasius neglectus* colony is approaching both bridges and once crossed, there is abundant habitat into which the ant can spread. The area around the footbridge is somewhat dank so I would expect the transition to be made via the road bridge first.

The Yorkshire Wildlife Trust's Brockadale Nature Reserve is approximately one kilometre to the North West of the colony. Whilst the habitat matrix between the village and the reserve is unfavourable to the spread of the ants, there is a risk that the ants could be transported there accidentally e.g. in garden waste.

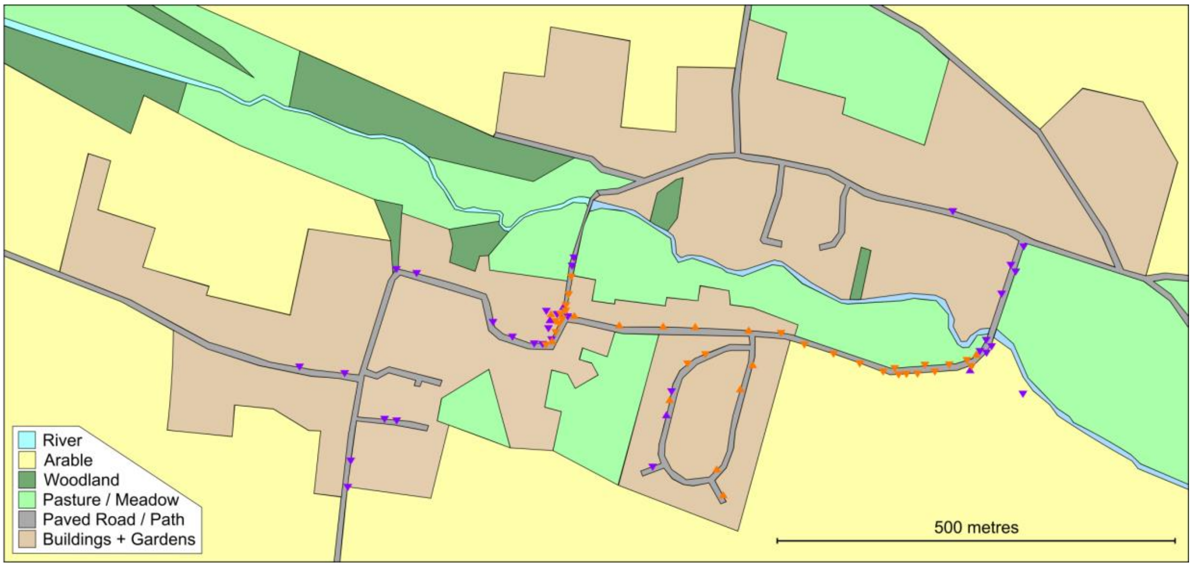


Figure 5.5 - A map showing Kirk Smeaton (south of the river) and Little Smeaton (north of the river). Native ants are indicated by purple and *Lasius neglectus* is indicated by orange. Triangles indicate records confirmed from voucher specimens using a microscope, inverted triangles indicate records only checked by eye in the field

5c – Sites in the UK where Lasius neglectus has been detected (as of December 2019)

Lasius neglectus has been identified at 11 separate sites in the UK table 5.4 although one is unconfirmed as the identification was based on a single worker. Of the 11 cases, two have been detected by naturalists, two by me, two by the National Trust and six via the pest control industry.

Table 5.4 – All known UK *Lasius neglectus* records and their status

Year Identified	Site	Site Description	Status
2009	Hidcote (Glostershire)	National Trust gardens and rural residential	Established and small satellite colony approximately 500m from main site
2010	Stowe (Buckinghamshire)	National Trust gardens	Eradicated
2011	Cambridge (Cambridgeshire)	Botanic Gardens and urban residential	Established colony
2014	Hendon (London)	Residential	Extent unknown
2016	Kirk Smeaton (North Yorkshire)	Rural residential	Established colony
2016	Rodmel (East Sussex)	Rural residential	Extent unknown but residents report ants very abundant
2016	Eastbourne (East Sussex)	Urban	Local pest controller reports ants very abundant – possibly 2 colonies?
2016	Holland Park (London)	Urban?	Extent unknown
2017	Brandscombe (Devon)	National Trust property and rural residential	Extent unknown
2018	Cheshunt (Hertfordshire)	Residential	Extent unknown
2019	St James' Park (London)	Urban park	Unconfirmed (identified from single specimen) – extent unknown

Chapter 6: General Discussion

In this thesis I have carried out a UK-wide risk assessment for this species (Chapter 2). Non-native species risk assessments are a stage in the **Cost and Benefit Analysis** when planning the response to an established non-native species (figure 6.1). After evaluating all the available evidence, I concluded that the overall threat from *Lasius neglectus* to Great Britain is high but that the confidence in this estimate was only medium. This confidence estimate was largely due to the shortage of evidence about the species' impacts and characteristics and the difficulty assessing its distribution. Subsequent chapters attempted to make a contribution to addressing this knowledge gap.

In Chapter 3 I presented a collaborative experiment investigating the potential use of tactile communication during foraging in *Lasius neglectus*. The foraging prowess of this species is one of its most notable **Biological Characteristics** (figure 6.1) and likely a significant contributor to its success as an invasive species. Understanding how the foraging is organised and regulated could have provided insights into this success or been useful for planning mitigation actions. However, we did not find any evidence for the use of tactile communication. Certain biological characteristics often underpin the success of invasive species and can affect their impact, spread and mitigation feasibility so require carful consideration.

We found, as described in Chapter 4, that the granular ant baits we tested are unlikely to be effective for controlling *Lasius neglectus*; this appears to be due to the low palatability of granular food substrates. Despite having a tool for localised control of *L.* neglectus, the **Mitigation Feasibility** (figure 6.1) is constrained by the lack of tools suitable for control over large areas or for eradication attempts. However, we demonstrated that *Lasius neglectus* will feed from hydrogels – although they have not yet been shown to feed from hydrogels in the presence of pesticide. Without suitable tools for the containment, control or eradication of an established invasive species, practical conservation efforts to tackle them stall.

We also carried out a large-scale survey to assess the distribution of this easily-overlooked species, concluding that while it is not as widely distributed in botanic gardens as expected, the number of urban sites where *Lasius neglectus* occurs is increasing (Chapter 5). This work contributes to the **Distribution/Abundance** box in figure 6.1 but the difficulties in trying to assess the distribution of *L. neglectus* on a national scale or at a fine resolution remain. Distribution data is crucial for prioritising action against invasive species and without it, threats can easily be underestimated.

In Appendix H we present a preliminary experiment with a novel design to assess the potential impact of *Lasius neglectus* on an important crop plant. This work looking at the **Impacts** of *L. neglectus* is important for understanding both the ecological and potentially the economic consequences of its invasive populations. Understanding the impacts of invasive species is crucial for prioritising the use of limited conservation resources to ensure the most harmful threats are targeted.

All of these areas would benefit from further work; below I detail some areas of particular interest.

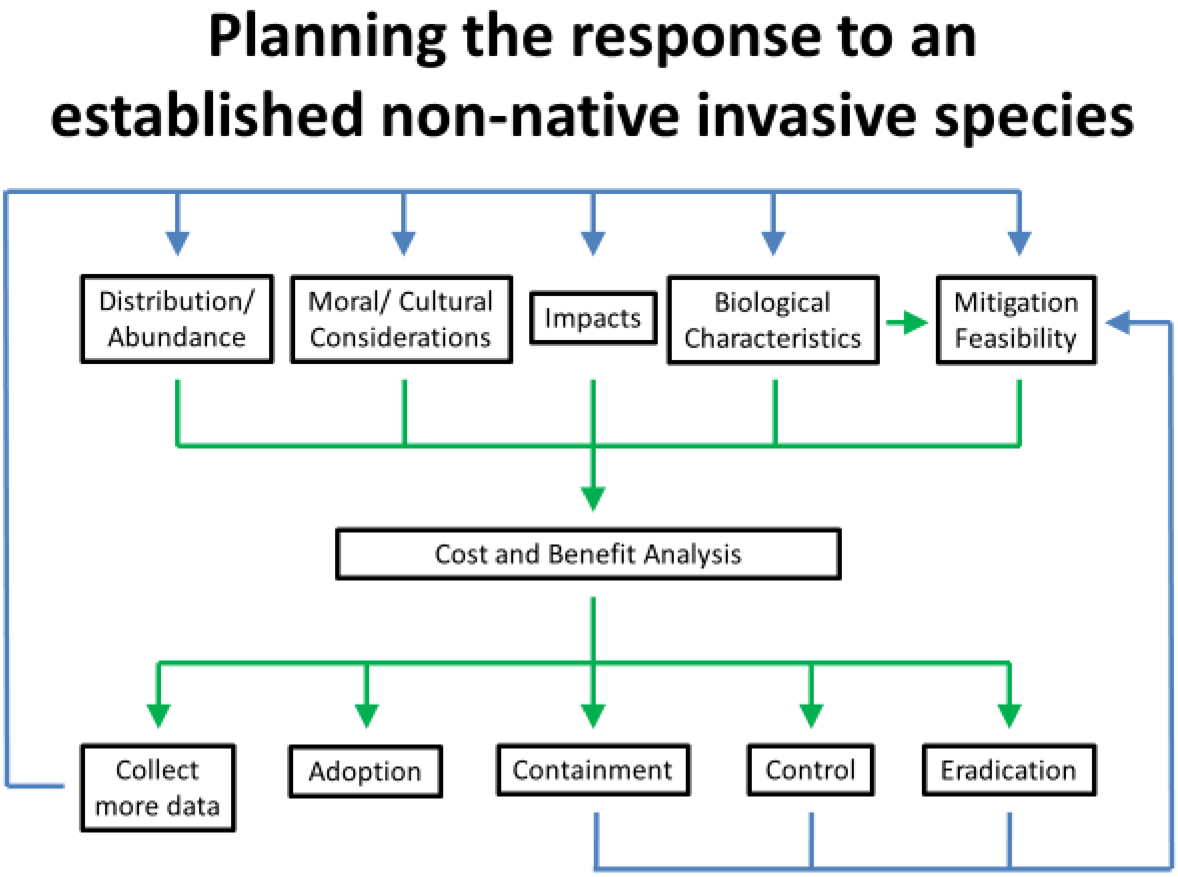


Figure 6.1 - A schematic of the stages and processes involved in planning and implementing the response to an invasive species (duplicate of figure 1.3).

Further work

Biological characteristics

While tactile communication does not appear to be key to the invasion success of this species, there are other biological characteristics that would merit further study. Indeed, it is not clear whether tactile communication would result in beneficial emergent properties in the foraging networks of species that also use mass recruitment. An agent-based modelling approach could be used to address this question. Of particular interest is *Lasius neglectus*' overwintering behaviour. As an invasive ant species, it is unusual in its ability to survive colder climates than most, and this gives it a greater potential climatic range than other ant species; understanding more about how it achieves this would be illuminating. For example, we do not know how deep over-wintering nests are, whether there is any temperature regulation as in some ant species, and what its physiological thermal tolerances are. Our current understanding of the potential geographical range of *Lasius neglectus* is based only on climatic conditions at existing known populations (Bertelsmeier et al., 2015a); the real potential population extent may reach greater thermal extremes; physiological data would make this clearer. Moisture requirements are another physiological constraint that it would be beneficial to quantify. It is important for understanding the species' potential range in drier regions such as large parts of Australia (Glynn Maynard, *pers. comm.*). There is also a data gap as regards what constitutes a minimum viable colony outside of laboratory conditions, i.e. what is the nature of a 'progagule' in this species – can a single queen found an entire population? Understanding this will have implications for predicting the frequency of dispersal to new sites and what is adequate in terms of containment measures.

Mitigation feasibility

Our work suggested that hydrogels may be a promising tool for pesticide delivery to *Lasius neglectus*. This technique has proved an effective pesticide delivery mechanism for an ecologically similar species, *Linepithema humile* (in Buczkowski et al., 2014b, 2014a; Tay et al., 2017), so future research should investigate the hydrogel delivery mechanism further, to see whether this is a workable method for *Lasius neglectus*. Our work could not draw any conclusions about the effectiveness of the active ingredients that were included in the baits, so future work should look explicitly at what active ingredients or combination of active ingredients are most effective.

Once a treatment strategy that is effective in the lab (delivery mechanism and active ingredient) has been identified, field trials will be required to ensure that performance is maintained under more ecologically relevant conditions. Research into the effects of the timing of application would likely increase the efficacy of pesticide application and decrease effects on non-target species. The idea here is that, particularly in a seasonal climate, colony growth will not be constant throughout the year, so there may be periods in the year when colonies are particularly susceptible. During these periods, the impact on the ants per unit of pesticide used will be greater, which means the impact on other invertebrates can be reduced. A mathematical model could be useful for assessing when would be the optimum time of year to treat *Lasius neglectus* colonies.

Distribution

Despite our extensive and systematic survey, the majority of UK records have actually come from the pest control industry, so providing information about the species' characteristics to pest controllers is likely to be an effective method of detecting new populations, as they are often the first to be informed when 'problem ants' occur. Such information could be based on the Information Sheet and Identification Sheet (Appendix I) which I produced for the Bees Wasps Ants Recording Society (BWARS), and are publicly available online (Appendix I). I also wrote an article about *Lasius neglectus* for a pest control magazine (Appendix I), to raise awareness amongst professional pest controllers.

Other than the pest controller records, the other main route through which records of *L. neglectus* have been made is naturalists. Naturalists are likely to be able to use the BWARS resources I have created, and send records directly to BWARS. To broaden awareness, contact with organisations such as Bug Life and the Royal Entomological Society could also be useful.

Over the course of this PhD project there has been considerable media coverage of *Lasius neglectus*. While this has sometimes been alarmist and often inaccurate, it has raised the profile of the species, resulting in multiple contacts (c25) from members of the public believing they have found new populations of the ants. In fact, none of these specimens sent by members of the general public have actually been *Lasius neglectus*, so this has not resulted in further records. However, perhaps this is encouraging, as it suggests a lack of problematic *L. neglectus* populations.

Another type of site with a potentially high propagule pressure for *Lasius neglectus* is sea ports through which potted plants are imported from Europe. The land in and around sea ports has been found to be a productive place to search for non-native ant species in New Zealand (Jacqueline Beggs, *pers. comm.*) and the high arrival rate of non-native species at ports is well documented

(Ward et al., 2006; Work et al., 2005). Applying the survey methods used in chapter 5 to ports would be a useful first step. Commercial garden centres would also be another useful place to target, as another waypoint on the import pathway. As the plants spend longer there, there is more chance of the ants spreading out of occupied plants and into the local environment. Training Plant Health Inspectors to identify *Lasius neglectus* would be valuable, and they could also use the trapping method from Chapter 5 to get an idea of whether more extensive searches would be useful.

Our thorough national survey gives us 'absence' data which is likely to be reliable for a large number of formal and botanic gardens throughout the UK. This provides valuable baseline data for future surveys, as any future *Lasius neglectus* populations can be anchored in time as establishing after this survey. Three of the UK *Lasius neglectus* sites (Hidcote, Cambridge and Kirk Smeaton) have been surveyed and we know the extent of the populations; but for the other sites listed in Chapter 5 we have only specimen data. It would be valuable to assess the extent of these populations, especially with a view to longer-term monitoring, i.e. to assess whether sites are contracting, stable or expanding, and whether control attempts would be useful or necessary.

Within the UK, discovery of new populations has been fairly linear over the last 10 years. It is unclear what stage of the invasion history we are at, but we can compare with mainland Europe where they appear to be at the steeply increasing part of the curve (Espadaler et al., 2007). Also, in Europe, several colonies appear to have undergone collapse – they have greatly reduced in size or disappeared altogether without human intervention. Several factors have been suggested as contributing to this including: resource over-exploitation, disease, insufficient nesting sites (Tartally et al., 2016). It is unclear how widespread this collapse phenomenon is.

In mainland Europe, work on the genetics, cuticular hydrocarbon profiles and behavioural interactions between populations has given an indication of the relationship between various populations (Ugelvig et al., 2008). A similar approach applied to the UK populations might provide an indication of whether there have been multiple introductions, or whether all our populations stem from a single source, and what that might be. If there have been multiple introductions, this makes prioritising prevention of further introductions crucial; if there has been only one, then attempts to reduce spread within the UK would be more important, and attempting complete eradication more justifiable.

Impacts

From a human perspective, over the course of this thesis, I have received personal communications describing the unpleasantness, and in some cases distress, of living with prolonged infestations of these ants. It would be beneficial to policy-makers to quantify the impact on humans who suffer from infestations.

From an ecological perspective, there is potential for disease transmission to native ant species. In France and Spain *Lasius neglectus* are affected by *Laboulbenia formicarum* a North American fungus (Tartally and Báthori, 2015), which appears to be generalist in its impact on ants. Non-native myrmecophiles may also be transported with *Lasius neglectus*. For example *Myrmecophilus fuscus,* a cricket is found in *Lasius neglectus* nests but is not native to the UK, (Stalling et al., 2015) and *Platyarthrus schoblii*, a woodlouse which has been co-introduced to Hungary with *L. neglectus* (in Tartally et al., 2004). The impacts these may have on native myrmecophiles are unknown.

There is a considerable body of work on the aggression shown by *L. neglectus* workers towards other ant species (Cremer et al., 2006; Frizzi et al., 2017; Santarlasci et al., 2014); this is considered a contributory factor for their success, but no work has been done on this in a UK context. Investigating conflict between common UK anthropophilic species such as *Lasius niger*, *Myrmica rubra* and *Myrmica scabrinodis* might provide insights into how *Lasius neglectus* colonies expand. For example, native ant colonies can often be found around the edge of *L. neglectus* colonies and it would be interesting to know whether these can resist the advance of *L. neglectus*, and if not, whether the native ants are displaced through mechanisms of competition or conflict.

Our study (Appendix H) is inconclusive with respect to the impact of *L. neglectus* on aphids and plant growth. I recommend future work should perform a laboratory study on the impact of *Lasius neglectus* on plants, to elucidate the mechanisms without the unpredictability of the field context. Determining what impacts the ants have on aphid populations and in turn what impact these then have on the plant performance would give insights into the ecological and potentially economic consequences of *L. neglectus* invasions. It would also be valuable to study the impact of the ants as agents of selection on the aphid populations, i.e. to find out whether the aphids evolve different characteristics in the presence of the ants (Depa et al., 2020) which could cause additional plant damage or help to sustain the ant invasion. In addition to aphids, *Lasius neglectus* workers also tend other Hemiptera, such as some psyllids (see figure 6.2), which can also be economically damaging pests.

Figure 6.2 - *Lasius neglectus* worker tending psyllid nymphs (*Cacopsylla* sp.) on an apple tree, Hidcote, Gloucestershire. Many *Cacopsylla* sp. are significant pests of fruit trees.

Cost and Benefit Analysis

Frameworks exist to help direct conservation management efforts to where they will be most feasible e.g. Booy et al. (2017). If suitable tools e.g. hydrogel delivered insecticides were developed for use on *Lasius neglectus*, the use of such tools would, in my opinion, be likely to score highly under many of the criteria in this framework indicating that the eradication of *Lasius neglectus* in the UK is feasible. However, given the limited availability of funds, an abundance of other non-native species, and the limited known distribution of *Lasius neglectus*, the scale of its impacts may not warrant prioritisation. Moreover, there are large gaps in our knowledge of this species that add uncertainty when selecting a response. Collecting more data to fill in these gaps would be beneficial.

Actions following Analysis

In addition to collecting more data, the remaining 4 options (Figure 6.1) are **Accept Presence** of *L. neglectus*, **Contain** it to prevent establishment of new populations, **Control** it to limit the extent, impact and spread of existing populations or **Eradication** of the species within the UK.

Based on the data collected in this thesis and the published literature, **containment** should be a minimum level of response to this species. Measures to prevent accidental transport of the ants from existing populations to new locations have been put in place at Hidcote (Boase, 2014; Chapter 2), and to some extent at Cambridge Botanic Gardens, in the form of restrictions on the disposal of soil waste (Sally Pettit, pers comm). At sites such as these which have a single manager, such measures are relatively easy to implement; however, as demonstrated in Chapter 5, most populations of *Lasius neglectus* in the UK are in residential areas. In these circumstances it is much more challenging to contain the ants, given variable levels of engagement from the people involved. In an ideal world, measures such as incineration of garden waste and an embargo on plant transport out of affected gardens should be implemented and enforced. There is a growing body of evidence that the horticultural trade needs more monitoring and regulation. For example, the economic cost of (a single plant disease) ash dieback in Great Britain over the next ten years is estimated to be around £7.6 billion whereas the entire horticultural trade only provides an economic benefit of around £300 million per year (Hill et al., 2019).

In the absence of active government-mandated containment measures, providing information to home-owners in affected areas about how to reduce the chances of inadvertent spread of the ant is a more practical response. I have contributed to the production of an NNSS information leaflet for home-owners, providing advice on how to recognise the ant and what to do if an infestation is detected. More general information approaches raising awareness are also valuable, which I have engaged in through the production of the BWARS Information Sheet (Appendix I) and through giving talks at natural history societies and pest control meetings.

In association with containment measures at Hidcote, **control** measures have also been implemented (Boase, 2014; Chapter 2). These have been used to mitigate impact where the infestation is most severe, e.g. around the buildings, particularly the kitchens, at Hidcote. Pest-control companies provide control for badly affected private home-owners, although sometimes with limited success (Sue Carrol, *pers comm*). This limited success in controlling the ants casts doubt on the feasibility of **eradication** at present. We need to develop a suitable tool before widespread eradication could be attempted; a tool that would be effective and would minimise suffering and impact on non-target species. We have promising leads with respect to the tools, for example the hydrogel approach (see Chapter 4 and Appendix E). Given the correct tools, eradication is actually very feasible because the population structure of this species results in isolated discrete concentrations of the species. This is a species where eradication could work, and locally the benefits of this would be great - in comparison with species with a national distribution but low population density, such as the Harlequin ladybird where targeting the invasive species is much more challenging and the potential for impact on non-target species is much greater. In comparison to species such as this, eradication is a potentially viable prospect, and could make for a positive conservation success story.

Such eradication would be futile if immediate reintroduction from outside of the UK occurred. With the exception of the population at Stowe, which is known to have arrived with stone imported from Italy, it is unknown whether the other UK populations have been seeded from within the UK or from abroad. Genetic work on the introduction history across Europe found 3 main groupings and allowed the construction of an (unrooted) phylogeny of European populations (Ugelvig et al., 2008); similar work might reveal whether our UK populations resulted from a single source, though it would be unlikely to be able to distinguish between multiple reintroductions from a single source in Europe and secondary spread after a single introduction to the UK. Preventing reintroductions is a different form of containment; containment facing outwards at a national level. This would require increased regulation of the plant trade, for example adopting a framework similar to the one presented in Hulme et al. (2018), and perhaps levels of biosecurity comparable to what is seen in Australia and New Zealand. As an island, we have a physical barrier to natural spread for many species, and stricter biosecurity controls have the potential to dramatically restrict not just this ant species, but invasive species across the board.

At the start of this PhD *Lasius neglectus* was the only invasive ant species known to have established outdoor colonies in the UK. However, two other polydomous pest species are now established. A large outdoor colony of the Argentine ant *Linepithema humile* is established in London (Fox and Wang, 2016) and *Tapinoma ibericum* is established at the Ventnor Botanic Gardens on the Isle of Wight (Seifert et al., 2017). Containment measure suitable for *Lasius neglectus* are very likely to be appropriate for these species as well.

Arguments for containment and control are based less on the severity of the ecological impacts (localised and in disturbed habitats) and more on the impact on humans. Mitigating this would be the main benefit of containment, control and any potential population eradications. From a biodiversity perspective, the impact of *Lasius neglectus* is, at least at a national level, minimal, and there is an argument that in this time of increased extinctions, the arrival of invasive species actually increases biodiversity (Thomas and Palmer, 2015). At a numerical level, invasive species do indeed increase biodiversity, but anthropogenic activities can dramatically alter the relative success of species, promoting species that cope with disturbed habitats (Byers, 2002; Irwin et al., 2010; Salomidi et al., 2013). Invasive species share a suite of characteristics that predispose them to invasion (see introduction) and to coping with human-dominated habitats, meaning that the increase in biodiversity provided by their arrival is unbalanced: they increase the number of species occupying these disturbed-environment niches, but biodiversity of other areas of niche space stays the same or is reduced. Further, number of species is not the best measure of a well-functioning ecosystem. Invasive species can reduce the ability of other species to perform ecosystem services, e.g. the Asian hornet’s predation on bees reduces pollination (Vanbergen et al., 2018), or the reduction in water clarity due to the trophic cascade triggered by the introduction of the spiny water flea (*Bythotrephes longimanus*) into North American Lakes (Walsh et al., 2016). In contrast other invasive species may take over unoccupied niches, such as the arrival of the tree bumblebee, *Bombus hypnorum*. This bee does not compete with native species for nesting sites, as it is our only arboreal-nesting bumblebee (Crowther et al., 2014; Lye et al., 2012), and provides pollination services.

Ultimately, the actions of humans are responsible for introduced non-native species, and preventing their introduction and spread is the best way to avoid the potential ethical, ecological and economic consequences of their impacts.

References

Bertelsmeier, C., Luque, G.M., Hoffmann, B.D., Courchamp, F., 2015. Worldwide ant invasions under climate change. Biodivers. Conserv. 24, 117–128.

Boase, C., 2014. *Lasius neglectus* (Hymenoptera : Formicidae) in the UK : status, impact and management. In: Proceedings of the Eighth International Conference on Urban Pests. pp. 223–228.

Booy, O., Mill, A.C., Roy, H.E., Hiley, A., Moore, N., Robertson, P., Baker, S., Brazier, M., Bue, M., Bullock, R., Campbell, S., Eyre, D., Foster, J., Hatton-Ellis, M., Long, J., Macadam, C., Morrison-Bell, C., Mumford, J., Newman, J., Parrott, D., Payne, R., Renals, T., Rodgers, E., Spencer, M., Stebbing, P., Sutton-Croft, M., Walker, K.J., Ward, A., Whittaker, S., Wyn, G., 2017. Risk management to prioritise the eradication of new and emerging invasive non-native species. Biol. Invasions 19, 2401–2417.

Buczkowski, G., Roper, E., Chin, D., 2014a. Polyacrylamide Hydrogels: An Effective Tool for Delivering Liquid Baits to Pest Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 107, 748–757.

Buczkowski, G., Roper, E., Chin, D., Mothapo, N., Wossler, T., 2014b. Hydrogel baits with low-dose thiamethoxam for sustainable Argentine ant management in commercial orchards. Entomol. Exp. Appl. 153, 183–190.

Byers, J.E., 2002. Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. Oikos 97, 449–458.

Cremer, S., Ugelvig, L. V, Lommen, S.T.E., Petersen, K.S., Pedersen, J.S., 2006. Attack of the invasive garden ant: aggression behaviour of *Lasius neglectus* (Hymenoptera: Formicidae) against native *Lasius* species in Spain. Myrmec. Nachr 9, 13–19.

Crowther, L.P., Hein, P.L., Bourke, A.F.G., 2014. Habitat and forage associations of a naturally colonising insect pollinator, the Tree Bumblebee Bombus hypnorum. PLoS One 9.

Depa, Ł., Kaszyca-Taszakowska, N., Taszakowski, A., Kanturski, M., 2020. Ant-induced evolutionary patterns in aphids. Biol. Rev.

Espadaler, X., Tartally, A., Schultz, R., Seifert, B., Nagy, C., 2007. Regional trends and preliminary results on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 54, 293–301.

Fox, M., Wang, C., 2016. Colony of the Argentine Ant, *Linepithema humile* (Hymenoptera : Formicidae), in Fulham, West London. Br. J. Entomolgy Nat. Hist. 29, 193–195.

Frizzi, F., Bartalesi, V., Santini, G., 2017. Combined effects of temperature and interspecific competition on the mortality of the invasive garden ant, Lasius neglectus: A laboratory study. J. Therm. Biol. 65, 76–81.

Hill, L., Jones, G., Atkinson, N., Hector, A., Hemery, G., Brown, N., 2019. The £15 billion cost of ash dieback in Britain. Curr. Biol. 29, R315–R316.

Hulme, P.E., Brundu, G., Carboni, M., Dehnen-Schmutz, K., Dullinger, S., Early, R., Essl, F., González-Moreno, P., Groom, Q.J., Kueffer, C., Kühn, I., Maurel, N., Novoa, A., Pergl, J., Pyšek, P., Seebens, H., Tanner, R., Touza, J.M., van Kleunen, M., Verbrugge, L.N.H., 2018. Integrating invasive species policies across ornamental horticulture supply chains to prevent plant invasions. J. Appl. Ecol. 55, 92–98.

Irwin, M.T., Wright, P.C., Birkinshaw, C., Fisher, B.L., Gardner, C.J., Glos, J., Goodman, S.M., Loiselle, P., Rabeson, P., Raharison, J.L., Raherilalao, M.J., Rakotondravony, D., Raselimanana, A., Ratsimbazafy, J., Sparks, J.S., Wilmé, L., Ganzhorn, J.U., 2010. Patterns of species change in anthropogenically disturbed forests of Madagascar. Biol. Conserv. 143, 2351–2362.

Lye, G.C., Osborne, J.L., Park, K.J., Goulson, D., 2012. Using citizen science to monitor Bombus populations in the UK: Nesting ecology and relative abundance in the urban environment. J. Insect Conserv. 16, 697–707.

Salomidi, M., Katsanevakis, S., Issaris, Y., Tsiamis, K., Katsiaras, N., 2013. Anthropogenic disturbance of coastal habitats promotes the spread of the introduced scleractinian coral *Oculina patagonica* in the Mediterranean Sea. Biol. Invasions 15, 1961–1971.

Santarlasci, A., Martelloni, G., Frizzi, F., Santini, G., Bagnoli, F., 2014. Modeling warfare in social animals: a “chemical” approach. PLoS One 9, e111310.

Seifert, B., D’Eustacchio, D., Kaufmann, B., Centorame, M., Lorite, P., Modica, M.V., 2017. Four species within the supercolonial ants of the *Tapinoma nigerrimum* complex revealed by integrative taxonomy (Hymenoptera: Formicidae). Myrmecological News 24, 123–144.

Stalling, T., Espadaler, X., Carrón, N.O.Z., 2015. First record of the ant-loving cricket *Myrmecophilus fuscus* Stalling, 2013 (Orthoptera: Myrmecophilidae) in mainland Spain. Sociobiology 62, 481–483.

Tartally, A., Antonova, V., Espadaler, X., Csősz, S., Czechowski, W., 2016. Collapse of the invasive garden ant, *Lasius neglectus*, populations in four European countries. Biol. Invasions 18, 3127–3131.

Tartally, A., Báthori, F., 2015. Does *Laboulbenia formicarum* (Ascomycota : Laboulbeniales) fungus infect the invasive garden ant , *Lasius neglectus* (Hymenoptera : Formicidae), in Hungary ? e-Acta Nat. Pannonica 8, 117–123.

Tartally, A., Hornung, E., Espadaler, X., 2004. The joint introduction of *Platyarthrus schoblii* (Isopoda: Oniscidea) and *Lasius neglectus* (Hymenoptera : Formicidae) into Hungary. Myrmecologische Nachrichten 6, 61–66.

Tay, J.W., Hoddle, M.S., Mulchandani, A., Choe, D.H., 2017. Development of an alginate hydrogel to deliver aqueous bait for pest ant management. Pest Manag. Sci. 73, 2028–2038.

Thomas, C.D., Palmer, G., 2015. Non-native plants add to the British flora without negative consequences for native diversity. Proc. Natl. Acad. Sci. U. S. A. 112, 4387–4392.

Ugelvig, L. V, Drijfhout, F.P., Kronauer, D.J.C., Boomsma, J.J., Pedersen, J.S., Cremer, S., 2008. The introduction history of invasive garden ants in Europe: integrating genetic, chemical and behavioural approaches. BMC Biol. 6.

Vanbergen, A.J., Espíndola, A., Aizen, M.A., 2018. Risks to pollinators and pollination from invasive alien species. Nat. Ecol. Evol. 2, 16–25.

Walsh, J.R., Carpenter, S.R., Van Der Zanden, M.J., 2016. Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc. Natl. Acad. Sci. U. S. A. 113, 4081–4085.

Ward, D.F., Beggs, J.R., Clout, M.N., Harris, R.J., O'Connor, S., 2006. The diversity and origin of exotic ants arriving in New Zealand via human-mediated dispersal. Divers. Distrib. 12, 601–609.

Work, T.T., McCullough, D.G., Cavey, J.F., Komsa, R., 2005. Arrival rate of nonindigenous insect species into the United States through foreign trade. Biol. Invasions 7, 323–332.

Appendix A (supplement 1 to Chapter 3)

Aim

The aim of this experiment was to determine whether *Lasius neglectus* can communicate the position of food in a simple T-maze as has previously been demonstrated in *Lasius niger*.

Methods

The experiment was performed on a population of approximately 400 *Lasius neglectus* workers without gynes or brood. The ants were housed in a gypsum-plaster-lined nest inside a 300mm x 210mm open box with sides coated in Fluon®. The population was maintained on a diet of a 50% honey solution and meal worm fragments (*Tenebrio molitor*) three times per week. Water was available *ad libitum*. The ants were maintained in a temperature controlled room at 22°C (+/- 3°C) under a 12:12 light:dark cycle.

The experiment was conducted using a simple T-maze (see figure A1 for dimensions) with sides coated in Fluon. The experiment was performed in a room with no external windows but multiple ceiling lights.

Before use, the population of ants was starved for between three and five days. Workers were not sorted into "scout" and "recruit" populations as they were in the *Lasius niger* equivalent version of this experiment. For details of the procedure, see figure A1. An interaction was deemed to have occurred if the two ants met head on (i.e. with antennal overlap) and exchanged antennal contact. If the two ants passed each other on the stem of the platform but did not interact, the replicate proceeded and the subsequent choice was deemed to be a control measurement. Results were accepted only if the interaction occurred more than 100mm from the bifurcation, in order to ensure that the removing of the paper cover did not influence the ant's branch choice.

Between replicates the apparatus was cleaned with 80% ethanol to remove any chemical residue that may affect the bifurcation decisions of subsequent ants. Once an ant had made a bifurcation decision, it was isolated from the main population of ants until all replicates had been completed. Between "successful" replicates (i.e. attempted replicates that contained both an interaction and a bifurcation choice) the feeder was switched to the other branch.

The experiment was videoed from above to allow the duration of the interaction and the distance from the bifurcation at which it occurred to later be determined if desired.

Statistical Analyses were performed using R (R Core Team, 2013). Where Generalised Linear Models (GLMs) were appropriate, a saturated model was fitted including all measured variables that might have impacted the response variable and all second order interactions. Variables that did not make a significant contribution to the fit of the model were removed (in order of ascending contribution), beginning with interaction terms. These models used a binomial framework and logistic link function.

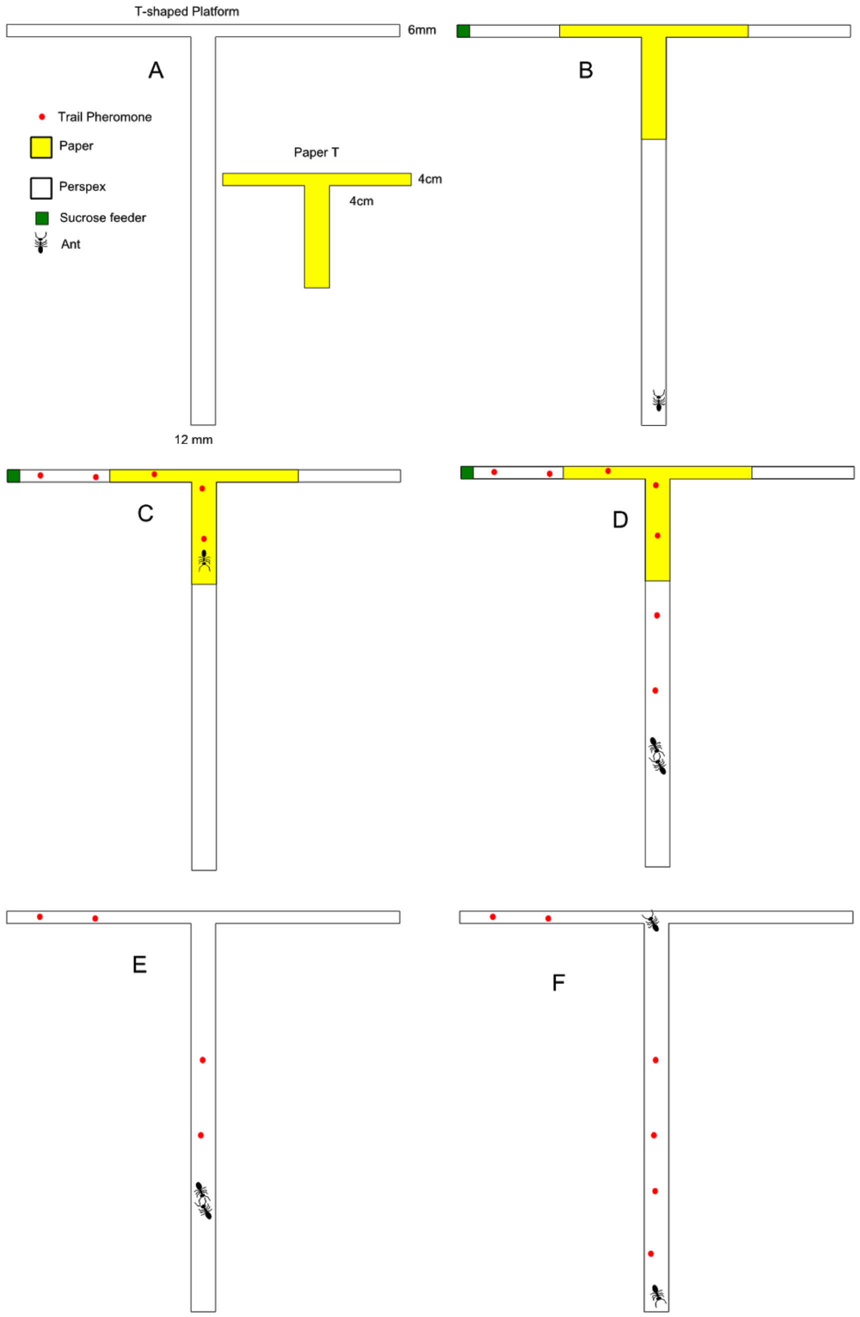


Figure A1 – Apparatus and Methods: A - Apparatus dimensions and figure Key. The vertical sides of the T-shaped platform are coated with Fluon. B – An ant is transferred to the base of the platform using a cocktail stick and is allowed to discover sucrose and feed. C- Ant returns towards the base of the platform, possibly laying a pheromone trail or other chemical marks. D – A naive ant is transferred to the base of the platform using a cocktail stick and allowed to interact with the experienced ant. E- Whilst the experienced and naive ants are interacting, the sucrose feeder and paper T (including any chemical deposit from the experienced ant) are removed from the apparatus. F – The naive ant is permitted to travel to the bifurcation and select a branch (by crossing a virtual line 40mm from the bifurcation on either branch.

Results

Interactions and branch choice

After an interaction on the trail, naive ants were more likely to choose the food branch than expected $X^2 = 4.16$, df = 1, p = 0.041 (see Table A1).

Table A1

Interaction Occurred (Test Treatment)	Food on Left Hand Branch	Food on Right Hand Branch
Chose Left Hand Branch	25	16
Chose Right Hand Branch	14	23

Where no interaction occurred before the naive ants arrived at the bifurcation, they were not more likely to choose the food branch than expected $X^2 = 0.019$, df = 1, p = 0.890 (see Table A2).

Table A2

No Interaction (Control Treatment)	Food on Left Hand Branch	Food on Right Hand Branch
Chose Left Hand Branch	12	9
Chose Right Hand Branch	11	9

Discussion

The data presented here support the hypothesis that after an interaction with an experienced forager, naive workers of the invasive garden ant are more likely to choose the food branch on an unmarked bifurcation than would be expected if branch choice were random. The effect is weaker than that shown for *Lasius niger*, but this could in part be due to differences in experiment design (particularly the use of scouts and recruits in the *L. niger* experiment). *Lasius neglectus* appear to be an easier species to work within these experiments both in terms of the ratio of attempts to results and the shorter interactions, making them more suitable for future detailed analysis.

References

R Core Team, 2013. R: A language and environment for statistical computing.

Appendix B (supplement 2 to Chapter 3)

Supplement to: No evidence of tactile communication of direction in foraging Lasius ants

Stefan Popp[1,2], Phillip Buckam-Bonnett[3], Sophie E. Evison[4], Elva J. H. Robinson[3], and Tomer J. Czaczkes[1,*]

[1] Institute of Zoology, Universität Regensburg, Universitätsstraße 31, D-93053 Regensburg, Germany

[2] Department of Behavioral Physiology and Sociobiology, Biocenter, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany

[3] Department of Biology and York Centre for Complex Systems Analysis, Heslington, York, YO10 5DD, UK

[4] Animal and Plant Sciences, University of Sheffield, Alfred Denny Building, Western Bank, Sheffield, S10 2TN, UK

[*] Corresponding author

This Supplement consists of a description of two additional experiments: an initial experiment by SP and TJC with a flawed methodology, and a confirmatory experiment carried out on *Lasius neglectus* by SP, PBB & EJHR in a different lab to the main experiment.

Experiment 0 - an Initial flawed experiment to test ant-ant physical communication of direction

Materials and methods

Study species and animal maintenance

This experiment was carried out directly prior to the experiment in the main text (experiment 1). Eight *Lasius niger* colonies were used. All 8 colonies were also used in the main experiment. Ant care was identical to that described in experiment 1.

General experimental procedure

The experimental procedure and analysis was largely similar to the one described in the main experiment, with some key differences. Below we provide a detailed account of the experimental methods, adapted from Popp (2015).

We tested the transfer of directional information via physical contact on a maze with one bifurcation (see figure B1). During the experiments the colony was connected to a paper bridge (40cm long × 2cm wide) leading to a “meeting section”, consisting of two moveable platforms (each 8cm × 0.5cm) arranged in a line. The last element was a T-shaped bifurcation. The stem of the T (15cm × 2cm) had a narrow part (2cm × 0.5cm) at the transition to the head of the T maze, to prevent a strong effect of ants cutting the corner (see below for details). At the end of one of the two bifurcation branches (9 × 2cm each), a feeder (a small piece of acetate sheet carrying a drop of 1 molar sucrose solution) was placed. All elements after the bridge were plastic platforms covered with an overlay of standard printer paper. To prevent ants from leaving the setup all sections were elevated on fluon coated plastic pillars standing in water-filled petri dishes. Lights and dark objects in the room served as possible landmarks to facilitate orientation of the ants. The observer was always located on the right side of the apparatus.

Ant-ant physical contact treatment

At the beginning of every trial ants were allowed to enter the apparatus and find the sucrose solution. The first two successful foragers were individually marked with a dot of acrylic paint on the abdomen. Marking did not influence the general behaviour of ants. These individuals served as “informed ants” for the rest of this trial and were allowed to repeatedly make trips between the nest and the sugar. All other ants on the apparatus were placed back into the nest and further ants were prevented from entering the setup. Testing of naïve ants began after the informed ants had made at least 4 trips to the sugar solution to ensure that the informed formed reliable memory of the location of the food (Grüter et al., 2011). The feeder was located on the same side of the T-maze for the entire trial, but was changed randomly between trials. When am informed ant that had made at least 4 visits to the feeder was about to return to the nest, several naïve ants were allowed onto the bridge and one of them was allowed onto the first platform of the meeting section. As soon as the informed ant stepped onto the second platform, the segments were connected to allow physical contact between the two ants. We only collected data from ants when they were contacted by the informed ant with both antenna on the head or the antenna. The informed could then proceed back to the nest and the outbound naive ant was immediately allowed to explore the T-maze. Whilst the naïve ant was on its way onto the maze, the feeder was removed to eliminate possible odour cues from the sugar solution and the overlay of the head was replaced with a fresh piece of paper to remove the trail laid by the teacher. The tested ants thus had to rely solely on information that was possibly transferred through physical contact with the informed ant. We recorded the initial decision of the forager using decision lines located 4cm away from the middle line (in the following this is referred to as “correct decision”). Additionally, we recorded on which side the ant reached the end of the maze and the times it took the ant from antennation to reaching the T-head and end of the maze. An ant was considered as having made a decision when both of her antenna crossed the decision line or the end of the T-maze head respectively. If an ant did not make a decision within 90 seconds, it was considered not to be motivated and thus rejected for data collection. 146 of our 898 tested ants (=16.3%) were rejected for this reason. After the ant reached the end of the maze it was removed from the experiment.

Control treatment

To assess the possibility of still lingering pheromone traces that could lead the naïve ants, controls were run identically to the treatment, except without having the informed and the naïve ant interact physically on the trail. While the returning informed ant and the naïve outgoing ant were each on one of the two platforms of the meeting section, they were moved past each other and allowed to proceed on their way without meeting each other. After a trainer made one trip, several (X ± SD = 2.65 ± 1.67, range 1-7, n = 63) ants were tested consecutively. Thus the 20th naïve ant was tested after both informed ants together made approximately 16 runs (X ± SD = 15.57 ± 6.40, range 9 – 29, n = 7) as opposed to 28 (X ± SD = 28.19 ± 2.40, range 24 – 33, n = 16) runs in the ant-ant physical contact treatment. This is important because it can explain the positive results we got for the first experiment (see below). Controls in the main experiment were run identically to the controls run here, but to account for effects that show only with a higher number of informed ant runs, controls were run until the informed ants had made approximately 25 (X ± SD = 24.85 ± 2.72, range 16 – 30, n = 20) visits to the feeder.

Differences between experiment 0 and experiment 1

In this experiment only the paper overlays covering the T-maze were changed to remove pheromone on the maze. The plastic T-maze was not exchanged. This method had previously been shown to provide strong and reliable differences in ant behaviour (Czaczkes et al., 2013, 2011). However, it seems that after many repeated returns some pheromone contamination of the plastic T-maze beneath the paper overlays can occur (see results and discussion below).

The stem of the T-maze was constricted to 5mm for the last cm before the head, but the rest of the stem was 20mm wide (see figure B1 below).

No buffer section was used between the communication section and the T-maze.

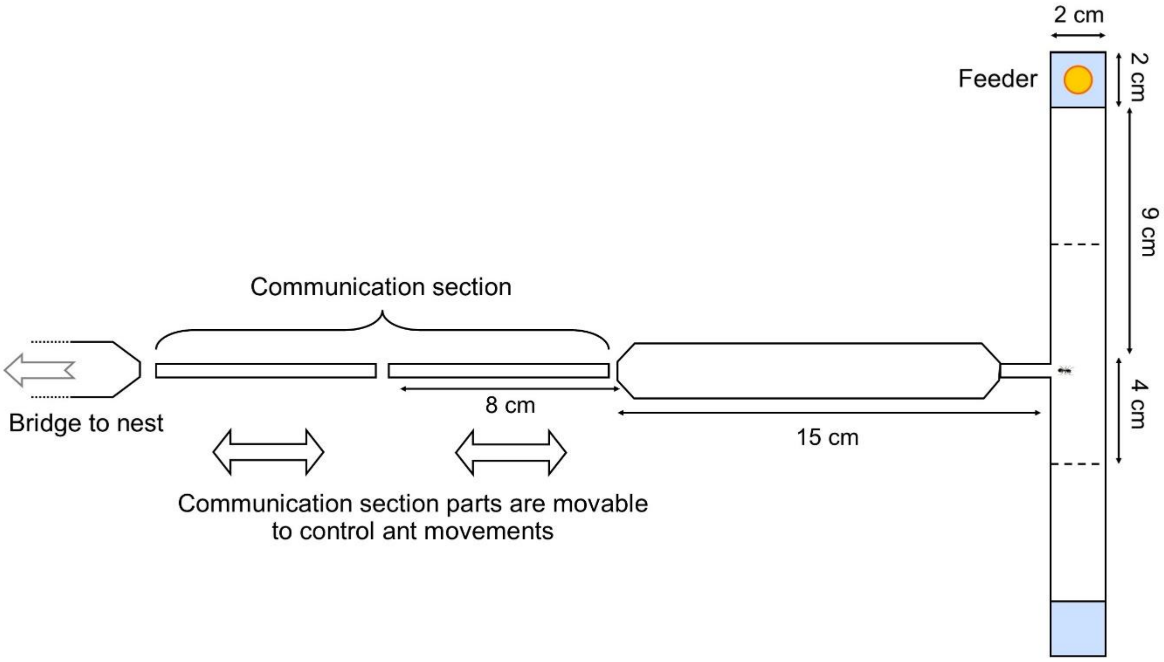


Figure B1 – Experimental setup for initial, flawed experiment. Two marked (=informed) ants with knowledge of the feeder location are allowed to make repeated return visits to the feeder. On their return visits they may be allowed to encounter naïve ants on the communication section, by allowing a naïve ant onto the first section and the informed ant onto the second section, then joining the two sections. The naïve ant is then allowed, via the buffer section, onto the maze, and its arm choice decision noted. Paper overlays covering the T-maze head are replaced whenever an ant walked over it. The T-maze itself remained in place, however, and may have become contaminated by trail pheromones. The figure, including an ant entering the T-maze head, is to scale.

Additional results to those provided in the main text

Using mixed-effect models, we could explore the effect of ant order (how late in the experimental run a specific ant was chosen), and control for trial-based random effects. Ants tested later in the trial, once informed ants had made more runs, showed greater path choice accuracy in their decision-line data (Z = 2.59, P = 0.0095, see figure B2). This effect disappears when the final decision of the ants is considered (Z = -0.299, P = 0.77). Control ants, which had been tested at lower trainer visit numbers, showed no effect of ant order either in their initial (Z = -0.214, P = 0.83) or final (Z = 350, P = 73) decisions. To control for this uneven distribution of data, we reran the mixed-effect model above but excluded later parts of the experiment, during which the informed ants had made more than 14 return visits. Once such later runs are excluded, the model finds no significant effect of treatment type (Z = -0.15, P = 0.88), and no significant effect of ant order (Z = -0.70, P = 0.88) on the proportion of correct initial decisions.

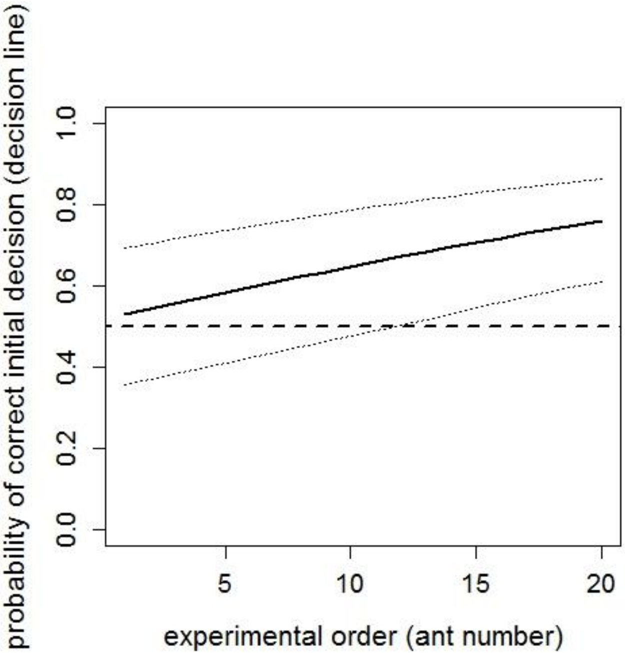


Figure B2 – Decision accuracy of ants according to experimental order. The predicted effect of experimental order on the choice accuracy of ants in the ant-ant contact treatment. The thick line is the prediction from the mixed effect model, and the dotted lines are 95% confidence intervals for the prediction. The dashed line shows random choice. Note that the predicted accuracy rises from random in the first visits to significantly better than random in later visits.

Discussion of the results from the initial, flawed experiment

The results from this experiment seem to suggest that ant-ant antennal contact can convey directional information. However, we believe these results to be spurious. There are several patterns in the data that cast doubt on these results.

Firstly, the apparent effect of the treatment is only apparent from the decision line data, i.e. over the first 4cm of the T-maze head. The effect disappears when we consider which arm of the maze the ants reach the end of first. This suggests that the directional effect is somehow localised at the T-maze head/stem junction – that is, where there is most likely to be pheromone contamination. Returning informed ants tend to 'cut the corner' of the maze head/stem junction (see figure B3). This is likely to result in pheromone being placed preferentially on one side of the T-maze stem. We attempted to mitigate this effect using a thin (5mm) stem section, but 5mm may still be enough space for such directional information to play out on. Moreover, even if a constriction is put in place on the final approach to the T-maze junction, the broader part of the stem nonetheless allows room for side-biased pheromone deposition. This in turn biases edge following by the naïve ants (Dussutour et al., 2005), which may continue through the narrow section. In 'cutting the corner' returning ants may also sometimes walk on the 1mm thick side of the T-maze, which is not covered by paper. Furthermore, if the paper overlays were not placed perfectly straight, it is possible that a small strip of T-maze would be uncovered near the junction.

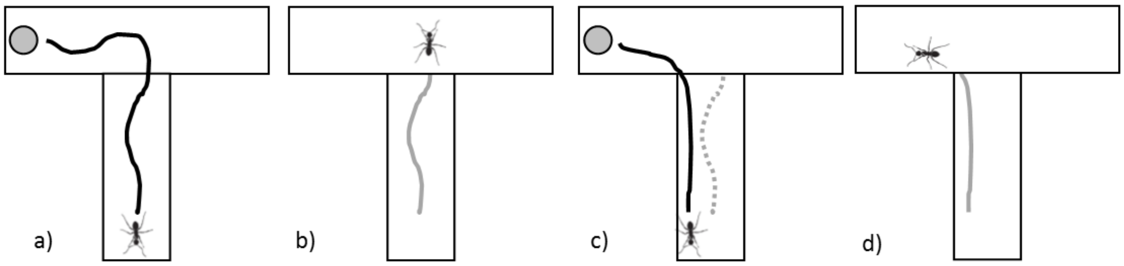


Figure B3 – Pheromone contamination due to corner cutting. Unbroken lines represent fresh pheromone trails, dotted lines old pheromone trails. Grey lines are trails from the previous informed ant. a) Early run of an informed (i.e. experienced) ant. As long as the ant has not yet acquired reliable route memory, it meanders on its nest-bound trip in order to find the branch leading to the nest. It may even walk to the end of the non-rewarded branch before finding the T-stem. No directional bias is left on the T-maze stem when the pheromone from the T-maze head is removed b)

Naïve ant tested in the beginning of the trial. Since the pheromone trail of the informed ant is located more or less in the middle of the T-stem, the naïve ant is not biased in her choice. c) Late run of an informed ant. As soon as an informed ant formed a reliable route memory, it may cut the corner and also continue walking on the respective edge of the T-maze stem, leaving a pheromone trail that leads to the edge of the rewarded branch. d) Naïve ant tested in a late stage of a trial. Naïve ants can now follow the late, corner-cutting pheromone trails and are biased to walk into the rewarded direction.

A second line of evidence suggesting that the results are spurious is the conspicuous increase in accuracy of the naïve tested ants over the course of a trial (see figure B2). This strongly suggests that some sort of signal – most likely pheromone – is building up over the course of the trial. While it is conceivable that this reflects the increasing confidence of the informed ants about their private information, and thus an increase in willingness to communicate (Czaczkes and Heinze, 2015), these effects play out over a much longer number of visits than is required for *L. niger* foragers to perfectly learn a single T-maze turn (Grüter et al., 2011).

Lastly, that these effects disappear if completely separate T-mazes are used for informed and naïve ants (see figure 3.2 in main manuscript) strongly suggests that the results found in this initial experiment are a result of pheromone contamination, rather than ant-ant antennal contact.

It is notable that uncontacted naïve ants (control ants) performed worse than the contacted naïve ants, at least in terms of their initial decision (see figure 3.4). This may be explained by the lower range of informed ant visits over which the control ants were tested in this experiment. As mentioned above, control ants on average interacted with an informed ant that had made 16 return visits, while test ants on average interacted with an informed ant that had made 28 visits. However, it may also be that meeting a successful returning forager might prime outgoing naïve ants to pay closer attention to pheromone trails. Behavioural differences can allow otherwise identical ants to either detect, or not detect, faint trails and follow them (Jackson et al., 2006). However, the effect of such behavioural states in *L. niger* has been found to be very weak or non-existent (Czaczkes et al., 2017).

Methods figure for experiment 2 - Confirmatory experiment testing ant-ant physical communication of direction

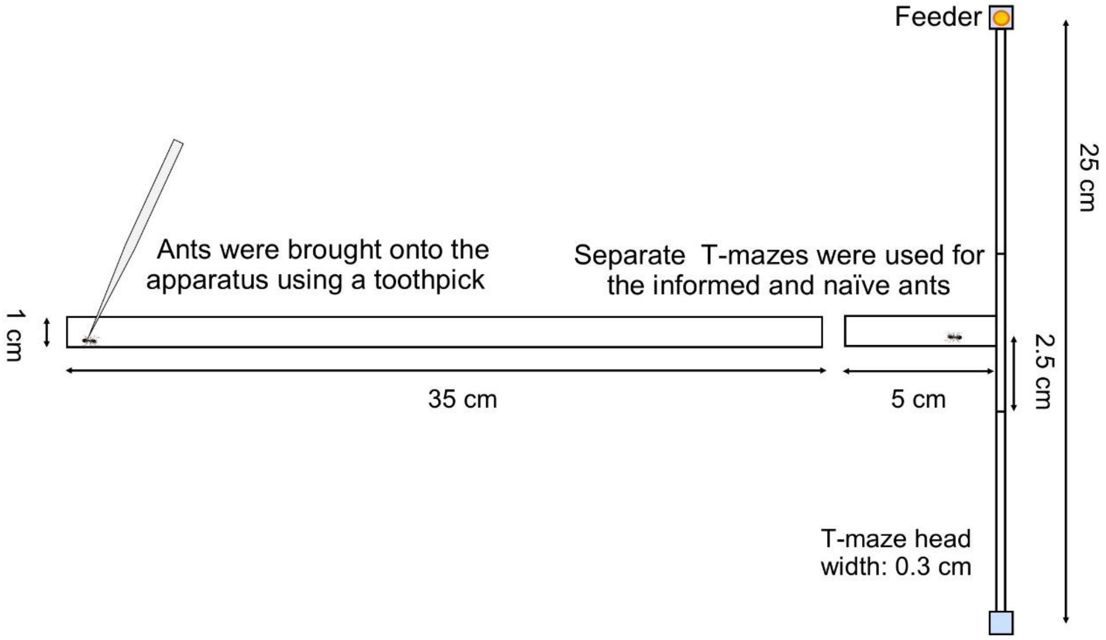


Figure B4 – Experimental setup for the confirmatory experiment. An ant was allowed to find the feeder and return. After leaving the T-maze, the T maze was replaced by a fresh one. A naïve ant was then brought onto the apparatus using a toothpick. If the ants interact, the trial is considered a test trial, and if the ants walk past each other without interacting the trail is considered a control.

References

Czaczkes, T.J., Castorena, M., Schürch, R., Heinze, J., 2017. Pheromone trail following in the ant *Lasius niger*: high accuracy and variability but no effect of task state. Physiol. Entomol. 42, 91–97.

Czaczkes, T.J., Grüter, C., Ellis, L., Wood, E., Ratnieks, F.L.W., 2013. Ant foraging on complex trails: route learning and the role of trail pheromones in *Lasius niger*. J. Exp. Biol. 216, 188–97.

Czaczkes, T.J., Grüter, C., Jones, S.M., Ratnieks, F.L.W., 2011. Synergy between social and private information increases foraging efficiency in ants. Biol. Lett. 7, 521–4.

Czaczkes, T.J., Heinze, J., 2015. Ants adjust their pheromone deposition to a changing environment and their probability of making errors. Proc. R. Soc. B Biol. Sci. 282, 20150679-.

Dussutour, A., Deneubourg, J.L., Fourcassié, V., 2005. Amplification of individual preferences in a social context: The case of wall-following in ants. Proc. R. Soc. B Biol. Sci. 272, 705–714.

Grüter, C., Czaczkes, T.J., Ratnieks, F.L.W., 2011. Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav. Ecol. Sociobiol. 65, 141–148.

Appendix C (supplement to Chapter 4 - Methods)

Supplementary Methods

Pesticide mortality effects experiment

Table C1 – The insecticidal ingredients and their mode of uptake and action

Insecticide	Mode of uptake	Mode of Action	Source
Acetamiprid	Ingestion and contact	Acetylcholine receptor agonist (Neonicotinoid)	http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/11.htm
S-methoprene	Ingestion and contact	Juvenile hormone analogue	http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/1457.htm
Imidacloprid	Ingestion and contact	Acetylcholine receptor agonist (Neonicotinoid)	http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/397.htm
Hydramethylnon	Ingestion	Mitochondrial complex III electron transport inhibitor	http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/386.htm
Indoxacarb	Ingestion and contact	Voltage-dependent sodium channel blocker	http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/399.htm

Table C2 - precise number of workers by colony and treatment. The table states the number of dead workers + survivors recovered from each box. All colonies started with 40 workers. Where final number >40, this could be due to brood developing; where final number <40 this could be due to destruction of an ant corpse by surviving workers before the count was made.

Treatment	Final total number of workers, live and dead			
	Colony A	Colony B	Colony C	Colony D
ACE	40	40	40	39
CON	40	41	40	39
GEL	41	40	40	40
HYD	41	41	39	40
IMI	39	40	40	41
IND	41	40	40	39

Effect of gypsum plaster and lid on humidity

A dummy treatment box was created to examine the best methods for increasing humidity. The two methods considered were placing a pot of moist gypsum plaster in the box and adding the gypsum plaster and a transparent acetate lid. As adding a transparent lid could lead to a small greenhouse effect, the impact of the treatments on temperature was recorded at the same time as the impact on humidity.

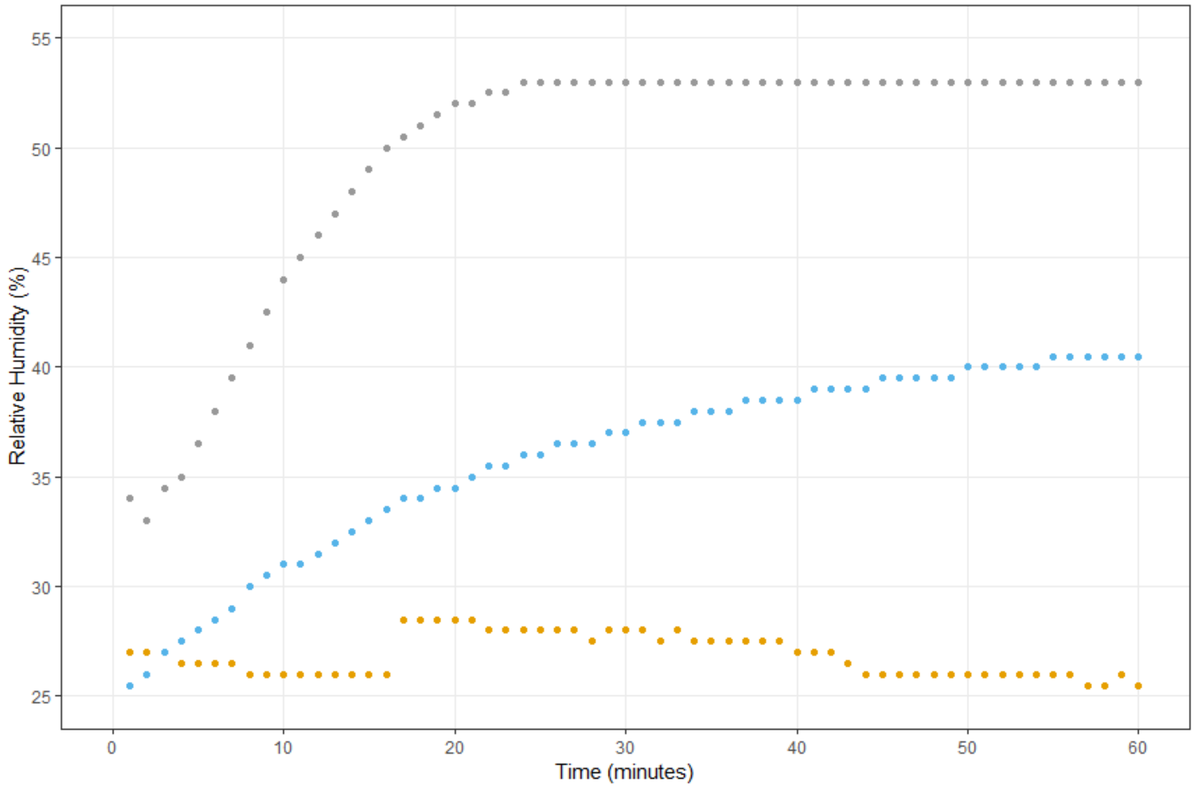


Figure C1 - Humidity in a dummy treatment box under different conditions. Orange represents open box without gypsum plaster (low humidity condition in experiment). Blue represents the effect of adding moist gypsum plaster. Grey represents the effect of having both moist gypsum plaster and a lid on the box (high humidity treatment in experiment). Treatments were applied sequentially: no plaster and no lid, plaster and no lid, plaster and lid.

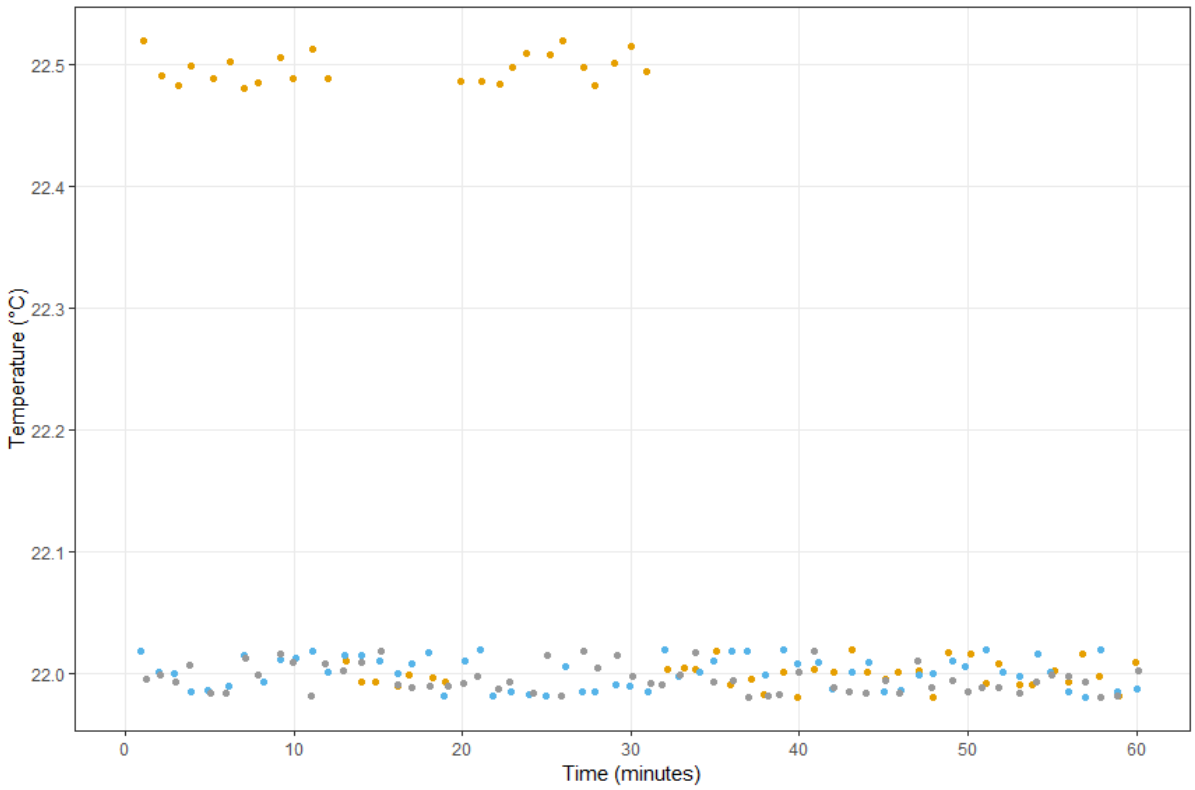


Figure C2 - Temperature in the dummy treatment box as measured concurrently with humidity. Orange represents open box without gypsum plaster (low humidity condition in experiment). Blue represents the effect of adding moist gypsum plaster. Grey represents the effect of having both moist gypsum plaster and a lid on the box (high humidity treatment in experiment). Treatments were applied sequentially: no plaster and no lid, plaster and no lid, plaster and lid. Points have been jittered (by +/- 0.2 on x-axis and +/- 0.02 on y-axis) to avoid overplotting and aid visual interpretation.

Timing of steps in methods for Pesticide mortality effects experiment

Table C4 – a summary of the steps for each test colony in Pesticide mortality effects

Day (relative to the introduction of pesticide baits)	Event duration per test colony	Event
-1	Once	Test colony set up (nest tube, water tube, honey solution, mealworm, ants).
0	Once	Pesticide bait station added. Number of ant visits to bait station in first five minutes recorded. Number of ants on the pesticide bait station, the mealworms and the honey solution at sixty minutes after introduction of pesticides recorded.
1	Once daily until end of experiment	Dead ants counted and removed. Number of (live) ants outside the nest counted. Number of ants on pesticide bait station counted.
1	Once daily until day 68	Number of ants on the honey solution and mealworm bait stations counted.
2	Once, three times per week until end of experiment	Mass of pesticide measured.
42	Once	(After data collection) Small pot of moist gypsum plaster and transparent acetate lid added to each test colony.
42	Once daily until end of experiment	1ml water added to gypsum plaster
68	Once	(After data collection) Honey solution and mealworm bait stations removed from all test colonies.
96	Once	Experiment end

Statistical Methods

Analyses were carried out using the R statistical environment (R Core Team, 2018). Modelling made use of functions in the 'survival' (Therneau, 2015) and 'multcomp' (Hothorn et al., 2008) packages. Graphs were produced with the aid of functions from the 'ggplot2' (Wickham, 2016), 'survminer' (Kassambara and Kosinski, 2018), 'gridExtra' (Auguie, 2017) and 'forestplot' (Gordon and Lumley, 2017) packages. Following Forstmeier and Schielzeth (2011), only terms and interactions that we a priori expected to explain the results were included in the statistical models, and these models were not simplified by removing non-significant terms.

The Kaplan-Meier estimator is a function for estimating survival from right-censored time to event data. As the sample size increases, the Kaplan-Meier estimator will tend towards the true survival curve. In this paper, we use the Kaplan-Meier estimator to aid visualisation of the raw data. Cox proportional hazard models were fitted to examine the relationship between different pesticide treatments on mortality rates. Where possible, ant colony was included as a frailty term (random effect) in the model; colony was omitted where a low sample size led to a high risk of model overfitting (Harrell Jr, 2015). The proportional hazards assumption for each model was assessed using the "cox.zph" function in the 'survival' package (Therneau, 2015) and the data were graphically assessed for influential points using dfbeta residuals (see supplementary materials).

The proportion of ants inside and outside of the nest in each treatment box daily was analysed using a generalised linear mixed effects model with a binomial structure and logistic link function. The proportion of ants outside the nest (weighted by the number alive in the box) was used as the response variable with time in days, treatment and their interaction as fixed effects. As the data were longitudinal in nature (i.e. repeated measures from each experimental box over time) treatment box (nested in colony) was included as a random intercept and a random slope. This model was found to be overdispersed (leading to an increased risk of Type I errors) so an observation-level random effects procedure (Harrison et al., 2018) was applied and the identity of each observation was included as a random factor. The model was fitted using the "glmer" function in the 'lme4' package (Bates et al., 2015) and the "ggpredict" function in the 'ggeffects' package (Lüdecke, 2018) was used to generate graphical representations of the marginal effects. Model adequacy was assessed by checking for overdispersion, graphically assessing Pearson residuals as a function of fitted values, and separately by the fixed effect terms and examining normal QQ plots for each of the random effect terms (see supplementary information part X) (Harrison et al., 2018).

The amount of time that individual ants spent interacting with or feeding on substrates in the palatability experiment was not suitable for Analysis of Variance technique due to non-normality of residuals (feeding and interacting) and non-equality of variances (interacting). Instead Kruskal-Wallis tests were performed. If these indicated that groups differed, the R package ‘dunn.test’ (Dinno, 2017) was used to apply a post hoc Dunn’s test, using the Benjamini-Hochberg procedure with a critical value of 0.05 to account for multiple comparisons, to identify which groups differed significantly. This conservative process was motivated by the high risk of type II errors when performing familywise error rate procedures on a large number of comparisons (Nakagawa, 2004).

References

Auguie, B., 2017. gridExtra: Miscellaneous Functions for “Grid” Graphics.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using {lme4}. J. Stat. Softw. 67, 1–48.

Dinno, A., 2017. dunn.test: Dunn’s Test of Multiple Comparisons Using Rank Sums.

Forstmeier, W., Schielzeth, H., 2011. Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner’s curse. Behav. Ecol. Sociobiol. 65, 47–55.

Gordon, M., Lumley, T., 2017. forestplot: Advanced Forest Plot Using “grid” Graphics.

Harrell Jr, F.E., 2015. 4.4 Sample Size, Overfitting and Limits on Number of Predictors. In: Regression Modeling Strategies. pp. 72–74.

Harrison, X.A., Donaldson, L., Correa-Cano, M.E., Evans, J., Fisher, D.N., Goodwin, C.E.D., Robinson, B.S., Hodgson, D.J., Inger, R., 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ 6, e4794.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous Inference in General Parametric Models. Biometrical J. 50, 346–363.

Kassambara, A., Kosinski, M., 2018. survminer: Drawing Survival Curves using “ggplot2.”

Lüdecke, D., 2018. ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. J. Open Source Softw. 3, 772.

Nakagawa, S., 2004. A farewell to Bonferroni: The problems of low statistical power and publication bias. Behav. Ecol. 15, 1044–1045.

R Core Team, 2018. R: A Language and Environment for Statistical Computing.

Therneau, T.M., 2015. A Package for Survival Analysis in S.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Appendix D (supplement to Chapter 4 - Results)

Supplementary Details of Results

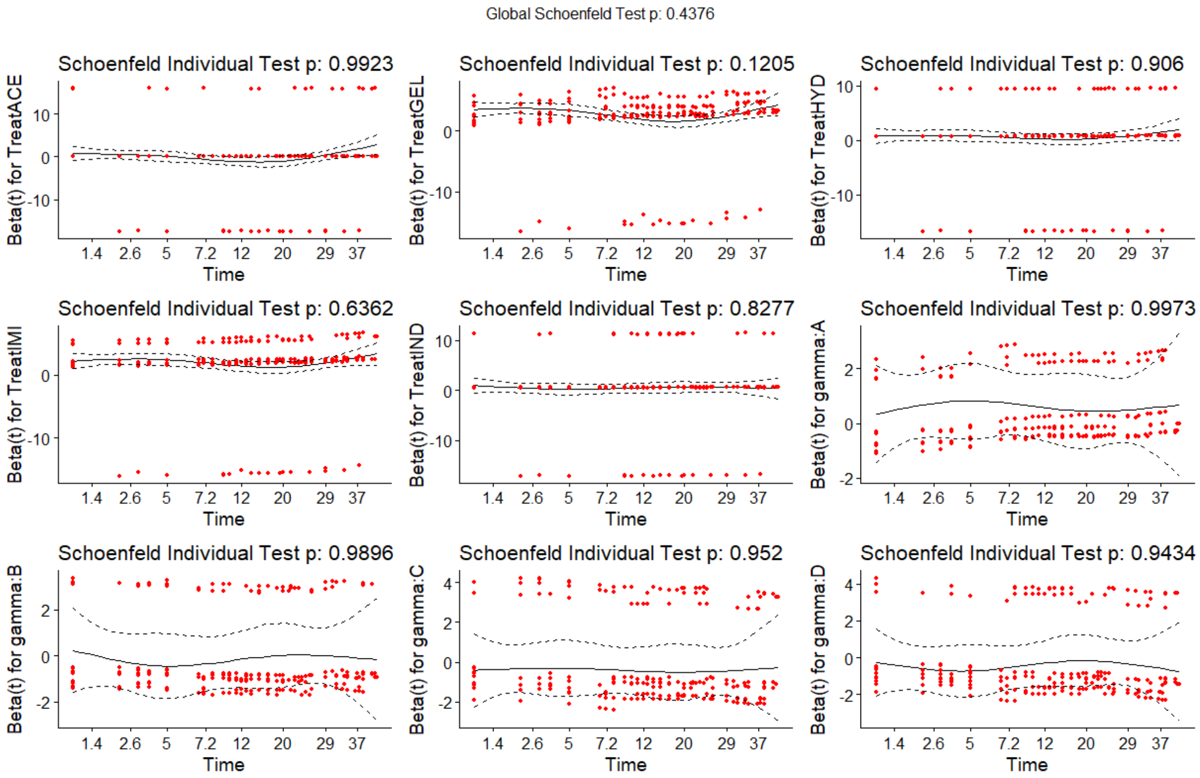
D1 - Initial Survival

The effect of Pesticide treatment on survival during the first 42 days. Fitted values for colony terms in bold

```
Call: coxph(formula = Surv(Time, Event) ~ Treat + frailty(Colony))

  n= 961, number of events= 332

                coef     se(coef)  se2      Chisq   DF     p
TreatACE        0.1094   0.3166    0.3166   0.12    1.00   7.3e-01
TreatGEL        2.8253   0.2504    0.2503   127.33  1.00   1.6e-29
TreatHYD        0.7378   0.2810    0.2810   6.89    1.00   8.7e-03
TreatIMI        2.0557   0.2521    0.2520   66.51   1.00   3.5e-16
TreatIND        0.5408   0.2914    0.2914   3.44    1.00   6.3e-02
frailty(Colony)                             55.37   2.93   5.1e-12

              exp(coef)  exp(-coef) lower .95 upper .95
TreatACE      1.1156     0.89634    0.5998    2.075
TreatGEL      16.8659    0.05929    10.3250   27.551
TreatHYD      2.0913     0.47818    1.2057    3.627
TreatIMI      7.8120     0.12801    4.7666    12.803
TreatIND      1.7174     0.58228    0.9701    3.040
gamma:A       1.7667     0.56603    0.8688    3.592
gamma:B       0.8973     1.11448    0.4350    1.851
gamma:C       0.6789     1.47289    0.3268    1.411
gamma:D       0.6571     1.52185    0.3161    1.366

Iterations: 10 outer, 25 Newton-Raphson
Variance of random effect= 0.5063467   I-likelihood = -2040.5
Degrees of freedom for terms= 5.0 2.9
Concordance= 0.769  (se = 0.016 )
Likelihood ratio test= 364.4  on 7.93 df,   p=<2e-16
```

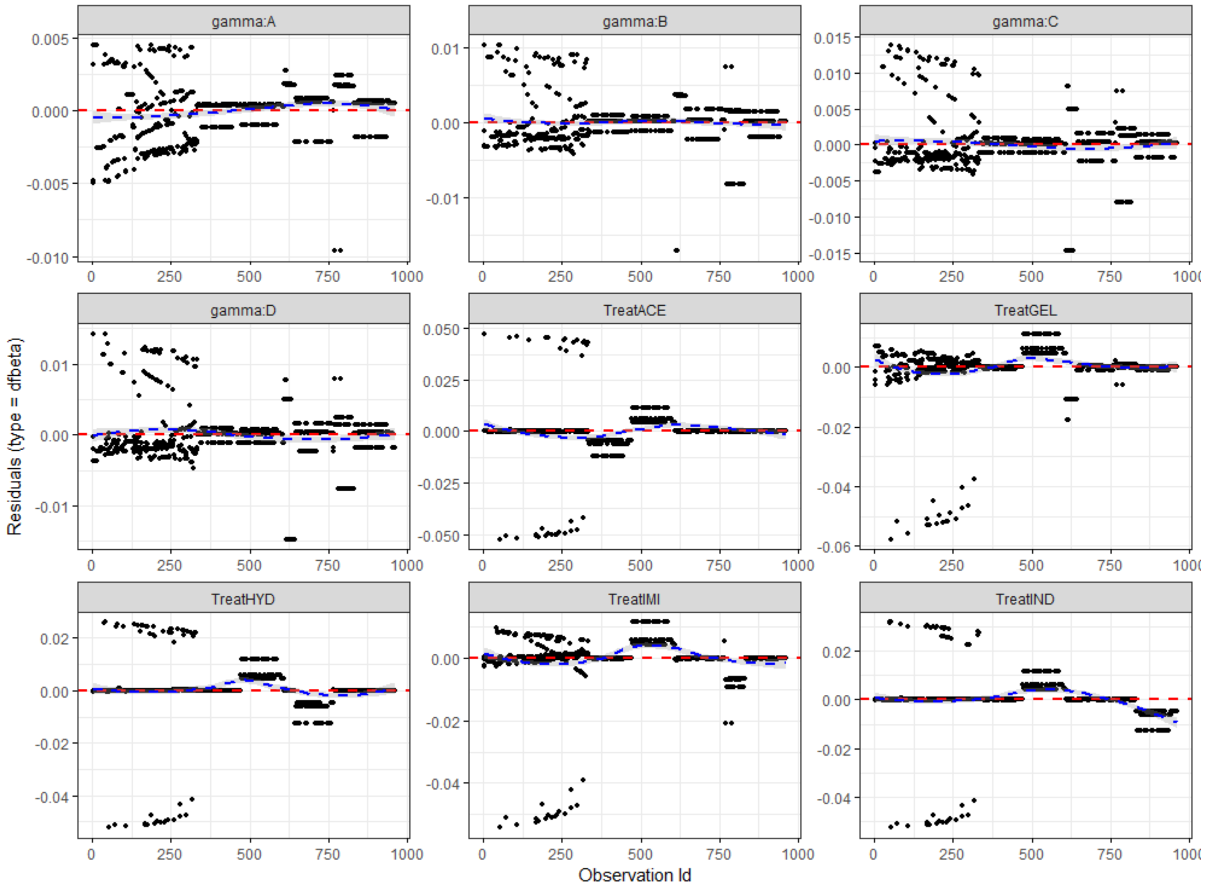
Assessment of proportional hazards assumption for the frailty model of the effect of Pesticide treatment on survival in first 42 days

```
              rho        chisq      p
TreatACE   -0.000526   9.21e-05   0.992
TreatGEL   -0.084032   2.41e+00   0.120
TreatHYD    0.006479   1.40e-02   0.906
TreatIMI   -0.025765   2.24e-01   0.636
TreatIND   -0.011938   4.74e-02   0.828
gamma:A     0.000953   1.14e-05   0.997
gamma:B    -0.002692   1.71e-04   0.990
gamma:C    -0.010765   3.62e-03   0.952
gamma:D     0.012668   5.04e-03   0.943
GLOBAL       NA         9.00e+00   0.438
```



A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.

Assessment of influential points for the frailty model of the effect of Pesticide treatment on survival in first 42 days



The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

D2 - Comparison of fitted survival parameters (and hazard ratios) between negative and positive controls and other treatment groups

Table D2.1 – summary of pairwise treatment comparisons and associated hazard ratios (p-values adjusted with a Bonferroni correction). CON = Control treatment (no pesticide present); ACE = granular Acetamiprid treatment; IMI = granular Imidacloprid treatment; IND = granular Indoxacarb treatment; HYD = granular Hydramethylnon treatment; GEL = Imidacloprid gel treatment (positive control).

Comparison	Estimate (β)	Standard error	z-value	p-value	Hazard ratio
CON - ACE	0.1094	0.3166	0.346	1.000	1.116
CON - IMI	2.0557	0.2521	8.155	<0.001	7.812
CON - IND	0.5408	0.2914	1.856	0.571	1.717
CON - HYD	0.7378	0.2810	2.626	0.078	2.091
CON - GEL	2.8253	0.2504	11.284	<0.001	16.866
GEL - ACE	-2.7159	0.2399	-11.321	<0.001	0.066
GEL - IMI	-0.7696	0.1379	-5.582	<0.001	0.463
GEL - IND	-2.2845	0.2049	-11.148	<0.001	0.101
GEL - HYD	-2.0875	0.1903	-10.972	<0.001	0.123

D3 - Survival under increased Humidity

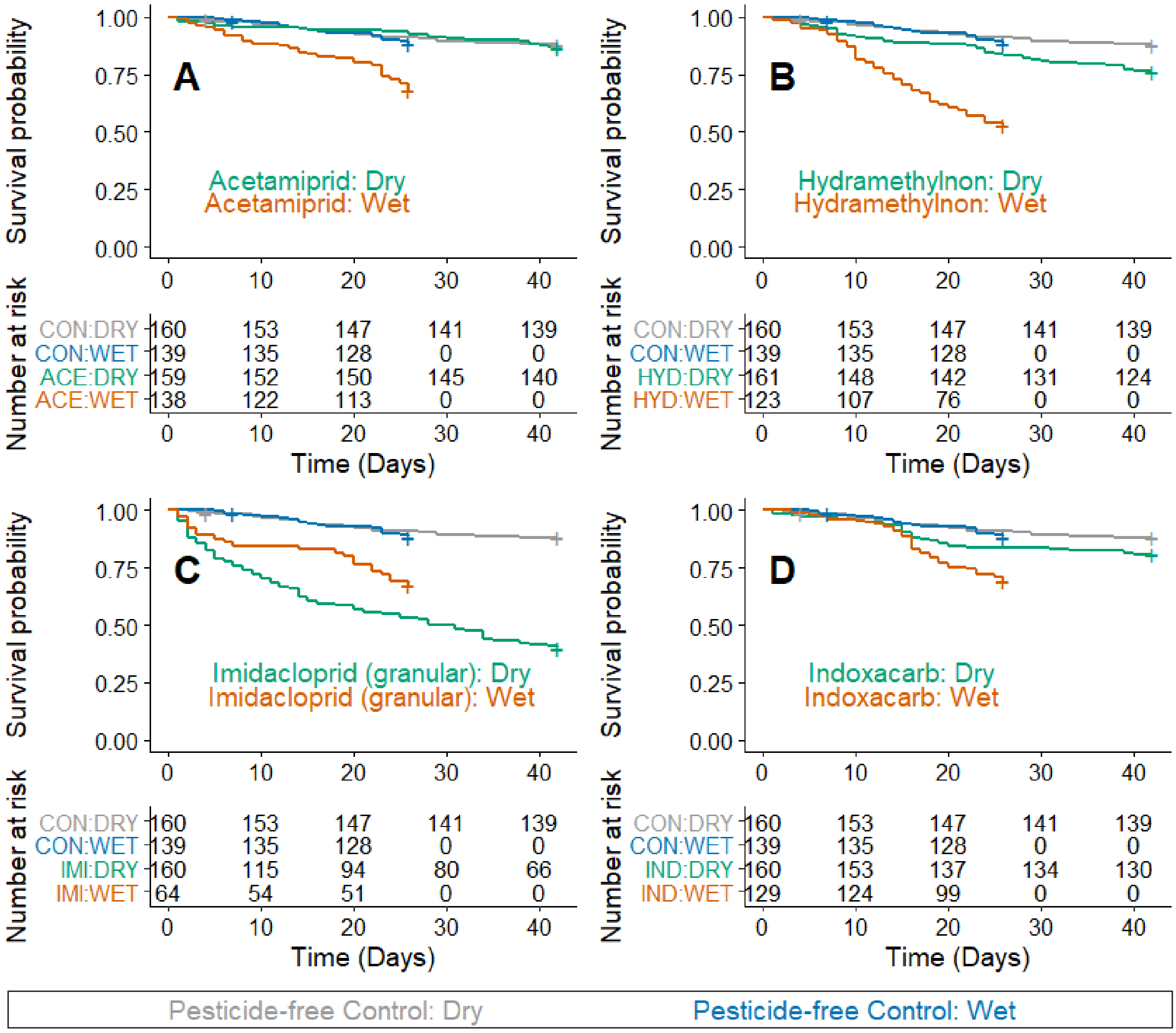


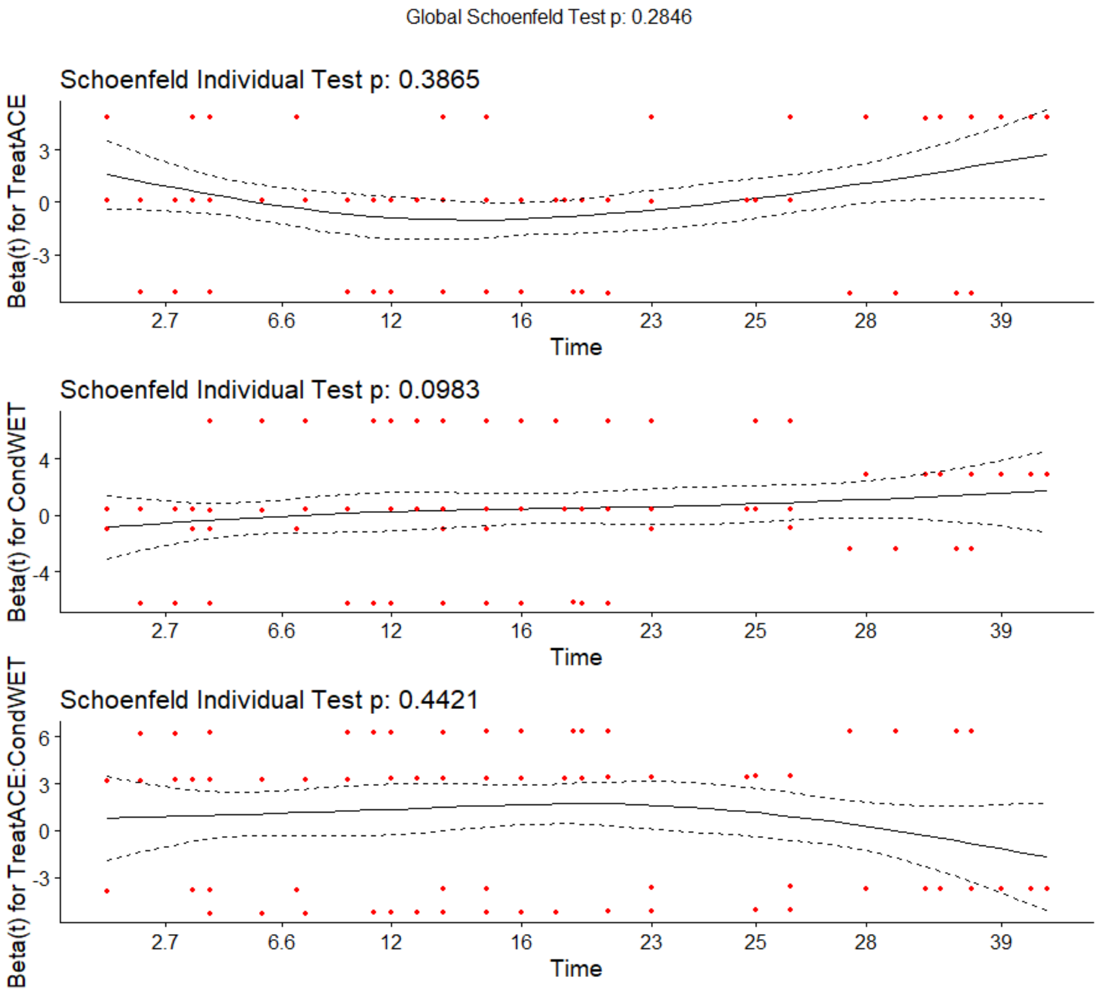
Figure D3.1 - Kaplan-Meier estimators of survival under high humidity (Wet) and low humidity (Dry) conditions, separated by pesticide treatment, pooled across all colonies. For each colony the Dry treatment was applied immediately before the Wet treatment, hence starting sample sizes differed between treatments. The key comparison is whether increasing the humidity affects the pesticide-treated colonies differently from the controls. Censored results indicated with a cross. Risk tables indicate the number of live ants present by group at ten-day time intervals (note number at risk at day zero is the starting sample size for that group).

Acetamiprid humidity model

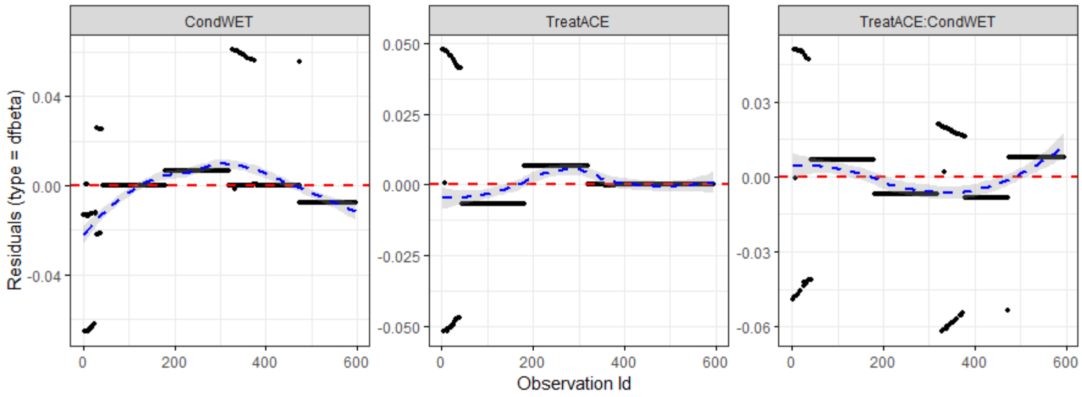
```
Call: coxph(formula = Surv(Interval_length, Event) ~ Treat * Cond)

  n= 596, number of events= 100

                    coef      exp(coef)  se(coef)   z        Pr(>|z|)
TreatACE            0.08799   1.09198    0.31663   0.278    0.7811
CondWET             0.39304   1.48148    0.35849   1.096    0.2729
TreatACE:CondWET 1.03958   2.82804    0.43075    2.413    0.0158

                    exp(coef)   exp(-coef)   lower .95   upper .95
TreatACE            1.092       0.9158       0.5871      2.031
CondWET             1.481       0.6750       0.7338      2.991
TreatACE:CondWET    2.828       0.3536       1.2157      6.579

Concordance= 0.653  (se = 0.029 )
Rsquare= 0.062   (max possible= 0.876 )
Likelihood ratio test= 38.4  on 3 df,   p=2e-08
Wald test            = 39.93  on 3 df,   p=1e-08
Score (logrank) test = 46.72  on 3 df,   p=4e-10
```

Test of proportional hazards assumption for Acetamiprid Humidity model

```
                    rho       chisq    p
TreatACE            0.0866    0.750    0.3865
CondWET             0.1612    2.733    0.0983
TreatACE:CondWET    -0.0769   0.591    0.4421
GLOBAL              NA        3.794    0.2846
```



Acetamiprid humidity model - A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.



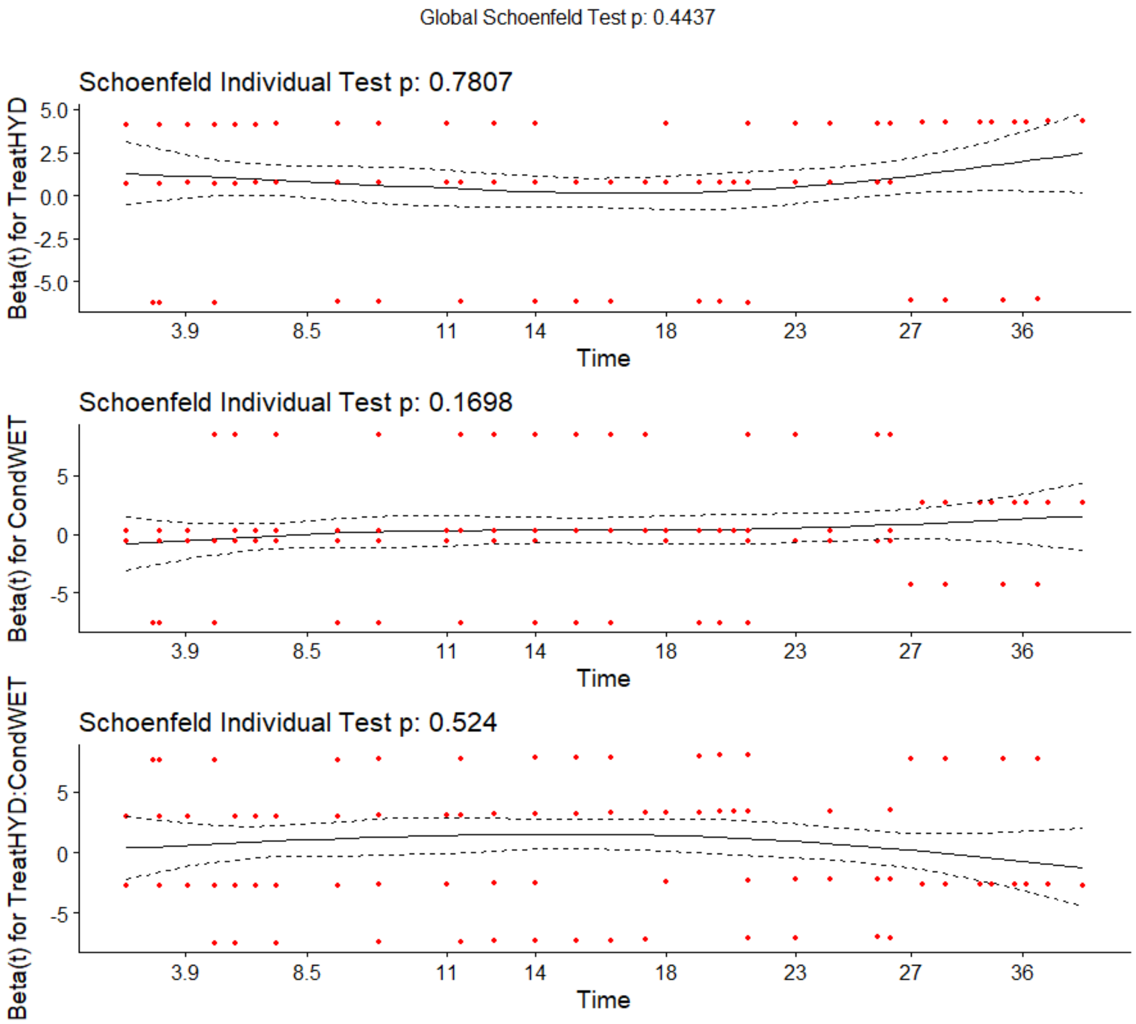
Acetamiprid humidity model - The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

Hydramethylnon humidity model

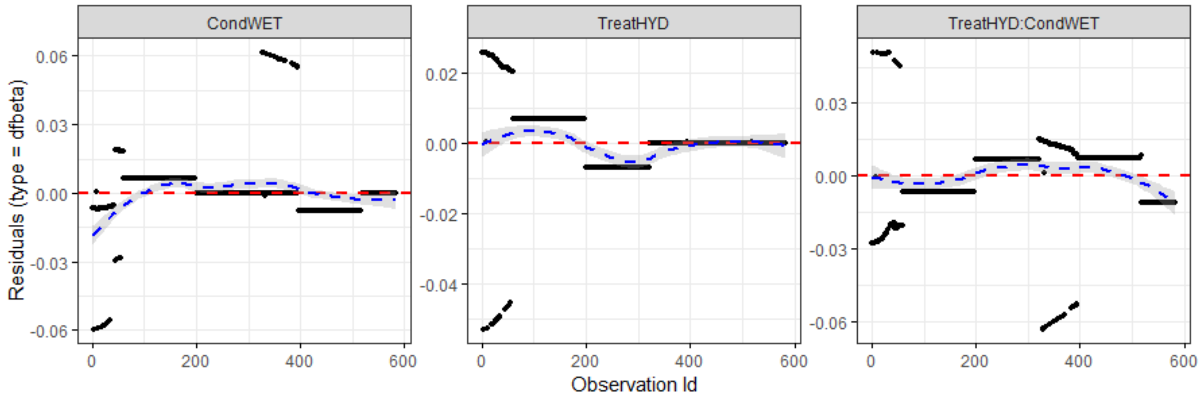
```
Call: coxph(formula = Surv(Interval_length, Event) ~ Treat * Cond)

  n= 583, number of events= 131

                      coef     exp(coef)  se(coef)     z        Pr(>|z|)
TreatHYD             0.7398   2.0955     0.2810     2.633    0.00847
CondWET              0.3025   1.3532     0.3498     0.865    0.38715
TreatHYD:CondWET     0.9031   2.4673     0.3986     2.266    0.02348

                     exp(coef)  exp(-coef)   lower .95   upper .95
TreatHYD             2.095       0.4772       1.2081      3.635
CondWET              1.353       0.7390       0.6818      2.686
TreatHYD:CondWET     2.467       0.4053       1.1296      5.390

Concordance= 0.689  (se = 0.025 )
Rsquare= 0.114   (max possible= 0.938 )
Likelihood ratio test= 70.73  on 3 df,   p=3e-15
Wald test            = 71.95  on 3 df,   p=2e-15
Score (logrank) test = 88.7  on 3 df,   p=<2e-16
```

Test of proportional hazards assumption for Hydramethylnon Humidity model

```
                     rho       chisq      p
TreatHYD             0.0243   0.0775    0.781
CondWET              0.1193   1.8843    0.170
TreatHYD:CondWET    -0.0557   0.4060    0.524
GLOBAL               NA       2.6797    0.444
```



Hydramethylnon humidity model - A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.



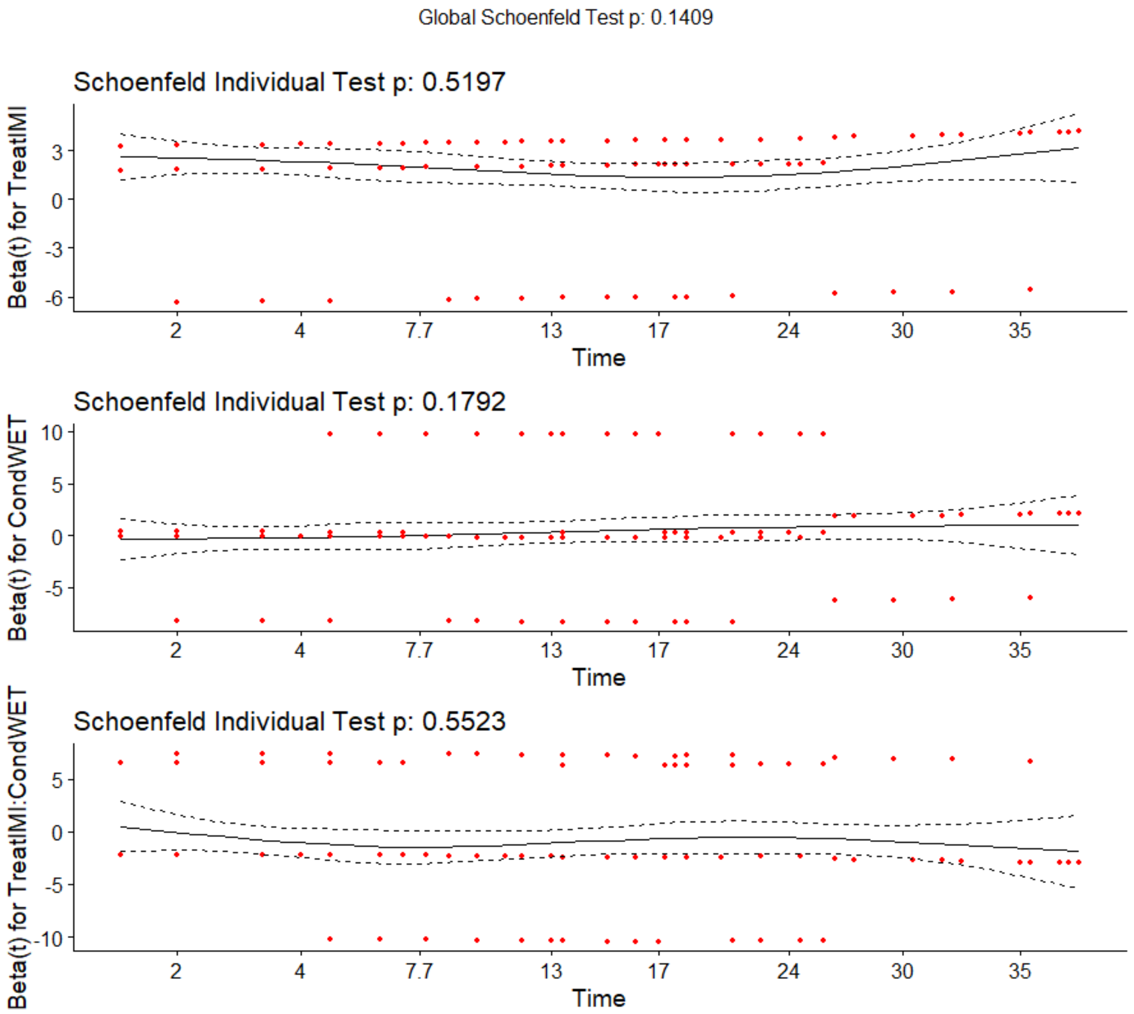
Hydramethylnon humidity model - The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

Imidacloprid granular humidity model

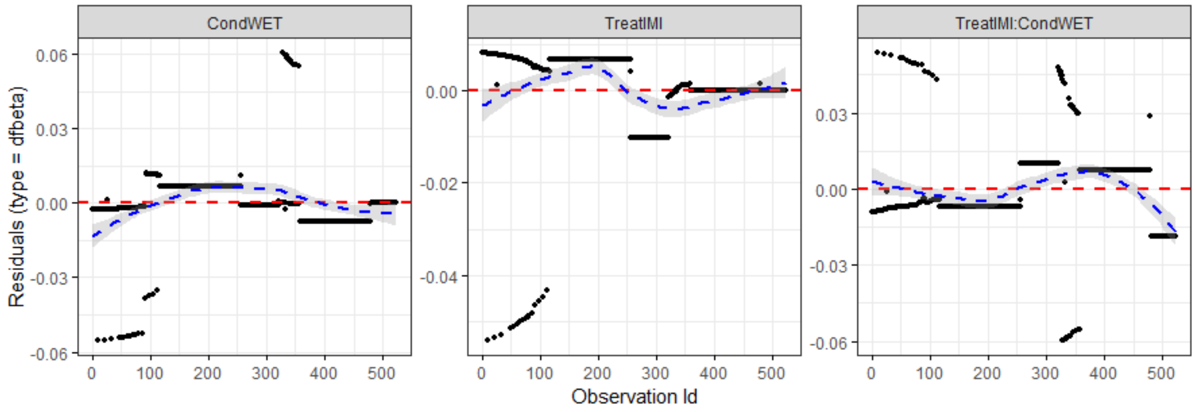
```
Call: coxph(formula = Surv(Interval_length, Event) ~ Treat * Cond)

  n= 523, number of events= 152

                  coef exp(coef)  se(coef)   z      Pr(>|z|)
TreatIMI         1.9907    7.3209   0.2517    7.908    2.61e-15
CondWET          0.2888    1.3349   0.3450    0.837    0.4025
TreatIMI:CondWET -0.8146    0.4428   0.4164    -1.956   0.0505

                 exp(coef) exp(-coef) lower .95 upper .95
TreatIMI            7.3209     0.1366    4.4698    11.991
CondWET             1.3349     0.7491    0.6788     2.625
TreatIMI:CondWET    0.4428     2.2583    0.1958     1.002

Concordance= 0.721  (se = 0.023 )
Rsquare= 0.187   (max possible= 0.969 )
Likelihood ratio test= 108.1  on 3 df,   p=<2e-16
Wald test            = 88.76  on 3 df,   p=<2e-16
Score (logrank) test = 115.7  on 3 df,   p=<2e-16
```

Test of proportional hazards assumption for Imidacloprid granular Humidity model

```
                     rho chisq     p
TreatIMI         -0.0523 0.415 0.520
CondWET           0.1088 1.804 0.179
TreatIMI:CondWET -0.0481 0.353 0.552
GLOBAL                NA 5.462 0.141
```



Granular Imidacloprid humidity model - A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.



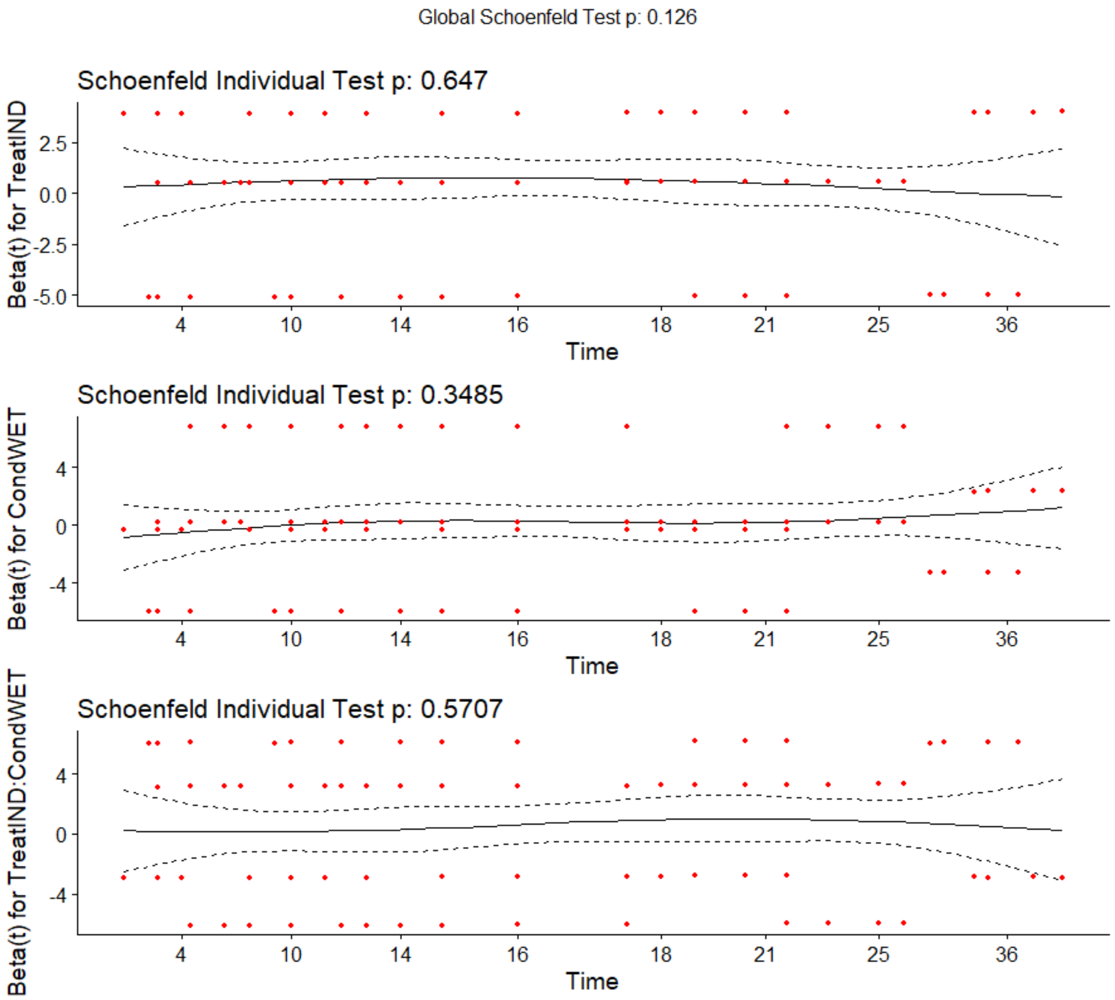
Granular Imidacloprid humidity model - The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

Indoxacarb humidity model

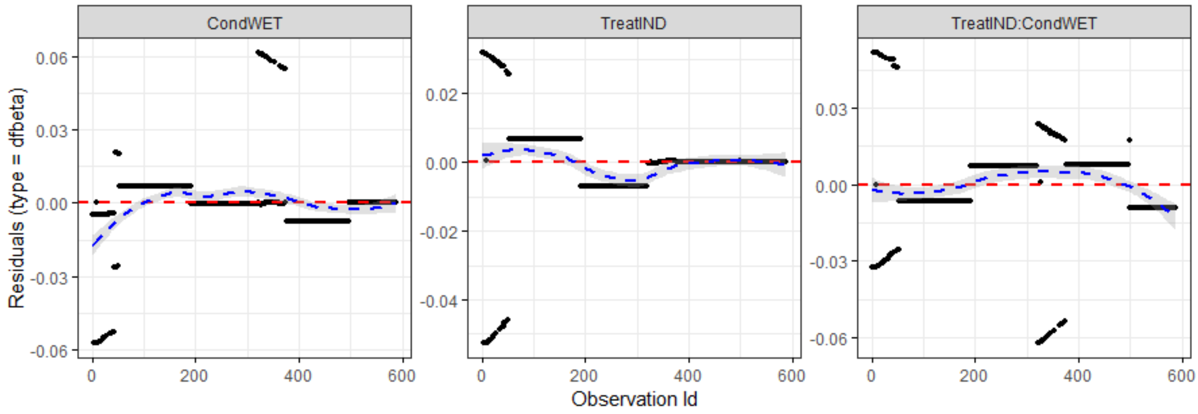
```
Call: coxph(formula = Surv(Interval_length, Event) ~ Treat * Cond)

  n= 588, number of events= 106

                  coef exp(coef) se(coef)     z Pr(>|z|)
TreatIND        0.5296    1.6983   0.2914 1.818   0.0691
CondWET         0.1898    1.2090   0.3468 0.547   0.5842
TreatIND:CondWET 0.5504    1.7340   0.4152 1.326   0.1850

                exp(coef) exp(-coef) lower .95 upper .95
TreatIND            1.698     0.5888    0.9594     3.006
CondWET             1.209     0.8271    0.6127     2.386
TreatIND:CondWET    1.734     0.5767    0.7684     3.913

Concordance= 0.63  (se = 0.028 )
Rsquare= 0.042   (max possible= 0.894 )
Likelihood ratio test= 25.06  on 3 df,   p=1e-05
Wald test            = 25.93  on 3 df,   p=1e-05
Score (logrank) test = 28.43  on 3 df,   p=3e-06
```

Test of proportional hazards assumption for Indoxacarb Humidity model

```
                    rho chisq     p
TreatIND        -0.0445 0.210 0.647
CondWET          0.0922 0.879 0.348
TreatIND:CondWET  0.0551 0.321 0.571
GLOBAL              NA 5.720 0.126
```



Indoxacarb humidity model - A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.



Indoxacarb humidity model - The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

Summary of models

Table D3.2 - Each model took the form mortality ~ treatment*condition where treatment was either the pesticide-free control group or one of the granular pesticides and condition was either high or low humidity. The interaction term allows the effect of condition to vary with humidity. All Wald tests performed on one degree of freedom.

Granular Pesticide	Term	coef	s.e	Wald-z	p - value
Acetamiprid	treatment	0.08799	0.31663	0.278	0.781
	condition	0.39304	0.35849	1.096	0.273
	interaction	1.03958	0.43075	2.413	**0.016**
Hydramethylnon	treatment	0.7398	0.2810	2.633	**0.008**
	condition	0.3025	0.3498	0.865	0.387
	interaction	0.9031	0.3986	2.266	**0.023**
Imidacloprid	treatment	1.9907	0.2517	7.908	**<0.001**
	condition	0.2888	0.3450	0.837	0.403
	interaction	-0.8146	0.4164	-1.956	**0.051**
Indoxacarb	treatment	0.5296	0.2914	1.818	0.069
	condition	0.1898	0.3468	0.547	0.584
	interaction	0.5504	0.4152	1.326	0.185

D4 - Survival under decreased Food Availability

S4a - The effects of reduced food availability on pesticide efficacy - graphs and survival tables

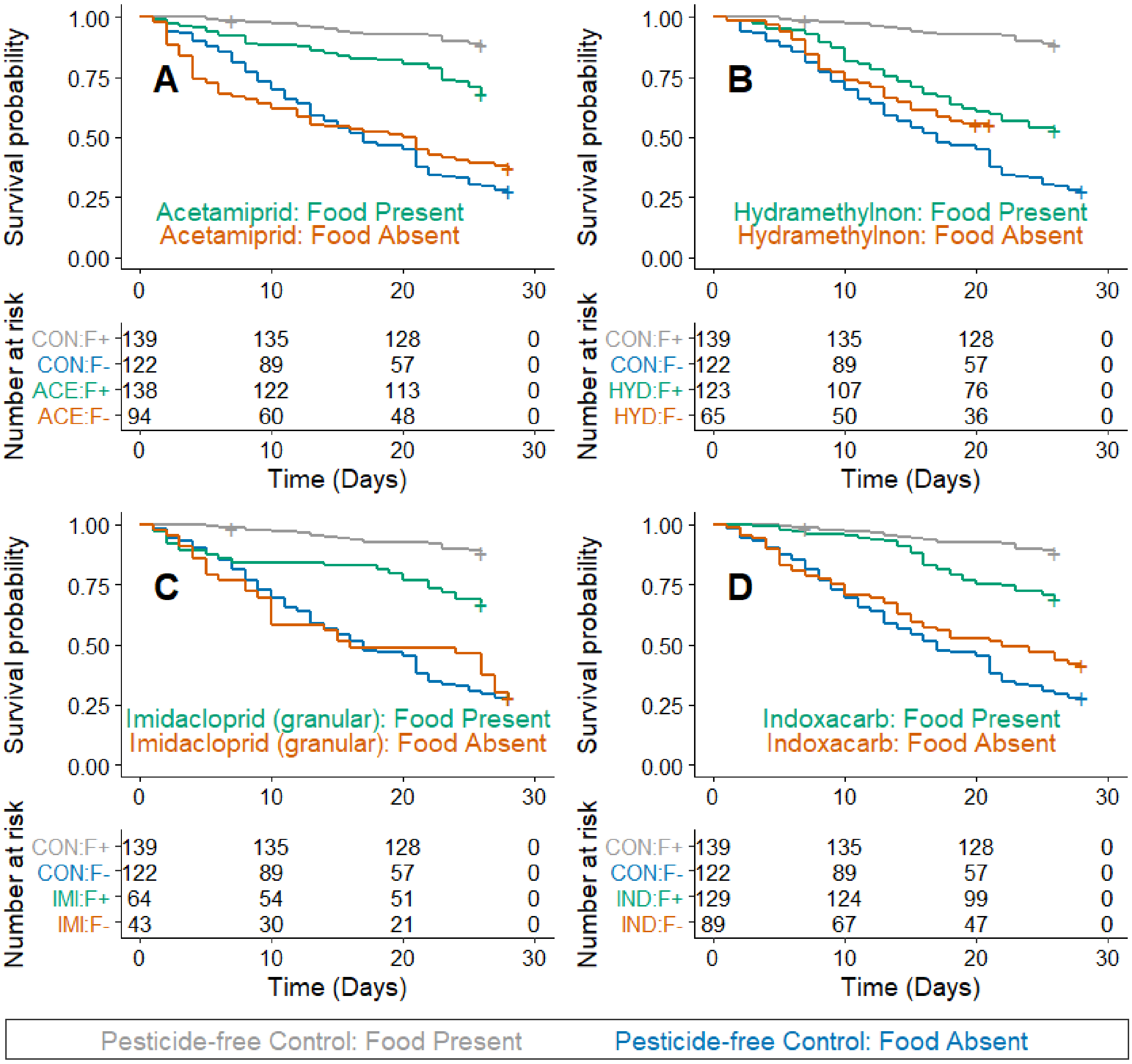


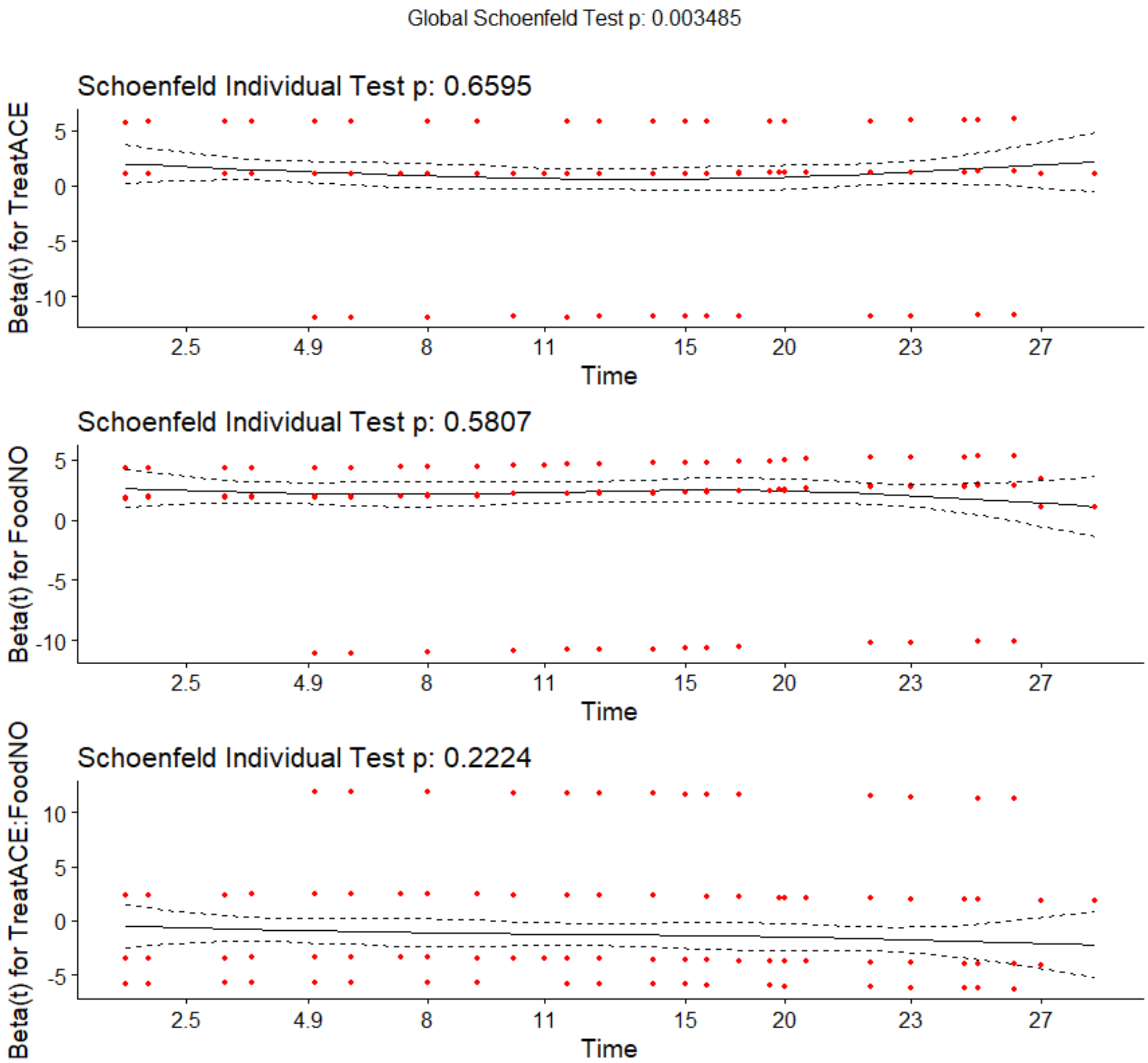
Figure D4.1 - Kaplan-Meier estimators of survival with food (sucrose solution and mealworms) present or absent, separated by pesticide treatment, pooled across all colonies. For each colony the food present treatment was applied immediately before the food absent treatment, hence starting sample sizes differed between treatments (see Supplementary Info). Analysis is only applied to ants surviving to the high humidity treatment. The key comparison is whether removing food affects the pesticide treated colonies differently from the controls. Censored results indicated with a cross. Risk tables indicate the number of live ants present by group at ten day time intervals (note number at risk at day zero is the starting sample size for that group).

Acetamiprid food model

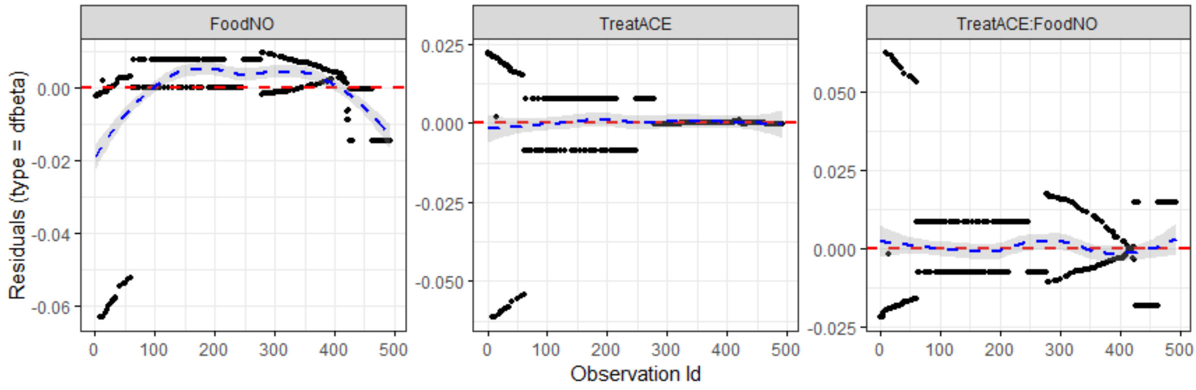
```
Call: coxph(formula = Surv(Interval_length, Event) ~ Treat * Food)

  n= 493, number of events= 207

                  coef exp(coef) se(coef)      z Pr(>|z|)
TreatACE         1.1242    3.0778   0.2920  3.851 0.000118
FoodNO           2.2716    9.6952   0.2731  8.317  < 2e-16
TreatACE:FoodNO -1.2494    0.2867   0.3372 -3.705 0.000212

                exp(coef) exp(-coef) lower .95 upper .95
TreatACE           3.0778     0.3249     1.737    5.4546
FoodNO             9.6952     0.1031     5.676   16.5592
TreatACE:FoodNO    0.2867     3.4882     0.148    0.5552

Concordance= 0.709  (se = 0.02 )
Rsquare= 0.229   (max possible= 0.993 )
Likelihood ratio test= 128  on 3 df,   p=<2e-16
Wald test            = 96.59  on 3 df,   p=<2e-16
Score (logrank) test = 126.3  on 3 df,   p=<2e-16
```

Test of proportional hazards assumption for Acetamiprid food model

```
                    rho  chisq       p
TreatACE        -0.0306  0.194 0.65950
FoodNO          -0.0385  0.305 0.58069
TreatACE:FoodNO -0.0845  1.489 0.22238
GLOBAL               NA 13.611 0.00349
```



Acetamiprid food model - A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.



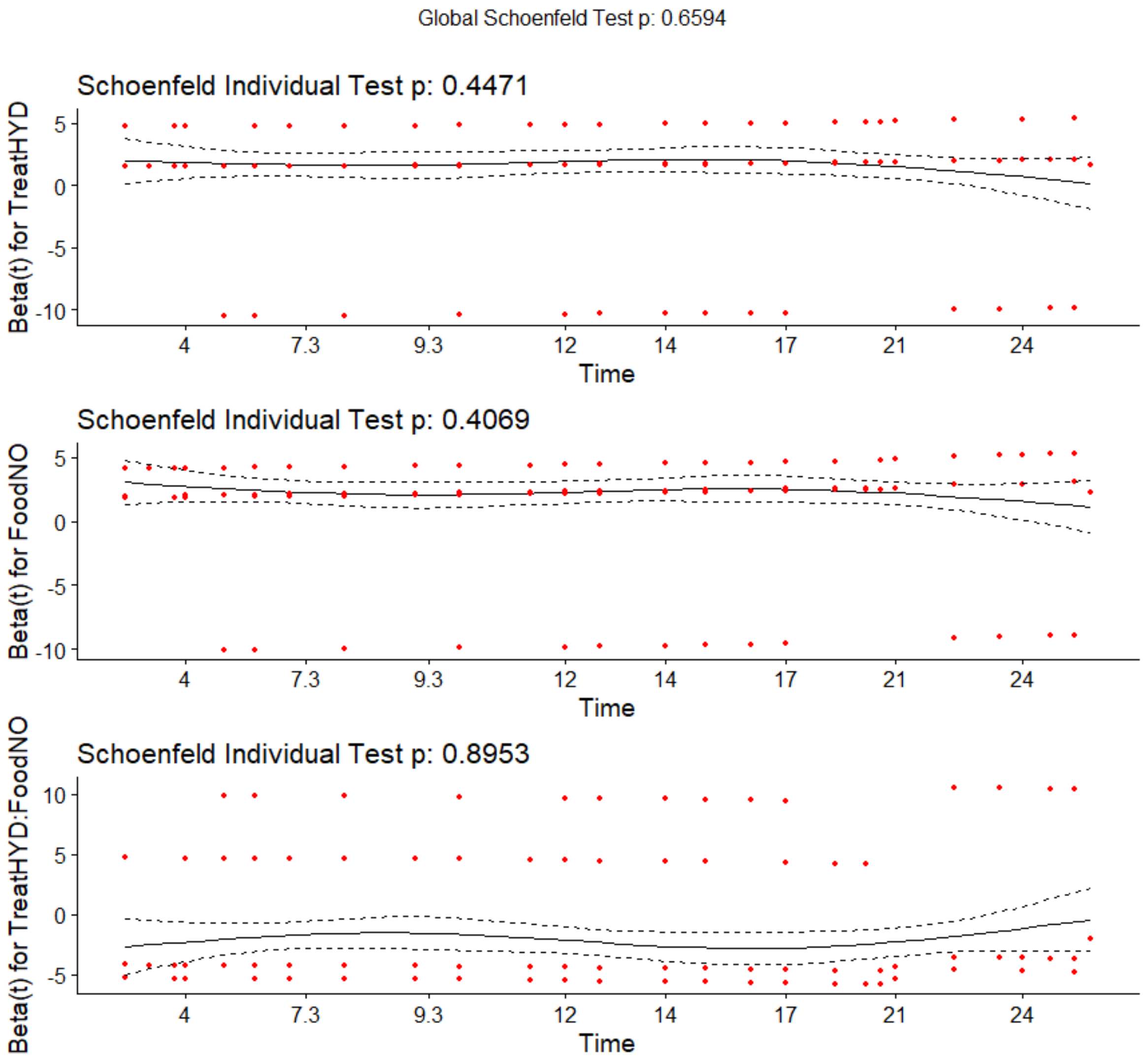
Acetamiprid food model - The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

Hydramethylnon food model

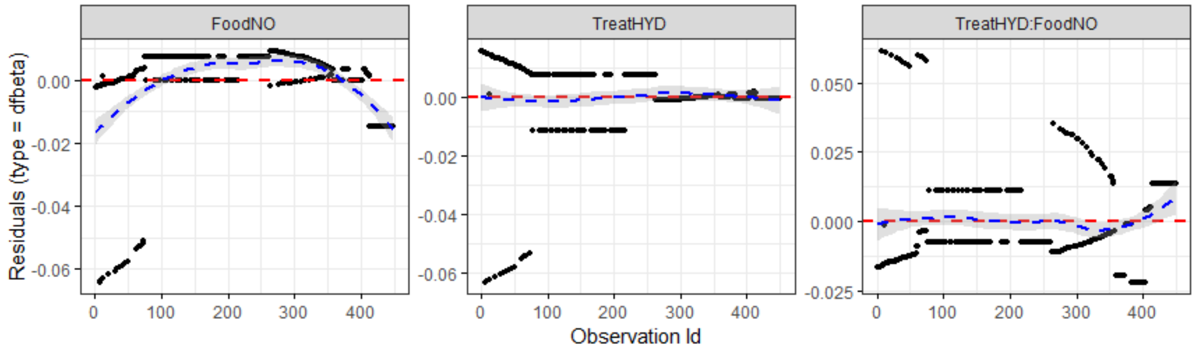
```
Call: coxph(formula = Surv(Interval_length, Event) ~ Treat * Food)

  n= 449, number of events= 191

                  coef exp(coef) se(coef)      z Pr(>|z|)
TreatHYD        1.6551    5.2335   0.2827  5.855 4.76e-09
FoodNO          2.2932    9.9067   0.2733  8.391  < 2e-16
TreatHYD:FoodNO -2.0456    0.1293   0.3554 -5.756 8.61e-09

               exp(coef) exp(-coef) lower .95 upper .95
TreatHYD          5.2335     0.1911   3.00737    9.1075
FoodNO            9.9067     0.1009   5.79839   16.9259
TreatHYD:FoodNO    0.1293     7.7341   0.06443    0.2595

Concordance= 0.697  (se = 0.021 )
Rsquare= 0.215   (max possible= 0.993 )
Likelihood ratio test= 108.5  on 3 df,   p=<2e-16
Wald test            = 73.22  on 3 df,   p=9e-16
Score (logrank) test = 98.53  on 3 df,   p=<2e-16
```

Test of proportional hazards assumption for Hydramethylnon food model

```
                    rho  chisq     p
TreatHYD        -0.05521 0.5779 0.447
FoodNO          -0.06045 0.6877 0.407
TreatHYD:FoodNO  0.00945 0.0173 0.895
GLOBAL                NA 1.6001 0.659
```



Hydramethylnon food model - A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.



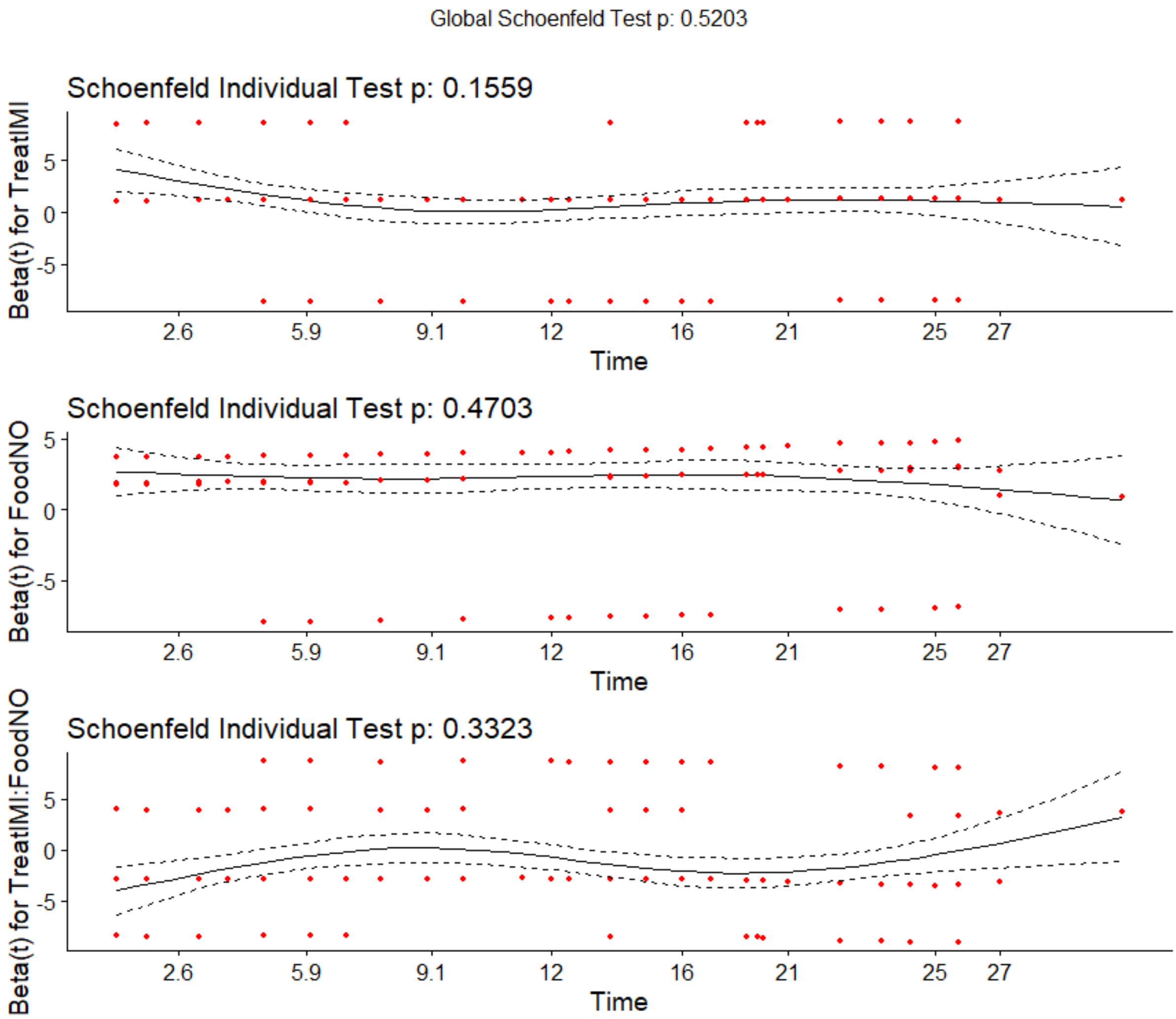
Hydramethylnon food model - The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

Imidacloprid granular food model

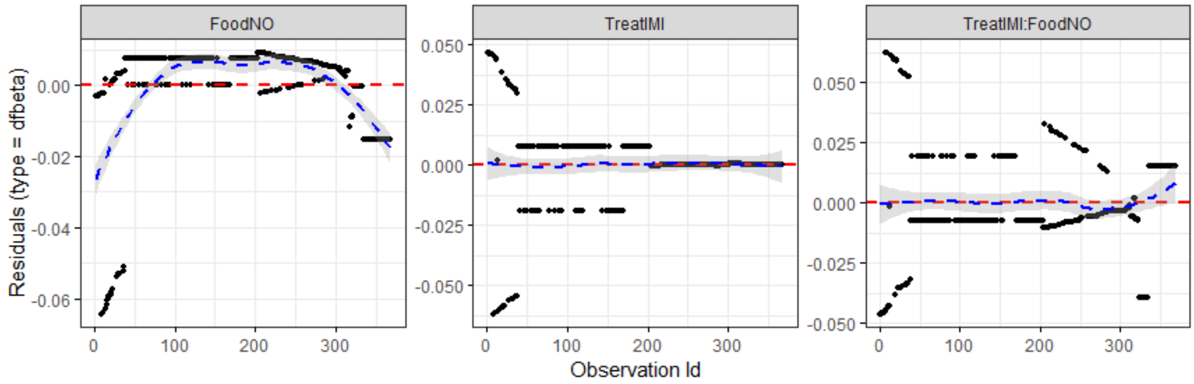
```
Call: coxph(formula = Surv(Interval_length, Event) ~ Treat * Food)

  n= 368, number of events= 156

                  coef exp(coef) se(coef)      z Pr(>|z|)
TreatIMI        1.1986    3.3153   0.3319  3.611 0.000305
FoodNO          2.2566    9.5505   0.2740  8.237  < 2e-16
TreatIMI:FoodNO -1.2360    0.2905   0.3925 -3.149 0.001636

                exp(coef) exp(-coef) lower .95 upper .95
TreatIMI          3.3153     0.3016    1.7299     6.354
FoodNO            9.5505     0.1047    5.5826    16.339
TreatIMI:FoodNO   0.2905     3.4419    0.1346     0.627

Concordance= 0.72  (se = 0.023 )
Rsquare= 0.265   (max possible= 0.991 )
Likelihood ratio test= 113.2  on 3 df,   p=<2e-16
Wald test            = 80.41  on 3 df,   p=<2e-16
Score (logrank) test = 110.7  on 3 df,   p=<2e-16
```

Test of proportional hazards assumption for Imidacloprid granular food model

```
                    rho chisq     p
TreatIMI        -0.1138 2.014 0.156
FoodNO          -0.0579 0.521 0.470
TreatIMI:FoodNO  0.0777 0.940 0.332
GLOBAL               NA 2.260 0.520
```



Granular Imidacloprid food model - A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.



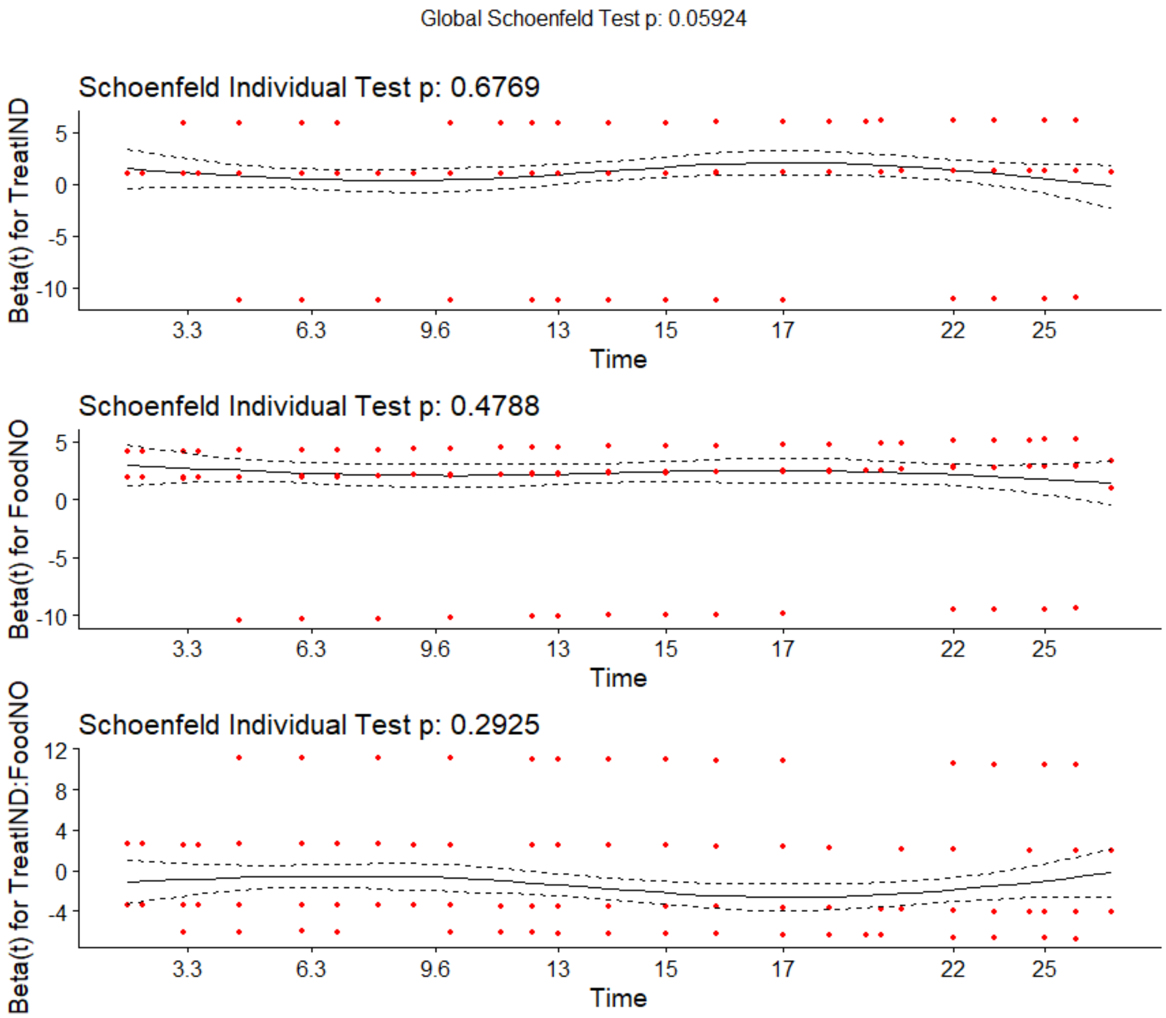
Granular Imidacloprid food model - The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

Indoxacarb food model

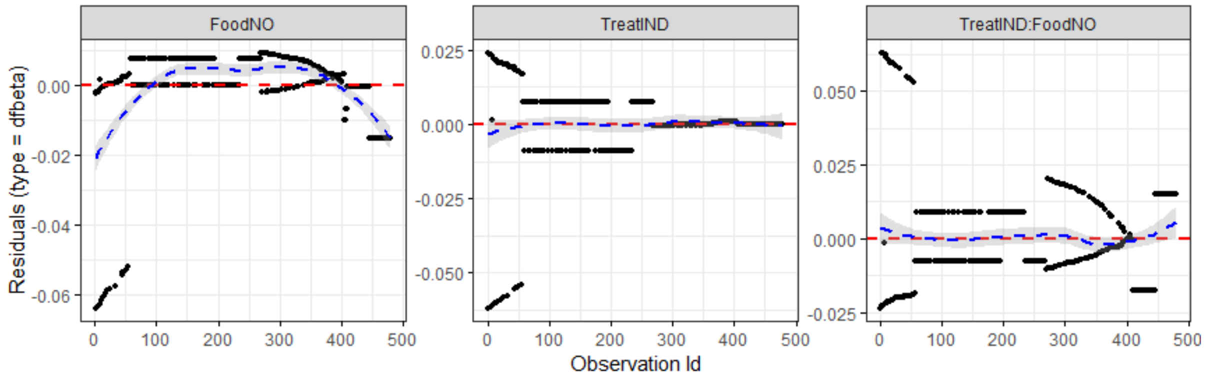
```
Call: coxph(formula = Surv(Interval_length, Event) ~ Treat * Food)

  n= 479, number of events= 196

                  coef exp(coef) se(coef)      z Pr(>|z|)
TreatIND        1.0813    2.9484   0.2959  3.654 0.000258
FoodNO          2.3000    9.9738   0.2730  8.424  < 2e-16
TreatIND:FoodNO -1.4089    0.2444   0.3440 -4.095 4.22e-05

                exp(coef) exp(-coef) lower .95 upper .95
TreatIND           2.9484     0.3392    1.6510    5.2655
FoodNO             9.9738     0.1003    5.8406   17.0320
TreatIND:FoodNO    0.2444     4.0914    0.1245    0.4797

Concordance= 0.718  (se = 0.021 )
Rsquare= 0.225   (max possible= 0.992 )
Likelihood ratio test= 121.8  on 3 df,   p=<2e-16
Wald test            = 94.33  on 3 df,   p=<2e-16
Score (logrank) test = 123.1  on 3 df,   p=<2e-16
```

Test of proportional hazards assumption for Indoxacarb food model

```
                    rho    chisq p
TreatIND         0.0298 0.174 0.6769
FoodNO          -0.0508 0.502 0.4788
TreatIND:FoodNO -0.0751 1.108 0.2925
GLOBAL               NA 7.435 0.0592
```



Indoxacarb food model - A slope not equal to zero would indicate that the proportional hazard assumption is violated. None of the slopes differ significantly from zero, so we can assume proportional hazards.



Indoxacarb food model - The removal of individual data-points has little effect on parameter fits relative to the magnitude of the parameter estimates

Summary of models

Table D4.2 - Each model took the form mortality ~ treatment*food where treatment was either the pesticide-free control group or one of the granular pesticides and food was either food present of absent. The interaction term allows the effect of condition to vary with food status. All Wald tests performed on one degree of freedom.

Granular Pesticide	Term	coef	s.e	Wald-z	p - value
Acetamiprid	treatment	1.1242	0.2920	3.851	**< 0.001**
	food	2.2716	0.2731	8.317	**< 0.001**
	interaction	-1.2494	0.3372	-3.705	**< 0.001**
Hydramethylnon	treatment	1.6551	0.2827	5.855	**< 0.001**
	food	2.2932	0.2733	8.391	**< 0.001**
	interaction	-2.0456	0.3554	-5.756	**< 0.001**
Imidacloprid	treatment	1.1986	1.1986	3.611	**< 0.001**
	food	2.2566	0.2740	8.237	**< 0.001**
	interaction	-1.2360	0.3925	-3.149	**= 0.002**
Indoxacarb	treatment	1.0813	0.2959	3.654	**< 0.001**
	food	2.3000	0.2730	8.424	**< 0.001**
	interaction	-1.4089	0.3440	-4.095	**< 0.001**

D5 - Sub-lethal effects

The sub-lethal effects model

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
Family: binomial  ( logit )
Formula: pout ~ treat * day + (day | col:box) + (1 | obs)
Control: glmerControl(optimizer = "Nelder_Mead", optCtrl = list(maxfun = 2e+07))

     AIC      BIC   logLik deviance df.resid
  4812.7   4890.7  -2390.3   4780.7      956

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.2532 -0.6089 -0.0221  0.5408  5.7457

Random effects:
 Groups  Name        Variance  Std.Dev. Corr
 obs     (Intercept) 2.931e-02 0.171214
 col:box (Intercept) 1.926e-01 0.438871
         day         6.815e-05 0.008255 -0.59
Number of obs: 972, groups:  obs, 972; col:box, 24

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)  -0.749383   0.227766  -3.290   0.0010
treatACE     -0.057288   0.322103  -0.178   0.8588
treatGEL     -0.017584   0.337918  -0.052   0.9585
treatHYD     -0.112485   0.322498  -0.349   0.7272
treatIMI     -0.838996   0.327002  -2.566   0.0103
treatIND     -0.123729   0.322271  -0.384   0.7010
day           0.003660   0.004824   0.759   0.4480
treatACE:day  0.005212   0.006816   0.765   0.4445
treatGEL:day -0.042334   0.008711  -4.860 1.18e-06
treatHYD:day  0.007612   0.006855   1.110   0.2668
treatIMI:day  0.002562   0.007379   0.347   0.7284
treatIND:day  0.005827   0.006836   0.852   0.3939
```

```
Correlation of Fixed Effects:
            (Intr) trtACE trtGEL trtHYD trtIMI trtIND day    trACE: trGEL: trHYD: trIMI:
treatACE    -0.707
treatGEL    -0.674  0.476
treatHYD    -0.706  0.499  0.477
treatIMI    -0.696  0.493  0.470  0.492
treatIND    -0.707  0.500  0.476  0.499  0.492
day         -0.609  0.431  0.410  0.430  0.424  0.430
treatACE:dy  0.431 -0.609 -0.290 -0.304 -0.300 -0.305 -0.708
treatGEL:dy  0.336 -0.238 -0.547 -0.239 -0.240 -0.238 -0.552  0.391
treatHYD:dy  0.428 -0.303 -0.290 -0.609 -0.298 -0.303 -0.704  0.498  0.391
treatIMI:dy  0.398 -0.281 -0.269 -0.281 -0.609 -0.281 -0.654  0.463  0.372  0.460
treatIND:dy  0.430 -0.304 -0.289 -0.303 -0.299 -0.609 -0.706  0.500  0.390  0.497  0.462
```

Sublethal effects model: random effects

```
$`col:box`
       (Intercept)          day
A:AACE -0.06349649  0.0112267597
A:ACON -0.07800975  0.0065536472
A:AGEL -1.31619210  0.0141020718
A:AHYD -0.60110470  0.0088513718
A:AIMI -0.07084110  0.0112901715
A:AIND -0.12734168  0.0008133135
B:BACE -0.10411757 -0.0025443170
B:BCON  0.05418902 -0.0085223517
B:BGEL  0.16680561 -0.0070199818
B:BHYD -0.39194181 -0.0059882948
B:BIMI -0.05520380 -0.0051936860
B:BIND  0.03550026 -0.0019026925
C:CACE  0.08504599 -0.0041087800
C:CCON -0.31295699  0.0047591265
C:CGEL  0.43035785 -0.0064045805
C:CHYD  0.39651033 -0.0024723499
C:CIMI -0.23668035  0.0050195122
C:CIND  0.23700686 -0.0026246667
D:DACE  0.10206284 -0.0047592971
D:DCON  0.35584743 -0.0028725914
D:DGEL  0.75688448 -0.0005120671
D:DHYD  0.61804376 -0.0006297325
D:DIMI  0.40639138 -0.0112350786
D:DIND -0.12336055  0.0035144662
```

Sublethal effects - models adequacy checks

Model assumptions were verified through the use of Normal QQ and Pearson's residual plots.

Sublethal effects - multiple comparisons

Table D5.1 - Hypothesis test of the fixed effect logistic parameters by treatment for the sublethal effects model (CON = pesticide free control, ACE = acetamiprid, GEL = imidacloprid gel, HYD = hydramethylnon, IMI = imidacloprid granular, IND = indoxacarb). All Wald tests on one degree of freedom with p-values adjusted for multiple comparisons.

Hypothesis	log likelihood	standard error	Wald - z	p - value
Intercept of CON - Intercept of ACE = 0	-0.0573	0.3221	-0.178	1.000
Intercept of CON - Intercept of GEL = 0	-0.0176	0.3379	-0.052	1.000
Intercept of CON - Intercept of HYD = 0	-0.1125	0.3225	-0.349	1.000
Intercept of CON - Intercept of IMI = 0	-0.8390	0.3270	-2.566	0.080
Intercept of CON - Intercept of IND = 0	-0.1237	0.3223	-0.384	1.000
Curve of CON - Curve of ACE = 0	0.0052	0.0068	0.765	0.987
Curve of CON - Curve of GEL = 0	-0.0423	0.0087	-4.860	<0.001
Curve of CON - Curve of HYD = 0	0.0076	0.0069	1.110	0.886
Curve of CON - Curve of IMI = 0	0.0026	0.0074	0.347	1.000
Curve of CON - Curve of IND = 0	0.0058	0.0068	0.852	0.974

D6 - Palatability experiment

During the palatability experiment, one ant in the Imidacloprid gel treatment became stuck in the bait. The consequence of this was that our measures of interaction and feeding time were inflated for this datapoint; it had a magnitude twice the size of the next nearest point. We excluded this datapoint from the analysis in the main text as it is clearly erroneous but incidentally, as rank statistical analyses were used its inclusion would have had very little impact on the results (see below).

D6.1 - Interaction with bate substrate - results - outlier excluded

Pairwise Mann-Whitney tests listed by increasing size of p-value for time spent interacting with the contents of a bait station. In order to account for multiple testing, the p-value must be lower than the alpha level provided next to it. (ACE = acetamiprid, GEL = imidacloprid gel, HYD = hydramethylnon, IMI = imidacloprid granular, IND = indoxacarb, SOL = sucrose solution, SUC = granular sucrose, WAT = water, MEA = chopped mealworms).

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 24.2265, df = 8, p-value = 0

Dunn's test with Benjamini-Hochberg adjustment at alpha = 0.05

List of pairwise comparisons: Z statistic (adjusted p-value)
-------------------------------
ACE - GEL : -1.947772 (0.0842)
ACE - HYD :  0.315125 (0.4105)
GEL - HYD :  2.254492 (0.0544)
ACE - IMI :  0.389530 (0.3920)
GEL - IMI :  2.326913 (0.0514)
HYD - IMI :  0.074404 (0.4838)
ACE - IND :  0.463935 (0.3856)
GEL - IND :  2.399333 (0.0493)
HYD - IND :  0.148809 (0.4668)
IMI - IND :  0.074404 (0.4703)
ACE - MEA :  1.960782 (0.0898)
GEL - MEA :  3.856257 (0.0021)*
HYD - MEA :  1.645656 (0.1198)
IMI - MEA :  1.571251 (0.1306)
IND - MEA :  1.496847 (0.1423)
ACE - SOL : -1.019781 (0.2409)
GEL - SOL :  0.955189 (0.2546)
HYD - SOL : -1.334907 (0.1559)
IMI - SOL : -1.409312 (0.1429)
IND - SOL : -1.483716 (0.1379)
MEA - SOL : -2.980564 (0.0173)*
ACE - SUC :  1.038969 (0.2445)
GEL - SUC :  2.911115 (0.0162)*
```

```
HYD - SUC :  0.732248 (0.3212)
IMI - SUC :  0.659828 (0.3396)
IND - SUC :  0.587408 (0.3580)
MEA - SUC : -0.869515 (0.2769)
SOL - SUC :  2.031552 (0.0844)
ACE - WAT : -1.461833 (0.1362)
GEL - WAT :  0.524928 (0.3722)
HYD - WAT : -1.776958 (0.0972)
IMI - WAT : -1.851363 (0.0888)
IND - WAT : -1.925768 (0.0812)
MEA - WAT : -3.422615 (0.0056)*
SOL - WAT : -0.442051 (0.3823)
SUC - WAT : -2.461813 (0.0498)
```

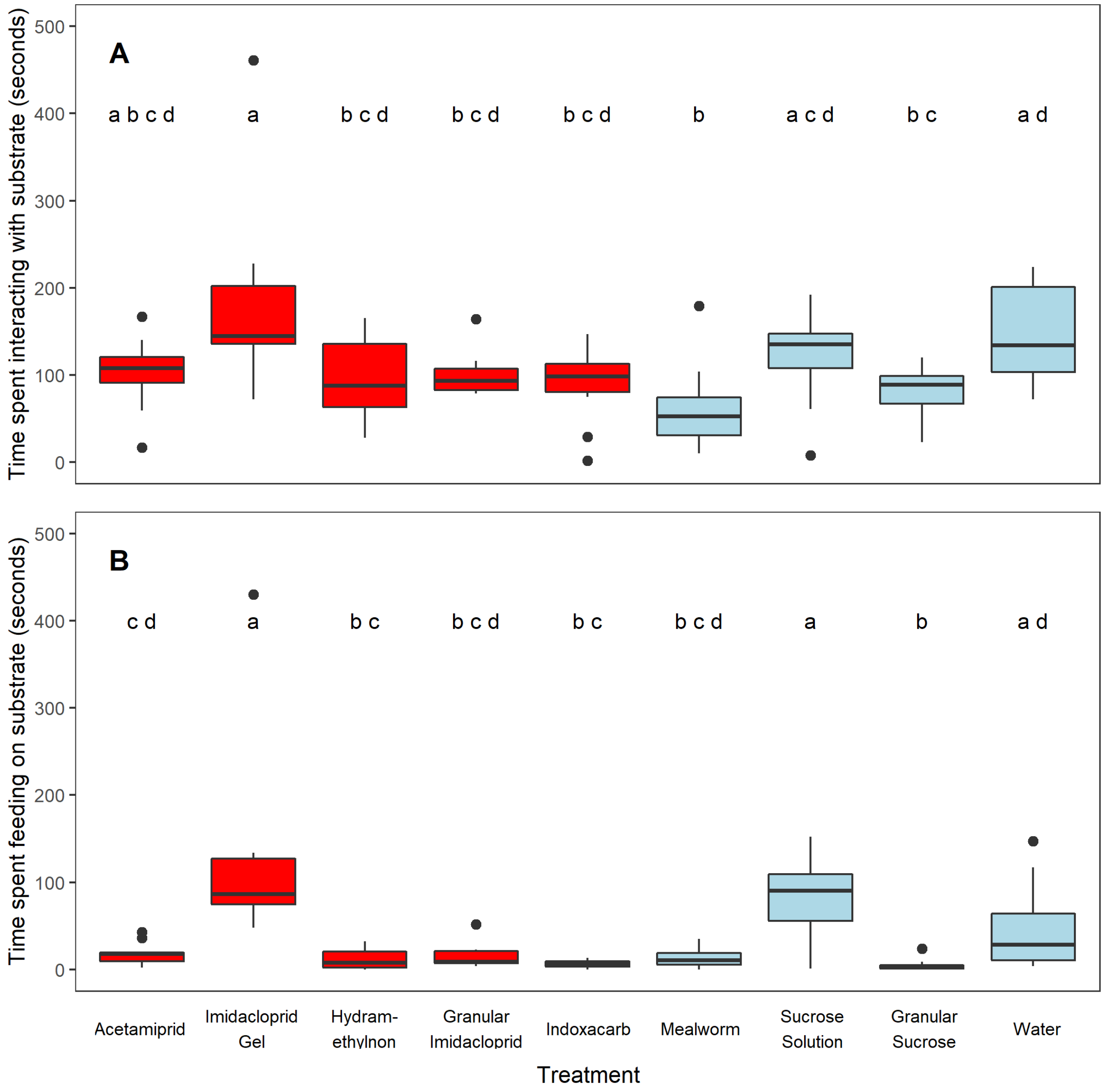
D6.2 - Feeding on bait substrate - results - outlier excluded

Kruskal-Wallis and Dunn’s test with Benjamini-Hochberg adjustment for time spent feeding on the contents of a bait station. In order to account for multiple testing, the p-value must be lower than the alpha level provided next to it. (ACE = acetamiprid, GEL = imidacloprid gel, HYD = hydramethylnon, IMI = imidacloprid granular, IND = indoxacarb, SOL = sucrose solution, SUC = granular sucrose, WAT = water, MEA = chopped mealworms).

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 44.1939, df = 8, p-value = 0

Dunn’s test with Benjamini-Hochberg adjustment at alpha = 0.05

List of pairwise comparisons: Z statistic (adjusted p-value)
-------------------------------
ACE - GEL : -2.716208 (0.0108)*
ACE - HYD :  1.164712 (0.1758)
GEL - HYD :  3.849855 (0.0004)*
ACE - IMI :  0.385318 (0.3600)
GEL - IMI :  3.091249 (0.0040)*
HYD - IMI : -0.779393 (0.2705)
ACE - IND :  1.681388 (0.0878)
GEL - IND :  4.352751 (0.0001)*
HYD - IND :  0.516676 (0.3302)
IMI - IND :  1.296070 (0.1526)
ACE - MEA :  0.674307 (0.3001)
```

```
GEL - MEA :  3.372530 (0.0019)*
HYD - MEA : -0.490405 (0.3303)
IMI - MEA :  0.288988 (0.3863)
IND - MEA : -1.007081 (0.2093)
ACE - SOL : -2.219958 (0.0340)
GEL - SOL :  0.555458 (0.3360)
HYD - SOL : -3.384670 (0.0021)*
IMI - SOL : -2.605277 (0.0127)*
IND - SOL : -3.901347 (0.0004)*
MEA - SOL : -2.894265 (0.0068)*
ACE - SUC :  2.170693 (0.0359)
GEL - SUC :  4.763162 (0.0000)*
HYD - SUC :  1.037045 (0.2075)
IMI - SUC :  1.795652 (0.0768)
IND - SUC :  0.534149 (0.3337)
MEA - SUC :  1.514371 (0.1114)
SOL - SUC :  4.331442 (0.0001)*
ACE - WAT : -0.950159 (0.2199)
GEL - WAT :  1.791390 (0.0732)
HYD - WAT : -2.114871 (0.0387)
IMI - WAT : -1.335478 (0.1487)
IND - WAT : -2.631548 (0.0127)*
MEA - WAT : -1.624466 (0.0938)
SOL - WAT :  1.269798 (0.1531)
SUC - WAT : -3.095511 (0.0044)*
```

D6.3 - Interacting and feeding with outlier included - Graph



Time spent interacting with (panel A) or feeding on (panel B) the contents of a bait station separated by treatment **including significant outlier (ant that got stuck in Imidacloprid Gel treatment - Panel A point at 461, Panel B point at 430 – c.f. figure 7 in main text)**. Red boxes are pesticide treatments and blue boxes are non-pesticide resource comparisons. Matching letters indicate no significant difference in population distributions (Kruskal-Wallis with Dunn's post hoc test using Benjamini-Hochberg false discovery rate adjustment to control for multiple testing - see supplementary for details).

D6.4 - Interacting results - outlier included (ant that got stuck in Imidacloprid Gel treatment)

Kruskal-Wallis and Dunn’s test with Benjamini-Hochberg adjustment for time spent interacting with the contents of a bait station **Including significant outlier (ant that got stuck in Imidacloprid Gel treatment)**. In order to account for multiple testing, the p-value must be lower than the alpha level provided next to it. (ACE = acetamiprid, GEL = imidacloprid gel, HYD = hydramethylnon, IMI = imidacloprid granular, IND = indoxacarb, SOL = sucrose solution, SUC = granular sucrose, WAT = water, MEA = chopped mealworms).

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 25.9757, df = 8, p-value = 0

Dunn’s test with Benjamini-Hochberg adjustment at alpha = 0.05

List of pairwise comparisons: Z statistic (adjusted p-value)
-------------------------------
ACE - GEL : -2.168242 (0.0603)
ACE - HYD :  0.311603 (0.4120)
GEL - HYD :  2.479845 (0.0338)
ACE - IMI :  0.385176 (0.3938)
GEL - IMI :  2.553418 (0.0320)
HYD - IMI :  0.073573 (0.4841)
ACE - IND :  0.458749 (0.3878)
GEL - IND :  2.626991 (0.0310)
HYD - IND :  0.147146 (0.4675)
IMI - IND :  0.073573 (0.4707)
ACE - MEA :  1.938867 (0.0859)
GEL - MEA :  4.107109 (0.0007)*
HYD - MEA :  1.627263 (0.1244)
IMI - MEA :  1.553690 (0.1353)
IND - MEA :  1.480117 (0.1470)
ACE - SOL : -1.008384 (0.2350)
GEL - SOL :  1.159858 (0.2014)
HYD - SOL : -1.319987 (0.1601)
IMI - SOL : -1.393560 (0.1471)
IND - SOL : -1.467133 (0.1423)
MEA - SOL : -2.947251 (0.0144)*
ACE - SUC :  1.027357 (0.2381)
GEL - SUC :  3.137769 (0.0102)*
HYD - SUC :  0.724064 (0.3247)
IMI - SUC :  0.652453 (0.3305)
```

```
IND - SUC :  0.580843 (0.3484)
MEA - SUC : -0.859797 (0.2807)
SOL - SUC :  2.008846 (0.0802)
ACE - WAT : -1.445494 (0.1405)
GEL - WAT :  0.722747 (0.3132)
HYD - WAT : -1.757098 (0.1014)
IMI - WAT : -1.830671 (0.0930)
IND - WAT : -1.904244 (0.0853)
MEA - WAT : -3.384361 (0.0064)*
SOL - WAT : -0.437110 (0.3844)
SUC - WAT : -2.434298 (0.0336)
```

D6.5 - Feeding results - outlier included

Kruskal-Wallis and Dunn’s test with Benjamini-Hochberg adjustment for time spent feeding on the contents of a bait station **Including significant outlier (ant that got stuck in Imidacloprid Gel treatment)**. In order to account for multiple testing, the p-value must be lower than the alpha level provided next to it. (ACE = acetamiprid, GEL = imidacloprid gel, HYD = hydramethylnon, IMI = imidacloprid granular, IND = indoxacarb, SOL = sucrose solution, SUC = granular sucrose, WAT = water, MEA = chopped mealworms).

```
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 45.9413, df = 8, p-value = 0

Dunn’s test with Benjamini-Hochberg adjustment at alpha = 0.05

List of pairwise comparisons: Z statistic (adjusted p-value)
-------------------------------
ACE - GEL : -2.866206 (0.0075)*
ACE - HYD :  1.151678 (0.1796)
GEL - HYD :  4.017884 (0.0003)*
ACE - IMI :  0.381006 (0.3616)
GEL - IMI :  3.247212 (0.0026)*
HYD - IMI : -0.770671 (0.2737)
ACE - IND :  1.662572 (0.0913)
GEL - IND :  4.528778 (0.0001)*
HYD - IND :  0.510894 (0.3324)
IMI - IND :  1.281566 (0.1565)
ACE - MEA :  0.666760 (0.2932)
GEL - MEA :  3.532967 (0.0012)*
HYD - MEA : -0.484917 (0.3323)
```

```
IMI - MEA :  0.285754 (0.3875)
IND - MEA : -0.995811 (0.2129)
ACE - SOL : -2.195115 (0.0362)
GEL - SOL :  0.671090 (0.3013)
HYD - SOL : -3.346793 (0.0021)*
IMI - SOL : -2.576121 (0.0138)*
IND - SOL : -3.857688 (0.0004)*
MEA - SOL : -2.861876 (0.0069)*
ACE - SUC :  2.146401 (0.0382)
GEL - SUC :  4.936161 (0.0000)*
HYD - SUC :  1.025440 (0.2113)
IMI - SUC :  1.775557 (0.0758)
IND - SUC :  0.528172 (0.3360)
MEA - SUC :  1.497424 (0.1151)
SOL - SUC :  4.282970 (0.0001)*
ACE - WAT : -0.939526 (0.2234)
GEL - WAT :  1.926679 (0.0572)
HYD - WAT : -2.091204 (0.0411)
IMI - WAT : -1.320533 (0.1527)
IND - WAT : -2.602099 (0.0139)*
MEA - WAT : -1.606287 (0.0974)
SOL - WAT :  1.255588 (0.1569)
SUC - WAT : -3.060869 (0.0044)*
```

D7 - Pesticide Mass Data

Data on the mass of the insecticides during the experiment was also collected for each test box and for a dummy box (for each pesticide) with no ants present. The mass data might indicate how the pesticides responded to environmental conditions (i.e. absorption of water from or loss of water to the atmosphere). Alternatively, a significant loss in mass could have indicated that the pesticide had been eaten by the ants. The balance was accurate to +/- 0.001g

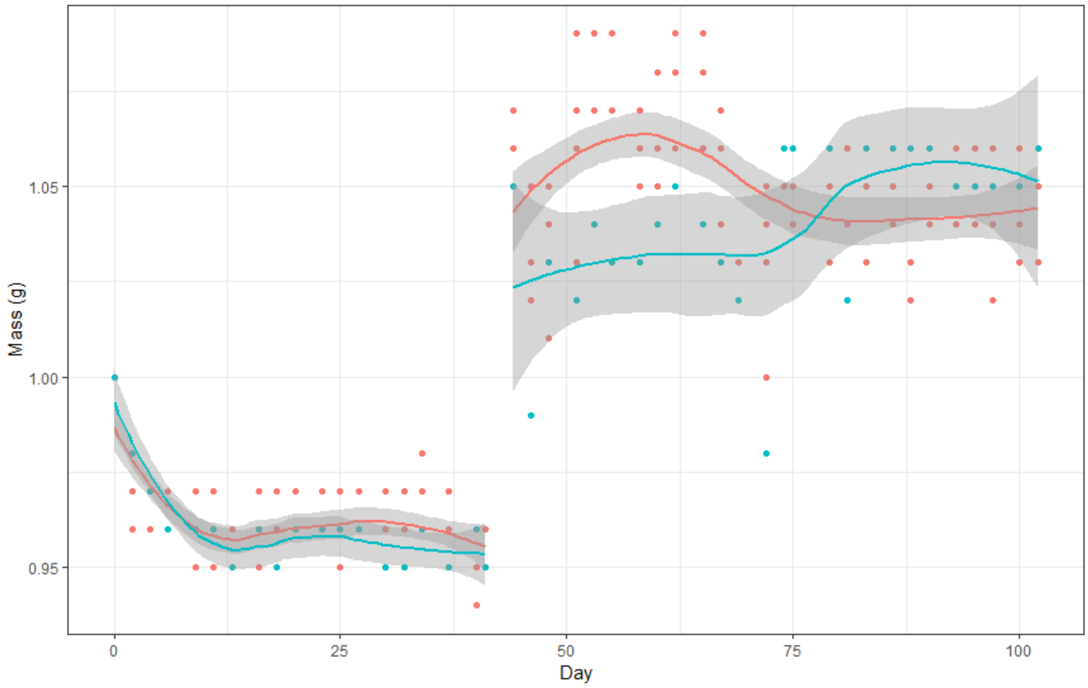


Figure D7.1 - Acetamiprid mass over the duration of the experiment. One ant-free box shown in blue and four ant-present boxes shown in red. Smooth fits for each group represented by the corresponding colour, low humidity phase on the left and high humidity (including starvation) on the right

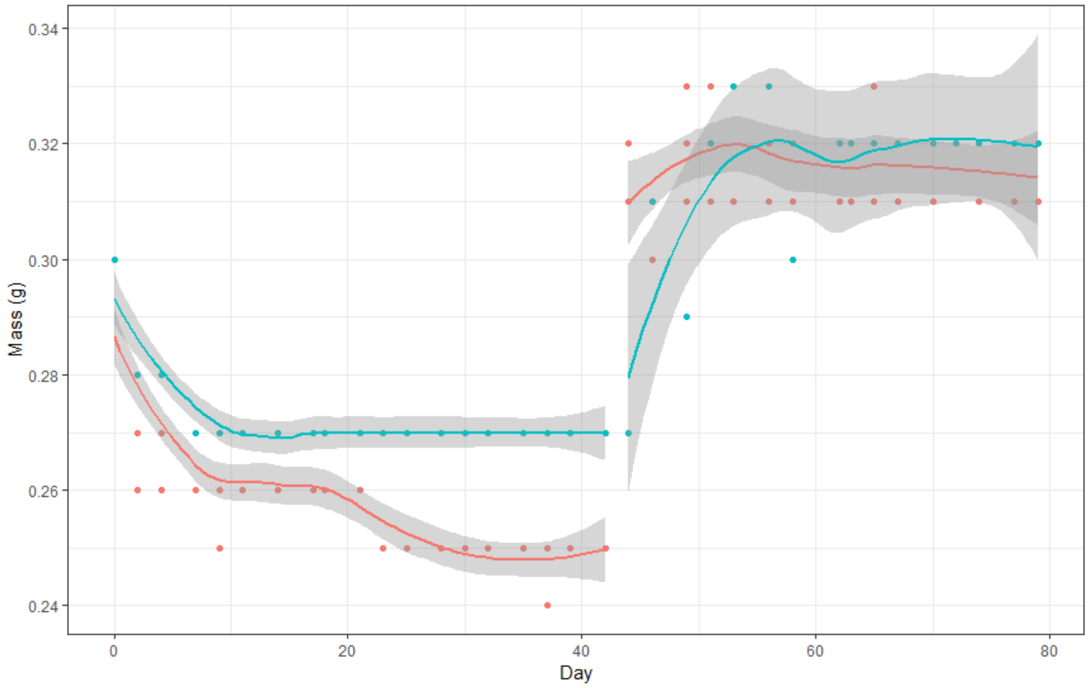


Figure D7.2 - Imidacloprid Gel mass over the duration of the experiment. One ant-free box shown in blue and four ant-present boxes shown in red. Smooth fits for each group represented by the corresponding colour, low humidity phase on the left and high humidity (including starvation) on the right. (If colony extinct mass data was not collected).

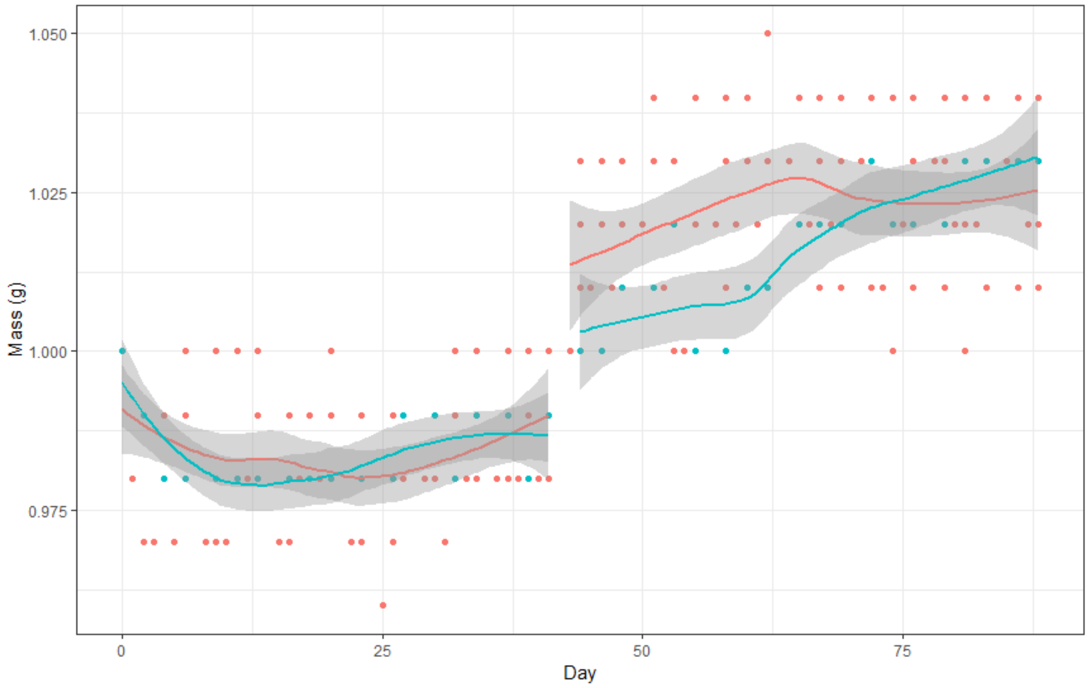


Figure D7.3 - Hydramethylnon mass over the duration of the experiment. One ant-free box shown in blue and four ant-present boxes shown in red. Smooth fits for each group represented by the corresponding colour, low humidity phase on the left and high humidity (including starvation) on the right

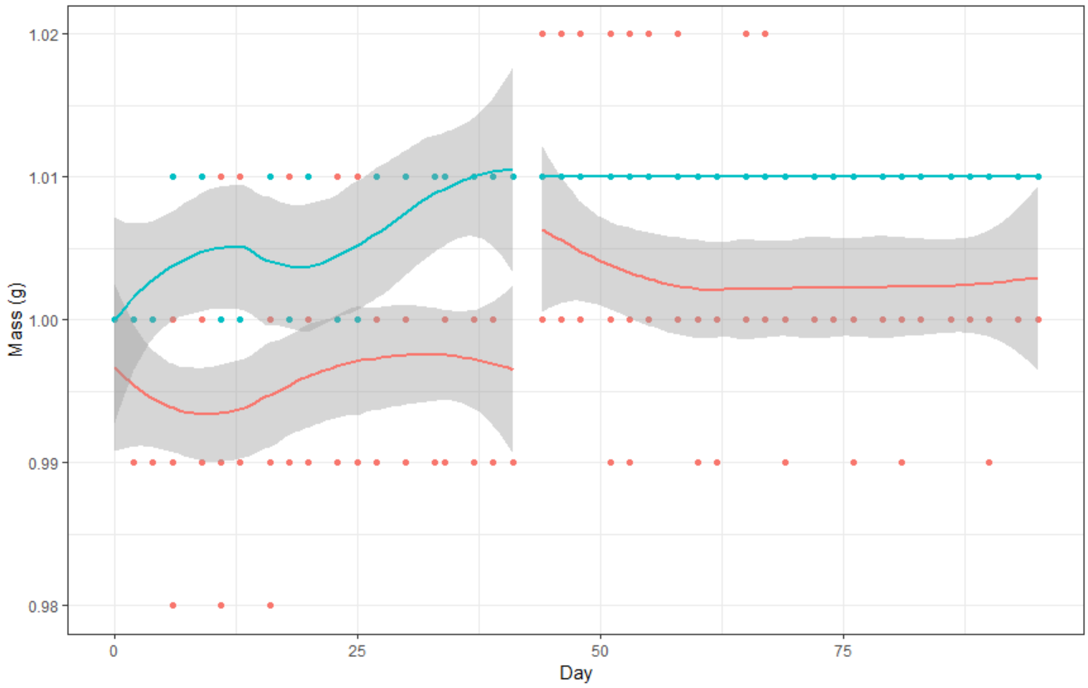


Figure D7.4 - Imidacloprid granular mass over the duration of the experiment. One ant-free box shown in blue and four ant-present boxes shown in red. Smooth fits for each group represented by the corresponding colour, low humidity phase on the left and high humidity (including starvation) on the right

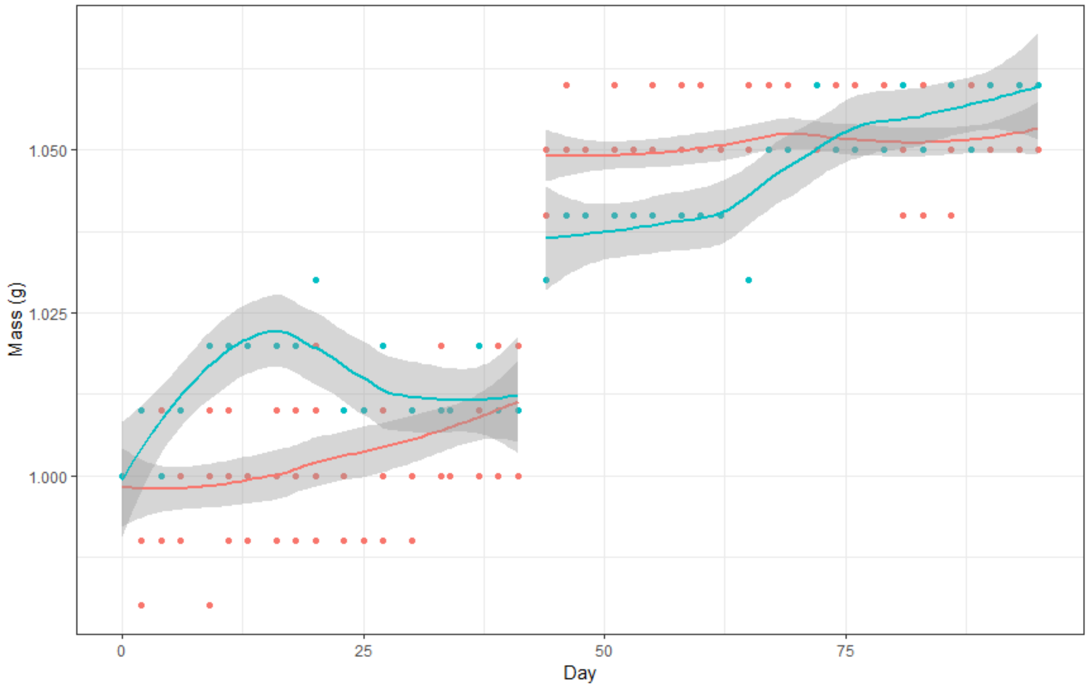


Figure D7.5 - Indoxacarb mass over the duration of the experiment. One ant-free box shown in blue and four ant-present boxes shown in red. Smooth fits for each group represented by the corresponding colour, low humidity phase on the left and high humidity (including starvation) on the right

Appendix E (supplement to Chapter 4 – Hydrogel Experiment)

Introduction

Water-absorbing crystals (hydrogels) are commercially available for use by gardeners to improve the water storage capacity of soil in plant pots. These crystals have been used to deliver soluble insecticides and aqueous sugar attractants for control of the Argentine ant *Linepithema humile* (Boser et al., 2014; Buczkowski et al., 2014a, 2014b). These crystals absorb liquids and present a moist surface film upon which the ants can feed. If *Lasius neglectus* will feed from polyacrylamide crystals, this delivery method could combine the implementation ease a granular product with the palatability of a gel.

Aim

To test whether *Lasius neglectus* workers will feed from commercially available "water absorbing crystals."

Materials and Methods

We tried two brands of commercially available polyacrylamide "water absorbing crystals". One was a Miracle-Gro product and the other was a Wilko own brand. The crystals were soaked in a 50% honey solution for ten minutes before being placed in a clean weighboat. The soaked crystals were then maintained in a room at 20°C (+/- 2) and approximately 25% relative humidity. The crystals were placed in a bait station in a foraging arena and ten starved extranest *Lasius neglectus* workers were transferred to the foraging arena using a cocktail stick. This was performed for each crystal type, within 30 minutes, 7 days and 15 days after hydration. Ten ants were tested per treatment per time point. Only one replicate was performed per treatment but this included multiple crystals. The presence/absence of feeding behaviour was recorded.

Results

Hydration increases the size of the crystals considerably and they become gelatinous in texture. The ants fed from both brands of crystal within 30 minutes after hydration, 7 days after hydration and 15 days after hydration (Figure E1). The Miracle-Gro product absorbed more liquid and appeared to attract more ants at the two later time steps but these differences were not quantified. Recruitment pheromone deposition behaviour was also observed by some of the ants feeding at each type of crystal within 30 minutes of hydration.

Figure E1 – Two *Lasius neglectus* workers feeding on Miracle-Gro crystals within 30 minutes after hydration with 50% honey solution. A video demonstrating the feeding behaviour is also available.

Discussion

We were not able to add pesticide to the hydrated crystals, as this is not currently a legally approved method of pesticide delivery in the UK. However, our results show that the ants are willing to feed from hydrated crystals, acting as a 'proof of concept' for the idea that this method could be used to control *Lasius neglectus*. The ants' willingness to feed from the crystals persisted for two weeks after hydration despite the crystals being stored in a warm and low humidity environment. This is promising for outdoor use, especially as, if used outside, the crystals would naturally rehydrate when it rains. When gel products are left outside, a dry skin can from preventing consumption by the ants (Buckham-Bonnett pers obs.). We suggest the long-term effectiveness of the crystals will be greater than the gel, because they have been designed to rehydrate and store water.

Next steps

As the crystals are highly likely to be an effective delivery mechanism it is worth trialling them with a range of different active insecticidal ingredients to identify the most effective pesticide (see Hoffmann et al., 2016). Our results from the main experiment (see Chapter 4) suggest that unpalatability, rather than lack of toxicity is responsible for the ineffectiveness of the pesticides that we tested in granular form, so these remain possibilities. Imidacloprid is effective when administered in gel form (see main text) so is a promising candidate for future work with hydrogels.

References

Boser, C.L., Hanna, C., Faulkner, K.R., Cory, C., Randall, J.M., Morrison, S.A., 2014. Argentine Ant Management in Conservation Areas: Results of a Pilot Study. Monogr. West. North Am. Nat. 7, 518–530.

Buczkowski, G., Roper, E., Chin, D., 2014a. Polyacrylamide Hydrogels: An Effective Tool for Delivering Liquid Baits to Pest Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 107, 748–757.

Buczkowski, G., Roper, E., Chin, D., Mothapo, N., Wossler, T., 2014b. Hydrogel baits with low-dose thiamethoxam for sustainable Argentine ant management in commercial orchards. Entomol. Exp. Appl. 153, 183–190.

Hoffmann, B.D., Luque, G.M., Bellard, C., Holmes, N.D., Donlan, C.J., 2016. Improving invasive ant eradication as a conservation tool: A review. Biol. Conserv. 198, 37–49.

Appendix F (supplement to Chapter 5)

Full Results

Table F1- List of sites that returned sets of traps with the ant species identified. Each set comprised 4 traps; the number of traps from each site returning each species is also shown. Some sites received two sets of traps; in these cases the site name is followed by "(A)" or "(B)". Some traps contained few individual ants and in some cases these specimens were too damaged to identify to species level. These are either reported at the sub-family level or, where possible for *Lasius* ants, reported as a morphological species complex (denoted by *s.l.*). Date deployed or deployment duration are given as "NA" when the information was not supplied by the site. OSGB36 grid references are supplied for each site at a 1km resolution if possible. If the site occurs in multiple 1km grid squares, a 10km grid reference is provided. If the site is on the boundary of 10km squares, all 10km squares in which it occurs are supplied.

Site	**Ant taxa (present in number of traps)**	**Date deployed**	**Deployment duration (days)**	**Grid References**
Attingham Park	*Lasius niger* (3); Myrmicinae (1)	15/09/16	5	SJ50, SJ51
Avebury Manor and Garden	*Lasius niger* (2)	08/09/16	5	SU0969
Bedgebury National Pinetum and Forest	No ants	06/10/16	7	TQ73
Belton House	*Lasius niger* (2)	15/09/16	2	SK9239
Benmore Botanic Garden	No ants	17/10/16	10	NS18
Benthall Hall	*Myrmica rubra* (1)	13/10/16	3	SJ6502
Berrington Hall	*Myrmica rubra* (2)	19/09/16	8	SO56
Biddulph Grange Garden	No ants	06/09/16	3	SJ8959
Birmingham Botanic Gardens	*Lasius niger* (1)	10/10/16	7	SP0485

Blickling Estate	No ants	01/10/16	26	TG12
Bodnant Garden	No ants	06/09/16	7	SH77, SH87
Branklyn Garden	No ants	05/10/16	15	NO12
Canons Ashby	*Myrmica rubra* (2)	NA	NA	SP5750
Castle Drogo	*Myrmica ruginodis* (2)	06/09/16	5	SX79
Charlecote Park	*Lasius niger* (3); *Myrmica rubra* (1)	28/09/16	3	SP2556
Chartwell House	No ants	NA	NA	TQ4551
Chelsea Physic Garden	No ants	13/10/16	4	TQ2777
Chirk Castle	No ants	NA	NA	SJ23
Cliveden	*Lasius niger* (2)	NA	NA	SU98
Colby Woodland Garden	*Lasius niger* (1)	12/09/16	2	SN1508
Cotehele	*Lasius niger* (2); Myrmicinae (1)	NA	NA	SX4268
Cragside	*Lasius niger* (2)	19/08/16	14	NU0702
Croft Castle	*Lasius niger* (1); *Myrmica rubra* (1)	01/10/16	2	SO4465
Dawyck Botanic Garden	No ants	10/10/16	6	NT1635
Dudmaston	*Lasius niger* (3)	04/09/16	2	SO7488
Dunham Massey	*Myrmica ruginodis* (1)	20/09/16	5	SJ7387
Durham University Botanic Garden	*Lasius niger* (1)	30/09/16	4	NZ2740
Dyrham Park	*Lasius niger* (1); *Myrmecina graminicola* (1)	10/10/16	12	ST7475
East Riddlesden Hall	No ants	15/09/16	4	SE0842
Felbrigg Hall	*Lasius niger* (3)	20/09/16	4	TG1939

Fenton House	No ants	18/10/16	6	TQ2686
Florence Court	*Myrmica rubra* (1); *Myrmica ruginodis* (1)	20/09/16	NA	NV20, SA20
Fountains Abbey & Studley Royal Water Garden	*Myrmica rubra* (1)	14/09/16	5	SE26
Gibside	*Lasius niger* (1)	02/10/16	9	NZ1758
Godolphin	*Lasius niger* (2)	06/09/16	3	SW6031
Greenway	Myrmicinae (1)	14/09/16	5	SX85
Greys Court	No ants	11/11/16	5	SU7283
Gunby Estate	*Lasius niger* (2); *Myrmica rubra* (1)	20/09/16	5	TF4666
Hanbury Hall	*Lasius niger* (4)	13/09/16	7	SO9463
Hardy's Birthplace	*Lasius niger* (1); *Myrmecina graminicola* (1)	08/09/16	7	SY7292
Hare Hill	No ants	06/09/16	6	SJ8776
Hatchlands Park	*Myrmecina graminicola* (1)	01/10/16	9 or 23	TQ05
Hidcote Manor Garden (A)	*Lasius neglectus* (4)	NA	NA	SP1742
Hidcote Manor Garden (B)	*Lasius neglectus* (4)	05/10/16	14	SP1742
Hinton Ampner	*Lasius niger* (2); *Myrmica ruginodis* (1); Myrmicinae (2)	08/09/16	4	SU5927
Ickworth	No ants	06/10/16	7	TL86
Ightham Mote	*Lasius niger* (3); *Myrmica scabrinodis* (1)	07/09/16	4	TQ5853
Inverewe gardens (NTS)	*Myrmica rubra* (1)	11/10/16	4	NG8681
Isabella Plantation (Richmond Park)	No ants	06/10/16	15	TQ17, TQ27

Killerton	*Lasius niger* (2)	09/09/16	5	SS9700
Kingston Lacy (A)	*Lasius niger* (3); Myrmicinae (2)	06/09/16	NA	ST90
Kingston Lacey (B)	No ants	06/09/16	NA	ST90
Knightshayes (A) - Kitchen Garden	*Lasius niger* (1); *Myrmica ruginodis* (1)	26/10/16	7	SS91
Knightshayes (B) - Woodland Garden	No ants	26/10/16	7	SS91
Lacock Abbey	*Lasius niger* (1); Myrmicinae (1); *Myrmecina graminicola* (1)	05/09/16	4	ST96
Lanhydrock	*Lasius niger* (2); *Myrmica scabrinodis* (1)	NA	NA	SX0863
Lytes Cary Manor	*Lasius flavus* (1); *Lasius flavus s.l.* (1); *Lasius niger* (2); *Lasius sabularum s.l.* (1); *Myrmica ruginodis* (1); *Myrmica scabrinodis* (1)	NA	NA	ST5326
Montacute House	*Myrmecina graminicola* (1)	19/10/16	5	ST4917
Morden Hall Park	*Tapinoma sp.* (4)	19/09/16	2	TQ2668
Mount Stewart (County Down)	*Myrmica rubra* (1); *Myrmica scabrinodis* (1)	09/09/16	4	NW6723
Mount Stuart Trust Gardens (Isle of Bute)	No ants	17/10/16	14	NS16
Myddelton House Gardens	*Myrmica rubra* (1); Myrmicinae (1)	25/10/16	3	TQ3499
Nostell	No ants	09/09/16	7	SE4017
Nymans	*Lasius niger* (1)	09/09/16	6	TQ2629
Overbeck's	*Lasius niger* (1); *Myrmica ruginodis* (1); *Tetramorium caespitum* (1)	12/09/16	4	SX7237
Oxburgh Hall	*Lasius niger* (3); *Myrmica scabrinodis* (1)	14/09/16	26	TF7401

Packwood House	*Lasius niger* (3)	06/09/16	3	SP1772
Peckover House	*Lasius niger* (2)	06/09/16	2	TF4509
Petworth	*Lasius niger* (2); *Myrmica rubra* (1); *Myrmica ruginodis* (1)	29/09/16	4	SU92
Plant Conservation Centre (National Trust)	No ants	26/09/16	4	SY09
Polesden Lacey	*Lasius niger* (1); *Myrmica scabrinodis* (1)	21/10/16	5	TQ1352
Powis Castle	*Lasius flavus* (1); *Lasius flavus s.l.* (1)	05/09/16	3	SJ2106
Quarry Bank	No ants	05/09/16	NA	SJ8383
RBG Kew	*Lasius niger* (2); Myrmicinae (1)	21/10/16	10	TQ17
RBGE Inverleith Edinburgh	*Tetramorium bicarinatum* (3); *Myrmica ruginodis* (1)	05/10/16	5	NT2475
Red House	*Lasius niger* (4)	07/09/16	14	TQ4875
RHS Harlow Carr	*Formica lemani* (1)	NA	NA	SE25
RHS Hyde Hall	*Lasius niger* (2)	08/10/16	7	TQ79
RHS Rosemoor	No ants	25/10/16	37	SS41
RHS Wisley	No ants	07/10/16	3	TQ05
Rowallane Gardens	*Formica fusca* (1); *Myrmica ruginodis* (2); Myrmicinae (1)	06/09/16	6	NW51
Rufford Old Hall	No ants	13/09/16	4	SD41
Saltram	*Myrmecina graminicola* (1)	14/09/16	5	SX5255
Scotney Castle	No ants	28/09/16	5	TQ6835
Sir Harold Hillier Gardens	*Myrmica ruginodis* (1)	07/10/16	5	SU32
Sizergh	No ants	06/10/16	4	SD4987
Snowshill Manor	*Lasius niger* (1); *Myrmica rubra* (1)	09/09/16	5	SP0933

St Andrews Botanic Gardens	*Lasius niger* (1)	11/10/16	6	NO5016
The Courts Garden	*Lasius flavus* (1); *Lasius niger* (3)	NA	NA	ST86
The Weir Garden	*Myrmica ruginodis* (1)	05/09/16	3	SO44
Tintinhull House and Gardens	*Lasius niger* (2); *Myrmica rubra* (1); *Myrmica ruginodis* (1)	29/09/16	4	ST5019
Tredegar House	No ants	04/10/16	3	ST2885
Tregrehan Gardens	*Lasius niger* (3)	03/10/16	11	SX0553
University of Dundee Botanic Gardens	*Lasius niger* (1)	07/10/16	6	NO32
Uppark House	*Myrmica rubra* (1); Myrmicinae (1)	13/10/16	6	SU71
The Vyne	*Lasius flavus* (1); *Lasius niger* (2)	20/10/16	5	SU65
Westbury Court Garden	*Lasius niger* (3); *Myrmica scabrinodis* (1)	19/10/16	2	SO7113
Westonbirt Arboretum	*Myrmica rubra* (1)	05/10/16	5	ST88
Wightwick Manor	*Lasius niger* (1); Myrmicinae (1)	NA	NA	SO8698
Wimpole Estate	*Lasius niger* (1); *Myrmica rubra* (1)	06/09/16	9	TL35
Winkworth Arboretum	*Lasius niger* (2); *Myrmica ruginodis* (2)	07/09/16	5	SU94
Winterbourne House and Garden	No ants	12/10/16	5	SP0583
Wordsworth House	*Lasius niger* (3)	23/09/16	4	NY1130

Preliminary survey - manual search

Aim

The aim of this survey was to assess the likelihood of finding non-native ants, particularly *Lasius neglectus,* in botanic gardens.

Methods

In 2015 ten sites were manually searched for the presence of ants. The search focussed on detecting *L. neglectus* or other non-native species (see table F2). Each site was searched for approximately one hour. Search effort was concentrated on the warmer areas of each site (open areas with exposed soil, a southerly aspect or glasshouses) as these areas are often favoured by ants in the UK. If the ants could be reached from a path, voucher specimens were taken for later identification.

Results and Discussion

Ants were detected at all sites visited except Dawyck Botanic Garden and non-native ant species were found at five of the sites (table F2). Native ants were identified to species level, non-native ants were identified to genus or species where possible. *Lasius neglectus* was found only at the Cambridge University Botanic Gardens, a site where its presence had already been confirmed. The results suggest that non-native ant species frequently occur in botanic gardens. Further surveying of sites with extensive plant collections may reveal new locations where *L. neglectus* is present.

Table F2 - Ant species detected by manual searching from ten UK sites with large plant collections in 2015. Non-native species are indicated in bold.

Site	**Date**	**Ant species found**
University of Cambridge Botanic Gardens	17/01/15	***Lasius neglectus*** *Lasius niger* ***Plagiolepis* sp.**
Plant Hunters Garden Pitlochry	03/09/15	*Myrmica ruginodis*
St Andrews Botanic Garden	04/09/15	*Lasius niger*
University of Dundee Botanic Gardens	04/09/15	*Lasius niger*
Royal Botanic Gardens Edinburgh	05/09/15	*Myrmica ruginodis* *Lasius niger* ***Tetramorium* sp. *(bicarinatum?)***
Bicton Park Botanic Gardens	08/09/15	*Lasius niger* *Myrmecina graminicola* ***Technomyrmex* sp. *(albipes?)***
Godinton House	10/09/15	*Lasius niger*
Oxford University Botanic Gardens	11/09/15	*Lasius niger* ***Plagiolepis* sp.**
Dawyck Botanic Gardens	22/09/15	None
Durham University Botanic Gardens	01/10/15	*Formica lemani*

Appendix G (supplement to Chapter 5)

Ant Survey – Guidance Notes

Thank you very much for agreeing to take part in the ant survey. The following provides guidance, but do contact me directly if you have any questions.

Equipment supplied:

- Black plastic ant traps.
- Adhesive card inserts for traps (no harmful components).
- Tube of honey, and spoon.
- Plastic bags
- Instructions and recording form.
- Stamps and address labels.

Selecting areas to place the monitors

Select warm, sunlit areas as these are preferred by ants. Here are some suggestions:

- Anywhere where numbers of small, active, dark-coloured ants have been seen.
- Under plants or trees that are prone to aphids or ants.
- Greenhouses.
- Rockeries.
- Base of sun-warmed south or west facing walls or buildings, where there is soil and vegetation at the foot of the wall.

Preparing the monitors

- Open the hinged lid of the black plastic monitor.
- Remove the protective film from an adhesive card and insert it, adhesive side up, into the plastic case. First slide it under the lugs inside the front of the monitor, and then rest it on the pins at the rear of the monitor.
- Using the spoon, put a small blob of honey in each monitor, **so it straddles the adhesive insert and the plastic case (i.e. runs off the edge of the adhesive insert).**
- Close the lid of the monitor.

Placing the monitors

- Place the monitor flat on the ground, out of sight of the public.
- Rest a stone or brick on top of the monitor, to protect and conceal it from animals, visitors, the weather etc.

Retrieving and despatching the monitors

- After 2 – 5 days, pick up the monitors and seal them individually in a plastic bag, *immediately each one is picked up*. **Place the bagged monitor in a freezer (below 0°c) for a minimum of 12 hours.**
- Place the bagged monitors in the card box, together with the completed form, and seal with tape. Stick the return address label and stamps on the box, and post.

Questions?

Contact: Phillip - pbb502@york.ac.uk

Thank you very much for your help

Ant trap location form

Site…………………………………….

Date monitors put down...

Date monitors collected..

Monitor number (on inside of lid)	**Location**
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

Please return this completed form with the traps. Thank you.

Appendix H (Ant-plant interactions)

Preliminary work and a novel experimental method to investigate the effect of *Lasius neglectus* on plant performance

Introduction

One of the main reasons invasive species are of concern to policy makers is the potential for significant negative impacts on economically important plants (Pimentel et al., 2005). Through their interactions with honeydew-producing insects such as aphids, the presence of ants can result in a reduction in the fecundity of human crop plants (Styrsky and Eubanks, 2007). Where *Lasius neglectus* has been studied in Europe, strong mutualisms with aphids have been reported and there are qualitative reports of these interactions having detrimental impacts on the aphids' host plants (Espadaler and Rey, 2001). In addition to effects resulting from the interactions with aphids, ants can also exploit plants directly (e.g. via extra floral nectaries) or conversely have a positive impact by reducing herbivore numbers (Lach, 2003; Stanley et al., 2012). Here we present a novel protocol for assessing the impact of ants on plant growth and reproduction in a field-based context.

Methods

Broad bean plants (*Vicia faba*) were germinated in a greenhouse and pairs were matched for size. Each plant was transferred to a 15-litre plant pot containing compost. These were placed on upturned plant saucers to reduce the potential variation from the pots coming into contact with different amounts or types of soil underneath. In each pair, one saucer had its underside painted with Fluon® and was placed on an X shaped wooden stand. The stand raised the saucer off the ground and the Fluon® prevented ants from accessing the plant (see figure H1). Each plant was seeded with five black bean aphids (*Aphis fabae*) each. Fifteen pairs of plants were deployed at *Lasius neglectus* sites and fifteen were deployed at *Lasius niger* sites. All the *Laisius neglectus* plant pairs were sited in an allotment (containing a mixture of grass and exposed soil). The *Lasius niger* plant pairs were split between two sites (a sheep meadow and a field margin) approximately 340m and 830m from the allotment respectively. Eighty-six days after being planted out the bean plants were collected and stored in paper bags. They were then later dried at 70^{0}c for 24 hours to determine their dry mass.

Figure H1 – Ant access (left pot) and ant excluded (right pot) plant pair design. The underside of the tray on the right is coated with Fluon® to prevent ants from accessing the plant. *Lasius neglectus* plant pair, Hidcote 2015.

Analysis

Analyses were performed in R (R Core Team, 2013). The plant mass data does not follow a Gaussian distribution so non-parametric methods were used to compare central tendency between groups. Type I errors were accounted for by using the False Discovery Rate approach (Benjamini and Hochberg, 1995). This approach calculates a new alpha-level for each comparison when these are ranked in order of magnitude. Null hypotheses can only be rejected when the p-value is less than or equal to the new alpha-level. New alpha levels were calculated to give an overall type I error rate for all comparisons of 5%.

Results

Broad bean plants (*Vicia faba*) visited by *Lasius neglectus* have a lower somatic, above-ground biomass than plants visited by *Lasius niger* (see Table H1, first row and Figure H2). There is an almost significant trend for the somatic, above-ground biomass of broad bean plants to be lower in the *Lasius neglectus* ant attended group than the paired control group without ant attendance (see Table H1, second row and Figure H2). There was no difference in mass between the two non ant-attended control groups (see Table H1, third row and Figure H2) or between the *Lasius niger*, ant attended group than the paired control group without ant attendance (see Table H1, fourth row and Figure H2). The number of beans produced did not vary between the four treatment groups (X^2 = 0.185, d.f. = 1, p = 0.667.

Table H1 – Statistical analysis of plant somatic above ground biomass between treatment groups.

Comparison	Test	Statistic	p-value	α - level
Native, ants: Invasive, ants	Wilcoxon rank sum test with continuity correction	W= 25.5	<0.001	0.0125
Invasive, no ants: Invasive, ants	Wilcoxon signed rank test	V=95	0.048	0.0250
Native, no ants : Invasive, no ants	Wilcoxon rank sum test with continuity correction	W = 73	0.106	0.0375
Native, no ants: Native, ants	Wilcoxon signed rank test	V = 48	0.525	0.0500

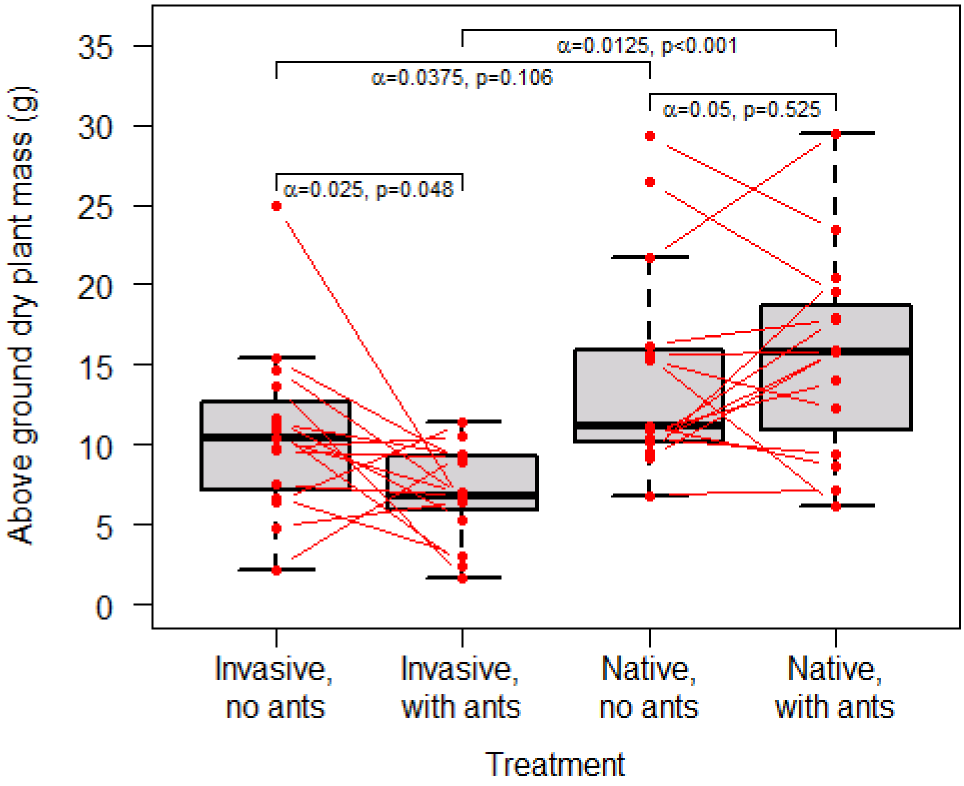


Figure H2 - Somatic above ground biomass in treatment groups. Paired data are linked by red lines and a False Discovery Rate approach is used to adjust alpha levels for multiple comparisons.

Discussion and Further Work

The results from this preliminary experiment suggested that *Lasius neglectus* might have a significant negative impact on plant growth but did not directly measure how the ants are impacting the plants so further work is required to elucidate this. In addition, the preliminary results suggest that a larger sample size may give a clearer indication of the potential difference in plant performance when tended by either the native or the non-native ant species. A power analysis using these effect sizes suggests that a sample size of 23 should be sufficient for investigating this relationship with a type II error rate of 5% (see table H2).

Table H2 – Power analysis based on preliminary data to determine required sample size for an acceptable type II error rate.

Comparison	Cohen’s d (effect size from prelim experiment)	Sample size (of each of the four group) required (for t-test) with a type II error rate of:				
		5%	10%	15%	20%	25%
Native, ants: Invasive, ants	1.317	17	13	12	11	10
Invasive, no ants: Invasive, ants	0.795	23	19	17	15	14
Native, no ants: Invasive, no ants	0.627	68	55	47	41	37
Native, no ants: Native, ants	0.159	516	418	358	313	277

Stock fencing or a similar approach should be used to protect the plants if this experiment is performed in field-based setting (see H3). Using data loggers to collect microclimate data would be an advantage as would detail observations of the ant’s behaviour and counts of aphid abundance. Whilst performing these experiments in the field provides a more realistic ecological context, it may be that field conditions are too variable to elucidate weak impacts of the ants’ behaviour in which case a laboratory-based approach might be preferable.

Figure H3 – Stock fencing to prevent sheep damaging experiment. *Lasius niger* plant pair Hidcote, 2015.

Acknowledgements

We would like to thank Hidcote Manor (National Trust) and John Righton for allowing us to perform these experiments on their land

References

Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57, 289–300.

Espadaler, X., Rey, S., 2001. Biological constraints and colony founding in the polygynous invasive ant *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 48, 159–164.

Lach, L., 2003. Invasive ants: unwanted partners in ant-plant interactions? Ann. Missouri Bot. Gard. 90, 91–108.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288.

R Core Team, 2013. R: A language and environment for statistical computing.

Stanley, M.C., Nathan, H.W., Phillips, L.K., Knight, S.J., Galbraith, J. a., Winks, C.J., Ward, D.F., 2012. Invasive interactions: can Argentine ants indirectly increase the reproductive output of a weed? Arthropod. Plant. Interact. 7, 59–67.

Styrsky, J.D., Eubanks, M.D., 2007. Ecological consequences of interactions between ants and honeydew-producing insects. Proc. Biol. Sci. 274, 151–64.

Appendix I (*Lasius neglectus* informational outputs)

Video

I produced a short video with information about *Lasius neglectus* in the UK. It can be found at the following link or QR code:

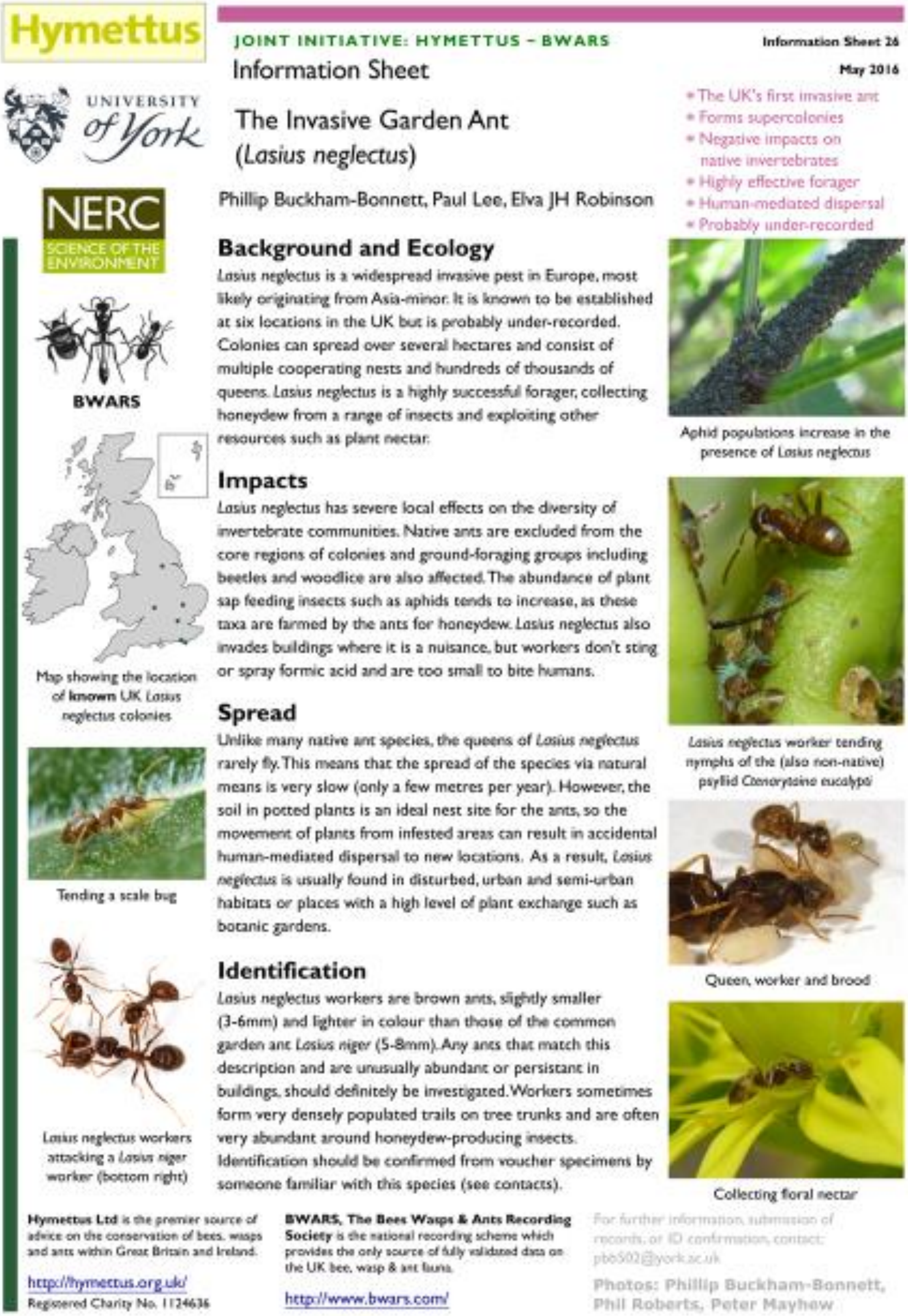
https://www.youtube.com/watch?v=oUG6g4-I1no

ID and information sheets

I produced an information sheet and an ID sheet about *Laisus neglectus* for BWARS/ Hymettus. Copies are in this appendix and they are also hosted on the BWARS website: https://www.bwars.com/ant/formicidae/formicinae/lasius-neglectus

Magazine Article

I also wrote a short article about *Lasius neglectus* for *Professional Pest Controller* Magazine. The article was the cover feature of that edition and the British Pest Control Association developed a short CPD module for those in the industry from it. A copy is below and it can also be found on the BPPCA website: https://bpca.org.uk/write/MediaUploads/Documents/PPC%20Back%20Issues/5596_BPCA_-_PPC87_DESKTOP_PRINT.pdf



Hymettus

JOINT INITIATIVE: HYMETTUS – BWARS

Information Sheet 26

May 2016

Information Sheet

The Invasive Garden Ant (*Lasius neglectus*)

Phillip Buckham-Bonnett, Paul Lee, Elva JH Robinson

- The UK's first invasive ant
- Forms supercolonies
- Negative impacts on native invertebrates
- Highly effective forager
- Human-mediated dispersal
- Probably under-recorded

Background and Ecology

Lasius neglectus is a widespread invasive pest in Europe, most likely originating from Asia-minor. It is known to be established at six locations in the UK but is probably under-recorded. Colonies can spread over several hectares and consist of multiple cooperating nests and hundreds of thousands of queens. *Lasius neglectus* is a highly successful forager, collecting honeydew from a range of insects and exploiting other resources such as plant nectar.

Aphid populations increase in the presence of *Lasius neglectus*

Impacts

Lasius neglectus has severe local effects on the diversity of invertebrate communities. Native ants are excluded from the core regions of colonies and ground-foraging groups including beetles and woodlice are also affected. The abundance of plant sap feeding insects such as aphids tends to increase, as these taxa are farmed by the ants for honeydew. *Lasius neglectus* also invades buildings where it is a nuisance, but workers don't sting or spray formic acid and are too small to bite humans.

Map showing the location of **known** UK *Lasius neglectus* colonies

Lasius neglectus worker tending nymphs of the (also non-native) psyllid *Ctenarytaina eucalypti*

Spread

Unlike many native ant species, the queens of *Lasius neglectus* rarely fly. This means that the spread of the species via natural means is very slow (only a few metres per year). However, the soil in potted plants is an ideal nest site for the ants, so the movement of plants from infested areas can result in accidental human-mediated dispersal to new locations. As a result, *Lasius neglectus* is usually found in disturbed, urban and semi-urban habitats or places with a high level of plant exchange such as botanic gardens.

Tending a scale bug

Queen, worker and brood

Identification

Lasius neglectus workers are brown ants, slightly smaller (3-6mm) and lighter in colour than those of the common garden ant *Lasius niger* (5-8mm). Any ants that match this description and are unusually abundant or persistant in buildings, should definitely be investigated. Workers sometimes form very densely populated trails on tree trunks and are often very abundant around honeydew-producing insects. Identification should be confirmed from voucher specimens by someone familiar with this species (see contacts).

Lasius neglectus workers attacking a *Lasius niger* worker (bottom right)

Collecting floral nectar

Hymettus Ltd is the premier source of advice on the conservation of bees, wasps and ants within Great Britain and Ireland.

http://hymettus.org.uk/

Registered Charity No. 1124636

BWARS, The Bees Wasps & Ants Recording Society is the national recording scheme which provides the only source of fully validated data on the UK bee, wasp & ant fauna.

http://www.bwars.com/

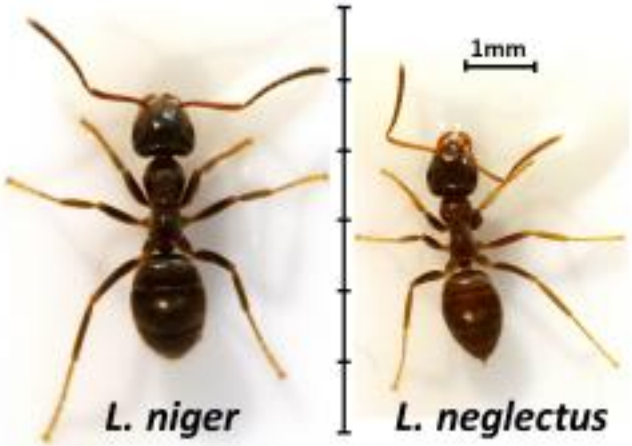
For further information, submission of records, or ID confirmation, contact: pbb502@york.ac.uk

Photos: Phillip Buckham-Bonnett, Phil Roberts, Peter Mayhew

Identifying the invasive garden ant (*Lasius neglectus*)

Phillip Buckham-Bonnett, Paul Lee, Elva JH Robinson   contact: pbb502@york.ac.uk

Field Characteristics



L. niger ***L. neglectus***

- Smaller than *L. niger*
- Lighter colour
- Often higher abundance

L. neglectus workers foraging on silver birch

Separating *L. neglectus* from UK *Lasius*

1. Body has a uniform brown colour (not yellow or black)

Lasius niger

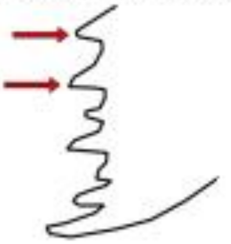
Lasius neglectus

2. Erect hairs on antennal scape and hind tibia absent in *L. neglectus*

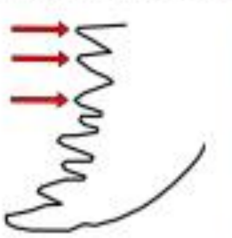
Antennal hairs and body colour separate *L. neglectus* from all UK *Lasius sp.* except *L. alienus* and *L. psammophilus* (which have a very different ecology). These can be eliminated by considering the dentition of the ants. The dentition can be variable (see photograph), so it is important to check multiple specimens.

3. Usually 7 teeth per mandible and 2 larger teeth at basal end. (c.f. 8 and 3) **Check at least 5 specimens.**

Typical *L. neglectus*



Typical *L. alienus*



Hymettus UNIVERSITY *of York* NERC SCIENCE OF THE ENVIRONMENT

Further information:
www.bwars.com/ant/formicidae/formicinae/lasius-neglectus

This image: CC antweb.org   All other images: Phillip Buckham-Bonnett

An online CPD quiz based on this feature is now available on the BPCA website.
Each quiz is worth three PROMPT CPD points
– register to take part at
www.bpca.org.uk/affiliate

LASIUS NEGLECTUS
THE INVASIVE GARDEN ANT

LASIUS NEGLECTUS IS A HIGHLY SUCCESSFUL FORAGER, COLLECTING HONEYDEW FROM A RANGE OF INSECTS AND EXPLOITING OTHER RESOURCES SUCH AS PLANT NECTAR

We thought it was time to have a proper look at one of the newest pests on the block: Lasius neglectus.What is the impact on the environment, how do you spot them and can we treat them? Phillip Buckham-Bonnett from the University of York shares some of his research with PPC.

BACKGROUND AND ECOLOGY

The invasive garden ant (Lasius neglectus) is a widespread invasive pest in Europe, most likely originating from Asia-minor. It is known to be established at seven locations in the UK as far apart as Yorkshire and East Sussex and is probably under-recorded. Unlike the native garden ant where a colony consists of a single nest with a single queen, Lasius neglectus colonies can have multiple queens per nest and many nests per colony. This allows colonies to spread over huge areas (12 hectares for the largest known UK colony) and be made up of thousands of cooperating nests and hundreds of thousands of queens.

Lasius neglectus is a highly successful forager, collecting or 'farming' honeydew from a range of insects and exploiting other resources such as plant nectar. It will readily form mutualisms with native and other non-native honeydew producing insects but is highly aggressive towards native ants. Lasius neglectus will often form very dense columns when foraging on trees, a behaviour that marks it out from native ant species.

IMPACTS

Lasius neglectus has severe local effects on the diversity of invertebrate communities. Native ants are excluded from the core regions of colonies and ground-foraging groups including beetles and woodlice are also negatively affected. Conversely, the abundance of plant sap-feeding insects such as aphids tends to increase dramatically as these taxa are farmed by the ants for honeydew and in return receive protection from ladybirds and other predators. There is some evidence that the presence of Lasius neglectus can result in an abundance of aphids, which in turn damages plants. Large aphid colonies are certainly extremely frustrating for gardeners.

Lasius neglectus readily invades buildings in large numbers, and while the workers don't sting or spray formic acid, and are too small to bite humans, the sheer abundance of ants can cause significant distress to the building's occupants. The ants are very happy living under paving slabs and in cavity walls but will also nest in plug sockets and electrical goods. The build up of ants in these places can lead to short circuits damaging the electrical equipment. Over time this can result in thousands of pounds worth of damage.

SPREAD

'Flying ant day' is probably familiar to most pest controllers. This is the period in late summer when queens of many native ant species disperse through flight to found new colonies, alarming people across the country. However, unlike many native ant species, the queens of Lasius neglectus rarely fly. The morphology of


Foraging on a silver birch


Queen and worker tending the brood


Aphids are farmed by the ants for honeydew

queens suggests that they are capable of flight, but this is rarely observed (I'm only aware of two instances). Instead, new colonies are usually founded through a process called budding. When a colony buds, a queen (or queens) and some workers from an existing nest depart on foot to found a new nest. Fortunately, from a human perspective, this means that the spread of the species via natural means is very slow (only a few metres per year).

However, Lasius neglectus often turns up at a novel location far from existing colonies, and this is due to accidental human-mediated dispersal. The ants will readily nest in the soil around potted plants, and the movement of these from an infected site can easily transport workers and importantly queens to a new site. The movement of large quantities of soil, building material and potentially even garden waste could also contribute to dispersal.

As a result of these dispersal patterns, Lasius neglectus is usually found in disturbed, urban areas or places with a high level of plant exchange such as botanic gardens, rather than natural sites. Most of the UK records are from urban/ semi-urban areas, and pest controllers have played a vital role in detecting many of these colonies.

PEST CONTROL RESPONSE

The sheer size of Lasius neglectus colonies and the number of queens they contain makes controlling them a significant challenge. Eliminating a few queens or even a few nests will not solve the problem. No eradication attempts of well-established colonies have ever been successful, but it is possible to reduce ant populations in and around buildings. The treatment of affected areas with a 0.03% imidacloprid ant gel can result in up to a 90% reduction in ant numbers (see Boase, 2014). Granular products may be less effective due to a low palatability resulting from the species' preference for liquid food sources.

CONTINUED >

SPEED VIEW

- A new invasive pest, Ladius neglectus is active in seven sites right across England, but spread is slow
- Colonies are massive, with multiple queens, forming dense columns when travelling
- If allowed to thrive, Ladius neglectus can short-circuit electrical machinery
- Invasive garden ants farm nectar and honeydew, and are aggressive to other ants and ground-foraging insects
- Lasius neglectus is usually found in disturbed, urban areas or places with a high level of plant exchange such as botanic gardens, rather than natural sites

Other invasive ants in the UK

While currently not as abundant as Lasius neglectus, other non-native ant species are found in the UK some of which have the potential to have even more serious impacts.

The Argentine ant (Linepthema humili) is native to South America and is invasive on several continents. Colonies have been reported from the London area. These ants also look very similar to the native garden ant but are slightly smaller and rather than having a round acidopore at the tip of their abdomen, the opening is a slit and is not encircled by hairs.

Ants from the Tapinoma nigerimmum group are native to Mediterranean Europe but are invasive at several countries in northern Europe. One species in the group (Tapinoma ibericum) is invasive in the UK and currently known from the Isle of Wight. These small dark ants can be identified from their deep clypeal notch and have two worker casts (large and small individuals).

Many other non-native ants species can be found in the UK, but these tend to be species that can only survive in buildings or very warm environments such as glasshouses. Examples include: pharaoh ants (Monomorium pharaonis); cork ants (Crematogaster scutellaris); white-footed ants (Technomyrmex albipes); and ghost ants (Tapinoma melanocephalum).

LASIUS NEGLECTUS
THE INVASIVE GARDEN ANT


L neglectus with a supply of nectar


Attending a scale bug


Lasius niger (left) compared to L neglectus

The Non-Native Species Secretariat is producing an 'advice for homeowners' leaflet **www.nonnativespecies.org**, and this can be used provide information for people living or working in affected areas. It is also vital to inform the relevant authorities if a suspected case of Lasius neglectus is detected. The feasibility of a national eradication programme is currently being considered, and the abundance of Lasius neglectus in the UK will directly affect this. Knowing where colonies are is also important for implementing strategies to reduce their spread.

IDENTIFICATION

The invasive garden ant is superficially similar to the native garden ant (Lasius niger) and, unfortunately, this means it is easily overlooked (hence the name 'neglectus'). However, workers of the invasive species are slightly smaller and slightly paler brown. When encountering any ants that match this description or are unusually abundant or persistent in a location at least five specimens should be collected, and their identity checked. An identification sheet is available on the Bees, Wasps and Ants Recording Society (BWARS) website or contact the author who is very happy to check specimens.

IDENTIFICATION SHEET

https://goo.gl/jpqYwS

WHAT TO DO IF YOU THINK YOU HAVE FOUND LASIUS NEGLECTUS

Please report any suspected cases of Lasius neglectus (or other non-native ants) by emailing...

✉ pbb502@york.ac.uk

References

This is a complete list for the whole thesis. Relevant references also appear at the end of each section for convenience.

Abbott, K.L., 2006. Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. Divers. Distrib. 12, 101–110.

Abbott, K.L., Green, P.T., 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. Oikos 116, 1238–1246.

Akino, T., Yamamura, K., Wakamura, S., Yamaoka, R., 2004. Direct behavioral evidence for hydrocarbons as nestmate recognition cues in *Formica japonica* (Hymenoptera: Formicidae). Appl. Entomol. Zool. 39, 381–387.

Alkassab, A.T., Kirchner, W.H., 2017. Sublethal exposure to neonicotinoids and related side effects on insect pollinators: honeybees, bumblebees, and solitary bees. J. Plant Dis. Prot. 124, 1–30.

Aron, S., Beckers, R., Deneubourg, J.L., Pasteels, J.M., 1993. Memory and chemical communication in the orientation of two mass-recruiting ant species. Insectes Soc. 40, 369–380.

Attewell, 2011. *Myrmica* Scapes [WWW Document]. www.bwars.com. URL http://www.bwars.com/sites/www.bwars.com/files/diary_downloads/myrmica-scabrinodis-group-scapes-images.pdf

Auguie, B., 2017. gridExtra: Miscellaneous Functions for “Grid” Graphics.

Baker, R.H.A., Black, R., Copp, G.H., Haysom4, K.A., Hulme, P.E., Thomas, M.B., Brown, A., Brown, M., Cannon, R.J.C., Ellis, J., Ellis, M., Ferris, R., Glaves, P., Gozlan, R.E., Holt, J., Howe, L., Knight, J.D., MacLeod, A., Moore, N.P., Mumford, J.D., Murphy, S.T., Parrott, D., Sansford, C.E., Smith, G.C., St-Hilaire, S., Ward, N.L., 2008. The UK risk assessment scheme for all non-native species. Biol. Invasions– from Ecol. to Conserv. 7, 46–57.

Baker, T.C., Van Vorhis Key, S.E., Gaston, L.K., 1985. Bait-preference Tests for the Argentine Ant (Hymenoptera: Formicidae). J. Econ. Entomol. 78, 1083–1088.

Balbuena, M.S., Molinas, J., Farina, W.M., 2012. Honeybee recruitment to scented food sources: Correlations between in-hive social interactions and foraging decisions. Behav. Ecol. Sociobiol. 66, 445–452.

Banks, C.J., Macaulay, E.D.M., 1967. Effects of *Aphis fabae* Scop, and of its attendant ants and insect predators on yields of field beans (*Vicia faba* L.). Ann. Appl. Biol. 60, 445–453.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using {lme4}. J. Stat. Softw. 67, 1–48.

Bates, D., Maechler, M., Bolker, B., Al, E., 2014. lme4: Linear mixed-effects models using Eigen and S4.

Beckers, R., Deneubourg, J.L., Goss, S., 1993. Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. J. Insect Behav. 6, 751–759.

Beckers, R., Deneubourg, J.L., Goss, S., Pasteels, J.M., 1990. Collective decision making through food recruitment. Insectes Soc. 37, 258–267.

Beckers, R., Goss, S., Deneubourg, J.L., Pasteels, J.M., 1989. Colony size, communication, and ant foraging strategy. Psyche (Stuttg). 96, 239–256.

Beggs, J., 2001. The ecological consequences of social wasps (*Vespula* spp.) invading an ecosystem that has an abundant carbohydrate resource. Biol. Conserv. 99, 17–28.

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate : A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57, 289–300.

Bertelsmeier, C., Blight, O., Courchamp, F., 2016. Invasions of ants (Hymenoptera : Formicidae) in light of global climate change. Myrmecological News 22, 25–42.

Bertelsmeier, C., Luque, G.M., Hoffmann, B.D., Courchamp, F., 2015a. Worldwide ant invasions under climate change. Biodivers. Conserv. 24, 117–128.

Bertelsmeier, C., Ollier, S., Avril, A., Blight, O., Jourdan, H., Courchamp, F., 2015b. Colony-colony interactions between highly invasive ants. Basic Appl. Ecol. 17, 106–114.

Bhatkar, A., Whitcomb, W.H., 1970. Artificial Diet for Rearing Various Species of Ants. Florida Entomol. 53, 229.

Boase, C., 2014. *Lasius neglectus* (Hymenoptera : Formicidae) in the UK : status, impact and management. In: Proceedings of the Eighth International Conference on Urban Pests. pp. 223–228.

Bonavita-Cougourdan, A., Morel, L., 1984. Les activités antennaires au cours des contacts trophallactiques chez la fourmi *Camponotus vagus* Scop. Ont-elles valeur de signal? Insectes Soc. 31, 113–131.

Boomsma, J.J., Brouwer, A.H., Van Loon, A.J., 1990. A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe. Insectes Soc. 37, 363–375.

Booy, O., Mill, A.C., Roy, H.E., Hiley, A., Moore, N., Robertson, P., Baker, S., Brazier, M., Bue, M., Bullock, R., Campbell, S., Eyre, D., Foster, J., Hatton-Ellis, M., Long, J., Macadam, C., Morrison-Bell, C., Mumford, J., Newman, J., Parrott, D., Payne, R., Renals, T., Rodgers, E., Spencer, M., Stebbing, P., Sutton-Croft, M., Walker, K.J., Ward, A., Whittaker, S., Wyn, G., 2017. Risk management to prioritise the eradication of new and emerging invasive non-native species. Biol. Invasions 19, 2401–2417.

Boser, C.L., Hanna, C., Faulkner, K.R., Cory, C., Randall, J.M., Morrison, S.A., 2014. Argentine Ant Management in Conservation Areas: Results of a Pilot Study. Monogr. West. North Am. Nat. 7, 518–530.

Bradshaw, C.J.A., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., Barbet-Massin, M., Salles, J.M., Simard, F., Courchamp, F., 2016. Massive yet grossly underestimated global costs of invasive insects. Nat. Commun. 7, 1–8.

Buckham-Bonnett, P., Aguión, A., Boase, C., Lee, P., Robinson, E.J.H., n.d. Evaluating the palatability and toxicity of commercially available pesticides for large scale use on the Invasive Garden ant (*Lasius neglectus*).

Buckham-Bonnett, P., Lee, P., Robinson, E.J.H., 2016. Identifying the invasive garden ant (*Lasius neglectus*) [WWW Document]. URL http://bwars.com/sites/www.bwars.com/files/info_sheets/L.neglectus_ID_sheet_v2.pdf

Buckham-Bonnett, P., Robinson, E.J.H., 2017. GB Non-native Species Rapid Risk Assessment : Rapid Risk Assessment of: *Lasius neglectus* (Invasive Garden Ant).

Buczkowski, G., Roper, E., Chin, D., 2014a. Polyacrylamide Hydrogels: An Effective Tool for Delivering Liquid Baits to Pest Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 107, 748–757.

Buczkowski, G., Roper, E., Chin, D., Mothapo, N., Wossler, T., 2014b. Hydrogel baits with low-dose thiamethoxam for sustainable Argentine ant management in commercial orchards. Entomol.

Exp. Appl. 153, 183–190.

Byers, J.E., 2002. Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. Oikos 97, 449–458.

Colautti, R.I., MacIsaac, H.J., 2004. A neutral terminology to define 'invasive' species. Divers. Distrib. 10, 135–141.

Collett, M., Chittka, L., Collett, T.S., 2013. Spatial memory in insect navigation. Curr. Biol.

Comont, R.F., Roy, H.E., Harrington, R., Shortall, C.R., Purse, B. V., 2014. Ecological correlates of local extinction and colonisation in the British ladybird beetles (Coleoptera: Coccinellidae). Biol. Invasions 16, 1805–1817.

Cook, S.C., Eubanks, M.D., Gold, R.E., Behmer, S.T., 2011. Seasonality directs contrasting food collection behavior and nutrient regulation strategies in ants. PLoS One 6, e25407.

Cremer, S., Ugelvig, L. V, Drijfhout, F.P., Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Hughes, D.P., Schulz, A., Petersen, K.S., Konrad, H., Stauffer, C., Kiran, K., Espadaler, X., d'Ettorre, P., Aktaç, N., Eilenberg, J., Jones, G.R., Nash, D.R., Pedersen, J.S., Boomsma, J.J., 2008. The evolution of invasiveness in garden ants. PLoS One 3, e3838.

Cremer, S., Ugelvig, L. V, Lommen, S.T.E., Petersen, K.S., Pedersen, J.S., 2006. Attack of the invasive garden ant: aggression behaviour of *Lasius neglectus* (Hymenoptera: Formicidae) against native *Lasius* species in Spain. Myrmec. Nachr 9, 13–19.

Crowley, S.L., Hinchliffe, S., McDonald, R.A., 2017. Conflict in invasive species management. Front. Ecol. Environ. 15, 133–141.

Crowther, L.P., Hein, P.L., Bourke, A.F.G., 2014. Habitat and forage associations of a naturally colonising insect pollinator, the Tree Bumblebee Bombus hypnorum. PLoS One 9.

Czaczkes, T.J., Castorena, M., Schürch, R., Heinze, J., 2017. Pheromone trail following in the ant *Lasius niger*: high accuracy and variability but no effect of task state. Physiol. Entomol. 42, 91–97.

Czaczkes, T.J., Czaczkes, B., Iglhaut, C., Heinze, J., 2015a. Composite collective decision-making. Proc. R. Soc. B Biol. Sci. 282.

Czaczkes, T.J., Grüter, C., Ellis, L., Wood, E., Ratnieks, F.L.W., 2013. Ant foraging on complex trails: route learning and the role of trail pheromones in *Lasius niger*. J. Exp. Biol. 216, 188–97.

Czaczkes, T.J., Grüter, C., Jones, S.M., Ratnieks, F.L.W., 2011. Synergy between social and private information increases foraging efficiency in ants. Biol. Lett. 7, 521–4.

Czaczkes, T.J., Grüter, C., Ratnieks, F.L.W., 2015b. Trail Pheromones: An Integrative View of Their Role in Social Insect Colony Organization. Annu. Rev. Entomol. 60, 581–599.

Czaczkes, T.J., Heinze, J., 2015. Ants adjust their pheromone deposition to a changing environment and their probability of making errors. Proc. R. Soc. B Biol. Sci. 282, 20150679-.

Czaczkes, T.J., Salmane, A.K., Klampfleuthner, F.A.M., Heinze, J., 2016. Private information alone can trigger trapping of ant colonies in local feeding optima. J. Exp. Biol. 219, 744–751.

Czaczkes, T.J., Schlosser, L., Heinze, J., Witte, V., 2014. Ants use directionless odour cues to recall odour-associated locations. Behav. Ecol. Sociobiol. 68, 981–988.

Czechowska, W., Czechowski, W., 2003. Further record of *Lasius neglectus* Van Loon, Boomsma et Andrasflavy (Hymenoptera:Formicidae) for Warsaw, with a key to the Polish species of the subgenus Lasius s.str. Fragm. faun. 46, 195–202.

Dacke, M., Srinivasan, M. V., 2008. Evidence for counting in insects. Anim. Cogn. 11, 683–689.

Daniels, M.J., Beaumont, M.A., Johnson, P.J., Balharry, D., Macdonald, D.W., Barratt, E., 2001.

Ecology and genetics of wild-living cats in the north-east of Scotland and the implicaitons for the conservation of the wildcat. J. Appl. Ecol. 38, 146–161.

Depa, Ł., Kaszyca-Taszakowska, N., Taszakowski, A., Kanturski, M., 2020. Ant-induced evolutionary patterns in aphids. Biol. Rev.

Department of Environment Food and Rural Affairs, 2018. Biodiversity 2020: A strategy for England's wildlife and ecosystem services Indicators.

Devigne, C., Detrain, C., 2006. How does food distance influence foraging in the ant *Lasius niger*: the importance of home-range marking. Insectes Soc. 53, 46–55.

Dinno, A., 2017. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums.

Drinkwater, E., Robinson, E.J.H., Hart, A.G., 2019. Keeping invertebrate research ethical in a landscape of shifting public opinion. Methods Ecol. Evol. 2041–210X.13208.

Duggan, J.M., Heske, E.J., Schooley, R.L., Hurt, A., Whitelaw, A., 2011. Comparing detection dog and livetrapping surveys for a cryptic rodent. J. Wildl. Manage. 75, 1209–1217.

Dussutour, A., Deneubourg, J.L., Fourcassié, V., 2005. Amplification of individual preferences in a social context: The case of wall-following in ants. Proc. R. Soc. B Biol. Sci. 272, 705–714.

EEA, 2012. The impacts of invasive alien species in Europe. EEA Technical report, No 16/2012.

Espadaler, X., 1999. *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy, 1990 (Hymenoptera, Formicidae), a potential pest ant in Spain. Orsis 14, 43–46.

Espadaler, X., Bernal, V., 2003. Exotic ants in the Canary Islands (Hymenoptera, Formicidae). Vieraea 31, 1–7.

Espadaler, X., Bernal, V., 2007. *Lasius neglectus*. Control Trials. [WWW Document]. URL http://www.creaf.uab.es/xeg/Lasius/Ingles/control.htm (accessed 2.18.16).

Espadaler, X., Bernal, V., 2015. *Lasius neglectus* a polygynous, sometimes invasive, ant: Distribution [WWW Document]. URL http://www.creaf.uab.es/xeg/lasius/Ingles/distribution.htm (accessed 2.2.16).

Espadaler, X., Bernal, V., 2018. *Lasius neglectus* Distribution [WWW Document]. URL http://www.creaf.uab.es/xeg/Lasius/Ingles/distribution.htm (accessed 11.20.18).

Espadaler, X., Lebas, C., Wagenknecht, J., Tragust, S., 2011. *Laboulbenia formicarum* (Ascomycota, Laboulbeniales), an exotic parasitic fungus, on an exotic ant in France. Vie Milieu 61, 41–44.

Espadaler, X., Rey, S., 2001. Biological constraints and colony founding in the polygynous invasive ant *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 48, 159–164.

Espadaler, X., Rey, S., Bernal, V., 2004. Queen number in a supercolony of the invasive garden ant, *Lasius neglectus*. Insectes Soc. 51, 232–238.

Espadaler, X., Santamaria, S., 2003. *Laboulbenia formicarum* Thaxt. (Ascomycota, Laboulbeniales) crosses the Atlantic. Orsis (Org. Sist.) 18, 97–101.

Espadaler, X., Tartally, A., Schultz, R., Seifert, B., Nagy, C., 2007. Regional trends and preliminary results on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera, Formicidae). Insectes Soc. 54, 293–301.

European Commission 2007/393/EC, 2007. Commission decision of 6 June 2007 concerning the non-inclusion of diazinon in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance.

Evans, T.A., Forschler, B.T., Grace, J.K., 2013. Biology of Invasive Termites: A Worldwide Review. Annu. Rev. Entomol. 58, 455–474.

Evison, S.E.F., 2008. Foraging Organisation in Ants. University of Sheffield.

Evison, S.E.F., Petchey, O.L., Beckerman, A.P., Ratnieks, F.L.W., 2008. Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. Behav. Ecol. Sociobiol. 63, 261–267.

Farina, W.M., Grüater, C., Díaz, P.C., 2005. Social learning of floral odours inside the honeybee hive. Proc. R. Soc. B Biol. Sci. 272, 1923–1928.

Forstmeier, W., Schielzeth, H., 2011. Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. Behav. Ecol. Sociobiol. 65, 47–55.

Fox, M., Wang, C., 2016. Colony of the Argentine Ant, *Linepithema humile* (Hymenoptera : Formicidae), in Fulham, West London. Br. J. Entomolgy Nat. Hist. 29, 193–195.

Fox, M.G., 2010. First incursion of Lasius neglectus (Hymenoptera: Formicidae), an invasive polygynous ant in Britian. Br. J. Ent. Nat. Hist. 23, 259–261.

Frizzi, F., Bartalesi, V., Santini, G., 2017. Combined effects of temperature and interspecific competition on the mortality of the invasive garden ant, Lasius neglectus: A laboratory study. J. Therm. Biol. 65, 76–81.

GADM, 2017. Database of Global Administrative Areas [WWW Document]. URL https://gadm.org/data.html

Gentz, M.C., 2009. A review of chemical control options for invasive social insects in island ecosystems. J. Appl. Entomol. 133, 329–335.

Gippet, J.M.W., Mondy, N., Diallo-Dudek, J., Bellec, A., Dumet, A., Mistler, L., Kaufmann, B., 2017. I'm not like everybody else: urbanization factors shaping spatial distribution of native and invasive ants are species-specific. Urban Ecosyst. 20, 157–169.

Godfray, H.C.J., Blacquière, T., Field, L.M., Hails, R.S., Petrokofsky, G., Potts, S.G., Raine, N.E., Vanbergen, A.J., McLean, A.R., 2014. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc. R. Soc. B Biol. Sci. 281.

Goodwin, K.M., Engel, R.E., Weaver, D.K., 2010. Trained Dogs Outperform Human Surveyors in the Detection of Rare Spotted Knapweed (*Centaurea stoebe*). Invasive Plant Sci. Manag. 3, 113–121.

Gordon, M., Lumley, T., 2017. forestplot: Advanced Forest Plot Using "grid" Graphics.

Goss, S., Aron, S., Deneubourg, J.L., Pasteels, J.M., 1989. Self-organized shortcuts in the Argentine ant. Naturwissenschaften 76, 579–581.

Green, P.T., O'Dowd, D.J., Abbott, K.L., Jeffery, M., Retallick, K., Mac Nally, R., 2011. Invasional meltdown: Invader-invader mutualism facilitates a secondary invasion. Ecology 92, 1758–1768.

Groom, Q., Desmet, P., Vanderhoeven, S., Adriaens, T., 2015. The importance of open data for invasive alien species research, policy and management. Manag. Biol. Invasions 6, 119–125.

Grüter, C., Balbuena, M.S., Farina, W.M., 2008. Informational conflicts created by the waggle dance. Proc. Biol. Sci. 275, 1321–7.

Grüter, C., Czaczkes, T.J., Ratnieks, F.L.W., 2011. Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav. Ecol. Sociobiol. 65, 141–148.

Gurevitch, J., Padilla, D.K., 2004. Are invasive species a major cause of extinctions? Trends Ecol. Evol. 19, 470–4.

Hara, A.H., Aoki, K.L., Cabral, S.K., Niino-DuPonte, R., 2014. Attractiveness of gel, granular, paste, and solid formulations of ant bait insecticides to the little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae). Proc. Hawaii. Entomol. Soc. 46, 45–54.

Harrell Jr, F.E., 2015. 4.4 Sample Size, Overfitting and Limits on Number of Predictors. In: Regression

Modeling Strategies. pp. 72–74.

Harrison, X.A., Donaldson, L., Correa-Cano, M.E., Evans, J., Fisher, D.N., Goodwin, C.E.D., Robinson, B.S., Hodgson, D.J., Inger, R., 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ 6, e4794.

Harrower, C.A., Rorke, S.L., Roy, H.E., 2019. England Biodiversity Indicators -Technical background document - 20. Pressure from invasive species.

Hee, J.J., Holway, D.A., Suarez, A. V., Case, T.J., 2000. Role of propagule size in the success of incipient colonies of the invasive Argentine ant. Conserv. Biol. 14, 559–563.

Heller, N.E., Ingram, K.K., Gordon, D.M., 2008. Nest connectivity and colony structure in unicolonial Argentine ants. Insectes Soc. 55, 397–403.

Herbers, J.M., Choiniere, E., 1996. Foraging behaviour and colony structure in ants. Anim. Behav. 51, 141–153.

Hill, L., Jones, G., Atkinson, N., Hector, A., Hemery, G., Brown, N., 2019. The £15 billion cost of ash dieback in Britain. Curr. Biol. 29, R315–R316.

Hoffmann, B.D., Abbott, K.L., Davis, P.D., 2010. Invasive Ant Management. In: Lach, L., Parr, C.L., Abbott, K.L. (Eds.), Ant Ecology. Oxford University Press.

Hoffmann, B.D., Andersen, A.N., Hill, G.J.E., 1999. Impact of an introduced ant on native rain forest invertebrates: *Pheidole megacephala* in monsoonal Australia. Oecologia 120, 595–604.

Hoffmann, B.D., Luque, G.M., Bellard, C., Holmes, N.D., Donlan, C.J., 2016. Improving invasive ant eradication as a conservation tool: A review. Biol. Conserv. 198, 37–49.

Hölldobler, B., 1971. Recruitment behavior in *Camponotus socius* (Hym. Formicidae). Z. Vgl. Physiol. 75, 123–142.

Hölldobler, B., 1976. Recruitment behavior, home range orientation and territoriality in harvester ants, *Pogonomyrmex*. Behav. Ecol. Sociobiol. 1, 3–44.

Hölldobler, B., Wilson, E.O., 1978. The multiple recruitment systems of the african weaver ant *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 3, 19–60.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Springer-Verlag Berlin Heidelberg.

Holway, D., Lach, L., Suarez, A., 2002. The causes and consequences of ant invasions. Annu. Rev. Ecol. Syst. 33, 181–233.

Hornung, E., Vilisics, F., Tartally, A., 2005. Occurrence of *Platyarthrus schoblii* (Isopoda, Oniscidea) and its ant hosts in Hungary. Eur. J. Soil Biol. 41, 129–133.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous Inference in General Parametric Models. Biometrical J. 50, 346–363.

Huang, D., Haack, R. a, Zhang, R., 2011. Does global warming increase establishment rates of invasive alien species? A centurial time series analysis. PLoS One 6, e24733.

Huber, P., 1810. Recherchez sur les Meœrs des Fourmis lndigenes. JJ Paschoud, Paris.

Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46, 10–18.

Hulme, P.E., 2017. Climate change and biological invasions: evidence, expectations, and response options. Biol. Rev. 92, 1297–1313.

Hulme, P.E., 2020. Plant invasions in New Zealand: global lessons in prevention, eradication and control. Biol. Invasions 22, 1539–1562.

Hulme, P.E., Brundu, G., Carboni, M., Dehnen-Schmutz, K., Dullinger, S., Early, R., Essl, F., González-Moreno, P., Groom, Q.J., Kueffer, C., Kühn, I., Maurel, N., Novoa, A., Pergl, J., Pyšek, P., Seebens, H., Tanner, R., Touza, J.M., van Kleunen, M., Verbrugge, L.N.H., 2018. Integrating invasive species policies across ornamental horticulture supply chains to prevent plant invasions. J. Appl. Ecol. 55, 92–98.

IPBES, 2019. Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.

Irwin, M.T., Wright, P.C., Birkinshaw, C., Fisher, B.L., Gardner, C.J., Glos, J., Goodman, S.M., Loiselle, P., Rabeson, P., Raharison, J.L., Raherilalao, M.J., Rakotondravony, D., Raselimanana, A., Ratsimbazafy, J., Sparks, J.S., Wilmé, L., Ganzhorn, J.U., 2010. Patterns of species change in anthropogenically disturbed forests of Madagascar. Biol. Conserv. 143, 2351–2362.

Jackson, D.E., Martin, S.J., Holcombe, M., Ratnieks, F.L.W., 2006. Longevity and detection of persistent foraging trails in Pharaoh's ants, *Monomorium pharaonis* (L.). Anim. Behav. 71, 351–359.

Jarić, I., Heger, T., Castro Monzon, F., Jeschke, J.M., Kowarik, I., McConkey, K.R., Pyšek, P., Sagouis, A., Essl, F., 2019. Crypticity in Biological Invasions. Trends Ecol. Evol. 34, 291–302.

Jolivet, P., 1986. Les fourmis et la télévision. L' Entomol. 42, 321–323.

Kassambara, A., Kosinski, M., 2018. survminer: Drawing Survival Curves using "ggplot2."

Keller, R.P., Geist, J., Jeschke, J.M., Kühn, I., 2011. Invasive species in Europe: ecology, status, and policy. Environ. Sci. Eur. 23, 23.

Kenis, M., Auger-Rozenberg, M.A., Roques, A., Timms, L., Péré, C., Cock, M.J.W., Settele, J., Augustin, S., Lopez-Vaamonde, C., 2009. Ecological effects of invasive alien insects. In: Ecological Impacts of Non-Native Invertebrates and Fungi on Terrestrial Ecosystems. pp. 21–45.

Kennedy, P., Baron, G., Qiu, B., Freitak, D., Helanterä, H., Hunt, E.R., Manfredini, F., O'Shea-Wheller, T., Patalano, S., Pull, C.D., Sasaki, T., Taylor, D., Wyatt, C.D.R., Sumner, S., 2017. Deconstructing Superorganisms and Societies to Address Big Questions in Biology. Trends Ecol. Evol.

Konrad, M., Grasse, A. V, Tragust, S., Cremer, S., 2015. Anti-pathogen protection versus survival costs mediated by an ectosymbiont in an ant host. Proc. Biol. Sci. 282, 20141976.

Lach, L., 2003. Invasive ants: unwanted partners in ant-plant interactions? Ann. Missouri Bot. Gard. 90, 91–108.

Lach, L., 2008. Floral visitation patterns of two invasive ant species and their effects on other hymenopteran visitors. Ecol. Entomol. 33, 155–160.

Lach, L., Parr, C., Abbott, K., 2010. Ant ecology. Oxford University Press.

Landau, I., Mueller, G., Schmidt, M., 2017. First occurrence of *Lasius neglectus* (Hymenoptera: Formicidae) in Zurich, Switzerland: distribution and control measures. In: Proceedings of the Ninth International Conference on Urban Pests. pp. 111–116.

Le Breton, J., Fourcassie, V., 2004. Information transfer during recruitment in the ant *Lasius niger* L. (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 55, 242–250.

Le Parisiene, 2015. Ardèche : un village envahi par les fourmis. Le Paris.

Lenoir, A., 1982. An informational analysis of antennal communication during trophallaxis in the ant *Myrmica Rubra* L. Behav. Processes 7, 27–35.

Lenoir, A., Depickère, S., Devers, S., Christidès, J.P., Detrain, C., 2009. Hydrocarbons in the ant *Lasius niger*: from the cuticle to the nest and home range marking. J. Chem. Ecol. 35, 913–21.

Lessard, J., Fordyce, J., Gotelli, N., Sanders, N., 2009. Invasive ants alter the phylogenetic structure of ant communities. Ecology 90, 2664–2669.

Lin, H., Chi, W., Lin, C., Tseng, Y., Chen, W., 2011. Fire Ant-Detecting Canines : A Complementary

Method in Detecting Red Imported Fire Ants. Househ. Struct. Insects 104, 225–231.

Linders, T.E.W., Schaffner, U., Eschen, R., Abebe, A., Choge, S.K., Nigatu, L., Mbaabu, P.R., Shiferaw, H., Allan, E., 2019. Direct and indirect effects of invasive species: Biodiversity loss is a major mechanism by which an invasive tree affects ecosystem functioning. J. Ecol. 107, 2660–2672.

Lindsjö, J., Fahlman, Å., Törnqvist, E., 2016. Animal Welfare From Mouse To Moose—Implementing the Principles of the 3Rs in Wildlife Research. J. Wildl. Dis. 52, S65–S77.

Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20, 223–228.

Lowe, S., Brownw, M., Boudjelas, S., De Poorter, M., 2000. 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. Invasive Species Spec. Gr. a Spec. Gr. Species Surviv. Comm. World Conserv. Union 12, 12.

Lüdecke, D., 2018. ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. J. Open Source Softw. 3, 772.

Lye, G.C., Osborne, J.L., Park, K.J., Goulson, D., 2012. Using citizen science to monitor Bombus populations in the UK: Nesting ecology and relative abundance in the urban environment. J. Insect Conserv. 16, 697–707.

Mabelis, A.A., van Loon, A.J., Dekoninck, W., 2010. Verovert de plaagmier Nederland? Entomol. Ber. 70(2), 30–36.

Mamiko, O., Ayako, W.-K., Kazuyo, F., Masayuki, I., Fumio, Y., Yuji, S., Tomoyosi, N., Ryohei, Y., 2005. Ant Nestmate and Non-Nestmate Discrimination by a Chemosensory Sensillum. Science (80-.). 309, 311–314.

McCabe, S., Farina, W.M., Josens, R.B., 2006. Antennation of nectar-receivers encodes colony needs and food-source profitability in the ant *Camponotus mus*. Insectes Soc. 53, 356–361.

McGlynn, T.P., 1999. The worldwide transfer of ants: Geographical distribution and ecological invasions. J. Biogeogr. 26, 535–548.

Mooney, H. a, Cleland, E.E., 2001. The evolutionary impact of invasive species. Proc. Natl. Acad. Sci. U. S. A. 98, 5446–51.

Mumford, J.D., Booy, O., BAKer, R.H.A., Rees, M., Copp, G.H., Black, K., Holt, J., Leach, A.W., Hartley, M., 2010. Invasive non-native species risk assessment in Great Britain. Asp. Appl. Biol. 104, 49–54.

Nagy, C., Tartally, A., Vilisics, F., Merkl, O., Szita, É., Szél, G., Podlussány, A., Rédei, D., Csösz, S., Pozsgai, G., Orosz, A., Szövényi, G., Markó, V., 2009. Effects of the invasive garden ant, *Lasius neglectus* VAN LOON, BOOMSMA & ANDRÁS-FALVY, 1990 (Hymenoptera: Formicidae), on arthropod assemblages: pattern analyses in the type supercolony. Myrmecological News 12, 171–181.

Nakagawa, S., 2004. A farewell to Bonferroni: The problems of low statistical power and publication bias. Behav. Ecol. 15, 1044–1045.

Ness, J., Bronstein, J., 2004. The effects of invasive ants on prospective ant mutualists. Biol. Invasions 6, 445–461.

NNSS, 2020. GB Non-native Species Secretariat - Risk Assessment [WWW Document]. URL http://www.nonnativespecies.org/index.cfm?pageid=143

Novgorodova, T. a., 2015. Organization of honeydew collection by foragers of different species of ants (Hymenoptera: Formicidae): Effect of colony size and species specificity. Eur. J. Entomol. 112, 688–697.

Novgorodova, T.A., 2006. Experimental investigation of information transmission in *Formica pratensis* (Hymenoptera, Formicidae) using “binary tree” maze. Entomol. Rev. 86, 287–293.

Nuñez, M.A., Moretti, A., Simberloff, D., 2011. Propagule pressure hypothesis not supported by an 80-year experiment on woody species invasion. Oikos 120, 1311–1316.

Paini, D.R., Sheppard, A.W., Cook, D.C., De Barro, P.J., Worner, S.P., Thomas, M.B., 2016. Global threat to agriculture from invasive species. Proc. Natl. Acad. Sci. U. S. A. 113, 7575–7579.

Paris, C.I., Espadaler, X., 2009. Honeydew collection by the invasive garden ant *Lasius neglectus* versus the native ant *L. grandis*. Arthropod. Plant. Interact. 3, 75–85.

Paris, C.I., Espadaler, X., 2012. Foraging Activity of Native Ants on Trees in Forest Fragments Colonized by the Invasive Ant *Lasius neglectus*. Psyche A J. Entomol. 2012, 1–9.

Pau, G., Fuchs, F., Sklyar, O., Boutros, M., Huber, W., 2010. EBImage---an R package for image processing with applications to cellular phenotypes. Bioinformatics 26, 979–981.

Pebesma, E.J., R.S.B., 2005. Classes and methods for spatial data in R. R news 5.

Pejchar, L., Mooney, H.A., 2009. Invasive species, ecosystem services and human well-being. Trends Ecol. Evol. 24, 497–504.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288.

Popp, S., 2015. Possibility of transfer of directional information via physical contact in *Lasius niger*. University of Würzburg.

Popp, S., Buckham-Bonnett, P., Evison, S.E.F., Robinson, E.J.H., Czaczkes, T.J., 2018. No evidence for tactile communication of direction in foraging *Lasius* ants. Insectes Soc. 65, 37–46.

Portha, S., Deneubourg, J.-L., Detrain, C., 2004. How food type and brood influence foraging decisions of *Lasius niger* scouts. Anim. Behav. 68, 115–122.

Pospischil, R., 2011. Role of tropical greenhouses for introduction and establishment of foreign ant species (Hymenoptera: Formicidae) in Central Europe. In: Proceedings of the Seventh International Conference on Urban Pests. pp. 59–66.

Prior, K.M., Adams, D.C., Klepzig, K.D., Hulcr, J., 2018. When does invasive species removal lead to ecological recovery? Implications for management success. Biol. Invasions 20, 267–283.

R Core Team, 2012. R: A Language and Environment for Statistical Computing.

R Core Team, 2013. R: A language and environment for statistical computing.

R Core Team, 2018. R: A Language and Environment for Statistical Computing.

Rabitsch, W., 2011. The hitchhiker’s guide to alien ant invasions. BioControl 56, 551–572.

Rey, S., Espadaler, X., 2004. Area-wide management of the invasive garden ant *Lasius neglectus* (Hymenoptera: Formicidae) in Northeast Spain. J. Agric. Urban Entomol. 21, 99–112.

Reznikova, Z., 2007. Dialog with black box: Using information theory to study animal language behaviour. Acta Ethol.

Reznikova, Z., 2008. Experimental paradigms for studying cognition and communication in ants (Hymenoptera: Formicidae). Myrmecol. News 11, 201–214.

Reznikova, Z., 2017. Studying animal languages without translation: An insight from ants, Studying Animal Languages without Translation: An Insight from Ants.

Reznikova, Z., Ryabko, B., 1994. Experimental study of the ants’ communication system, with the application of the information theory approach. Memorab. Zool. 48, 219–236.

Reznikova, Z.I., Ryabko, B., 2001. A study of ants’ numerical competence. Comput. Inf. Sci. 5, 111–126.

Ricciardi, A., Palmer, M.E., Yan, N.D., 2011. Should Biological Invasions Be Managed as Natural Disasters? Bioscience 61, 312–317.

Rice, E.S., Silverman, J., 2013. Propagule pressure and climate contribute to the displacement of *Linepithema humile* by *Pachycondyla chinensis*. PLoS One 8, e56281.

Robinson, E.J.H., Green, K.E., Jenner, E.A., Holcombe, M., Ratnieks, F.L.W., 2008. Decay rates of attractive and repellent pheromones in an ant foraging trail network. Insectes Soc. 55, 246–251.

Robinson, E.J.H., Jackson, D.E., Holcombe, M., Ratnieks, F.L.W., 2005. Insect communication: “no entry” signal in ant foraging. Nature 438, 442.

Roces, F., 1990. Olfactory conditioning during the recruitment process in a leaf-cutting ant. Oecologia 83, 261–262.

Roces, F., 1994. Odour learning and decision-making during food collection in the leaf-cutting ant *Acromyrmex lundi*. Insectes Soc. 41, 235–239.

Roy, H.E., Adriaens, T., Isaac, N.J.B., Kenis, M., Onkelinx, T., Martin, G.S., Brown, P.M.J., Hautier, L., Poland, R., Roy, D.B., Comont, R., Eschen, R., Frost, R., Zindel, R., Van Vlaenderen, J., Nedvěd, O., Ravn, H.P., Grégoire, J.-C., de Biseau, J.-C., Maes, D., 2012. Invasive alien predator causes rapid declines of native European ladybirds. Divers. Distrib. 18, 717–725.

Roy, H.E., Peyton, J., Aldridge, D.C., Bantock, T., Blackburn, T.M., Britton, R., Clark, P., Cook, E., Dehnen-Schmutz, K., Dines, T., Dobson, M., Edwards, F., Harrower, C., Harvey, M.C., Minchin, D., Noble, D.G., Parrott, D., Pocock, M.J.O., Preston, C.D., Roy, S., Salisbury, A., Schönrogge, K., Sewell, J., Shaw, R.H., Stebbing, P., Stewart, A.J. a, Walker, K.J., 2014a. Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. Glob. Chang. Biol. 20, 3859–71.

Roy, H.E., Preston, C.D., Harrower, C.A., Rorke, S.L., Noble, D., Sewell, J., Walker, K., Marchant, J., Seeley, B., Bishop, J., Jukes, A., Musgrove, A., Pearman, D., Booy, O., 2014b. GB Non-native Species Information Portal: documenting the arrival of non-native species in Britain. Biol. Invasions 16, 2495–2505.

Salo, O., Rosengren, R., 2001. Memory of location and site recognition in the ant *Formica uralensis* (Hymenoptera: Formicidae). Ethology 107, 737–752.

Salomidi, M., Katsanevakis, S., Issaris, Y., Tsiamis, K., Katsiaras, N., 2013. Anthropogenic disturbance of coastal habitats promotes the spread of the introduced scleractinian coral *Oculina patagonica* in the Mediterranean Sea. Biol. Invasions 15, 1961–1971.

Sanders, N.J., Gotelli, N.J., Heller, N.E., Gordon, D.M., 2003. Community disassembly by an invasive species. Proc. Natl. Acad. Sci. 100, 2474–2477.

Santarlasci, A., Martelloni, G., Frizzi, F., Santini, G., Bagnoli, F., 2014. Modeling warfare in social animals: a “chemical” approach. PLoS One 9, e111310.

Sato, J.J., Yasuda, S.P., Hosoda, T., 2009. Genetic Diversity of the Japanese Marten (*Martes melampus*) and Its Implications for the Conservation Unit. Zoolog. Sci. 26, 457–466.

Schultz, R., Busch, T., 2009. The northernmost record of the invasive garden ant, *Lasius neglectus* (Hymenoptera: Formicidae). Myrmecological News 12, 183–186.

Schultz, R., Seifert, B., 2005. *Lasius neglectus* (Hymenoptera: Formicidae)–a widely distributed tramp species in Central Asia. Myrmecol News 7, 47–50.

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Van Kleunen, M., Winter, M., Ansong, M., Arianoutsou, M., Bacher, S., Blasius, B., Brockerhoff, E.G., Brundu, G., Capinha, C., Causton, C.E., Celesti-Grapow, L., Dawson, W., Dullinger, S., Economo, E.P., Fuentes, N., Guénard, B., Jäger, H., Kartesz, J., Kenis, M., Kühn, I., Lenzner, B., Liebhold, A.M., Mosena, A., Moser, D., Nentwig, W., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H.E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., Walker, K., Ward, D.F., Yamanaka, T., Essl, F., 2018. Global rise in emerging alien species results from increased accessibility of new source pools. Proc. Natl. Acad. Sci. U. S. A. 115, E2264–E2273.

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., Kartesz, J., Kenis, M., Kreft, H., Kühn, I., Lenzner, B., Liebhold, A., Mosena, A., Moser, D., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H.E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., Van Kleunen, M., Walker, K., Weigelt, P., Yamanaka, T., Essl, F., 2017. No saturation in the accumulation of alien species worldwide. Nat. Commun. 8, 1–9.

Seifert, B., 2000. Rapid range expansion in *Lasius neglectus* (Hymenoptera, Formicidae) – an Asian invader swamps Europe. Mitt. Mus. Nat. kd. Berl., Dtsch. entomol. Z. 47, 173–179.

Seifert, B., 2007. Die Ameisen Mittel-und Nordeuropas. lutra.

Seifert, B., 2010. Intranidal mating, gyne polymorphism, polygyny, and supercoloniality as factors for sympatric and parapatric speciation in ants. Ecol. Entomol. 35, 33–40.

Seifert, B., D'Eustacchio, D., Kaufmann, B., Centorame, M., Lorite, P., Modica, M.V., 2017. Four species within the supercolonial ants of the *Tapinoma nigerrimum* complex revealed by integrative taxonomy (Hymenoptera: Formicidae). Myrmecological News 24, 123–144.

Shackleton, R.T., Shackleton, C.M., Kull, C.A., 2019. The role of invasive alien species in shaping local livelihoods and human well-being: A review. J. Environ. Manage. 229, 145–157.

Shik, J.Z., Dussutour, A., 2020. Nutritional Dimensions of Invasive Success. Trends Ecol. Evol. 35, 691–703.

Skinner, G.J., Allen, G.W., 2013. Ants (Naturalists' Handbooks). Pelagic Publishing.

Smith, J.D., Beran, M.J., Couchman, J.J., Coutinho, M.V.C., 2008. The comparative study of metacognition: Sharper paradigms, safer inferences. Psychon. Bull. Rev. 15, 679–691.

Stalling, T., Espadaler, X., Carrón, N.O.Z., 2015. First record of the ant-loving cricket *Myrmecophilus fuscus* Stalling, 2013 (Orthoptera: Myrmecophilidae) in mainland Spain. Sociobiology 62, 481–483.

Stanley, M.C., Nathan, H.W., Phillips, L.K., Knight, S.J., Galbraith, J. a., Winks, C.J., Ward, D.F., 2012. Invasive interactions: can Argentine ants indirectly increase the reproductive output of a weed? Arthropod. Plant. Interact. 7, 59–67.

Strayer, D.L., Eviner, V.T., Jeschke, J.M., Pace, M.L., 2006. Understanding the long-term effects of species invasions. Trends Ecol. Evol. 21, 645–51.

Stukalyuk, S. V., 2020. Uzbekistan — the alleged native range of the invasive ant *Lasius neglectus* (Hymenoptera, Formicidae): geographical, ecological and biological evidences. Zoodiversity 54, 111–122.

Stukalyuk, S. V., Radchenko, A.G., 2018. *Lasius neglectus* Van Loon et al. (Hymenoptera, Formicidae), an Invasive Ant Species in Crimea. Entomol. Rev. 98, 690–701.

Styrsky, J.D., Eubanks, M.D., 2007. Ecological consequences of interactions between ants and honeydew-producing insects. Proc. Biol. Sci. 274, 151–64.

Suarez, A. V., McGlynn, T.P., Tsutsui, N.D., 2010. Biogeographic and Taxonomic Patterns of Introduced Ants. In: Ant Ecology. p. 233.

Sudd, J.H., 1967. An introduction to the behaviour of ants. Edward Arnold, London.

Sumner, S., Bevan, P., Hart, A.G., Isaac, N.J.B., 2019. Mapping species distributions in 2 weeks using citizen science. Insect Conserv. Divers.

Tartally, A., 2000. Notes on the coexistence of the supercolonial *Lasius neglectus* Van Loon, Boomsma et Andrásfalvy 1990 (Hymenoptera: Formicidae) with other ant species. Tiscia 1990, 43–46.

Tartally, A., 2006. Long term expansion of a supercolony of the invasive garden ant, *Lasius neglectus* (Hymenoptera: Formicidae). Myrmecologische Nachrichten 9, 21–25.

Tartally, A., Antonova, V., Espadaler, X., Csősz, S., Czechowski, W., 2016. Collapse of the invasive garden ant, *Lasius neglectus*, populations in four European countries. Biol. Invasions 18, 3127–3131.

Tartally, A., Báthori, F., 2015. Does *Laboulbenia formicarum* (Ascomycota : Laboulbeniales) fungus infect the invasive garden ant , *Lasius neglectus* (Hymenoptera : Formicidae), in Hungary ? e-Acta Nat. Pannonica 8, 117–123.

Tartally, A., Hornung, E., Espadaler, X., 2004. The joint introduction of *Platyarthrus schoblii* (Isopoda: Oniscidea) and *Lasius neglectus* (Hymenoptera : Formicidae) into Hungary. Myrmecologische Nachrichten 6, 61–66.

Tay, J.W., Hoddle, M.S., Mulchandani, A., Choe, D.H., 2017. Development of an alginate hydrogel to deliver aqueous bait for pest ant management. Pest Manag. Sci. 73, 2028–2038.

Team, F., 2016. FFmpeg.

Therneau, T.M., 2015. A Package for Survival Analysis in S.

Thiel, S., Köhler, H.-R., 2016. A sublethal imidacloprid concentration alters foraging and competition behaviour of ants. Ecotoxicology.

Thienen, W. Von, Metzler, D., Choe, D.H., Witte, V., 2014. Pheromone communication in ants: A detailed analysis of concentration-dependentdecisions in three species. Behav. Ecol. Sociobiol. 68, 1611–1627.

Thomas, C.D., Palmer, G., 2015. Non-native plants add to the British flora without negative consequences for native diversity. Proc. Natl. Acad. Sci. U. S. A. 112, 4387–4392.

Todd, J.H., Barratt, B.I.P., Tooman, L., Beggs, J.R., Malone, L.A., 2015. Selecting non-target species for risk assessment of entomophagous biological control agents : Evaluation of the PRONTI decision-support tool 80, 77–88.

Tragust, S., Feldhaar, H., Espadaler, X., Pedersen, J.S., 2015. Rapid increase of the parasitic fungus *Laboulbenia formicarum* in supercolonies of the invasive garden ant *Lasius neglectus*. Biol. Invasions 17, 2795–2801.

UE, 2014. Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species, Official Journal of the European Union.

Ugelvig, L. V, Drijfhout, F.P., Kronauer, D.J.C., Boomsma, J.J., Pedersen, J.S., Cremer, S., 2008. The introduction history of invasive garden ants in Europe: integrating genetic, chemical and behavioural approaches. BMC Biol. 6.

Van Loon, A.J., Boomsma, J.J., Andrasfalvy, A., 1990. A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe description and general Biology. Insectes Soc.

37, 348–362.

Vanbergen, A.J., Espíndola, A., Aizen, M.A., 2018. Risks to pollinators and pollination from invasive alien species. Nat. Ecol. Evol. 2, 16–25.

Vanderhoeven, S., Branquart, E., Casaer, J., D'hondt, B., Hulme, P.E., Shwartz, A., Strubbe, D., Turbé, A., Verreycken, H., Adriaens, T., 2017. Beyond protocols: improving the reliability of expert-based risk analysis underpinning invasive species policies. Biol. Invasions 19, 2507–2517.

Von Frisch, K., 1923. Über die" Sprache" der Bienen. Zool Jb Physiol 40, 1–186.

Von Frisch, K., 1967. The dance language and orientation of bees. Belknap Press, Cambridge.

Wada-Katsumata, A., Silverman, J., Schal, C., 2013. Changes in taste neurons support the emergence of an adaptive behavior in cockroaches. Science (80-.). 340, 972–975.

Walsh, J.R., Carpenter, S.R., Van Der Zanden, M.J., 2016. Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc. Natl. Acad. Sci. U. S. A. 113, 4081–4085.

Wang, L., Zeng, L., Chen, J., 2015. Sublethal Effect of Imidacloprid on *Solenopsis invicta* (Hymenoptera: Formicidae) Feeding, Digging, and Foraging Behavior. Environ. Entomol. 44, 1544–1552.

Ward, D.F., Beggs, J.R., Clout, M.N., Harris, R.J., O'Connor, S., 2006. The diversity and origin of exotic ants arriving in New Zealand via human-mediated dispersal. Divers. Distrib. 12, 601–609.

Warner, M.R., Lipponen, J., Linksvayer, T.A., 2018. Pharaoh ant colonies dynamically regulate reproductive allocation based on colony demography. Behav. Ecol. Sociobiol. 72, 1–13.

Wasmann, E., 1905. Comparative studies in the psychology of ants and of higher animals, 2nd ed, Comparative studies in the psychology of ants and of higher animals / by Eric Wasmann. B Herder, London.

Wehner, R., Menzel, R., 1990. Do Insects Have Cognitive Maps. Annu. Rev. Neurosci. 13, 403–414.

Whitehead, H., Rendell, L., Osborne, R.W., Würsig, B., 2004. Culture and conservation of non-humans with reference to whales and dolphins: Review and new directions. Biol. Conserv. 120, 427–437.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wittman, S., 2014. Impacts of invasive ants on native ant communities (Hymenoptera: Formicidae). Myrmecological News 19, 111–123.

Work, T.T., McCullough, D.G., Cavey, J.F., Komsa, R., 2005. Arrival rate of nonindigenous insect species into the United States through foreign trade. Biol. Invasions 7, 323–332.

Yongo, E., Outa, N.., 2015. Effects of Water Hyacinth (Eichhornia Crassipes Solm) Infestation on Water Quality , Fish Diversity and Abundance in the. Lakes, Reserv. ponds 9, 67–74.

Zhu, Y.C., Yao, J., Adamczyk, J., Luttrell, R., 2017. Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (*Apis mellifera*). PLoS One 12, 1–16.