TO THE SKIES AND UNDERGROUND

Ecological and behavioural aspects of dispersal and pupation of the small hive beetle (*Aethina tumida* Murray; Coleoptera: Nitidulidae) as an invasive species

Bram Cornelissen

Propositions

1. The dispersal capacity by flight of the small hive beetle (*Aethina tumida* Murray) is adapted to the distribution and migration behaviour of its natural host *Apis mellifera* L.
(this thesis)
2. Due to global warming, the ecological and economic impact of the small hive beetle will increase.
(this thesis)
3. Achieving global food security requires an increase in plant-based food production at the expense of fodder production.
4. Although urban environments can harbour many species of plants and animals, they neither compensate for nor mitigate the loss of biodiversity.
5. Re-allocating public resources used for space travel to earth travel will reduce the carbon footprint of the latter.
6. Public registration of rare bird sightings leads to an increase in carbon dioxide emission by birders.

Propositions belonging to the thesis, entitled

To the skies and underground: Ecological and behavioural aspects of dispersal and pupation of the small hive beetle (*Aethina tumida* Murray; Coleoptera: Nitidulidae) as an invasive species

Bram Cornelissen

Wageningen, 6 November 2023

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To the skies and underground

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Bram Cornelissen

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Contents

Chapter 1

General introduction

The process of biological invasion

Biological invasion is the movement of animals and plants across natural biogeographical boundaries (Elton, 1958). Although it occurs naturally, the growth of global trade and transport has increased the frequency of biological invasions (Hulme, 2009; 2021). Human-mediated biological invasion can be intentional and beneficial. For instance, the introduction of food crops has increased food security (Bradshaw & Bonierbale, 2010) and beneficial organisms are applied globally to control pests in agricultural crops (van Lenteren et al., 2006). However, most invasion incidences occur accidentally of which some have harmful environmental or socio-economic consequences (Crosby, 1986; Jarić & Cvijanović, 2012; Schweiger et al., 2010). Such incidences have increased exponentially over the past decades (Mormul et al., 2022). Depending on the impact a species has outside of its natural range, it is considered an invasive alien species. Pimentel et al. (2000) calculated the economic impact of invasive alien species as a result of environmental damage and losses in the United States alone at US$ 120 billion annually. A more recent study estimates the annual costs associated with invasive insect species alone at US$ 70 billion globally, and US$ 6.9 billion for the US (Bradshaw et al., 2016). Estimated costs for the EU are €20 billion per year in damage and control, while estimated costs for science-based strategies aimed at addressing invasive alien species pre-emptively are estimated to be less than a tenth of that figure (Shine et al., 2009).

A complex array of mechanisms govern biological invasion with many potential outcomes, but a defined set of phases has been described for the process itself (Blackburn et al., 2011; Colautti & MacIsaac, 2004; Lewis et al., 2016). First, through transport, for instance, tourism, transhumance or natural dispersal, organ isms are introduced into a new environment (Figure 1). This stage requires the organism to survive in an alien and sometimes adverse environment and to start reproducing. About 10% of the species introduced survive in their newly found habitat (Williamson & Fitter, 1996) and establish a population (Lockwood et al., 2013). Becoming established depends on species traits as well as on local biotic and abiotic conditions. Also, the unintentional nature of invasions usually limits the number of individuals of an incipient population, thereby making it vulnerable to inbreeding and stochasticity, for instance due to weather extremes or climate (Lewis et al., 2016). When the life cycle can be completed and the incipient population grows numerically, it can expand beyond the initial range. This stage is defined as spread. A population can spread in a diffusive way when adjacent areas are colonized. Alternatively, it can also occur through jump-dispersal. This entails the spread to distant sites, often vectored naturally, for instance by water or wind, but also through human-mediated transport (Lockwood et al., 2013; Suarez et al., 2001). Once a species spreads, the impact it causes is subjectively described ranging from low to high (Lockwood et al., 2013). It is often based on the assessment of the ecological, social and economic impacts that a species is considered an invasive alien species. Considering the costs and effort involved in prevention, management, monitoring and control of invasive alien species, it seems highly advantageous to invest time and effort in understanding biological traits and environmental conditions, which govern the process of biological invasion (Andersen et al., 2004; Stohlgren & Schnase 2006).

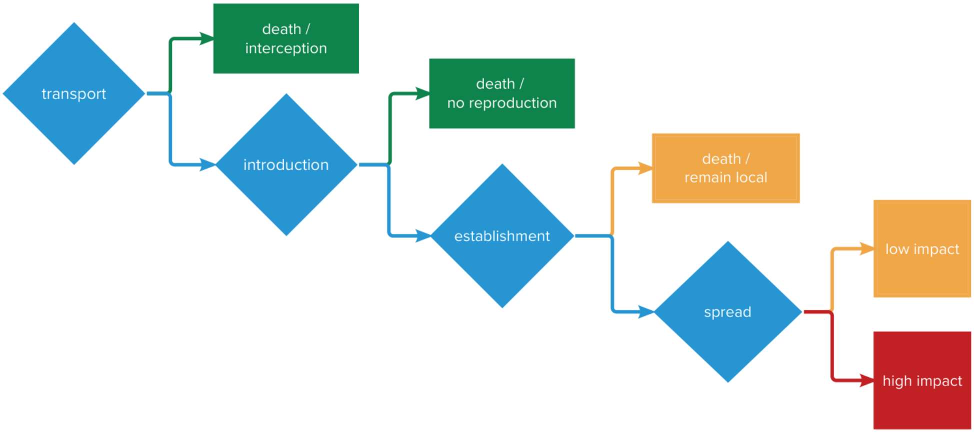

Figure 1. A simplified overview of the stages of invasion based on Blackburn et al. (2011), Lewis et al. (2016) and Lockwood et al. (2013). The blue diamonds show the stages of invasion, which follow one another if an organism survives. During transport for example through trade, an organism can be intercepted (i.e., during import inspection) or die, because of a lack of resources or adverse conditions. If an organism survives, it can be introduced, for instance by dispersal of the organism itself or through human-mediated transport. Depending on local environmental conditions (e.g., season, weather, food availability, etc.) and aspects of the life history of the individual(s), for instance mating status, the organism can reproduce, or fail to do so. Successful reproduction will lead to the completion of the life cycle after which the incipient population will become established. It can either remain local and cause little to no obvious impact, or it can spread to other areas, either human-mediated or through natural dispersal. The impact of a species that survives all stages of invasion can be perceived as low or high, depending on the environmental, economic and societal impact. This perception can be subjective and is therefore debated (Colautti & MacIsaac, 2004; Lockwood et al., 2013).

A host for invaders

Native species can facilitate the establishment of introduced species and often do so (Traveset & Richardson, 2014). The western honey bee (*Apis mellifera* L. subspecies; Hymenoptera: Apidae) is such a native species and over the past seven decades, this has led to at least five invasive alien species becoming established in the endemic range of the western honey bee (*Vespa velutina* Lepeletier: Arca et al., 2015; *Tropilaelaps spp.* Delfinado & Baker: COLOSS, 2023; *Nosema ceranae* Fries: Fries, 2010; *Aethina tumida* Murray: Neumann & Elzen, 2004; *Varroa destructor* Anderson and Trueman: Rosenkranz et al., 2010). These are all species that have a symbiotic relationship with honey bee species (*Apis* spp.) in their native range. A prime example is the parasitic varroa mite (*Varroa destructor* Anderson & Trueman; Mesostigmata: Varroidae), which is entirely dependent on honey bees for its survival (de Ruijter, 1987). It has utilised the western honey bees' comparatively similar biology to that of the mite's natural host the Eastern honey bee (*Apis cerana* Fabricius) to infest colonies in almost the entire range of the Western honey bee (Blacquière et al., 2009). This has led to increased mortality rates of managed colonies and the extinction of feral populations of the Western honey bee in Europe (Jaffé et al., 2010; Neumann & Carreck, 2010). Only now, *ca.* 50 years after its introduction are feral populations in some parts of Europe slowly recovering (Panziera et al., 2022), and for beekeepers it remains a constant threat, leading to increased costs and effort (Brodschneider, 2023). In most cases, the symbiotic nature between honey bees as a host and associated invasive species is not as firm as it is with the varroa mite. This is for example because these species have alternative food sources or because they are only partially dependent on the honey bee for the completion of their life cycle. The presence or absence of honey bees in the invaded range, can nonetheless define the success these species have as invaders.

The small hive beetle

Such is the case with the small hive beetle (*Aethina tumida* Murray; Coleoptera: Nitidulidae, from here on referred to as SHB) (Schäfer et al., 2019, Chapter 2 of this thesis). Native to sub-Saharan Africa (Lundie, 1940), the SHB is considered an ecological generalist (Neumann et al., 2016). Although various hosts and food sources can be utilised for reproduction (Arbogast et al., 2010; Buchholz et al., 2008; Spiewok & Neumann, 2006b), the SHB is most commonly associated with western honey bees (*A. mellifera subspecies*.) as this species acts as the natural host in native and invaded ranges (Lundie, 1940; Neumann et al., 2016; Schmolke, 1974). After emergence from the soil, male and female adult SHBs search for a host colony to invade. SHBs are attracted to olfactory cues associated with honey bee colonies and conspecifics (Hayes et al., 2015; Mustafa et al., 2015; Suazo et al., 2003). The SHB is an income breeder, as it can utilize stored energy resources to fly shortly after emergence, but needs to acquire additional resources for the development of its reproductive capacity (de Guzman et al., 2015; Jervis et al., 2008; Langlands et al., 2021). Some reports suggest mating can take place in the soil after emergence (Neumann & Elzen, 2004), but considering the chance of finding a sexual partner is highest in a host colony, the place of action is likely to be situated there. This is supported by observations that attraction to the opposite sex is highest 18 days after emergence, and that aggregation of the SHB stimulates reproduction (Mustafa et al., 2015). In the host colony, (aggregations of) SHBs hide in corners, brood cells, cracks and crevices, but are also forcibly imprisoned by worker bees in corrals, where they are fed and kept alive (Ellis, 2005; Neumann et al., 2001). Their defensive turtle-like behaviour mostly prevents SHBs from being killed or removed from the colony by patrolling bees, but it does suppress their natural reproductive behaviour (Neumann et al., 2016; Schmolke, 1974). In the colony, SHB females acquire protein-rich food to advance ovary activation (de Guzman, 2015; Langlands et al., 2021). A female may lay *ca*. 1500 eggs in her life time depending on temperature and diet (Meikle & Patt, 2011). Eggs are laid in batches of irregular numbers at a time (Lundie 1940; Neumann & Elzen, 2004) (Figure 2). The frequency of egg-laying and the quantity of eggs laid, very much depends on the opportunity. Larvae hatch from eggs after approximately 3 days, after which they start feeding on hive constituents. The reproduction rate is generally at low levels, with low numbers of larvae feeding for instance in debris at the bottom of the nest or hive (Spiewok & Neumann, 2006a). In some cases though, the aggregation of SHBs can lead to a mass laying of eggs. The host colony can then be overrun by feeding larvae, ultimately leading to the colony to abscond, or collapse (Hepburn & Radloff, 1998; Neumann et al., 2010). Larval development may take somewhere between 8 and 17 days, depending on nutritional as well as environmental conditions (Meikle & Patt, 2011). Once fully grown, wandering larvae leave the hive or nest and seek a suitable place to pupate (Lundie, 1940). Pupation takes place in the soil, where edaphic conditions affect survival and duration of the metamorphosis (Bernier et al., 2014; Ellis et al., 2004; Meikle & Diaz 2012; Meikle & Patt, 2011). Once fully developed the life cycle starts again.

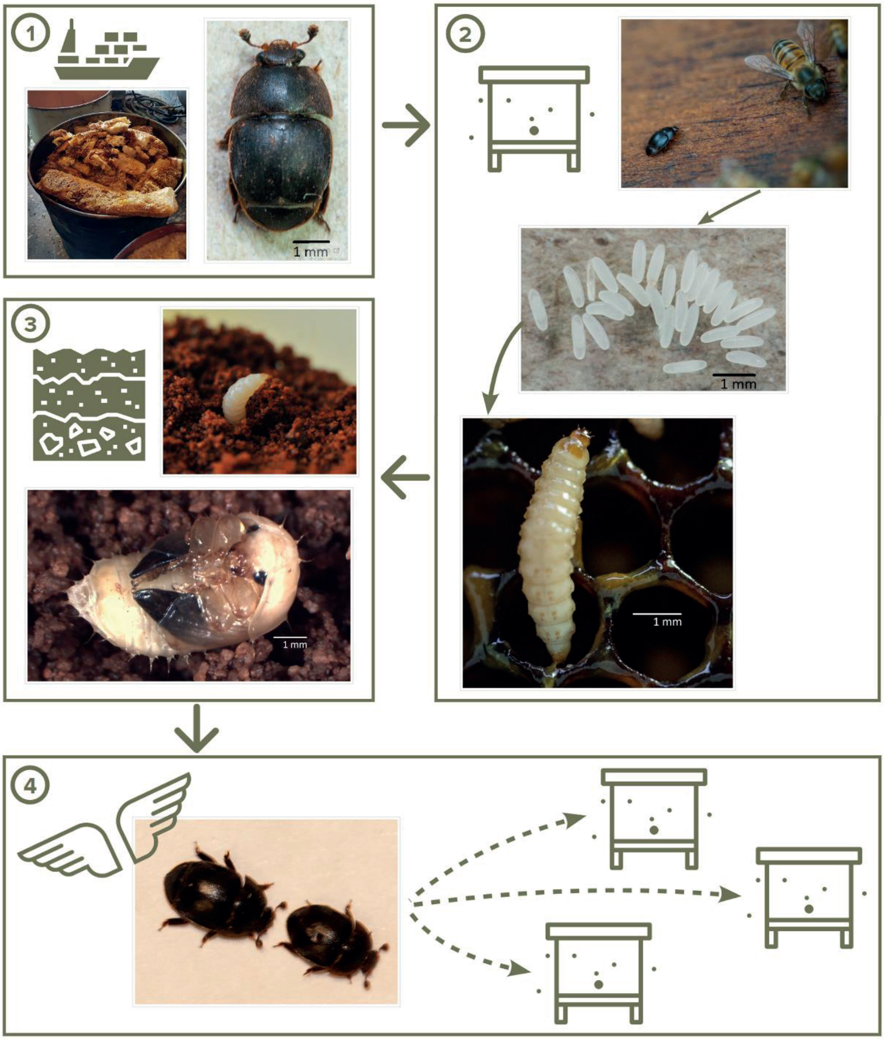

Figure 2. Integrated schematic overview of the invasion ecology and life cycle of the small hive beetle (SHB, Aethina tumida Murray; Coleoptera: Nitidulidae). See Neumann et al. (2016) for a detailed overview of the life cycle. This figure is divided in four sections. ***1) Transport and introduction****: SHBs can be transported with honey bee related commodities such as bees' wax (top left corner: steel barrel containing bees' wax, see Idrissou et al., 2019), or other unrelated commodities. Theoretically, all stages of development of the SHB can survive transport, depending on the type of commodity and other conditions, such as temperature. Here, an adult is depicted and the assumed invading life stage. Once transported, adults will commence host finding, either facilitated by human introduction (hive material) and/or through dispersal by flight.* ***2) Inside the hive****: both males and (mated) females will enter a host colony. SHBs hide in cells, cracks and crevices in the hive, but are also imprisoned by worker bees in corrals. If the opportunity arises, females start laying eggs in small batches. Eggs can be found in small cracks, but also in brood cells. Larvae emerge within 3 days, after which they start feeding. Depending on the condition of the colony, larvae feed off hive debris in the bottom of the nest, on brood, honey, pollen and dead bees. The larval stage can take between 8 and 17 days, depending on food availability and environmental conditions such as temperature. Fully grown larvae, called wandering larvae, leave the hive and search for a suitable place to pupate.* ***3) Underground:*** *Pupation takes place in the soil and can take 14 days up to 78 days, depending on edaphic conditions. Once metamorphosis is completed the adult SHB emerges from the soil.* ***4*** *)* ***Spread:*** *Emerged adults will need to mate and seek a host colony. Dispersal can be human-mediated or take place by flight. If successful, the life cycle is completed and an incipient population has become established.*

The small hive beetle as an invader

As recent as 1996 the SHB has become an invasive species and by now can be found on all continents except Antarctica (Cornelissen & Neumann, 2022; Figure 3). Trade data combined with SHB genetics implicate global honey bees' wax trade as a transport route facilitating introductions of SHB (Idrissou et al., 2019). Seaports have been indicated as likely entry points expanding possible pathways to other commodities or even bee swarms residing on sea faring ships. A third pathway is provided by the global trade in package bees and queens (Neumann et al., 2016). Historically, successful introductions are without exception first noticed in honey bee colonies and apiaries, which underlines that survival beyond the natural range is likely to be dependent on the presence of *A. mellifera* subspecies. Whilst the SHB is considered a minor pest species in the native range (Lundie 1940; Neumann et al., 2016), as an invasive species it can cause serious damage to (sub)species of western honey bees and managed colonies in particular (Elzen et al., 1999). Although the western honey bee is not a novel host, the range expansion of the SHB has led to the incursion of nests of other social insect species. These include *Apis cerana* native to Asia (Bai et al., 2022; Zhao et al., 2020), stingless bee species in Australia, Asia and South America (Cervancia et al., 2016; Greco et al., 2010; Halcroft, 2007; Pereira et al., 2021) and bumble bees (*Bombus impatiens*) in North America (Ambrose et al., 2000; Spiewok & Neumann, 2006b). Nest incursion does not always lead to negative effects on the presumed new host, but given the opportunistic and generalist nature of the SHBs, it is possible that in some cases it will.

Besides a handful of exceptions (Portugal, 2004: Murilhas; Cosenza, 2018 and Sicily 2019: Istituto Zooprofilattico Sperimentale delle Venezie, 2015) most known introductions of the SHB have led to the establishment of populations and spread to other areas (Cornelissen & Neumann, 2022; Figure 3). Contingency plans have contributed, for instance in southern Italy, to the containment of an invasive population (Formato et al., 2021; Granato et al., 2017), but based on experience, eradication seems impossible once incipient populations are established. This can be explained in general terms by a lag time effect, which allows incipient populations to become established before they are detected (Lockwood et al., 2013; Richardson et al., 2011) and for the SHB specifically because detection is obscured by non-detection in unidentified apiaries and feral colonies, which could harbour SHBs (Neumann et al., 2016). Although containment from a national or international perspective is preferred, the social and economic costs for local stakeholders can undermine the support base for policies aimed at containment (Schäfer et al., 2019, Chapter 2). For instance, long-term movement restrictions and untimely compensation for destroyed materials and honey bee colonies have been shown to negatively affect the willingness of beekeepers to cooperate with authorities. For this and other reasons, preventive strategies and strategies aimed at early detection are more sustainable and cost-effective. Successful strategies however, require a better understanding of the biological mechanisms involved and the environmental conditions that affect the SHB as an invasive alien species.

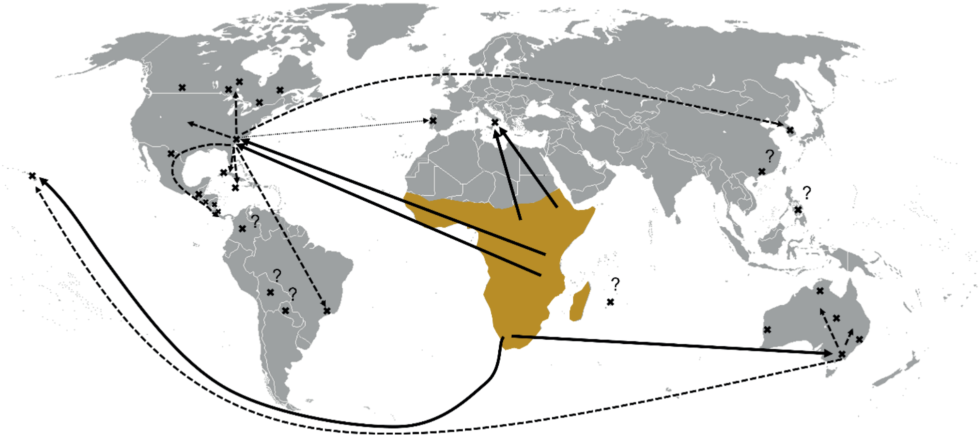

Figure 3. World map of small hive beetle (SHB, Aethina tumida Murray; Coleoptera: Nitidulidae) invasions and invasion routes based on Bai et al. (2022), Bulacio Cagnolo et al. (2023), Cornelissen & Neumann (2022) and Idrissou et al. (2019). The brown colour shows the approximate area in which the SHB is endemic. Solid lines show introduction routes from the endemic range to other parts of the world. Thick dashed lines show the spread from established invasive SHB populations to other areas either through jump dispersal or by the expansion of established populations. The thin dotted line shows the known introduction into Portugal (2004) that did not result in an established population. Crosses mark locations with confirmed introductions (Cornelissen & Neumann, 2022) with established (incipient) populations (except for Portugal). Question marks indicate locations where the SHB has been introduced, but of which the origin of the introduction has not been clarified at the time of writing. Note that the introduction of the SHB in Hawaii, has two possible origins (Australia and South Africa). Due to the genetic relatedness, one or the other cannot be excluded, therefore both are shown. Also note that due to the global scale, details regarding the exact introduction locations and population ranges are lost. See Bai et al. (2022), Bulacio Cagnolo et al. (2023), Cornelissen & Neumann (2022) and Idrissou et al. (2019) for detailed information on locations, invasion pathways and the genetic background of SHB invasions.

Several countries have implemented the use of honey bee trap hives as part of a monitoring strategy around high risk areas or sites such as transport hubs (Chauzat et al., 2015; Formato et al., 2021; Keeling et al., 2017). The concept entails a ring of honey bee trap hives placed around such sites, which are supposed to attract introduced SHB individuals. These hives are then inspected regularly for the presence of SHBs. However, the effectiveness of trap hives as part of an early detection strategy is unclear. The primary reason is limited knowledge of the flight capacity and behaviour of the SHB during invasion, making it difficult to anticipate SHB dispersal. SHBs are considered good flyers, with anecdotal reports suggesting a distance of over 10 km per day can be covered (Wenning, 2001) and freshly emerged beetles have been released and recaptured using honey bee colonies up to a distance of 200 m (Neumann et al., 2012). However, no dedicated study has been performed to investigate the flight capacity at a larger spatial scale, more relevant to invasion. Also, many contextual factors can affect flight patterns in insects, including weather, season and physiology (Johnson, 1969). Furthermore, flight is a costly and risky activity (Tobin & Robinett, 2022), and although SHBs are probably capable of long distance flights, it is unlikely that they will do so when host colonies are in proximity. Several studies highlight the ability of the SHB to respond to host and conspecific cues. However, the attractiveness of host and conspecific cues has only been tested in comparative laboratory assays in relation to mating and reproductive behaviour (Hayes et al., 2015; Mustafa et al., 2015; Papach et al., 2022; Suazo et al., 2003). Attraction to hosts and conspecifics has hardly been studied in field experiments and its relevance to invasion ecology of the SHB is largely unknown. One possible outcome could be that movement mostly takes place within the incipient population's range as both conspecific and host cues are present and thus outward expansion is less likely. On the other hand, newly emerged beetles might expand outward more readily than experienced flyers, due to a dispersal syndrome, which has been described for other insect species, initially neglecting conspecific cues altogether (Johnson, 1969). It is clear that the dynamics involved in dispersal are understudied. Therefore, increasing our understanding of SHB flight and dispersal and the factors involved are pivotal in anticipating invasions and could favour the management options for early detection and contingency.

Interacting with a changing climate, impact of an invasive alien species can vary, often resulting in an altered likelihood to become established and a possible range expansion to areas otherwise deemed uninhabitable (Dukes & Mooney, 1999; Early et al., 2016; Sutherst et al., 1996). In particular ectotherms, including insects, are often reliant on thermal conditions to pertain within a certain range in order to survive and develop (Barbet-Massin et al., 2013; Roura-Pascual et al., 2004). Environmental conditions can limit SHB reproduction and ultimately its distribution and impact both in their native range and beyond (Bernier et al., 2014; Lundie, 1940). From oviposition to the post-feeding stage, SHB can be found in honey bee colonies (Neumann et al., 2016), although other hosts such as fruits and bumble bee colonies can be utilized (Arbogast et al., 2010; Buchholz et al., 2008; Spiewok & Neumann, 2006b). Adult SHB are able to hibernate together with the bees in the winter cluster (Schäfer et al., 2011). This implies that wherever honey bee colonies are present, these stages in the life cycle can be completed successfully. Pupation, however, is considered a particularly vulnerable stage due to edaphic conditions of the soil being highly variable. Soil texture, humidity and temperature, affect pupal development time and survival (Bernier et al., 2014; Ellis et al., 2004; Meikle & Diaz 2012). Therefore, irrespective of host presence, abiotic factors seem to overrule other factors with regard to the survival and development of SHB populations and could affect the potential impact of invasive populations. Moreover, if edaphic conditions change, for instance due to global warming, this could alter the impact and survival of SHBs. For SHBs, there is also an alternative invasion opportunity which could counter adverse environmental conditions. Greenhouses provide a subset of conditions ideal for invasive alien insects. Over 60 Invasive alien species have been found in covered crops in 2002 in Europe alone (Hanafi, 2005). Honey bees, stingless bees and bumble bees are used as pollinators of greenhouse crops (Dag, 2008; Kiatoko et al., 2022). These crops include aubergine, strawberry, courgette, tomato and bell pepper (Blacquière et al., 2009). Approx. 40.000 – 50.000 bumble bee pollination units are used in The Netherlands for tomato pollination alone. The worldwide value of greenhouse tomato crops pollinated by bumble bees was estimated to be worth €12 billion annually in 2006 (Velthuis & van Doorn, 2006). With optimal growing conditions for up to 11 months a year and a host species present for 9 months, greenhouses could serve as hotspots for SHBs, defying environmental limitations set by the predominant environmental and edaphic constraints. For pollinator units, greenhouse conditions are suboptimal and they need to be replaced regularly (Sabara & Winston, 2003). For example, honey bee colonies used for greenhouse pollination are generally small, consisting of only a few frames of bees and brood. Bumble bee units generally survive for 6 weeks after which they are destroyed and if needed replaced. Stressed or weak honey bee colonies may be overrun by SHB infestations (Mustafa et al., 2014). Therefore, the SHB could pose a threat, not only to pollinators, but to pollination in greenhouses itself. As in a natural environment, a limiting factor for the SHB in greenhouses could be the ability to complete its life cycle. Different types of substrates are used to grow the crops on. It is unclear if SHBs can pupate in these substrates and thus if greenhouses could potentially provide a safe haven for invading SHBs in an otherwise hostile environment.

Thesis outline

Although the first human-mediated biological invasion of the SHB happened decades ago, our understanding of vital aspects of its invasion ecology have remained virtually untouched by science. The aim of this thesis was therefore to improve our understanding of the SHBs' flight capacity and of the environmental factors influencing its dispersal. A further exploration is aimed at understanding the role of edaphic factors in the establishment and impact of the SHB as an invasive alien species under different and changing environmental conditions.

This thesis is a culmination of research that has been executed in various locations and settings between 2015 and 2023. In **Chapter 2** an overview of imminent actions, required to further our knowledge of the SHB as an invasive species is presented. The chapter highlights the practical complications of dealing with introductions and established invasive populations of the SHB from the perspective of various stakeholders, including policy makers and beekeepers.

Chapter 3 deals with the flight capacity of adult SHBs. The main goal of this research was to improve our understanding of the potential distance SHBs can cover by flight and what factors influence initiation of flight. Based on the principle that honey bee colonies attract dispersing SHBs, a mark, release and recapture experiment was performed in collaboration with the University of Florida. A follow-up study is described in **Chapter 4**. Here, the main goal was to understand the relative attractiveness of host cues, host spatial configuration and conspecific cues on SHB dispersal under field conditions. Again, a mark, release, recapture study was designed, but now in the more confined setting of an apiary.

Chapters 5 and 6 deal with other aspects of the invasion ecology of the SHB by investigating pupation success and duration under varying edaphic conditions at varying scales. In Chapter 5, I investigated the potential of the greenhouse environment as a habitat for SHBs. With suitable temperatures and the host present, the question was if substrates used for crop production under glass are suitability for SHBs to pupate in. Four different substrates were tested and compared under laboratory conditions. In Chapter 6, data regarding the success and development of the SHB under variable edaphic conditions was compiled to draw a global picture of the potential spread and impact of the SHB under current and future climate scenarios. This research involved obtaining additional data from published and newly executed laboratory experiments. Based on the compiled data, a basic fitness model explaining marginal to optimal edaphic conditions for pupation of the SHB was developed. The fitness model was then applied to global edaphic parameter data and extended to include future climate change scenarios.

Finally, **Chapter 7** provides the synthesis of the results obtained during the past years. Here, I discuss the contributions of this thesis to our understanding of the biology of the SHB as an invasive alien species. Furthermore, I highlight how the results obtained are of value for a science-based approach to monitoring and management strategies for early detection of the SHB. I conclude my thesis with suggestions for future scientific endeavours.

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Chapter 2

How to slow the global spread of the small hive beetle

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reproduced with minor adaptations

Abstract

Small hive beetles (SHBs) are parasites of social bee colonies endemic to sub-Saharan Africa and have become a widespread invasive species. In the new ranges, SHBs can cause damage to apiculture and wild bees. Although the further spread seems inevitable, eradication of new introductions and containment of established ones are nevertheless urgently required to slow down the invasion speed until better mitigation options are available. However, at present there is no adequate action plan at hand. Here, we propose to take advantage of SHB invasion history and biology to enrol a feasible plan involving all stakeholders. Raising awareness, education and motivation of stakeholders (incl. adequate and timely compensation of beekeepers) is essential for success. Moreover, sentinel apiaries are recommended in areas at risk, because early detection is crucial for the success of eradication efforts. Given that introductions are detected early, SHB eradication is recommended, incl. destruction of all infested apiaries, installation of sentinel colonies to lure escaped SHBs and a ban on migratory beekeeping. If wild perennial social bee colonies are infested, eradication programs are condemned to fail and a strategic switch to a containment strategy is recommended. Containment includes adequate integrated pest management and a strict ban on migratory beekeeping. Despite considerable gaps in our knowledge of SHBs, the proposed action plan will help stakeholders to slow down the global spread of SHBs.

Introduction

Biological invasions typically follow a jump-dispersal pattern (Canning-Clode, 2015) and eradication of recent introductions combined with containment of established ones have been proven as a means to slow down the global spread for a number of species (eg. Boser et al., 2017; Campbell & Donlan, 2005). Efficient approaches for both eradication and containment require measures based on the biology of the invasive species in question, the local situation as well as adequate stakeholder involvement (Anonymous, 2017). Here, we focus on the small hive beetle (SHB), *Aethina tumida* (Coleoptera: Nitidulidae). This beetle has originally been described as a parasite and scavenger of honey bee colonies native to sub-Saharan Africa (Lundie, 1940). In 1996, it emerged as an invasive species, which has now reached all continents except Antarctica (DePaz, 2017; Lee et al., 2017; Neumann et al., 2016). The spread of SHBs is ongoing, as evidenced by a number of recent introductions (2014: Italy, Brazil; 2015: Philippines; 2017: Belize, Canada, South Korea; 2018: Mauritius; cf. DePaz, n.d.; Lee et al., 2017; Muli et al., 2018; Neumann et al., 2016; Sturgeon, 2017). Outside of its endemic range, SHBs can cause severe damage to apiculture as well as wild honey bee colonies and may also endanger other social bees serving as alternative hosts (bumble bees: Spiewok & Neumann, 2006; Hoffmann et al., 2008; stingless bees: Greco et al., 2010; Halcroft et al., 2008, 2011; Mutsaers, 2006; Peña et al., 2014; Wade, 2012). Accordingly, a range of pest mitigation measures has been developed, but at present SHB diagnosis and control are not sufficient (Neumann et al., 2016). In particular, control outside of managed apiaries is not available yet, which is urgently required to limit the impact of SHBs on wild bees. Thus, there is an urgent and apparent need to slow down the continuing global spread of SHBs until better mitigation methods are available. However, at present, there is no international strategy for SHBs. Therefore, we here propose a best-practice action plan for *A. tumida* invasions (Figure 1).

Suggested contingency measures

Stakeholder awareness and engagement

A science-based approach should be taken to raise stakeholder awareness of all relevant impacts of SHBs on managed and wild social bees (Table 1). All stakeholders should be aware that anytime SHBs might arrive in their country or region. Therefore, it is important to take adequate actions (Table 1), before new introductions occur. All stakeholders should be provided with information on how to access tools and guidelines that enable recognition of new infestations. However, stakeholders may value the costs of invasive species differently according to their business (Simberloff, 2003). Moreover, the degree of stakeholder engagement varies between countries due to resource constraints (finances, knowledge, etc.) possibly leading to a weakest link scenario (Stokes et al., 2006). Nevertheless, success is only possible if stakeholders agree and share a common view on the planned measures and respective consequences on their businesses and apiculture in general. Stakeholders, in particular beekeepers, should regard integrated pest management (IPM) of SHBs, incl. adjusted management and adequate sanitation of apiaries and other apicultural facilities (Hood, 2011; Neumann et al., 2016). In particular, adequate and fast compensation for beekeepers appears to be central for success.

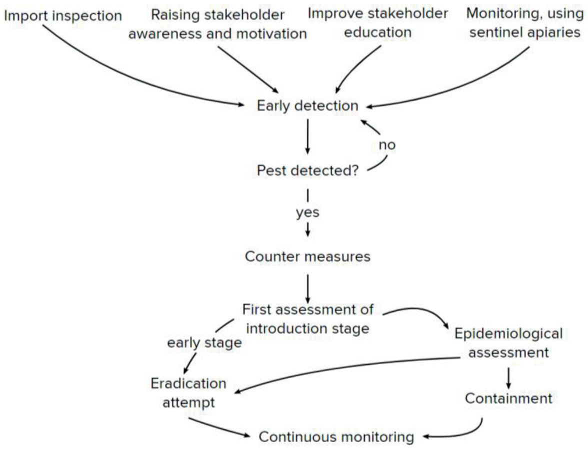

Figure 1. Flow diagram that demonstrates the proposed action plan to limit the global spread of the small hive beetle (SHB, Aethina tumida*). Import inspection is essential to limit introductions. Raise awareness and improve education among all stakeholders (especially beekeepers) to detect new infestations faster. Furthermore, the installation of regularly visited sentinel apiaries, in places chosen according to higher risks of importation, will enlarge the chances of early detection. Immediately after detection of SHB counter measures like the establishment of protection zones have to be implemented and an assessment on the stage of the introduction must be determined. Depending on the size and the location of the infested area, it must be decided if eradication is possible or if the outbreak has to be limited through containment. For both scenarios, continuous monitoring, including the immediate installation of sentinel colonies (trap hives) at the actual outbreak sites is necessary to prevent the further spread of adult SHBs that might escape the control measures.*

Early detection

Evidence strongly suggests that only if a new introduction of SHBs is detected early, eradication can be successful (Mutinelli, 2016; Neumann et al., 2016). In the USA, the first unidentified SHB specimens were collected in November 1996 (Neumann & Elzen, 2004), but it took 2 years until *A. tumida* was officially confirmed (Hood, 2000). By then, SHBs were already well established and widespread (Neumann & Elzen, 2004), rendering an eradication impossible. The same holds true for the introduction of SHBs into Australia (Neumann & Elzen, 2004).

Border control and quarantine measures within a suitable legal framework are clearly the first line of defence against SHB invasion and should be implemented by every country. Moreover, all stakeholders should be able to recognize *A. tumida* infestations via adult and larval morphology as well as clinical signs at the colony level (Neumann et al., 2016). In particular, beekeepers should be cognizant during their routine practice and apiary inspections. Competent laboratories are required to confirm or reject suspicion by beekeepers. This holds especially true for eggs and larvae, which cannot be assigned to *A. tumida* based on morphometrics alone (Neumann et al., 2016). Any apiary under suspicion should be taken under strict and immediate sanitary restrictions and all movements of bees (colonies and queens), bee products and used bee equipment, into and out of such apiaries must be banned until the competent authority has investigated the case.

At present, neither pheromones nor host kairomones have yet been identified for host finding SHBs. Therefore, the only known efficient attractants for adult SHBs are functional honey bee colonies. To be able to identify the pest early and to investigate a new outbreak in detail, it is therefore recommended to install sentinel apiaries, which consist of fully functional queenright honey bee colonies, to attract and trap SHBs in zones at risk for new introductions (Chauzat et al., 2016; Keeling et al., 2017; Mutinelli, 2016). Sentinel apiaries enable a cheaper and more efficient monitoring compared to active surveillance of hobby or commercial apiaries (Rivera-Gomis et al., 2017). Respective sentinel hives should be composed of one hive-box only with fewer frames than actually fit, to foster efficient and quick diagnosis. The hives should also be equipped with SHB traps that can be quickly checked (reviewed by Neumann et al., 2016). Inspectors are advised to regularly scale down these colonies by replacing brood frames with empty ones and to visit the hives at least every two weeks when local weather conditions foster SHB pupation (reviewed by Neumann et al., 2016). Surveillance in temperate and subtropical zones should therefore be reinforced from spring to autumn and all year long in tropical zones. During winter in temperate climates those traps should be preferentially used that allow monitoring but limit disturbance of the colonies (Neumann et al., 2016).

*Table 1 Dissemination of all relevant small hive beetle (*Aethina tumida*) information to all stakeholders.*

Whom to address?	How and what should be implemented?
1. Beekeepers, veterinarians, honey bee health laboratories (NRL, diagnostic laboratories)	a) General information through popular articles, case study reports, guidelines (e.g. diagnosis, Integrated Pest Management, incl. sanitation) and articles in local beekeeping and veterinary journals in the local language b) Awareness training and talks by SHB-experts at local beekeeping and veterinarian meetings c) Newsletters, conferences, workshops, seminars, training schools
2. Beekeepers' and veterinarians' associations	In addition to 1a, b, c a) Specific seminars with representatives of all country associations and local experts b) Periodical meetings to ease communication and discussion among beekeepers and veterinarians
3. Researchers, extension specialists, research networks	In addition to 1a and 2 a) Research and development (R&D) b) Articles in peer-reviewed journals c) Talks, posters, conference proceedings, national and international conferences d) Open access to training schools, seminars and conferences
4. Industry (incl. bumble bee and stingless bee breeders)	In addition to 1a, 2, 3 a) R&D meetings with local authorities and ministries b) Quality assurance system
5. Veterinary authorities	In addition to 1a, 2, 3 a) National guidelines and manuals (contingency plan) b) EU regulations, guidelines c) OIE standards (Animal Health Code and Terrestrial Manual) d) Surveillance programs and early warning systems e) Spoken person f) Accreditation g) Availability of extra resources (practitioners and extra work hours) h) Epidemiological analysis i) Inspection on apiary registrations j) Inspection on hive movements (migratory beekeeping and hive trade) k) Inspection on pollination service
6. Government and policy makers	In addition to 1a, 2, 3, 4, 5 Suggestions for guidelines and regulations: a) Prepare a detailed contingency plan in advance b) Apiary registration database c) Bumble bee registration database d) Pollination service database e) Traceability of hive movements (migratory beekeeping) f) Traceability of hive products (wax) and beekeeping equipment (supers) g) Study of the migratory beekeeping flows h) Monitoring of wild bees
7. General public	In addition to 1a a) Newspaper b) Broadcasts c) Contribution to visual media d) Interviews (national and international) e) Teachers information package on bee health

Official confirmation of infestation with *A. tumida* should be given, if the competent authority identifies SHB eggs, larvae, pupae or adults. Then, a protection zone should be installed immediately to limit further spread, including a strict ban on movements of bees and beekeeping equipment, because migratory beekeeping poses the highest risk of spreading *A. tumida* (Neumann & Elzen, 2004). The ban on movement requires an appropriate and permanent official control system to ensure application and compliance as well as traceability. The protection zone should be large enough to detect all possible natural spread, but small enough to be manageable with the local human resources available. As the maximum flight range of adult SHBs is still unknown, it is obviously impossible to define a safe distance. However, since all apiaries and beekeeping facilities (including abandoned ones) in the protection zone have to be investigated as quickly as possible and will potentially be placed under permanent restrictions, the size of the protection zone should initially be rather small (~ 10 km radius). It can subsequently be enlarged if required by the population status (e.g. when infested colonies are confirmed outside of the initial zone). In order to enhance chances of detecting new infestations, visual diagnosis should only be performed by trained staff (Neumann et al., 2013; OIE, 2017b; Spiewok et al., 2007) effective traps should be installed (cf. Neumann et al., 2016), and hive debris samples, workers or other hive matrices should be investigated with specific DNA diagnostics (Idrissou et al., 2018; Ward et al., 2007). To ensure that the locations of all beekeeping facilities inside the protection zone are known, every local beekeeper should be obliged to register and to update the competent authority about apiaries (in operation or not), honey houses, storage rooms and beeswax rendering or royal jelly plants. It is apparent that registration and control of movements might at present be unmanageable in some countries. Respective legislation and law enforcement is therefore recommended.

It is further recommended to establish a surveillance zone surrounding the protection zone, in which sentinel apiaries should be implemented (see above), movements of hives be registered and all apiaries sampled to detect any further spread as soon as possible (Chauzat et al., 2016; Granato et al., 2017).

In conclusion, a combination of sentinel sites as well as highly motivated (adequately compensated) and well-educated beekeepers appears to be the optimal approach fostering early SHB detection.

Eradication

The population status of every new SHB case should be investigated as soon as possible and the potential economic and environmental consequences should be evaluated, before starting an eradication process. These investigations are indispensable, as it seems to be possible to eradicate SHBs only if a new introduction is recognized early and the spread is still limited to a manageable area. Apicultural trade and migratory beekeeping activities, abandoned or not registered apiaries, commercial bumble bee hives as well as possible infestations of wild host populations must be considered (e.g. in Australia, feral honey bee nests in trees were inspected, cf. Neumann et al., 2016). As soon as wild social bees with perennial colonies (i.e. *Apis spp.*) are infested, eradication seems impossible, as such colonies might host SHBs over several years and cannot be entirely discovered. Likewise, unsuccessful eradication measures of managed apiaries over two seasons strongly suggest reservoirs outside of the reach of control. Then, it appears prudent to switch from eradication to containment.

Given that the introduction of SHBs appears to be early and infestations are still localized in a few managed apiaries, then it appears worthwhile starting eradication. Examples for successful eradications have been Perth, Australia and Sicily, Italy (cf. Mutinelli, 2016; Neumann et al., 2016). Most important is that eradication must begin as soon as the population status is sufficiently clear. Every day counts as adult SHBs may disperse into the wild bee populations, thereby escaping eradication. It is mandatory to stamp out all colonies of the SHB-infested apiaries as soon as possible, because false negative diagnostics are especially likely in case of low infestation levels. For eradication, the entrances of all colonies should be sealed in the early afternoon (before the peak flight activity of adult SHBs, Neumann et al., 2016). Then, the bees should be killed (e.g. with sulphur dioxide) and all the material destroyed (e.g. burning) without any delay under the supervision of the competent authorities (Mutinelli, 2016; Mutinelli et al., 2014). The soil surrounding SHB-infested colonies poses the risk of reinfection due to SHB-pupation and should be treated adequately (Hood, 2011; Mutinelli et al., 2014; Neumann et al., 2016). Continuous monitoring has to be considered and sentinel colonies must be installed before all colonies of an infested apiary are destroyed, as a considerable number of adult SHBs may stay outside of hives (Annand, 2011). These sentinel colonies in situ will attract free-flying SHBs that may have survived the local eradication effort.

Even though SHBs are able to reproduce on fruits and other food in the laboratory and in semi-field assays (Buchholz et al., 2008), field surveys so far were not able to confirm any association of SHBs with alternative food sources outside of social bee colonies (Mutinelli et al., 2015; Mutinelli & Ponti, 2017; Willcox et al., 2017). It appears most effective to focus on control measures for well-known major routes for SHB transmission and reproduction (cf. Neumann et al., 2016). In the case of eradication, any movement of managed bees into and out of the protection zone must be prohibited at all costs. Costs for stakeholders due to inadequate pollination should be covered by the competent authority. If one makes the decision to eradicate, one must be very strict to be successful. If one weakens the restrictions, the potential for further spread of the SHB rises in the area, which increases the possibility for the SHB to establish outside of managed beekeeping, making eradication impossible.

For the 5 years following the last report of the presence of *A. tumida* an annual survey, supervised by a competent authority, should be carried out on a representative sample of apiaries in the concerned country or zone, and no signs of the presence of *A. tumida* should be detected. Only if this condition is met, the free status as a result of an eradication program can be recognized (OIE, 2017a).

To enhance chances of eradication success, substantial efforts should be made to ensure that all stakeholders work closely together and communicate necessary measures clearly and on a fair base. In particular, adequate and timely compensation of beekeepers is highly recommended. It must be prevented that beekeepers feel forced to handle the problem by themselves, in view of substantial financial losses.

Containment

If eradication is no longer an option, SHB containment must be applied to limit further spread. Protection zones have to be established (see above) and inside these zones, measures have to be applied to limit SHB reproduction, especially destructive mass reproduction (Neumann et al., 2016). The measures should include the use of in-hive traps (Bernier et al., 2015; Levot et al., 2015; Neumann & Hoffmann, 2008), a beekeeping management that is adapted to the presence of SHBs, incl. adequate sanitation of apiaries and other apicultural facilities (Hood, 2011; Neumann et al., 2016) and the treatment of the surrounding soil, if damage by SHB larvae or other clear signs (i.e. slime traces of wandering larvae) have been observed inside the colonies (Neumann et al., 2016).

However, it is indispensable that stakeholders have legitimate access to products for SHB pest treatment (e.g. in-hive treatment and/or adequate soil drenching products; reviewed by Neumann et al., 2016). Most unfortunately, no such products are currently available in the EU. Furthermore, implications for declaring SHBs established in a region/country should be considered, particularly the possible impact on international commercial movement (i.e. restrictions, ban, etc.) of honey bees, bee equipment and bee products.

Gaps in our knowledge of *A. tumida*

There is an obvious and urgent demand for more research to improve contingency planning due to considerable gaps in our knowledge of SHBs (Neumann et al., 2016). For example, an optimal approach would be efficient SHB traps outside of managed apiaries, which would safeguard bumble bees, stingless bees as well as feral honey bees. Basic research is required on the general biology of *A. tumida* to foster improved diagnosis, control and prevention, incl. anatomy, behaviour, physiology as well as on pheromones and kairomones governing SHB host finding. Finally, sentinel apiaries and the various SHB-traps should be assessed for their effectiveness and efficiency.

Summary

- Successful eradication is only possible if new introductions are detected very early, before SHBs infest wild social bee populations.
- Motivation and education of stakeholders (especially beekeepers) is crucial for success (detection, eradication and containment), which requires early response capacity and continuity of resources (financial and human resources).
- Adequate border control and sentinel sites are fundamental for early detection and success.
- After epidemiological investigations, the competent authorities have to decide between eradication or containment based on a cost–benefit analysis.
- Sentinel colonies have to be installed at outbreak apiaries to lure free-flying SHBs that might have escaped eradication.
- Irrespective of eradication or containment strategy, a surveillance system should be activated and maintained.
- Movement restriction and/or ban of honey bees and beekeeping equipment in protection and/or surveillance zone requires the activation of an appropriate and permanent official system of registration and control (traceability should also be guaranteed).

- A legal basis for appropriate actions of the competent authorities is needed (e.g. adoption of restrictive measures, destruction of apiaries, compensation after stamping out).
- There is a need for effective and scientifically based tools for training, detection/diagnosis, control and management intended for the beekeepers, bee-inspectors, veterinarians and other responsible entities.
- The gaps in basic knowledge of *A. tumida* biology currently limit the effectiveness of SHB contingency planning.

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Chapter 3

Catch me if you can: dispersal of the small hive beetle by flight

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Abstract

The spread of invasive species often follows a jump-dispersal pattern. While jumps between distribution ranges are typically fostered by humans, local dispersal can occur due to the specific traits of a species, which are often poorly understood. This holds true for small hive beetles (SHB, *Aethina tumida*), which are free-flying parasites of social bee colonies native to sub-Saharan Africa. They have become a widespread invasive species and can cause significant damage to honey bee populations where introduced, but their dispersal capacity by flight is unknown. In 2017, a mark-release-recapture experiment was conducted in six replicates (A-F) using laboratory reared, dye-fed adults (N=15,690). Honey bee colonies were used to attract flying SHBs at fixed spatial intervals from a central release point. SHBs were recaptured (N=770) at a maximum distance of 3.2 km after 24 hours and 12 km after one week. Most SHBs were collected closest to the release point at 0 m (76%, replicate A) and 50 m (52%, replicates B to F) suggesting that SHBs prefer nearby host colonies, thereby limiting flight investment. Temperature and wind deviation had significant effects on dispersal, with more SHBs being recaptured when temperatures were high and confirming the role of wind for odour modulated dispersal of flying insects. Our findings show for the first time that SHBs are capable of long-distance flights, which should be considered for monitoring and mitigation efforts.

Introduction

Due to increased global trade, the number of biological invasions has increased exponentially from the Middle Ages onwards (Hulme, 2009, 2021). This has accelerated during the past decades (Mormul et al., 2022), affecting the environment and society as a whole (Pimentel et al., 2000; Jarić & Cvijanović, 2012). For a species to invade new territory, it has to transition through the various stages of biological invasion, including transport, introduction and reproduction, ultimately leading to establishment of a population (Blackburn et al., 2011). A successful invasion hinges on the ability of a species to survive and reproduce in a newly found habitat. Most introduced species simply do not become established (Williamson & Fitter, 1996), as the areas into which they are introduced offer biotic and abiotic challenges that counter their survival (Lewis et al., 2016). Still, some introduced species make it and thrive in their newly found environment and can become known as Invasive Alien Species, which are considered major global biological threats to biodiversity (Clavero & Garcia-Berthou, 2005; Maxwell et al., 2016). Understanding what mechanisms make these few species successful is important to help mitigate their impact (Stohlgren & Schnase, 2006).

Since 1996, the small hive beetle (SHB; *Aethina tumida* Murray, Coleoptera: Nitidulidae) has spread from its native range of sub-Saharan Africa to the Americas, Asia, Australia and Europe (Boncristiani et al., 2022) and is considered an invasive alien species (Cornelissen & Neumann, 2022; Schäfer et al., 2019). It has established populations on all these continents and global warming is predicted to result in further range expansion (Cornelissen et al., 2019). This parasite of western honey bee (*Apis mellifera* L.) colonies utilizes its host for feeding, hiding and reproduction (Ellis & Hepburn, 2006; Lundie, 1940; Neumann et al., 2016). In particular, mass reproduction events with often thousands of larvae can cause severe damage to seemingly healthy colonies often leading to the full structural collapse of the entire nest (Hepburn and Radloff 1998) within two weeks' time (Neumann et al., 2010). Both within and beyond the natural range, the SHB has also been associated with other host species, such as honey bee species native to Asia, stingless bees in Africa, South America and Australia, and bumble bees and solitary bees in North America (Cervancia et al., 2016; Gonthier et al., 2019; Pereira et al., 2021; Spiewok & Neumann, 2006; reviewed by Neumann et al., 2016). These host shifts could potentially have dire consequences for managed and wild host populations, the extent of which is yet to be documented (Bai et al., 2022; Neumann et al., 2016).

Past biological invasions of the SHB show a strong correlation between genetics and international honey bee wax trade data, thereby suggesting that commercial apicultural imports constitute the main gateway for crossing biogeographical boundaries (Idrissou et al., 2019). Furthermore, there is clear evidence that trade with queen cages, package bees and entire honey bee colonies had resulted in a number of non-intentional SHB introductions (reviewed by Neumann et al., 2016). In the introduced ranges, migratory beekeeping has shown to be a key for local spread (reviewed by Neumann and Elzen 2004). Therefore, it seems obvious that human-mediated jump dispersal plays an important role in the outward spread of the SHB. But since adult SHBs do fly, active flight may also contribute to dispersal within the introduced ranges. However, very little is known about the flight and dispersal capacity of the SHB and its ability to find hosts and possibly conspecifics under field conditions (Spiewok et al., 2008). Nevertheless, the SHB is considered an able flyer with anecdotal reports suggesting a flight range of 10 km a day (Somerville, 2003) and the ability to detect colonies 13 to 16 km away (Wenning, 2001). Also, SHB-free apiaries are readily (re)infested by SHBs in areas where they are well-established, showing a correlation with the density of colonies and mass-reproduction events (Spiewok et al., 2008; Neumann et al., 2010). Nevertheless, there are no dedicated studies investigating flight capacity over distances of more than 200 m (Neumann et al., 2012).

SHBs are attracted to honey bee and bumble bee colony odours when exposed in laboratory and field assays (Suazo et al., 2003; Spiewok and Neumann, 2012) and likely use these cues to detect host colonies, which they then invade (Ellis & Hepburn, 2004; Lundie 1940; reviewed by Neumann & Elzen, 2004). Host-, but also mate-finding has been shown under field conditions with mark-release-recapture experiments for other flying Nitidulidae which use chemotaxis (Foott, 1976; Okada et al., 2007). Such odour-modulated flight of insects can be affected by wind and temperature. For instance, host odour plumes are shaped and distorted by wind, requiring insects to apply host finding strategies (Cardé & Willis 2008; David et al., 1982) and temperature can affect the mobility of flying insects (Taylor, 1963). Moreover, intrinsic characteristics of individuals such as size, sex, age and physiological development have all been shown to affect the flight capacity and dispersal of insects (Candy et al., 1997; Johnson, 1963). Larger mountain pine beetles (*Dendroctonus ponderosae*) fly longer and farther than smaller individuals (Evenden et al., 2014) and mated *Arhopalus rusticus* beetles fly farther than unmated ones (Grilli and Fachinetti, 2017). Also *A. rusticus* females, which are bigger than males, were stronger flyers than males. To what extent this applies to SHBs under field conditions is currently unknown. This information would be relevant for understanding dispersal and the invasion biology of the SHB. It would support contingency plans aimed at preventing or eliminating introductions of this beetle pest (Schäfer et al., 2019, Chapter 2). Honey bee colonies are used as sentinels for early detection monitoring of SHB introductions, for instance near sea ports (Calderón-Fallas and Sanchez-Chaves 2013; Formato et al., 2021). However, there is no knowledge of the efficacy of such measures or the spatial interval required for effective monitoring purposes.

To better understand the dispersal of the SHB by flight, we here performed a mark-release-recapture experiment in the field. Based on anecdotal reports and from what is known from other flying beetle species, we hypothesized that SHBs would fly further than the currently known distance of 200 m (Neumann et al., 2012) and that temperature, wind and humidity will affect SHB dispersal similar to other insects. Warm temperatures and humid conditions might lead to longer distances flown, while wind direction is expected to affect flight direction, assuming that SHBs fly upwind to find a host (Spiewok et al., 2008; Suazo et al., 2003).

Materials & Methods

Between May and December 2017, an experiment was performed at the Plant Science and Research and Education unit, located in Citra, FL. (29°24'30.7"N 82°10'15.8"W), where 32 *A. mellifera* colonies of mixed European origin were housed in five-frame nucleus Langstroth hives and placed in the field (29°24'30.7"N 82°10'15.8"W) to attract free-flying SHBs. To limit potential variance in attractiveness, all colonies contained four frames covered with bees and three frames with brood. Additionally, the frames contained honey and pollen. The colonies were placed on pallets and sheltered underneath a white shade cover, because free-flying SHB are known to prefer shaded colonies over sun-exposed ones (Akinwande and Neumann 2018). All colonies were fed with sugar water (saccharose, 40%, mass ratio) *ad libitum*. If found queenless during inspection, colonies were requeened or replaced with new nucleus colonies of similar composition within two weeks.

The experiment was performed once in May (replicate A) and five times between October and December (replicates B to F). For replicate A, 32 colonies were used, which were transported and placed at the experimental site between 22 and 25 May 2017. Single colonies were placed at 50, 100, 200 and 400 m in all wind directions (N, E, S, W) and three colonies each spread out on a North-South alignment at 800, 1200, 1600, 2400 and 3200 m eastward from the release point. One colony was placed right at the release point at 0 m (Supplemental Figure S1).

On 25 September 2017, colonies were again placed at the same locations for replicates B to D as for replicate A, but no colony was placed at the release point. Due to a hurricane, the southernmost recapture point at 2400 m was flooded and therefore moved east by 100 m to a distance of 2500 m from the release point. For replicates E and F, a mirrored westward orientation layout was used with no colonies at 1200 m, but two at 1600 m and additional four nucleus colonies at 3600 m from the release point (Supplemental Figure S2). In order to get information on dispersal over distances > 3.6 km, two additional non-experimental apiaries away were included during replicates D to F. Depending on the location of the release point for replicate D, *vs.* E and F, these apiaries were located in Island Grove (N= 2 ten-frame honey bee colonies) at 5.9 and 5.1 km respectively and in Lochloosa (N = 12 ten-frame honey bee colonies) at 13.6 and 12.0 km respectively (Supplemental Figure S3).

Experimental SHBs were reared in the USDA-ARS laboratory in Gainesville, Florida, USA (Stuhl, 2022). Emerged adults were collected from the pupation containers twice a week and maintained in mixed-sex groups of 250 each in 3 L plastic containers with sugar water (saccharose, 40%, mass ratio) *ad libitum* at 25°C under constant darkness. Sugar water was refreshed and dead specimen were removed twice a week. The average age of adult SHBs was 16 days old (SD 5.4) at the time of release and can therefore be regarded as sexual mature (Schmolke, 1974) and likely mated.

Table 1. Mark, Release, Recapture of adult small hive beetles (SHBs, Aethina tumida). The replicates, dates of release, minimum, maximum and average temperatures (°C) for 24 h after release, the number of released and recaptured marked SHBs, recapture rates and the number of captured unmarked SHBs are shown. For replicate A, the numbers between brackets show the results when the (re)captures at the release point are included.

Replicate	**Date**	**Temperature °C (min-max)**	**Released**	**Recaptured**	**Recapture rate**	**Unmarked**	**Total**
A	29 May 2017	28.3 (23.9 – 34.5)	3105	145 (605)	4.7% (19.5%)	161 (182)	306 (787)
B	9 Oct 2017	27.7 (24.5 – 33.0)	889	29	3.26%	78	107
C	23 Oct 2017	20.9 (15.9 – 25.1)	3573	22	0.62%	19	41
D	6 Nov 2017	21.7 (17.3 – 28.3)	3079	41	1.33%	110	151
E	20 Nov 2017	15.6 (8.1 – 23.5)	1619	22	1.36%	45	67
F	4 Dec 2017	18.8 (14.7 – 24.7)	3425	51	1.49%	35	86
Total			15690	310	1.98%	448	760

In order to mark *en masse*, SHBs were fed three consecutive days a sucrose solution 40% with Rhodamine B (Sigma-Aldrich) dye (1%) using cotton dental rolls dipped in the solution for ten minutes prior to release. Rhodamine B is a dye commonly used for internal marking of insects (Schellhorn et al., 2004; Trewin et al., 2021). The drenched dental rolls were placed in a partially covered Petri dish to limit evaporation, but still allowing access by SHBs. Pilot studies indicated that the survival rate of dye fed SHBs eleven days post-feeding was 100% (n = 11), similar to SHBs fed only sugar water (40% solution; n = 11). In the laboratory, the dye could be observed in contained SHBs for 15 days after feeding (data not shown). In order to establish pre-release body mass and sex ratio for each replicate, three SHBs (in total 31 to 64 per replicate) were collected from every feeding container prior to each release, then sexed, weighed and squashed to check if the dye could be observed. In all but one sampled SHB (n = 230), the dye could be observed in the abdomen.

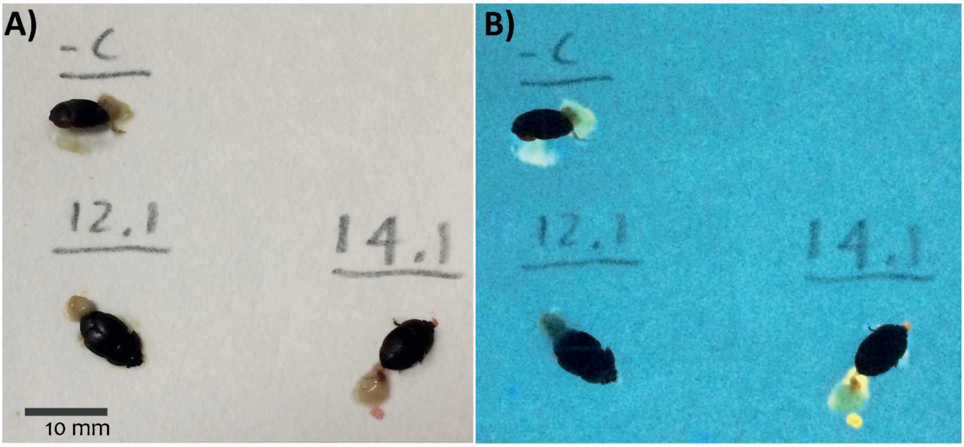

Figure 1. Small hive beetles (SHBs, Aethina tumida*), squashed on a Whatman filter for the purpose of establishing the presence of fed dye markings. SHBs were dye-fed with a sugar solution containing Rhodamine B. SHB '-C' is a negative control, SHB '12.1' is a field-captured unmarked SHB, and SHB '14.1' is a field captured dye-marked SHBs. The left picture (A) shows the SHBs under laboratory tubular lighting. The right picture (B) shows SHBs under UV-light. Pictures taken with an iPhone S5 by J. Elmquist.*

Dye-marked SHBs were released for replicates A to F (Table 1) at a minimum of 14 days from previous release dates. The SHBs were transferred to the release point in plastic containers, placed together on a pallet underneath a white shade and rain cover and then opened. The date, time and temperature at the time of release are given in Table 1. SHBs were released in dry weather conditions only. SHBs could be seen flying away from the release point in various directions. The following day, the SHBs remaining in the containers were counted and removed. This constituted less than 1% of the total number of SHBs released. SHBs were recaptured from the colonies by visually screening (Cornelissen & Neumann, 2018; Neumann et al., 2013) one, two, four and seven or eight days after release. Due to unfavourable weather conditions, day four inspections for replicate E and F were skipped. Sampled SHBs were kept in vials within a cool box containing ice packs and transferred into a fridge until further processing. Within 24 h after collection, all captured SHBs were weighed, sexed, and then frozen at -20°C until they were checked for dye marking via squashing them on a filter paper (Whatman, ∅ = 10 cm). SHBs were considered positive, when red staining of the gut content could be observed with the eye or a stereomicroscope (Leica, M205C, Wetzlar, Germany). As Rhodamine B has fluorescent properties, we also checked samples under UV light (Fotodyne, RM-0026-0, Heartland, Wisconsin). An unmarked control SHB was used each time for comparison with autofluorescence naturally present in SHB tissue. Fluorescent staining of dye-marked SHBs was recognisable as red to yellow-greenish coloured compared to faint green for autofluorescence (Figure 1).

Weather data for the experimental site were obtained from the Florida Automated Weather Network (https://fawn.ifas.ufl.edu/) and collected at 15 min intervals: temperature (°C, 2 m above ground), relative humidity (%), rainfall (cm), wind speed (km/h) and wind direction (0° to 360°, collected 10m above ground). Averages were calculated for the actual time intervals between release and recapture on day one and for the interval between recaptures for day two, four, seven and eight for replicates B to F. For replicate A, the exact time of recapture was not recorded and therefore 24 h averages prior to noon on recapture days were calculated except for day one. Since SHBs were released at 17.30 the previous evening, averages for day one entailed an 18.5 hour interval. Data on wind direction were converted from degrees to eight units of 45° representing the cardinal and intercardinal directions. Furthermore, wind deviation (units of 45°) was calculated by relating the position of recapture locations relative to the release point to the wind direction. Minimum and maximum values for wind deviation were 0 (wind blowing from same direction as position of recapture location relative to release point), and 2 (wind blowing from opposite direction).

Statistical analyses

For replicate A, descriptive statistics were calculated only and due to the different setup, this replicate was omitted from further statistical analyses. For replicate B to F, two datasets were analysed. The first constituted of the number of recaptured dyed and undyed SHBs per nucleus colony for the given time and location during the experiment. The second dataset contained data related to the characteristics (sex and body mass) of pre-release and recaptured dyed individual SHBs. All analyses were performed using R software (R version 3.6.1, R Core Team, 2019). First, a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution was fitted with the count of recaptured marked SHBs as the response variable. The following fixed explanatory variables were included in the model. We used the number of released SHBs (log) and time since the last observation (log) as offset variables. We used distance from the release point (log), time since release (log) as regressors for space and time. Also, we included colony position expressed as the cardinal direction in relation to the release point as well as the location of the release point. The latter reflects the alternate location of the release point and colonies for replicate B to D, compared to E and F (see Supplemental figures S1 and S2). The climate variables temperature, relative humidity, rainfall, wind speed (all standardized variables, i.e. with mean zero and standard deviation of one) and wind deviation were also included in the model. Explanatory variables with random effects were replicate and hive location, to account for the multiple observations from the same physical location. Additionally, we used function dredge from R package MuMIn (Barton, 2019) which fits all-possible subsets, ordering the resulting model fits by (corrected)

Akaike's Information Criterion. We wanted to know if males and females responded differently. Therefore, we used the same model set up for marked SHBs, but took either marked males or marked females as response variable. All other conditions were the same.

We ran a similar model (GLMM, negative binomial distribution) for SHBs we captured that were unmarked, and were thus not released. These represent free-flying SHBs that were entering the colonies and were collected under the same environmental circumstances as the marked SHBs. The model for unmarked SHBs differed in that we included only climate variables and time since last observation as an offset. We excluded captures on time points when colonies had been replaced (n=6), as these colonies were not checked for SHBs prior to use. For fitting the negative binomial GLMMs, we applied the function glmer.nb from package lme4 (Bates et al., 2015).

The second dataset was used to analyse differences in body mass and sex of recaptured marked SHBs and to compare them with those of marked SHBs that were sampled prior to release. The assumption was that flight affects body mass, due to energy consumption. In particular, the hypothesis is that pre-release SHBs are likely to have higher body mass than SHBs that were recaptured. Moreover, with an increasing distance from the release point, SHBs are likely to have a lower body mass. To test this hypothesis, we fitted a GLMM for the body mass of individual beetles, as measured before release and after recapture, assuming a normal distribution for the body mass, fixed effects for source (pre-release/recaptured) and sex, and random effects for replicate and location. Hereafter, we modelled, for recaptured SHBs only, the body mass with fixed effects for distance from the release point (log), day since release, sex, and climate variables (temperature, relative humidity, rainfall and wind speed, and random effects for replicate and hive location). For this, again a GLMM was used, assuming a normal distribution for body mass. To fit these models, we applied package glmmTMB because of its ability to model both means and variability simultaneously, as we found considerable differences in variability of SHB body mass between replicates and between pre-release and recaptured groups. To check model assumptions, we made residual plots and checks using R-package DHARMa (Hartig, 2022). In none of the analyses the residual plots and checks showed remarkable deviations from the model assumptions.

Results

During spring (replicate A), a total of 605 of 3105 marked SHBs were recaptured over the course of a week, of which 460 were found in the colony that was placed at the release point (0 m, Figure 2). Another 145 marked SHBs were recaptured up to 3200 m from the release point, of which 54 SHBs were found at a distance of 50 m from the release point. Within a day after release, two marked SHB females were recovered from a colony at a distance of 3200 m from the release point. During replicates B to F, 454 SHBs were collected of which 165 (out of 12585 released) were recaptured marked SHBs (Table 1). The remainder (n=287) were unmarked and represented free-flying SHBs that entered colonies during observations. Recapture rates varied between 0.62% to 3.26% per replicate.

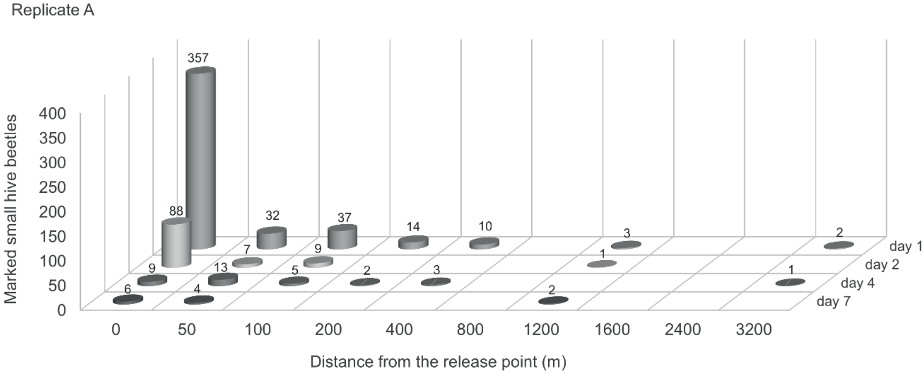

Figure 2. Marked small hive beetles (SHBs, Aethina tumida) recaptured during replicate A (May-June 2017) in honey bee (Apis mellifera) colonies. The x-axis shows the distance in meters in all directions from the release point at which SHBs were recaptured. The y-axis shows the number of marked SHBs recaptured and the Z-axis at which day after release the SHBs were recaptured.

Dredge results show that some explanatory variables are always recurring in a selected subset of models with marked SHBs as target variable (Table 2). These include day since release, distance, orientation, temperature, wind deviation and the offset variable #SHBs released.

Table 2. Dredge results for GLMM with marked adult small hive beetles (SHB, Aethina tumida) as target variable for replicates B to F. The table shows all possible subsets up to delta 2.5 for the resulting model fits by (corrected) Akaike's Information Criterion (AIC). Either the value for the slope is given, or inclusion (+) for binomial and ordinal variables. A missing value indicates the variable is not part of the subset. The model with the lowest AIC value has the best fit.

Model	**2031**	**1775**	**2032**	**1743**	**1776**	**1744**	**2047**	**1791**	**1999**
Intercept	-17.15	-17.00	-16.37	-16.93	-16.24	-16.14	-17.13	-16.99	-17.02
Direction			+		+	+			
Day since release (log)	-1.56	1.90	-1.58	-1.79	-1.92	-1.82	-1.62	-1.91	-1.56
Distance (log)	-1.43	-1.42	-1.40	-1.43	-1.39	-1.39	-1.43	-1.43	-1.43
Orientation	+	+	+	+	+	+	+	+	+
Rain							0.03	0.02	
Relative Humidity	-0.29	-0.24	-0.28		-0.22		-0.30	-0.24	
Temperature	1.01	1.03	0.98	0.90	0.99	0.88	1.02	1.03	0.87
Wind speed	-0.21		-0.22				-0.20		-0.13
Wind Deviation	0.47	-0.46	-0.63	-0.47	-0.62	-0.64	-0.47	-0.46	-0.47
Offset # SHBs released (log)	+	+	+	+	+	+	+	+	+
Offset Time since last observation (log)	+	+	+	+	+	+	+	+	+
df	11	10	14	9	13	12	12	11	10
Log Likelihood	-298.26	-299.42	-295.66	-301.03	-296.91	-298.24	-298.24	-299.42	-300.52
AIC	619.0	619.2	620.0	620.4	620.5	621.0	621.0	621.3	621.4
Delta	0.00	0.26	1.07	1.40	1.49	2.05	2.06	2.33	2.44
Weight	0.15	0.13	0.09	0.07	0.07	0.05	0.05	0.05	0.04

Fewer SHBs were recaptured with an increasing distance from the release point (Figure 3; GLMM: slope = -1.40, SE = 0.17, Z = -8.29, $P < 0.001$). The farthest distance from the release point where marked SHBs (n = 2) were recaptured was 12 km away, but most SHBs were caught at 50 m from the release point. Also, fewer SHBs were recaptured as days since the release passed (slope = -1.590, SE = 0.466, Z = -3.415, $P < 0.001$). The GLMM analysis showed that temperature affected the recapture of marked SHBs (slope = 0.982, SE = 0.169, Z = 5.808, P < 0.001). More SHBs were recaptured when temperatures were higher. No significant effects of either relative humidity, rain or wind speed were found (P > 0.05). However, a significant effect of wind deviation was found (slope = -0.634, SE = 0.219, Z = -2.894, P = 0.04). The higher the deviation, the lower the number of recaptured SHBs. For instance, if a nucleus colony was located to the north of the release point, more beetles were found when the wind was blowing from the north (deviation 0) compared to any other wind direction.

When only marked males were used as a target in the analysis, no significant effect of wind deviation on the number of males recaptured was found (P > 0.05). Also, no significant effect of days since release on male recaptures was found. Fewer marked females were recaptured as time since release progressed, and also wind deviation had a significant explanatory value (slope = -0.561, SE = 0.225, Z = -2.43, P = 0.01) with regard to the recapture of marked females. An increased deviation resulted in fewer marked female SHBs recaptured.

Unmarked SHB captures showed a partly different response to weather variables than marked ones. Similar to marked SHBs, temperature had a significant effect, with a positive coefficient. No significant effect of relative humidity was observed, but rain fall and wind speed affected unmarked SHB captures. The more it rained, the fewer SHBs were captured (slope = -0.303, SE = 0.112, Z = -2.71, P = 0.007). Furthermore, high wind speeds led to lower captures of unmarked SHBs (slope = -0.563, SE = 0.010, Z = -5.66, P < 0.001).

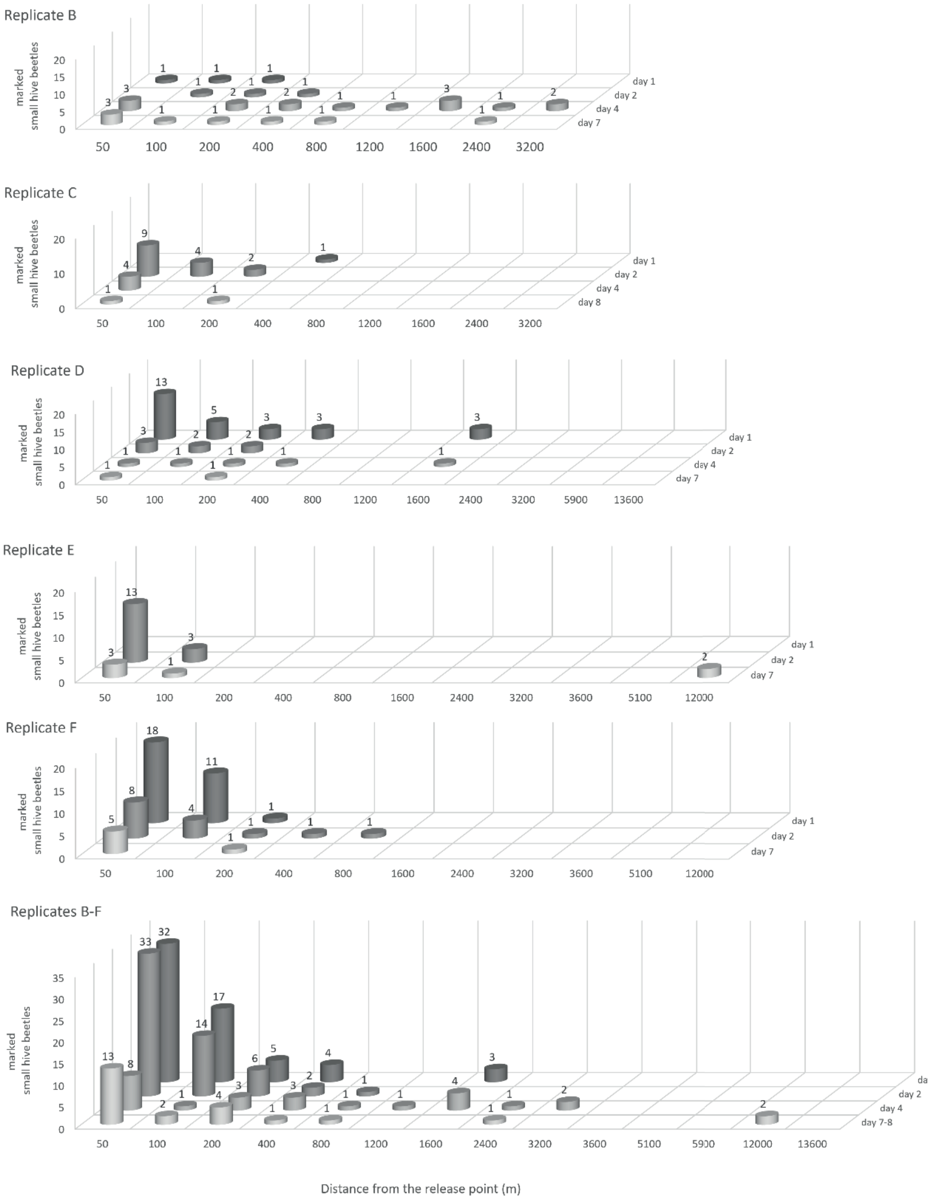

Figure 3. The number of marked recaptured adult small hive beetles (SHBs, Aethina tumida) for replicates B to F and the total in honey bee (Apis mellifera) colonies recaptured in autumn. The x-axis shows the distance in meters in all directions from the release point at which SHBs were recaptured. The y-axis shows the number of marked SHBs recaptured and the Z-axis at which day after release the SHBs were recaptured. The number of marked SHBs recaptured decreased as distance from (GLMM: slope = -1.40, SE = 0.17, Z = -8.29, P < 0.001) and time since release increased (GLMM: slope = -1.590, SE = 0.466, Z = -3.415, P < 0.001).

Discussion

Our data clearly show that flying SHBs are able to cover a distance of at least 3.2 km a day and 12 km within a week. However, dispersal seems to be limited because the majority of SHBs were recaptured in colonies closest to the release point. Further, SHB flight is significantly promoted by warm temperature. SHBs, in particular females, prefer to fly upwind, likely using host cues to locate a colony (Suazo et al., 2003). The results obtained appear to be relevant for our understanding and mitigation of this invasive species.

Neumann et al. (2012) performed a release- recapture experiment using unmarked, freshly emerged SHBs and recaptured individuals in honey bee colonies at a distance of up to 200 m. No other studies have thus far recorded flight distance, although anecdotal reports suggested that SHBs may fly several kilometres with flights over 10 km (Somerville 2003; Wenning, 2001). Systematic studies showed that apiaries were (re)infested after SHBs were removed from these apiaries, suggesting dispersal over several kilometres (Neumann et al., 2010; Spiewok et al., 2008). Similar results have been obtained using traps over shorter distances (Arbogast et al., 2007). Reinfestation from unknown sources, however, could not be excluded and exact distances could not be ascertained. Potentially, the distance SHBs can travel by flight is even higher than what was found in this study. Indeed, even longer flights of other insects have been recorded with the assistance of wind (downwind) and vertical lift (Johnson, 1969; Reynolds et al., 2006). It is likely that in most cases, SHBs will limit flight as it is considered a costly and risky undertaking (Johnson, 1969; Candy et al., 1997). This is supported by the data showing that most recaptured SHBs were found closest to the release point, e.g. during replicate A (76%). This can be explained by the attractiveness of the host (Elzen et al., 2000; Graham et al., 2011; Suazo et al., 2003; Torto et al., 2005) and the close association of the SHB with *A. mellifera* (Lundie 1940). SHBs can be considered an income breeder (Jervis et al., 2008), which needs to acquire resources for reproduction in the adult stage (Ellis et al., 2002; de Guzman et al., 2015; Langlands et al., 2021; Schmolke 1974). The sooner a colony is invaded, the sooner beetles get fed and reproduction can be initiated. This strategy also explains the significant negative correlation between the time since release and the number of recaptured females. Most females in the experiment were sexually mature, had likely already mated, and were subsequently in need of finding a host to provide resources for survival and reproduction. For males, no significant effect was found between time since release and the number recaptured, suggesting that males are more ambiguous towards host finding than females, which could possibly be aggravated if they have already mated. Suazo et al. (2003) showed that females were indeed more responsive to honey bee and bee-hive products than were males. This is further supported by the significant effect of wind deviation found in this study in favour of SHBs, in particular females, flying upwind towards a colony. Also, conspecific cues such as aggregation pheromones could play a role (Stuhl & Teal, 2020). Although we removed SHBs from the colonies at regular intervals, it is likely that conspecific cues were still present and could be detected by released SHBs. To what extent conspecific cues play a role in dispersal is still largely unknown for SHBs (Neumann et al., 2016), yet very much relevant as the absence of congener cues during SHB invasions could affect the dispersal pattern.

Nevertheless, a limited number of SHBs (n=7) still performed long distance flights and ended up over three kilometres away from the release point due to unknown reasons. A migration syndrome (Dingle, 1972) of none-sexual mature individuals initially ignoring obvious host cues before settling (Johnson, 1963) may come into play. Mürrle and Neumann (2004) showed that adult SHBs can remain in the soil for up to 35 days and thus could have mated already well before taking flight. Others report just several days of dwelling in the soil (de Guzman & Frake, 2007; Noor-ul-Ane & Jung, 2022; Schmolke, 1974), in which case it is likely that not all emerging SHBs have yet reached sexual maturity, but are able to fly. Mustafa et al. (2015) showed that mating is most frequent when SHBs are aggregated and around 18 days old. The SHBs used in the experiment varied in age and had the opportunity to mate, but it is well possible that not all had mated. Therefore, the few long-distance migratory SHBs may have still been unmated. Controlled flight studies with SHBs of known age and physiological development are needed to deeper understand long range flight capacity and the possible relationship between dispersal and reproduction as in other insect species (Grilli & Fachinetti, 2017; Jahant-Miller et al., 2022).

Weather variables have been suggested to affect SHB flight and flight initiation (Elzen et al., 2000). This is supported by the dredge analysis in which temperature features in all shown models with best fits (Table 2). Significantly more SHBs were (re)captured under high temperatures compared to low ones within the local ranges of 10.7°C to 26.6°C (for replicates B to F). Night time temperatures dropped below 20°C from replicate C onward during autumn, significantly reducing recaptures and thus apparently limiting SHB flight. Insects have a lower and upper temperature threshold for flight (Taylor, 1963). Our study provides a first indication of that range for SHBs, although it is likely that the range is narrower than the range of recorded temperatures. In particular, the lower threshold is likely to be higher than the lowest recorded temperature. Given the natural distribution of the SHB in Sub-Sahara Africa, it is unlikely that the SHB has developed any specific physiological cold-tolerance adaptation (Sheikh et al., 2017). This notion is supported by the observation that the SHB has adapted a more general cold tolerance strategy by living within its hosts' nest for most of its life stages (Lundie, 1940), specifically in the colony cluster (Atkinson & Ellis, 2012), where temperatures at the core of the cluster can be maintained at 25°C – 31°C even if ambient temperatures reach -20°C (Southwick & Heldmaier, 1987). Furthermore, other studies highlight the temperature dependency of other SHB traits such as oviposition and pupation (Meikle & Patt, 2011; de Guzman et al., 2015; Noor-ul-Ane & Jung 2022). Pupation occurs in the soil, where temperature is one of the key limiting factor for pupation success (Cornelissen et al., 2019). However, no such studies have been dedicated to flight performance. This is striking as temperature is a key factor explaining the activity and distribution of insects (Sheikh et al., 2017) and dispersal is an important feature of invasion biology and distribution patterns of newly established populations (Tobin & Robinett, 2022). In particular in temperate climates, the spread of the SHB might well be limited by the capacity to fly, due to low temperatures (Mellandy, 1939). Likewise, it shows the opportunity at hand, when SHBs are introduced to (sub)tropical climates. Further experimental studies are needed to determine the thermal limits of SHB flight and the implications for invasion.

In our study, rain and relative humidity did not lead to a significant increase in the number of marked SHBs that were recaptured. Flight initiation of SHBs upon emergence from the soil is associated with rainy conditions preceding flight (Elzen et al., 2000; Schmolke 1974), and colony infestation levels in savannah and forest conditions correlate with seasonal rains (Akinwande & Neumann, 2018), although SHB abundance in honey bee colonies in the US was not correlated to rainfall (de Guzman et al., 2010). The outcome we obtained could in part be related to the experimental set up, as marked SHBs had already been taken from the soil and placed in plastic containers. Thus the conditions under which flight was initiated were not comparable to naturally occurring conditions. Also, no rain was observed in the hours prior to release.

Rainy conditions did lead to fewer captures of unmarked free flying SHBs, compared with captures of the same during dry weather conditions. The differences with the results obtained for marked SHBs are likely explained by the different spatial scales and diffused distribution in the surrounding environment in relation to the locations where unmarked SHBs were captured. While rainfall could be a trigger or predictor of flight initiation and explain seasonal population dynamics, it is likely that rainfall as a weather condition in itself has an adverse effect on flight (Wainright et al., 2023). Similarly, wind speed affected the number of unmarked SHBs captured. Fewer were recaptured when wind speeds were high. Wind speed is known to affect upwind flight and flight initiation of other insect species with high wind speeds leading to less activity (Davies, 1936; Vanwoerkom et al., 1983).

Female SHBs were heavier than males, which is in line with earlier research studies (Ellis et al., 2002). The average body mass of SHBs did not change when they had flown, and no effect of the distance flown on body mass of SHBs could be found for either sex. For insects, flight is an energetically costly process (Candy et al., 1997), which in most cases leads to a decrease in body mass due to the exploitation of body components such as glycogen and lipids (Chapman, 2012). It is likely that the SHBs recaptured in the experiment lost body mass as a consequence of flying. However, the body mass loss could have been compensated by the intake of food in the hive soon after arrival (Schmolke, 1974). Furthermore, our results showed that rain and relative humidity led to heavier SHBs being recaptured. A possible explanation could be that heavier, more robust SHBs are better equipped to deal with such weather conditions than are SHBs of lower body mass.

Dispersal is a key factor of established and incipient invasive SHB populations and this study shows to what extent dispersal can play a role in the outward spread of SHBs. Most noteworthy is the distance SHBs are able to cover, but also that SHBs tend to limit flight when they can. This study has also increased our understanding of the effect of weather variables on free-flying SHBs. The results of this study complement existing knowledge relevant for containing, mitigating and anticipating SHB as an invasive species.

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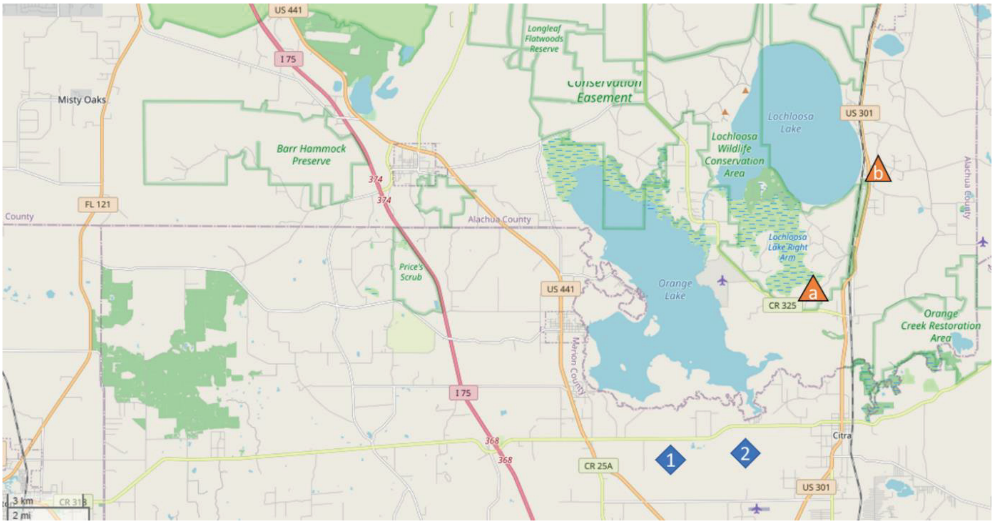
Supplemental files

Supplemental Figure S1 *Map showing the spatial design of the mark-release-recapture experiment for replicates A-D at the Plant Science Research and Education Unit (Citra, Florida, US) experimental farm of the university of Florida. Indicated with a blue cross-sectioned circle is the release point at which location marked small hive beetles (SHBs,* Aethina tumida*) were released. The location of honey bee colonies used to recapture SHBs are marked with a yellow diamond. Note that a honey bee colony was also present at the release point for replicate A. In the southern part of the prairie a blue arrow indicates the relocation of a recapture colony due to the flooding of the prairie in August 2017.*

Supplemental Figure S2 *Map showing the spatial design of the mark-release-recapture experiment for replicates E and F at the Plant Science Research and Education Unit (Citra, Florida, US) experimental farm of the University of Florida. Indicated with a cross-sectioned blue circle is the release point at which location marked small hive beetles (SHBs,* Aethina tumida*) were released. The location of honey bee colonies used to recapture SHBs are marked with a yellow diamond.*

Supplemental Figure S3 *Map showing the location of the apiaries used for collecting small hive beetles (*Aethina tumida*) at distances exceeding 3.6km. The orange triangles indicate the apiaries at (a) Island Grove and (b) Lochloosa. The location of the release points are indicated with a blue diamond: (1) release point for replicates A B, C and D, (2) release point for replicates E and F. Map source: ©Contributors, openstreetmap under licence (CCBY-AT 2.0).*



Chapter 4

Should I stay or should I go? Relative importance of honey bee host colony, conspecifics and alternative food for small hive beetle dispersal

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Abstract

Small hive beetles (SHBs, *Aethina tumida*) are free-flying parasites of western honey bee (*Apis mellifera*) colonies. SHBs are able to detect honey bee, conspecific and alternative food cues, but how these contribute to host finding and dispersal in the field is poorly understood. Here, an experiment was performed to assess the relative importance of host colony strength, conspecifics and cues from alternative food on dispersal. SHBs were released in an apiary consisting of identical hives with either unmanipulated or weakened honey bee colonies or alternative food (ripe bananas). Prior to release marked SHBs were introduced to half of the colonies and banana boxes to a priori provide conspecific cues. The data show that significantly more SHBs were found in unmanipulated colonies compared to weakened ones. No significant effect was observed for conspecific cues, except for alternative food which had more incoming SHB when conspecifics were present. Further, the number of SHBs leaving a colony was not significantly different between unmanipulated and weakened colonies. The majority of SHBs remained in the colony they were released in providing food and shelter without the need for any further flight. Our results show that SHB dispersal is governed by host colony cues rather than conspecific ones with strong colonies being preferred. Alternative food sources were not attractive. Further, SHB appear to show host colony fidelity despite their flight ability. In conclusion, the data highlight the importance of the honey bee host colony for this parasitic beetle.

Introduction

The small hive beetle (SHB, *Aethina tumida* Murray) is a parasite of western honey bees (*Apis mellifera* L.), which has established populations on all continents where honey bees occur (Cornelissen & Neumann, 2022). SHBs naturally inhabit sub-Saharan Africa where they are closely associated with native *A. mellifera* subspecies as their host (Lundie, 1940). SHBs reside in honey bee colonies, where adults feed off honey and pollen or get fed by honey bees through trophallaxis (Langlands et al., 2021; Neumann et al., 2016). Worker bees actively try to kill and remove adult SHBs, but this is mostly rendered unsuccessful due to the turtle-like defensive behaviour of the SHBs (Ellis & Hepburn, 2006; Lundie, 1940). The larvae feed off all honey bee colony constituents and generally occur in cryptic low number (Lundie, 1940; Spiewok & Neumann, 2006). Incidentally, mass reproduction can take place, caused by an aggregation of adults (Lundie, 1940; Neumann et al., 2016). This can lead to colony collapse within a matter of weeks (Neumann et al., 2010). Many countries have implemented policy measures to prevent introductions and eliminate or contain incipient populations (EFSA, 2015; Mutinelli, 2016). Monitoring and containment strategies incorporate the current knowledge of SHB ecology. For instance, sentinel honey bee colonies are used as monitoring units near locations considered a high risk for introduction, such as sea ports or border areas (Calderón-Fallas & Sánchez-Chaves, 2023; Schäfer et al., 2019). Small colonies consisting of three frames are often used, which are easier to visually inspect for SHBs compared to larger colonies (Formato et al., 2021; Schäfer et al., 2019), but using small colonies might limit the attractivity to SHBs. There are indications that host finding and dispersal are affected by colony phenotype (Mustafa et al., 2014). A large, healthy colony could provide a better chance of SHB survival compared to a small, weak colony. Furthermore, it is unclear if host colony phenotype affects the decision to stay put or move to another host. Knowledge of relevant features of honey bee colonies could improve monitoring strategies for the prevention of introductions of the SHB.

Once introduced, incipient populations of invasive alien species such as the SHB spread outward, often through jump-dispersal and facilitated by human traffic (Suarez et al., 2001). However, many different inherent dispersal strategies are known for invasive alien insect species, which can be influenced by environmental conditions, movement capabilities such as flight, reproductive strategies and host preference (Johnson, 1969; Tobin & Robinet, 2022). Individual SHBs are capable of dispersing by flight for more than 10 kilometres (Chapter 3, this thesis). Most SHBs however, limit flight to a minimum, which can be explained by the high risk and energy expenditure involved. When the SHB was introduced into Calabria, Italy in 2014, it was anticipated that it would spread naturally to other regions within several years after it was initially found. To this day, it has not spread to neighbouring districts, except through human-mediated transport. This is partially explained by a successful eradication and containment strategy, which incorporated the destruction and sanitation of infected apiaries and colonies (Mutinelli, 2016). Assumably, this has negatively affected the SHB population locally. However, this does not prevent natural spread of the SHB from undetected infections and therefore only partially explains the lack of outward dispersal of the species. A possible explanation could be the attractiveness to conspecific cues as component of mating strategies (Mustafa et al., 2015). Aggregation of SHBs is a trigger for mating. The most likely place for that to occur under natural conditions is within a honey bee hive. Thus, the assumption is that colonies that contain SHBs are more attractive than colonies void of SHBs, which could explain the lack of outward dispersal of incipient invasive SHB populations.

To investigate the effect of host colony phenotype and conspecific cues on SHB dispersal, an experimental set up was devised using honey bee colonies in different configurations in a single apiary. Two treatment factors were studied (Table 1). Firstly, host colony strength was either normal or weakened. Secondly, SHB presence and cues in honey bee colonies were diminished or enhanced. In order to further differentiate the effect of the host as a food source and the host cues, hives were placed in the apiary without a colony, but with ripe bananas as a known alternative food source for SHBs (Buchholz et al., 2008). Here again, conspecific cues were also diminished or enhanced. We hypothesized that when SHBs are host finding, they prefer strong colonies over weak ones. Strong colonies provide better conditions for SHBs to survive, as there are more food sources which the SHB needs as an income breeder. Furthermore, they are less prone to collapse due to a calamity. It is unclear exactly how SHBs discriminate strong colonies from weaker ones, but this could be due to the quantity and quality of host cues (Suazo et al., 2003), or colony phenotype. Similarly, adult SHBs searching for a host colony are more attracted to colonies that already harbour conspecifics, due to aggregation pheromones present, signalling an opportunity to mate (Mustafa et al., 2015). Furthermore, we hypothesized that colonies are more attractive to adult SHBs than alternative food sources or breeding substrates, as these provide no protection against adverse environmental conditions such as low temperatures or predation. Lastly, we hypothesized that even though strong colonies are preferred, adult SHBs will opportunistically remain in a colony once they have entered it, even in the vicinity of stronger colonies than the host colony they reside in, due to the relatively high risk of dispersal. However, they are more likely to leave weak host colonies compared to strong ones. Adult SHBs residing on alternative food sources, are likely to move to the more suitable environment of a honey bee colony.

Materials and methods

Between 13 September 2018 and 17 November 2018, a field experiment was carried out in Gainesville, Florida (29°37'38.6"N 82°21'22.2"W). A total of 30 experimental hives were randomly assigned to a treatment group (TG, Table 1) and placed in a 10 x 10 grid (Figure 1).

*Table 1. Treatment groups (TG) of the experiment. Each TG consisted of five hives, with either a honey bee (*Apis mellifera*) colony or a box carrying a ripe banana. Host strength as a factor only applied to honey bee colonies, with 'normal' alluding to colonies with no modulations, and 'weakened' to colonies where adult worker populations were artificially reduced. Small hive beetles (SHB,* Aethina tumida*) were either added to hives or not.*

Treatment group	Host	Host strength	SHB added
TG1	honey bee colony	normal	yes
TG2	honey bee colony	normal	no
TG3	honey bee colony	weakened	yes
TG4	honey bee colony	weakened	no
TG5	banana	-	yes
TG6	banana	-	no

Per TG, five hives were assigned. Each square of the grid measured 3 x 3 ft (0.91 x 0.91 m). Twenty hives contained a honey bee colony (TG 1 to TG4) and ten hives were used to place bananas in.

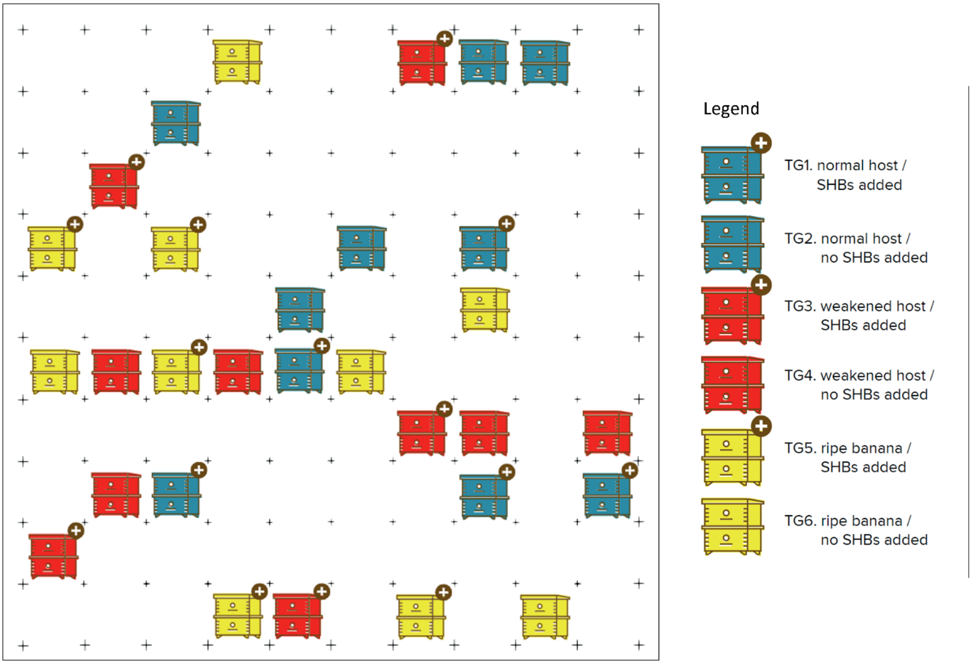

Figure 1. 10x10 grid layout of the experimental apiary. Each square measures 3x3 ft. Blue hives (TG1 and TG2) host honey bee colonies (Apis mellifera) *with normal (unmodified) host strength. Red hives (TG3 and TG4) host honey bee colonies weakened by removing worker bees. Yellow hives (TG5 and TG 6) host bananas as alternative food source. Additionally, to hives with plus signs (TG1, TG3 and TG5), adult small hive beetles (SHBs,* Aethina tumida*) were added.*

Prior to the experiment, all colonies were treated against *Varroa destructor* with Apistan strips (fluvalinate) according to the manufacturers' instruction. Throughout the experiment, colonies were fed with sugar water (saccharose, 40%, mass ratio) *ad libitum*. Half of the colonies (TG3 and TG4) were artificially weakened by reducing the adult worker population. This was done by removing worker bees in excess of two frames covered with bees from the colonies. Excess bees were shaken off the frames into a swarm box and removed from the apiary.

SHBs were reared according to standard methods (Neumann et al., 2013; Stuhl, 2022). After adult emergence, small hive beetles were collected and kept in 3 L plastic containers. Up to 50 beetles were kept per container and fed *ad libitum* with sugar water (mass ratio 40%) until release. The SHBs were divided in two subgroups, of which one was designated for release in the open air in the apiary, whilst the second group was designated for release in hives. Beetles to be released in hives were externally marked by scarring the thorax (Figure 2). SHBs were held down on a piece of Styrofoam, with the thumb and index finger. Then a scalpel with a surgical blade, (Swann-Morton ltd., Sheffield, UK) was used to scar the thorax superficially, though deep enough to leave a permanent marking. Scar markings were applied under a stereomicroscope (10x magnification) or using a headband magnifier (2.5x magnification). The scar marking symbols were specific for the treatment groups in which the SHBs were released. In a preliminary test 9 out of 12 scar-marked SHBs survived eight days after marking, compared to 3 out of 4 unmarked SHBs.

All colonies were assessed for size and queen presence on day 0 of each replicate (N=5). Colony size was estimated by assessing the number of frames containing bees and brood. A frame side was defined as 'covered with bees', if half or more of the total surface area of the frame side was covered with bees. For brood, all stages of brood were considered according to the same principle. The presence of a queen was assessed by visual observation of the queen herself, emergency cells, and/or the presence of eggs. If a queen was absent and not naturally superseded within three weeks, a laying queen was introduced into the colony, or the colony was replaced. During the assessment, all SHBs observed were collected from the colonies using an aspirator (Cornelissen & Neumann, 2018).

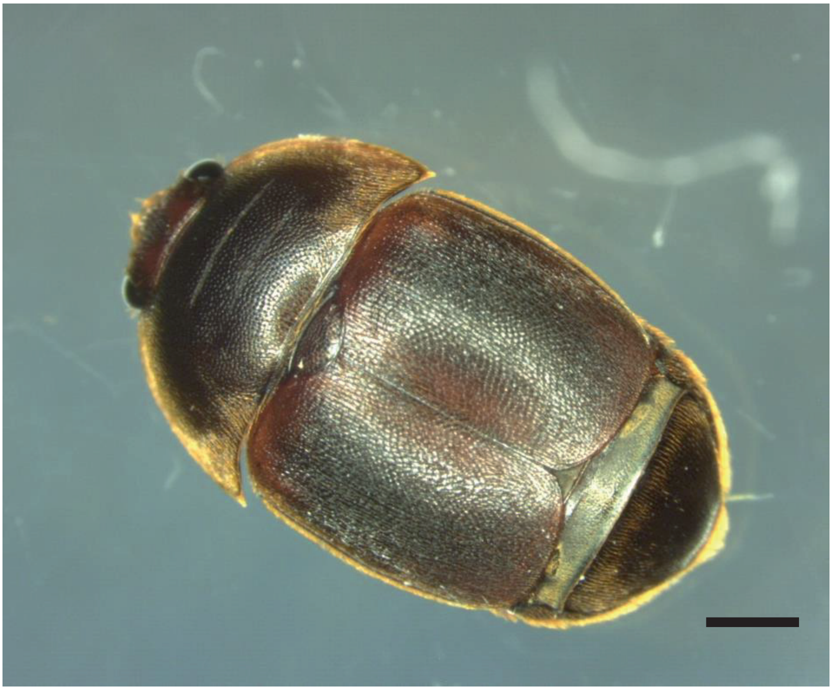

*Figure 2. Adult small hive beetle (*Aethina tumida*) with a double horizontal dash scar marking on the thorax. Size standard: black bar = 1 mm (Photo: Marc Hendriks).*

On day 1, a 3 L plastic lid-covered container holding a peeled ripe banana was placed in hives designated to TG5 and TG6. Three holes (1 cm diameter) were punched in the lids to facilitate the exit and entrance of SHBs. Hereafter, scar-marked SHBs were released into the banana boxes of TG5 and into colonies of TG1 and TG3 (Table 2) through a hole in the lid of the hive or banana box.

Also on day 1, unmarked SHBs were released in the apiary (Table 2). For each replicate eight plastic release containers were prepared carrying equal numbers of SHBs. For each replicate, the location of release was randomly assigned to a square within the 10x10 grid, which did not contain a colony. On day 7, SHBs were collected from the colonies and banana boxes using an aspirator. Collected SHBs were brought to the laboratory, where they were checked for scar markings and sex. This was done using a stereomicroscope (10x magnification). In some cases the sex of a SHB could not be determined.

Table 2. Specifications of small hive beetles (SHBs, Aethina tumida*) released per replicate during the experiment. Given are the number of scar-marked SHBs released per treatment group TG1, TG3 and TG5, the total number of SHBs released in the apiary and the sex ratio of a representative sample of the SHBs used. Dead SHBs remaining in release containers after release in the apiary, are subtracted from the number of SHBs released.*

Serial replicate	**Release date**	**Sex ratio F/M**	**Scar-marked SHBs TG 1 and 3**	**Scar-marked SHBs TG 5**	**SHBs released in the apiary**
1	12 Oct 2018	1.11 (n=57)	24 (n=10)	18 (n=5)	479
2	19 Oct 2018	1.24 (n=76)	35 (n=10)	35 (n=5)	799
3	26 Oct 2018	1.52 (n=53)	25 (n=10)	25 (n=5)	745
4	2 Nov 2018	1.74 (n=50)	40 (n=9)	40 (n=5)	799
5	9 Nov 2018	1.08 (n=50)	40 (n=8)	40 (n=5)	797
Total	-	-	1520 (n=47)	750 (n=30)	3619 (n=5)

Statistical analyses

Observations of colonies that collapsed or were robbed out, were excluded from the analysis. On three occasions, observations on SHBs were missing. All other data was considered for statistical analysis. The mean number of frames containing bees and brood were calculated per treatment group and replicate. In a similar fashion the number of collected SHBs were calculated. To investigate the effect of relevant explanatory variables on dispersing SHBs, a Generalized Linear Mixed Model (GLMM), with a negative binomial distribution and log link function was fitted with 'incoming SHBs' as a dependent variable. Replicate and the factors host strength (normal / weakened / none (i.e. banana)) and SHB (added / not added) and their interaction were added as explanatory variables with fixed effects. Hives were added as a random variable. To correct for spatial correlation, the average distances of hives to colonies of diverse treatment groups were added as covariates. Because of strong collinearities among these distances, only two were taken into the model: distance to TG1 and TG5 (identified as most important through a forward stepping approach). Subsequently, an analysis of deviance was performed and estimated means were calculated for significant treatment effects. To distinguish between treatment groups consisting of colonies and hives with banana's, contrast statements were defined. A second model (GLMM, binomial distribution, logit link function) was used to investigate the effect of relevant variables on the fraction of male SHBs in the hives. Hives with SHBs of an unknown sex were excluded from the analysis, as well as TG6 as only one SHB was recovered from this treatment group in total. Replicate and treatment group entered the model as fixed factors and hive as random factor. A third model (GLMM, beta-binomial distribution, logit link function) was used to analyse the effect of relevant variables on the fraction of SHBs that remained in the colony into which they were released (TG1, TG3 and TG5). In this model the dependent variable was the fraction remaining SHBs, while replicate and colony strength were factors with fixed effects, and hives with random effects. Furthermore, the whereabouts of scar-marked SHBs was accumulated per treatment group or categorised as unrecovered to calculate fractions of the total number released.

Results

Colony parameters

The average number of frames containing bees ranged between 1.6 for treatment groups TG4 and TG3 (SD ± 0.5 and 0.4 respectively, both weakened host strength) and 4.6 (SD ± 0.6) and 4.9 (SD ± 0.4) for TG2 and TG1 respectively (normal host strength, Table 3). The average number of frames containing brood was highest in TG1 (3.8 SD ± 1.1), followed in descending order of size by TG2, TG4 and TG3.

*Table 3. Mean number of frames (± SD) covered with honey bees (*Apis mellifera*) and containing brood, per colony for treatment groups 1 to 4. The data is grouped per treatment and serial replicate. Treatments consisted of TG1: normal host strength with small hive beetles (SHBs,* Aethina tumida*) added; TG2: normal host strength with no SHBs added; TG3: weakened host strength with SHBs added; TG4: weakened host strength with no SHBs added. The number of replicate hives is given between brackets.*

	Serial Replicate					
	1	2	3	4	5	Total
Treatment	Frames covered with bees					
TG1. Normal host strength / SHBs added	5.0 ±0.0 (n=5)	5.0 ±0.0 (n=5)	5.0 ±0.0 (n=5)	4.8 ±0.4 (n=5)	4.6 ±0.9 (n=5)	4.9 ±0.4 (n=25)
TG2. Normal host strength / No SHBs added	5.0 ±0.0 (n=5)	4.2 ±0.4 (n=5)	5.0 ±0.0 (n=5)	4.8 ±0.4 (n=5)	3.8 ±0.4 (n=5)	4.6 ±0.6 (n=25)
TG3. Weakened host strength / SHBs added	1.5 ±0.0 (n=5)	2.1 ±0.2 (n=5)	1.3 ±0.3 (n=5)	1.8 ±0.3 (n=4)	1.0 ±0.0 (n=3)	1.6 ±0.4 (n=22)
TG4. Weakened host strength / No SHBs added	1.7 ±0.3 (n=5)	2.1 ±0.5 (n=5)	1.2 ±0.3 (n=5)	2.1 ±0.2 (n=5)	1.0 ±0.0 (n=5)	1.6 ±0.5 (n=25)
	Frames with brood					
TG1. Normal host strength / SHBs added	4.6 ±0.5 (n=5)	4.2 ±0.8 (n=5)	3.8 ±1.3 (n=5)	3.3 ±0.7 (n=5)	2.9 ±1.5 (n=5)	3.8 ±1.1 (n=25)
TG2. Normal host strength / No SHBs added	4.2 ±0.4 (n=5)	3.6 ±0.9 (n=5)	2.6 ±0.5 (n=5)	3.0 ±0.7 (n=5)	2.2 ±0.4 (n=5)	3.1 ±0.9 (n=25)
TG3. Weakened host strength / SHBs added	2.0 ±0.0 (n=5)	1.9 ±0.2 (n=5)	2.1 ±1.2 (n=5)	1.4 ±0.9 (n=4)	2.7 ±0.6 (n=3)	2.0 ±0.8 (n=22)
TG4. Weakened host strength / No SHBs added	2.0 ±0.0 (n=5)	2.1 ±0.5 (n=5)	2.6 ±0.9 (n=5)	2.1 ±0.2 (n=5)	1.8 ±0.3 (n=5)	2.1 ±0.5 (n=25)

SHB collection

A total of 5116 SHBs were collected during the experiment of which 1511 (out of 2270 released) scar-marked SHBs. The remaining 3605 collected SHBs had no markings and thus originated from outside of the colonies. Another three SHBs flew away during observations, hence the origin of these SHBs could not be established.

Only one SHB was found in a hive in TG6 (banana / no SHBs) during the entire experiment

(Figure 3). The mean number of SHBs collected from hives in TG5 (banana / SHB added) varied between 0.2 to 3.8 per hive, for replicate 1 and 4 respectively. The mean number of SHBs collected in TG1 to TG4 varied between 19.2 (TG4, replicate 1) and 125 (TG1, replicate 4) per colony.

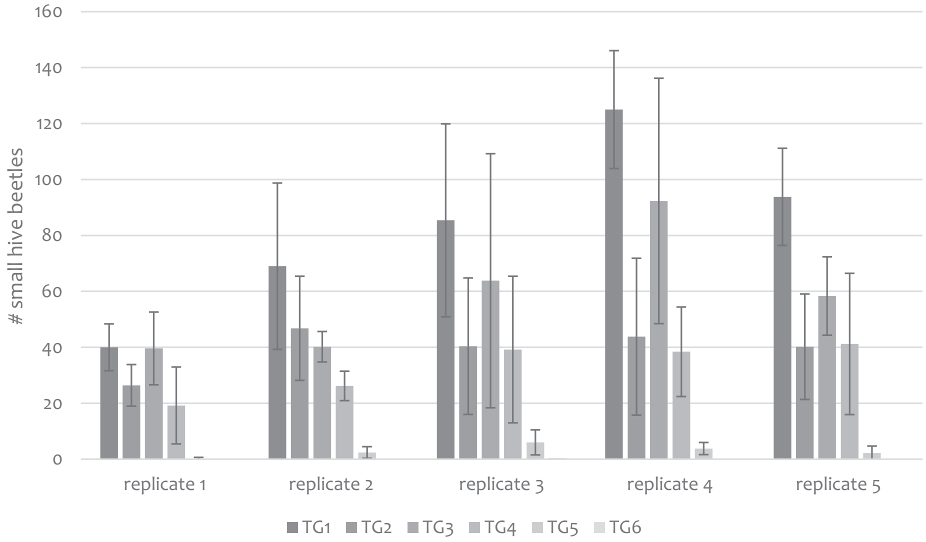

Figure 3. Average number of small hive beetles (SHBs, Aethina tumida) collected per colony during each serial replicate for the treatment groups. Error bars show the standard deviation of the mean. Treatments consisted of TG1: normal host strength with SHBs added; TG2: normal host strength with no SHBs added; TG3: weakened host strength with SHBs added; TG4: weakened host strength with no SHBs added.; TG5: banana, with SHBs added; TG6: banana with no SHBs.

Incoming SHBs

GLMM analysis showed highly significant joint results of the two treatment factors host strength and SHB presence (Likelihood ratio test: LRT = 124.3, df = 5, P < 0.001). Replicate effects and spatial variables were significant (Wald tests, all P < 0.05).

To explore the treatment effects further, we first split treatment groups into colony treatments (TG1,2,3,4) and banana treatments (TG5,6), because SHB behaviour was very different between these two groups. We found that colony treatments (averaged over TG1,2,3,4) had much higher mean counts than banana treatments (averaged over TG5,6): (t = -11.0, df = 130; P < 0.0001). Within the banana group we found 8.2 times higher mean count (95% CI: 1 - 67) for enhanced cues compared to diminished cues (t = 1.98, df = 13; P = 0.05). Within the colony group we found no significant interaction between strength and cues (t = 1.50, df = 130; P = 0.14). There was no main effect of cues (t = 1.15, df = 130; P = 0.25). On the other hand, the main effect of strength was highly significant (t = 5.11, df = 130; P < 0.0001). Normal strength colonies showed a 1.64 times higher mean count than weakened colonies (95% CI: 1.35-1.98).

A pairwise comparison of the strength categories, showed significant differences in incoming number of SHBs between all three groups (P < 0.001). Estimated means of incoming SHBs for colony strength categories are given in Figure 4.

Results for fraction of male SHBs

The binomial GLMM analysis for fraction males revealed no significant differences between the five treatments (Wald test, $X^2 = 2.62$, df = 4; P = 0.62, Supplemental figure S1). Significant differences were found among serial replicates (Wald test, $X^2 = 10.62$, df = 4; P = 0.03).

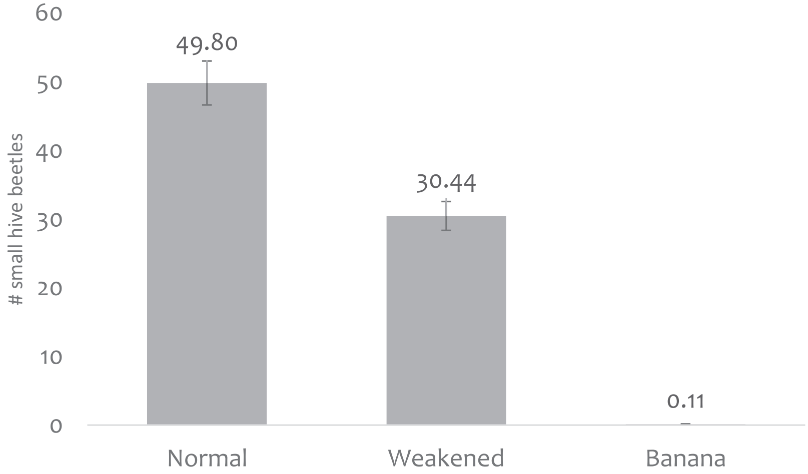

Figure 4. Estimated mean number (± SEM) of incoming small hive beetles (SHBs, Aethina tumida*) grouped for the treatment colony strength (normal, n = 49 / weakened, n = 45 / banana, n = 50) and averaged for replicate and cues. Weakened strength colonies were downsized to contain two frames covered with bees. The banana group consisted of a ripe banana placed in a plastic box within the hive. Group means differed significantly (pairwise comparisons by Wald tests, Tukey method, P < 0.001).*

Fraction of SHBs that remain

The fraction of SHBs that remained in the colony they were released in (TG 1,3,5), revealed significant differences between the three treatments (Wald test, $X^2 = 136.2$, df = 2; P < 0.0001). Pairwise comparison (Tukey method) showed no significant difference between TG1 (normal colony) and TG3 (weakened colony) (t = 1.12, df = 62; P = 0.51,), but the difference between TG1 and TG5 (banana group) was significant (t = 11.2, df = 62; P < 0.0001; odds ratio = 28.4 with 95% CI 13.8 - 58.2), as was the difference between T3 and T5 (t = 11.2, df = 62; P < 0.0001; odds ratio = 21.7 with 95% CI 10.5 - 45.1). Significant differences were also found between serial replicates (Wald test, $X^2 = 10.3$, df = 4; P = 0.04). Table 4 shows the recovery rate (fraction) of SHBs that were released in hives of TG1, 3 and 5. A total of 641 out of 820 SHBs that were released inside colonies of TG1 were collected from hives in the experimental apiary. 179 SHBs (21.8% of the total) remained unaccounted for. The majority (n = 586, 71.5%) of the SHBs released in TG1 was collected from colonies belonging to the same treatment group. On average a fraction of 0.01 (n = 25, range 0 - 0.08) was recovered from other treatment groups. Similarly, SHBs released in TG3 (n = 697), were mostly found in colonies of this treatment group (n = 438, 62.8%), and an average fraction of 0.02 (n = 25, range 0 – 0.12) was collected in hives of other treatment groups. A total of 177 SHBs (25.4% of the total) remained unrecovered. Finally, over half (n = 440, 55.6%) of the SHBs released (n = 790) in TG5 were unrecovered, whilst a total of 64 (8.1% of the total) remained. An average fraction of 0.08 per treatment group (n = 25, range 0 – 0.17) was recovered in hives of other treatment groups.

Table 4. Recovery as fraction of the total number of small hive beetles (SHBs, Aethina tumida) released into hives of treatment groups TG1, TG3 and TG5. Treatments consisted of TG1: normal host strength with SHBs added; TG2: normal host strength with no SHBs added; TG3: weakened host strength with SHBs added; TG4: weakened host strength with no SHBs added.; TG5: banana, with SHBs added; TG6: banana with no SHBs. Fractions are given per serial replicate. Green: highlights the fraction of SHBs which was recovered in hives belonging to the same treatment group as they originated from.

		Recovery (fraction)						
	Origin	TG1	TG2	TG3	TG4	TG5	TG6	Unrecovered
Replicate 1	TG1 (n=120)	0.66	0.04	0.08	0.03	0.00	0.00	0.20
	TG3 (n=118)	0.03	0.03	0.47	0.02	0.00	0.00	0.46
	TG5 (n=90)	0.12	0.16	0.12	0.11	0.01	0.00	0.48
Replicate 2	TG1 (n=175)	0.58	0.05	0.02	0.03	0.00	0.00	0.32
	TG3 (n=175)	0.04	0.01	0.84	0.02	0.00	0.00	0.09
	TG5 (n=175)	0.03	0.09	0.02	0.05	0.05	0.00	0.76
Replicate 3	TG1 (n=125)	0.83	0.01	0.01	0.02	0.00	0.00	0.14
	TG3 (n=125)	0.07	0.05	0.62	0.06	0.00	0.00	0.21
	TG5 (n=125)	0.12	0.06	0.10	0.17	0.22	0.00	0.34
Replicate 4	TG1(n=200)	0.66	0.01	0.01	0.01	0.00	0.00	0.32
	TG3 (n=160)	0.12	0.01	0.52	0.05	0.00	0.00	0.30
	TG% (n=200)	0.10	0.06	0.09	0.09	0.10	0.00	0.57
Replicate 5	TG1(n=200)	0.85	0.03	0.01	0.01	0.02	0.00	0.10
	TG3 (n=1190)	0.05	0.02	0.64	0.02	0.00	0.00	0.28
	TG5 (n=200)	0.14	0.09	0.08	0.12	0.04	0.00	0.54
Mean	TG1 (n=820)	0.72	0.03	0.02	0.02	0.00	0.00	0.21
	TG3 (n=697)	0.06	0.02	0.62	0.03	0.00	0.00	0.27
	TG5 (n=790)	0.10	0.09	0.08	0.11	0.08	0.00	0.54

Discussion

The results show that free-flying SHBs are more attracted to strong host colonies, compared to weakened ones. We did not observe an effect of the presence of SHB conspecifics within honey bee colonies on host colony attractiveness. Furthermore, SHBs that reside in a colony are likely to stay there, irrespective of colony strength. The results attest to the complex relationship of parasite and host, but also to the opportunistic nature of the SHB and give insight into choice behaviour of free-flying, host-bound and settled SHBs in the context of dispersal.

As a highly mobile species, the SHB can detect a host while dispersing over long distances (Chapter 3). In doing so, it seems likely that an encounter with a host will result in the invasion by SHBs. Whilst all host colonies are potentially attractive, our results point to normal colonies being more prone to invasion in comparison with weakened ones. These results contradict earlier findings (Spiewok et al., 2007), but are in line with more recent results by Mustafa et al. (2014), who showed that full-sized honey bee colonies attracted more SHBs than smaller colony configurations when left undisturbed. In their study, they also showed that there was no difference when colonies were screened for SHBs regularly. Both Spiewok et al. (2007) and Mustafa et al. (2014) relied on natural SHB populations for their experiments, but in our study, we actively released SHBs within the experimental apiary and left colonies undisturbed for a week after. Our design mostly resembles the setup of Mustafa et al. (2014) for unscreened colonies, and the results are similar. It is likely that the attractiveness of colonies to SHBs depends on strength of host cues (Graham et al., 2011; Suazo et al., 2003; Torto et al., 2005), although the quantity of volatiles emitted by colonies, and the relative attractiveness to SHBs has not been explored in this study or other studies. Furthermore, other factors such as host size itself can play a role in the attractiveness of hosts to parasites (Langeloh & Seppälä, 2018; Lo et al., 1998; Valera et al., 2004), but for honey bee colonies this is likely an interactive effect as larger colonies release higher amounts of odours than smaller ones. In our experiment, we removed worker bees to create weakened colonies. This leads to a disruption in colony demographics and a partial depletion of the workforce, while the demand for labour remains (Barron, 2015). Brood was not removed, in order to create a lower bee to brood area ratio, which has been suggested as a possible factor in explaining higher SHB abundance and reproduction in colonies (Neumann et al., 2016). To which extent this could be of importance to dispersal remains to be seen. A related factor could be a shift in odour profiles of honey bee colonies when stressed, possibly altering the attractiveness to SHBs. SHBs, but possibly also other symbionts may, however, change the odour profiles of honey bee colonies.

The results reaffirm the strong association between honey bees and SHBs, in comparison with other food sources (Buchholz et al., 2008). Although SHBs were found on bananas, a strong preference for honey bee colonies was apparent. It is, however, this comparison that highlights differential patterns in terms of attractiveness to conspecifics. More incoming SHBs were found on bananas when SHBs were added, compared to bananas to which no SHBs were added. Aggregation pheromones are likely to play a role in mating behaviour of SHBs (Mustafa et al., 2015) and SHBs are known to congregate in colonies (Lundie, 1940). For dispersal, conspecific cues of adult SHBs are probably overruled in the presence of host colonies. But when the preferred host is absent, it is likely that conspecific chemical cues can play a role in dispersal. This could be the case for novel hosts with less attractive odour profiles, such as stingless bees or alternative food sources as shown in this study. Still, it remains to be seen to what extent conspecific cues are used by SHBs for distances that extend beyond an apiary.

For SHBs released into colonies, we expected a similar response to that of free-flying SHBs. However, no difference was observed in the fraction of SHBs that remained in normal or weakened colonies. The majority of marked SHBs released in colonies stayed put. Although we cannot entirely exclude the shift to other colonies within the same treatment group, as SHBs were marked on treatment level and not on colony level. We assume that, based on the proportion of recovery of SHBs in other treatment groups (Table 5), most SHBs stayed in the colony they were released into. Dispersal is risky and energetically costly for insects and there is a potential trade-off with reproduction (Candy et al., 1997; Chapman, 2012). Avoiding the investment in dispersal could therefore be beneficial to individual SHBs.

Individual female SHBs can remain alive within a colony for long periods of time and apply a sit-and-wait strategy, only reproducing in colonies when the opportunity arises in the form of easily available protein rich resources, due to host defence being compromised (Neumann et al., 2016). Thus, host condition on the short time scale as observed in our study, will likely not show an effect on displacement, unless a colony completely collapses. Although SHB aggregation pheromones are likely not relevant for dispersal, they are involved in SHB clustering within colonies (Mustafa et al., 2015). This could also contribute to the lack of displacement of SHBs. Nevertheless, in the absence of a colony, but the presence of a suitable substrate for reproduction (banana), the majority of SHBs left, underlining the importance of the host in this regard.

Our results contribute to a further understanding of SHB dispersal, and in particular the relationship with its host and conspecifics under field conditions. This is relevant information in the context of policies to tackle invasions (Schäfer et al., 2019). When designing monitoring strategies for early detection, it is advised to use large colonies instead of the commonly used small sentinel colonies (Formato et al., 2021). Furthermore, it is likely that the displacement of SHBs is limited once a colony is invaded and if a colony is left undisturbed for a certain period (Mustafa et al., 2014). This means that outward spread in the early stages of an introduction is limited. Our results therefore improve policy decision making with regard to dealing with this invasive species.

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Supplemental file

Supplemental Table S1 *Estimated mean (± SE) male to female ratio of small hive beetles (SHBs,* Aethina tumida*) collected from hives per treatment group. Treatments consisted of TG1: normal host strength with SHBs added; TG2: normal host strength with no SHBs added; TG3: weakened host strength with SHBs added; TG4: weakened host strength with no SHBs added.; TG5: banana, with SHBs added; TG6: banana with no SHBs.*

Treatment group	Male to female ratio
TG1 (n=25)	0.45:1 (SE±0.12)
TG2 (n=24)	0.43:1 (SE±0.16)
TG3 (n=24)	0.43:1 (SE±0.15)
TG4 (n=21)	0.42:1 (SE±0.17)
TG5 (n=14)	0.49:1 (SE±0.06)
TG6 (n=0)	n/a

Chapter 5

Successful pupation of the small hive beetle in greenhouse substrates

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Abstract

The small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), is an invasive pest that has spread globally. Western honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), are considered the most important host and infestations can lead to collapse of colonies. Larvae feed on honey, pollen, and brood inside the hive and leave the hive as post feeding wandering larvae to pupate in the surrounding soil. Other host species include bumble bees, stingless bees, and solitary bees, all of which can facilitate small hive beetle reproduction and are used for greenhouse crop pollination worldwide. Here, we investigated if small hive beetles can complete their life cycle when soil is absent by pupating in plant root-supporting substrates commonly used in greenhouses. Wandering small hive beetle larvae were introduced into containers with coconut fiber, perlite, a mixture of both and stone wool substrates to investigate pupation success and development time. Sand was used as control substrate. In all but one substrate (perlite), small hive beetles developed into adults equally well as they did in the sand. Development time ranged between 23 and 37 days and was not different from that of the control. We showed that small hive beetles can pupate in greenhouse substrates. This could constitute a problem for greenhouse pollination as well as it could facilitate small hive beetle survival in areas which otherwise would be deemed unsuitable or marginal environments for small hive beetles to become established. Our study highlights the opportunistic nature of the small hive beetle as an invasive species.

Introduction

The small hive beetle, *Aethina tumida* Murray, is an invasive pest of social bee colonies (Ellis & Hepburn, 2006; Neumann et al., 2016), which has spread from its native range in sub-Saharan Africa to all continents except Antarctica (Cornelissen et al., 2019; Schäfer et al., 2019). Small hive beetles reproduce in honey bee nests, usually at cryptic levels that do not damage host colonies (Spiewok & Neumann, 2006). Occasionally, reproduction occurs as a mass event, whereby the small hive beetle larvae devour honey bee nest components (bee brood, bee bread, honey, and dead adult bees), often leading to total colony collapse (Ellis, 2012). Once reaching the post feeding stage, small hive beetle larvae migrate out of a hive and burrow into neighbouring soil where they pupate in chambers they excavate (Neumann et al., 2016). Small hive beetles can also reproduce in association with nests of bumble bees, stingless bees, and solitary bees (Gonthier et al., 2019; Hoffmann et al., 2008; Neumann et al., 2016).

Honey bees, stingless bees, bumble bees, and solitary bees are all used as pollinators for greenhouse crops. These crops include aubergine, strawberry, courgette, tomato, and bell pepper (Guerra-Sanz, 2008), among others. With optimal growing conditions maintained for up to 11 months a year and the presence of host species for many months, greenhouses could serve as hotspots for small hive beetles, especially in areas where they would otherwise meet environmental constraints limiting their reproduction and survival (Cornelissen et al., 2019). The small hive beetle's potential use of greenhouses could be limited if the beetle is unable to complete its lifecycle within the structure. Many greenhouse crop systems include substrates other than soil in which plants are grown. Even though small hive beetles can pupate in a variety of soil types, as long as the soils are sufficiently moist (Ellis et al., 2002), their ability to pupate successfully in these greenhouse substrates is currently unknown. Here, we investigated if substrates commonly used in greenhouses are suitable pupation media for small hive beetles. We hypothesized that small hive beetle larvae would be able to pupate in these substrates, making greenhouses potentially suitable sites for small hive beetle reproduction and survival.

Materials and Methods

Adult small hive beetles (n = 44) were manually collected 11 August 2016 from naturally infested local honey bee colonies managed according to standard practices for the region at the University of Florida, Honey Bee Research and Extension Laboratory, Bee Biology Unit (Gainesville, FL, 29°37′37.1″N 82°21′22.6″W). The adult small hive beetles were divided into two groups and placed into separate cubic plastic boxes (approximately 2 litre per box), each containing 400 g of standard small hive beetle food mixture (Neumann et al., 2013). The adults were left to oviposit and the boxes were maintained at 25°C and constant darkness during the experiment. The boxes were checked twice weekly, at which time moist tissue paper and additional food were added ad libitum. The tissue paper was moistened to near saturation with tap water and was used to raise the humidity in the breeding boxes to facilitate larval hatching (Neumann et al., 2013). On 1 September 2016, ample wandering larvae (Neumann et al., 2013) were available to start the pupation experiment.

Three greenhouse substrates were selected for this investigation (purchased in dry form, at Gator Hydroponics, Gainesville, FL): one organic (coir or coconut fibre - Cocogro) and two inorganic substrates (stone wool and perlite), all of which are commonly used in soilless plant cultures (Bar-Tal et al. 2019). Furthermore, we used a mixture of coconut fibre and perlite in a 70/30 ratio by mass, as it is also used in greenhouse cultures (Bar-Tal et al., 2019). We used sand (Quickrete premium play sand) as a positive control because small hive beetles readily pupate in sand (Ellis et al., 2002). The substrates were put into transparent plastic pupation containers (1.6 litre), with a minimum depth of 10 cm of substrate available for small hive beetle pupation. A total volume of 1,100–1,200 ml of substrate was used. The moisture levels of the different substrates varied due to the differences in the water holding capacity of the substrates (Table 1). Principally, the substrates were saturated with tap water, after which excess water was left to soak for 10 min and then drain for another 10 min.

Table 1. Substrate composition per container used for the in vitro pupation of small hive beetles (Aethina tumida)*. Substrate weight and water volume summed to a total volume of 1,100 to 1,200 ml per container.*

Substrate	Weight of substrate (g)	Water (ml)
Perlite	100	236
Coconut fiber / perlite	70 / 30	411
Coconut fiber	100	600
Stone wool	90	766
Sand	1250	100

Twenty-five small hive beetle wandering larvae were added to each pupation container, totalling 100 larvae per substrate distributed over four replicate containers per each of the five treatments. Thereafter, the containers were placed in an incubator at 25°C and total darkness for the duration of pupation. The containers were checked daily, for 37 d, for dead larvae and emerged adults until 9 October 2016, on which day all containers were checked for remaining live adult small hive beetles by filtering the soil. A generalized linear model (GLM) with a beta binomial distribution was used to compare emergence rates aggregated for containers with substrate as the fixed factor. We then performed pairwise comparisons between emergence rates of the substrates tested. Small hive beetle larvae that drowned within the first 5 d after exposure to the substrates were omitted from the analysis. All hypothesis tests were likelihood ratio tests (LRT). Similarly, development time (day adult emerged minus day larvae added to substrate) was compared between the different substrates with a univariate GLM with containers as a random factor. Pairwise comparisons between substrates were performed and estimated means generated from the models.

Results

Within 5 d after introduction to the substrates, 44 larvae (range: 5 – 13) drowned in the excess water accumulated at the bottom of the perlite containers. Similarly, four larvae had drowned in one of the containers of stone wool. The GLM analysis for the emergence of small hive beetles showed significant differences among substrates (df = 4, LRT = 27.267, $P < 0.001$). The pairwise comparison showed that emergence rates of small hive beetles were similar for all substrates, except for the perlite substrate (Table 2). The emergence rates in stone wool, coconut fibre, and the mixture were not different from that in the control substrate (sand, $P > 0.05$). Typical small hive beetle pupation chambers could be observed through the container wall in all substrates, except perlite. Here, the remaining larvae did not pupate and eventually died. Significant differences were observed in the development time of small hive beetles pupating in the various substrates ($F = 6.355$, $df = 3$, $P < 0.001$, see Table 2). Development time in coconut fibre and stone wool substrates were significantly shorter than in the control substrate ($P < 0.05$), but similar to the coconut fibre/perlite mixture. The latter was not different from the control substrate ($P > 0.05$). The first adult small hive beetles emerged from the coconut fibre substrate after 23 d. The last adults to emerge were observed in the sand, 37 d after the larvae were exposed to the substrate.

*Table 2. Estimated mean emergence rates (%) and development time (days) of small hive beetles (*Aethina tumida*), pupae in the tested greenhouse substrates and in sand as a positive control (emergence rate: GLM, $df = 4$, $LRT = 27.267$, $P < 0.001$, development time: GLM, $F = 6.355$, $df = 3$, $P < 0.001$). $n = 4$ replicate containers and $n = 456$ and $n = 317$ larvae for emergence rate and development time, respectfully. Larvae that drowned in the first 5 days were omitted from the analysis. Column means with the same letter are not different at $P \leq 0.05$.*

Substrate	Emergence rate (± SE) (%)	Development time (± SE) (days)
Perlite	0 (0.0)^{a}	Not applicable
Coconut fiber / perlite	68.1 (12.0)^{b}	28.5 (0.42)^{ab}
Coconut fiber	79.2 (10.4)^{b}	27.7 (0.43)^{b}
Stone wool	87.6 (8.1)^{b}	27.8 (0.33)^{b}
Sand	81.5 (8.6)^{b}	29.5 (0.31)^{a}

Discussion

Our data clearly show that small hive beetles can pupate in substrates used in greenhouse settings. In fact, small hive beetles pupated equally well in coconut fibre, coconut fibre + perlite, stone wool, and sand (the control substrate) with the emergence rates varying between 68.1 and 87.6%. The development time for rockwool and coconut fibre was shorter than the control substrate. Several studies obtained comparable results in various substrate types at similar temperatures (Cornelissen et al., 2019), thereby indicating that all of the tested substrates, except perlite, are suitable for completion of the small hive beetle life cycle. In comparison with the other substrates, perlite (an amorphous volcanic glass) has an extremely low density and does not form a consistent mass. The perlite substrates were composed of separate lumps of up to 1 cm in size, which probably could not support certain physical aspects required by small hive beetles for pupation. For instance, wandering larvae were unable to make pupation chambers (Neumann & Elzen, 2004) and would fall to the bottom, unable to crawl back into the substrate. Furthermore, several days into the experiment, water would accumulate at the bottom of the containers, in which several larvae drowned. Drowning of larvae ($n = 4$) also occurred in one container with stone wool. The cause of this was not related to the suitability of the substrate, but rather because the stone wool did not touch the bottom of the container entirely.

Our data further suggest that small hive beetles could cause problems to bees used to pollinate horticultural crops grown in greenhouses. However, no such large-scale problems have been reported in bumble bee pollinated greenhouse crops in the United States because the small hive beetle became established in 1996. At the same time, no information exists on the potential risks for other bee species, such as *Bombus terrestris* Linnaeus (Hymenoptera: Apidae), used for crop pollination outside of the United States. Furthermore, the risk for honey bee pollination units could be higher, as small hive beetles could already be residing in a colony before they enter a greenhouse. Although the use of honey bees for greenhouse pollination in the United States might be minimal, they are used at a larger scale in other countries. For instance, approximately 5,000 honey bee colonies are used annually for greenhouse and seed pollination in the Netherlands (Blacquière et al., 2009). Moreover, social bee colonies could be prone to small hive beetle infestation in greenhouses given that foraging and environmental conditions in greenhouses are generally suboptimal for pollinator units (Guerra-Sanz, 2008).

In a broader context, our data suggest an increased ability and likelihood for small hive beetles to complete their life cycles in greenhouses, thereby constituting potential hot spots for this beetle in regions, which would otherwise be marginal or unsuitable for establishment of this invasive species (Cornelissen et al., 2019). In conclusion, we demonstrated that the small hive beetle can pupate in a variety of substrates, which is consistent with the opportunistic nature of this invasive species (Gonthier et al., 2019).

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Chapter 6
Global warming promotes biological invasion of a honey bee pest

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Abstract

Climate change and biological invasions are two major global environmental challenges. Both may interact, e.g. via altered impact and distribution of invasive alien species. Even though invasive species play a key role for compromising the health of honey bees, the impact of climate change on the severity of such species is still unknown. The small hive beetle (SHB, *Aethina tumida* Murray) is a parasite of honey bee colonies. It is endemic to sub-Saharan Africa and has established populations on all continents except Antarctica. Since SHBs pupate in soil, pupation performance is governed foremost by two abiotic factors, soil temperature and moisture, which will be affected by climate change. Here, we investigated SHB invasion risk globally under current and future climate scenarios. We modelled survival and development time during pupation (= pupal performance) in response to soil temperature and soil moisture using published and novel experimental data. Presence data on SHB distribution were used for model validation. We then linked the model with global soil data in order to classify areas (resolution: 10 arcmin; i.e. 18.6 km at the equator) as unsuitable, marginal and suitable for SHB pupation performance. Under current climate, the results show that many areas globally yet uninvaded are actually suitable suggesting considerable SHB invasion risk. Future scenarios of global warming project a vehement increase in climatic suitability for SHB and corresponding potential for invasion, especially in the temperate regions of the Northern hemisphere, thereby creating demand for enhanced and adapted mitigation and management. Our analysis shows for the first time effects of global warming on a honey bee pest and will help areas at risk to prepare adequately. In conclusion, this is a clear case for global warming promoting biological invasion of a pest species with severe potential to harm important pollinator species globally.

Introduction

Human-mediated biological invasions are one of the most serious threats for biodiversity (Dyer et al., 2017; Early et al., 2016; McGeoch et al., 2010) which may even cause the breakdown of classical biogeographic regions (Capinha et al., 2015). Moreover, invasive species can cause considerable social, economic and ecological damage (Blackburn et al., 2011; Pimentel et al., 2000), altering ecosystems and endangering food security (Schweiger et al., 2010; Veldtman et al., 2011; Ziska et al., 2011). The invasiveness and impact of invasive species is a complex interplay between biotic and abiotic factors (D'Antonio, 1993; Thuiller et al., 2006; Tobin, 2015) with varying consequences (Cuthbert et al., 2018; Rejmánek & Richardson, 1996; Ricciardi & Cohen, 2007). Changing climates can impact the current status of alien species, often resulting in an increased probability to become established or to spread to areas currently deemed environmentally unsuitable (Dukes & Mooney, 1999; Early et al., 2016; Sutherst et al., 1996). This is particularly true for ectotherms, which depend on climatic conditions to permit survival and development within the thermal limitations a habitat poses (Barbet-Massin et al., 2013; McCann et al., 2017; Roura-Pascual et al., 2004).

The impact of invasive species is of major concern for society when the provision of ecosystem services is affected (Pejchar & Mooney, 2009). The Western honey bee, *Apis mellifera,* Linnaeus, is a particularly important species for providing pollination services globally (Calderone, 2012; Hung et al., 2018). However, managed honey bees have been facing severe colony losses in recent decades (Neumann & Carreck, 2010; vanEngelsdorp & Meixner, 2010; Brodschneider et al., 2018; Jacques et al., 2017). Even though the number of managed honey bee colonies is increasing globally, the demand for pollination is growing at a much higher rate (Aizen & Harder, 2009; Gallai et al., 2009). Amongst the many factors potentially impacting honey bee health and thus pollination services, invasive parasitic species, e.g. introduced by global trade in honey bees and related products such as wax and honey (Chanpanitkitchote et al., 2018; Krongdang et al., 2018; Neumann et al.,2016; Idrissou et al., 2019; Schäfer et al., 2019), can play a key role (Potts et al., 2010; Rosenkranz et al., 2010; Neumann et al., 2016; van Dooremalen et al. 2018). However, knowledge on potential effects of climate change on such species is currently lacking (Le Conte & Navajas, 2008). The small hive beetle (SHB, *Aethina tumida*, Murray) is a long known parasite of social bee colonies (honey bees: *Apis mellifera* (Lundie, 1940); *Apis cerana* (Cervancia et al., 2016), bumble bees: *Bombus impatiens* (Spiewok & Neumann, 2006), stingless bees: (Greco et al., 2010) native to sub-Saharan Africa, which can also infest nests of solitary bees (*Megachile rotundata*, Gonthier et al. 2019). Since 1996, SHB has become an invasive species and has established local populations on every continent except Antarctica (Neumann et al., 2016; Schäfer et al., 2019). Despite comprehensive elimination and contingency efforts, it is likely to continue spreading (Schäfer et al., 2019). The impact of SHBs on honey bee colonies in the invasive ranges is well documented (Neumann & Elzen, 2004) and depends on infestation levels, with higher infestation levels more likely leading to host colony collapse (Spiewok et al., 2007). When SHBs mass reproduce with often thousands of larvae (Neumann and Elzen, 2004), they can kill even strong colonies of European honey bee subspecies within 10 days (Neumann et al., 2010), often resulting in the full structural collapse of the entire nest (Hepburn and Radloff, 1998). This is very rare in the native range of SHB in Africa in colonies of the respective local honey bee subspecies (Lundie, 1940; Schmolke, 1974; Neumann 2017), where SHBs probably mostly rely on non-destructive low-level reproduction (Idrissou et al., 2018). The higher susceptibility of European honey bee subspecies is probably due to quantitative differences in a range of social immunity traits compared to the African ones (e.g. aggression (Elzen et al. 2001), absconding (non-reproductive swarming (Neumann et al. 2018) and social encapsulation of SHBs (Neumann et al., 2001)).

Besides biotic factors, abiotic factors may also contribute to the invasion success of SHBs. In contrast to other beetles, which can complete an entire life cycle within host colonies (Krishnan et al., 2015), SHBs have to pupate in the soil to complete their life cycle (Lundie, 1940; Ellis et al., 2004). SHB pupation success (survival rate) and the duration of pupation are governed by soil humidity and temperature (Ellis et al., 2004; Meikle & Patt, 2011; Meikle & Diaz, 2012; Bernier et al., 2014; Akinwande & Neumann, 2018). It is therefore apparent that abiotic factors can play a key role in explaining the performance and thus invasion success of this species. Indeed, under favourable environmental conditions, i.e. high humidity and temperature, SHBs can cause significant damage to apiculture outside its endemic range. For instance, in 1998 SHB caused damage of more than 3 million USD in Florida (Neumann & Elzen, 2004). There may be up to six SHB generations per year under US and South African climatic conditions (Neumann & Elzen, 2004), and de Guzman and Frake (2007) showed that almost 16 complete life cycles can be achieved within a year under a constant soil temperature of 34°C.

Given the importance of social bees for pollination services and their economic value (Gallai et al., 2009; Klein et al., 2006; Velthuis & Van Doorn, 2006), assessing the risks of SHB invading currently uninvaded areas and potential changes in the severity of SHB impacts is of utmost importance, especially under changing climatic conditions. This information is urgently needed (EFSA, 2015), as it will define management strategies during different stages of invasion (Cook et al., 2007; Schäfer et al., 2019). Therefore, identifying environmental limitations and their changes are key to assess the invasiveness of alien species and their biotic interactions (Schweiger et al., 2010).

Applying a common correlational approach of assessing the climatic niche of a species based on distributional data (Araújo & New, 2007; Thuiller et al., 2009) is particularly difficult for alien species that are still spreading and thus not in equilibrium with the environment and for which data from the native range are scarce (Václavík & Meentemeyer, 2012). Since this all applies to SHB, we developed a mechanistic niche model relying on physiological tolerances to environmental conditions and the corresponding effects on performance. However, the impact of environmental factors can vary among life stages, and thus it is important to focus on the most sensitive ones (Bowler & Terblanche, 2008). The part most sensitive to environmental conditions during the life cycle of SHB is the pupal stage outside the host colony and therefore we focused on measures of pupal performance. We used empirical data on the response of survival rate and development time to soil moisture and temperature conditions and assessed the global invasiveness and severity of SHB under current and projected future climatic conditions. We assume that pupal performance is one key aspect related to the invasion risk of SHB at a global scale and we predict that SHB invasion risk will increase as climate change and global warming in particular, promotes the chances of SHB to survive and thrive in many areas of the world.

Materials and Methods

SHB pupal performance data

SHB pupal performance data are here defined as survival rate and developmental time and were collected from peer-reviewed literature with focus on the impact of soil temperature and moisture (Supplemental Tables S1a – S1c). Since soil moisture was provided either as weight (moisture) or volume ratios (soil water content), the gravimetric measures were converted into volumetric measures according to the bulk density (kg/m^3) of the used soil types (Supplemental Tables S1a – S1c). To fill identified data gaps in the published studies, additional laboratory experiments were performed (Supplemental Tables S1a – S1c and Supplemental Method S1).

SHB pupal performance curves

To assess the potential global distribution and invasiveness under given and projected future climatic conditions, we quantified the responses of pupal survival rate and developmental time to varying soil temperatures and moisture conditions and combined them into a composite measure of pupal performance. Performance curves for many physiological processes are well described and they typically rise to an optimum and then decline more or less steeply to zero performance (e.g. Huey & Kingsolver, 1993). For survival rate and soil temperature, we applied a performance function ($S(T)$), used for ectothermic invertebrates, e.g. in Deutsch et al., (2008) or Vasseur et al., (2014), where the rise is described by a Gaussian function and the decline by a parabolic function:

$$S(T) = \begin{cases} \mathrm{Exp}\left[-\left(\frac{T-T_{\mathrm{opt}}}{2\sigma_p}\right)^2\right], & T \leq T_{\mathrm{opt}} \\ 1-\left[\frac{T-T_{\mathrm{opt}}}{T_{\mathrm{opt}}-\mathrm{T}_{\mathrm{max}}}\right]^2, & T > T_{\mathrm{opt}} \end{cases}, \tag{1.1}$$

where S is the performance metric of pupal survival, T is the soil temperature, T_{opt} is the temperature with maximum performance, T_{max} is the upper critical temperature at which performance is zero and σ_p is a shape parameter determining the steepness of the Gaussian function.

For survival rate and soil moisture ($S(M)$), we used the same function, but due to a larger plateau in the response curve, we had to re-parameterize the exponent of the parabolic function. Therefore, we let the exponent increase from 2 onwards and selected the best fitting model according to the lowest value of Akaike's information criterion (AIC; see Supplemental Figure S1 for visual assessment and Supplemental Tables S2a and S2b for AIC values) leading to the following model:

$$S(M) = \begin{cases} \mathrm{Exp}\left[-\left(\frac{M-M_{\mathrm{opt}}}{2\sigma_p}\right)^2\right], & M \leq M_{\mathrm{opt}} \\ 1-\left[\frac{M-M_{\mathrm{opt}}}{M_{\mathrm{opt}}-\mathrm{M}_{\mathrm{max}}}\right]^{10}, & M > M_{\mathrm{opt}} \end{cases}, \tag{1.2}$$

where S is the performance metric of pupal survival, M is the soil moisture, M_{opt} is the moisture with maximum performance, M_{max} is the upper critical moisture at which performance is zero and σ_p is a shape parameter determining the steepness of the Gaussian function.

To allow for varying shapes of the thermal performance curve under different soil moisture conditions and *vice versa*, we combined both performance curves ($S(TM)$) via an interaction term:

$$S(TM) = zS(T) \times S(M), \tag{1.3}$$

where z is a scaling factor.

We used a nonlinear regression approach to derive performance curves by fitting observed pupal survival rates (S(T) and S(M)) at the respective temperature (T) and moisture conditions (M) to the equations (1.1) and (1.2) and estimated the parameters T_{opt}, T_{max}, M_{opt}, M_{max} and σ_p. Starting values for the iterative estimation approach, by minimizing sum of squares, were obtained by visual inspections of plotting survival rate against temperature and soil moisture. We compared models including only one function (either $S(T)$ or $S(M)$), models including their additive and their interactive effects. The lowest AIC values indicated that the interactive effect performed best (Supplemental Tables S2a – S2b).

Development times often follow a u-shaped relationship with temperature, but if there is no indication of an increase in development times at very high temperatures, an asymptotic exponential function can be used (see e.g. Kingsolver 2013). We used a three-parameter asymptotic exponential function ($D(T)$) for both soil temperature and soil moisture:

$$D(T) = a - b\text{Exp}[-\text{Exp}[c]T], \tag{2.1}$$

where a is the horizontal asymptote on the right hand side (i.e. at high temperatures) and defines the minimum number of days for pupal development at high temperatures, b is given by a – R0, where R0 is the intercept, and c is the rate constant defining the shape of the curve. Parameters were also estimated with nonlinear regressions. Model comparison based on AIC indicated that only soil temperature is relevant for development time (Supplemental Tables S2a – S2b).

Finally, we combined performance measures of pupal survival rate and development time into a composite measure of pupal performance (P_i) by:

$$P_i = [S(TM)_i/D(T)_i]/\max[S(TM)/D(TM)], \tag{3.1}$$

where dividing by the maximum we let the measure vary between zero and one.

Global soil temperature and moisture data

We predicted global distribution and invasiveness of SHB under current climatic conditions using the composite measure of pupal performance (P_i) based on environmental information on soil temperature and soil moisture. For soil temperature, we used data provided by microclim (Kearney et al., 2014). Microclim provides hourly estimates from the surface to 1 m depth for the middle day of each month at a resolution of 10 arcmin (i.e. 18.6 km at the equator) including six shade levels and three substrate types (soil, rock and sand). According to the biology of SHB (De Guzman et al., 2010; Pettis & Shimanuki, 2000), we extracted data for soil at a depth of 10 cm and for a subset of every third hour. To assess the level of shading by vegetation cover, we used data on the Normalized Difference Vegetation Index (NDVI) as a proxy. NDVI data were obtained from the Global Inventory Modelling and Mapping Studies (GIMMS) (Pizon, 2005; Tucker et al., 2005) of the years 1981-2010 (Tucker et al., 2016) at a biweekly interval and at a resolution of 5 arcmin (9.3 km at the equator). These data were aggregated to mean monthly values at the 10 arcmin resolution of the soil temperature data and averaged across the thirty year period. NDVI values were equally binned into five classes and assigned to the respective levels of shading for the microclim data (0%, 25%, 50%, 75%, 100%). Based on the NDVI values, we extracted the respective soil temperature data for each grid cell leading to global soil temperature estimates for the middle day of each month for every third hour.

Soil moisture data were obtained from the ESA CCI Surface Soil Moisture (ESA CCI SM) project v2.2 (Liu et al., 2012, 2011; Wagner et al., 2012) on a daily basis for the period from 1985-2014 at a resolution of 15 arcmin (27.9 km at the equator). We calculated monthly means across the 30 year period and disaggregated the data to the 10 arcmin resolution. Since ESA CCI SM data did not cover the tropics, we filled these gaps with data from NASA SMAP L4_SM data product (Reichle et al., 2017) on a 3-hourly basis for the period from 2015-2017 at a 9 km resolution (provided on a global cylindrical equal area grid). We calculated mean monthly values for the three-year period and after re-projection we aggregated the data to the 10 arcmin resolution.

Since SHB has only been observed in vegetated areas we masked non-vegetated areas using the NASA Land Cover Type Climate Modelling Grid (CMG) product (MCD12C1) (Nasa Lp Daac, 2013). These data on dominant land cover types, originally provided at a 3 arcmin (5.6 km at the equator) resolution, were again aggregated to the 10 arcmin grid.

To assess potential consequences of global warming on the future invasion of SHB, we used current mean monthly surface temperature averaged across the period from 1960-1990 from WorldClim (Hijmans et al., 2005) provided at the 10 arcmin resolution and future projections obtained from the HadGEM2-ES general circulation model of the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Stocker, 2014). We used two scenarios of representative concentration pathways (RCPs) for 2060 (averaged over 2041-2060) and 2080 (averaged over 2061-2080) resulting in an average global increase of 2.88°C and 2.98°C (RCP2.6) or 4.24°C and 6.09°C (RCP8.5). To estimate future soil temperatures, we first performed linear regression models for each of the three-hourly soil temperature data as a function of mean surface temperature in a particular month. In this way we captured diurnal and seasonal variation in these relationships. We found strong relationships (mean $R^2 = 0.97$, range = 0.90 to 0.99) but with varying slopes (mean = 1.00, range = 0.60 to 1.13) and intercepts (mean = 0.44, range

= -2.22 to 12.21). Subsequently, we used these models to predict future soil temperatures per grid cell for each three-hour interval for the middle day of each month. To preserve grid-cell specific deviations from these overall relationships, e.g. caused by slope, aspect or precipitation, we added the respective residuals from the regression analyses under current conditions to the projections under future conditions, assuming that these deviations are constant over time. We also tested this approach for soil moisture and its relationship to monthly precipitation, but very low R^2 values (mean = 0.13, range = 0.04 to 0.24) indicated low reliability. We therefore relied on scenarios of temperature change only.

Global predictions and future projections of SHB pupal performance

We used performance curves of pupal survival rates ($S(TM)$) and development time ($D(T)$) to predict both processes separately based on current soil temperature and moisture conditions. To account for the strong geographic differences in diurnal variation of soil temperature and the corresponding consequences for pupal performance we predicted performance for each of the three-hour intervals per month and integrated them in a second step by averaging. We then used equation (3.1) to calculate the composite index of pupal performance per month, whereas we used the maximum of $S(TM)/D(TM)$ across all grid cells and months (global maps for monthly survival rate, development time and pupal performance are provided in Supplemental Figures S2 - S4). Monthly pupal performance was further condensed in two ways: (i) it was averaged across the months per grid cell assuming that pupal performance accumulates across the varying conditions within a year, and (ii) by extracting the highest level of performance across the months per grid cell assuming that invasiveness depends on maximum performance during shorter periods.

To assess the predictive ability of the model in general and to discriminate the relevance of mean annual climatic conditions from short-term optimal conditions for pupal performance, we used actual reported georeferenced occurrences in the native and invaded range (Supplemental Table S3). We included only established populations by focusing on observations of three years or longer. Since these data represent presence-only data, we used the continuous Boyce index (Boyce et al., 2002; Hirzel et al., 2006) to assess the quality of our predictions. This index varies from -1 (worse than expected by chance) to 0 (not better than expected by chance) to 1 (perfect predictions). The Boyce index compares the predicted frequency distribution of evaluation points with their expected frequency based on the distribution within a selected area and is thus sensitive to the spatial extent of the selected area. To overcome a potential bias by selecting a too large area, we analysed occurrences in different regions separately by calculating convex hulls for three areas in Africa, three in North America and one in Australia with sufficient data points. Further, we considered some uncertainty in the georeferences of the observations and used the maximum of pupal performance across all grid cells within a 25 km buffer. For evaluation of the Boyce index, we used an analogy to the categorisation recommended by Landis and Koch (1977) for the true skill statistic, which also ranges between -1 and 1, the following: excellent, Boyce index > 0.75; good, 0.40 < Boyce index < 0.75; and poor, Boyce index < 0.40.

Future projections of pupal survival rates, development time and pupal performance were calculated analogous to current conditions but for means of comparison, we used the same value of max[*S(TM)*/*D(TM)*] for equation (3.1) as for current conditions.

All analyses were performed in the statistical environment R (R Core Team, 2016) using the packages colorRamps (Keitt, 2012), ecospat (Broennimann et al., 2016), gdalUtils (Greenberg & Mattiuzzi, 2015), gtools (Warnes et al., 2015), maptools (Bivand & Lewin-Koh, 2016), minpack.lm (Elzhof et al. 2016), ncdf4 (Pierce, 2015), raster (R.J. Hijmans, 2017), RColorBrewer (Neuwirth, 2014), rgdal (Bivand et al., 2016) and sp (Pebesma & Bivand, 2005).

Results

Climatic factors defining pupal performance of SHB

Models for both SHB pupal survival rate and developmental time fitted the experimental data very well (Figure 1). Interestingly, we found an unusual positive skew of the thermal performance curve for survival rate with a very steep slope for the rising part of the curve and a less steep decreasing part (Figure 1a). Estimated temperature optimum for survival rate was at 18.9°C and the upper critical temperature was 40.9°C (all parameter estimates are provided in Supplemental Tables S4a – S4b). Pupal survival rate of SHB also showed a broad range of suitable soil moisture conditions reaching optimal conditions already at 0.04 m^3/m^3, followed by a larger plateau and reaching critical moisture conditions at 0.77 m^3/m^3 (Figure 1b). Pupal development time was well described by the asymptotic exponential relationship with temperature, with the asymptote approaching a minimum time for development of 15 days (parameter a in Supplemental Table S4b). Survival rate and development time indicated a temperature-related trade-off with longer development times at optimal temperatures for survival. The composite index of pupal performance consequently led to a shift of optimal temperature from 18.9°C for survival rate to 27.5°C when development time was additionally considered (Figure 1d). At this temperature, development time decreased from 43 to 18 days (Figure 1c), while survival rate was still 87% (Figure 1a).

Global predictions and future projections of SHB pupal performance

Boyce indices for the selected regions and all regions combined show that highly beneficial, but short-term climatic conditions, i.e. conditions in the 'best' month, explain the distribution and invasiveness of SHB much better than long-term conditions averaged across the year (Table 1). Predictive ability was generally good with one exception for the region around Lake Victoria in Africa.

For a better interpretation of the continuous pupal performance index, we identified two thresholds using the predicted/expected ratio used to calculate the Boyce index (Figure 2). A predicted/expected ratio higher than one indicates a better prediction than expected by chance (Hirzel et al., 2006) and occurred at pupal performance values higher than 0.64. In such areas climatic conditions are considered as highly suitable.

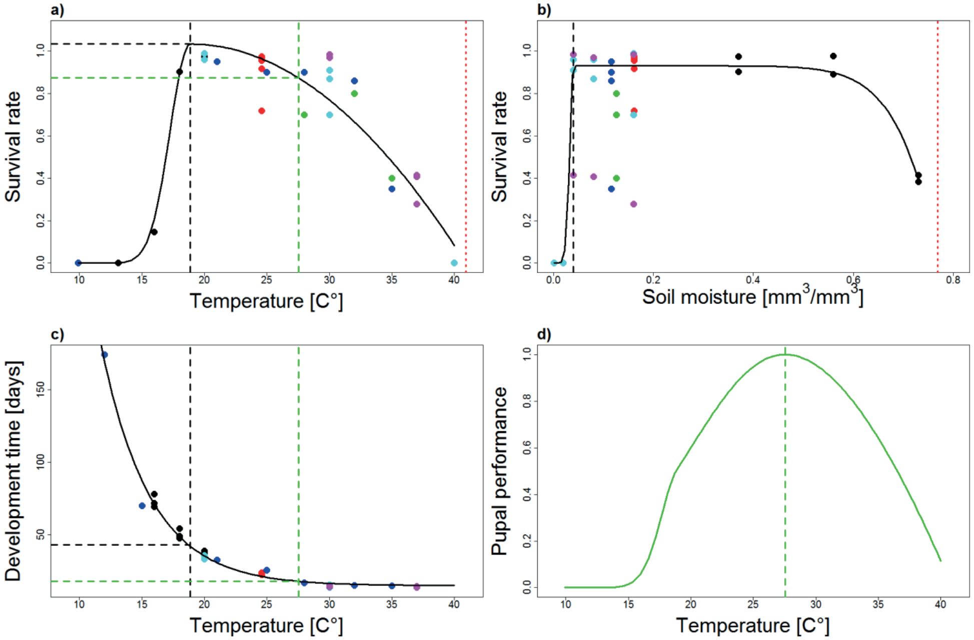


*Figure 1. Performance curves of small hive beetle (*Aethina tumida*) pupal survival rate in response to temperature (a) and soil moisture (b), pupal development time (c) and composite pupal performance (d) as a function of temperature. Since pupal survival was calculated based on interactive effects between thermal and moisture performance (equation 1.3), visualised curves are based on constant average conditions of the respective environmental measure, i.e. soil moisture of 0.21 mm³/mm³ for thermal performance (a) and soil temperature of 25.8°C for moisture performance (b) and respective upper and lower 10% quantiles of the data points are not displayed, i.e. data points with values lower than 0.02 and values higher than 0.56 mm³/mm³ for thermal performance (a) and data points with values lower than 16.0°C and values higher than 37.6°C for moisture performance (b). Black dashed lines in (a) and (b) indicate optimal conditions (Topt and Mopt in equations 1.1. and 1.2), red dotted lines indicate the upper critical temperature Tmax (a) and the upper critical moisture Mmax (b). Green dashes lines indicate the optimal temperature for composite pupal performance (d) and visualise a decrease in development time from 43 days according to Topt to 18 days (c) while survival rate decreased only to 87% (a). Point colours indicate data sources: black, Bernier et al. (2014); red, Ellis et al. (2004); green, Meikle & Diaz (2012); dark blue, Meikle & Patt (2011); light blue, unpublished data from new experiment 1; magenta, unpublished data from new experiment 2 (see Supplemental Tables S1a – S1c and Supplemental Method S1).*

Table 1. Boyce index calculated for three regions in Africa (af), three regions in North America (na), one region in Australia (aust) and all regions combined (all; see Figure 3). Average: Boyce index computed for pupal performance measures based on annual averages, assuming that overall pupal performance accumulates across the varying conditions within a year; Best: Boyce index computed for pupal performance measures based on the highest value across the months, assuming that invasiveness depends on maximum performance during shorter periods.

Region	Average	Best
af1	-0.44	0.67
af2	0.58	0.66
af3	-0.55	-0.72
na1	0.74	0.83
na2	0.67	0.46
na3	1.00	1.00
aust	0.94	0.76
all	-0.02	0.70

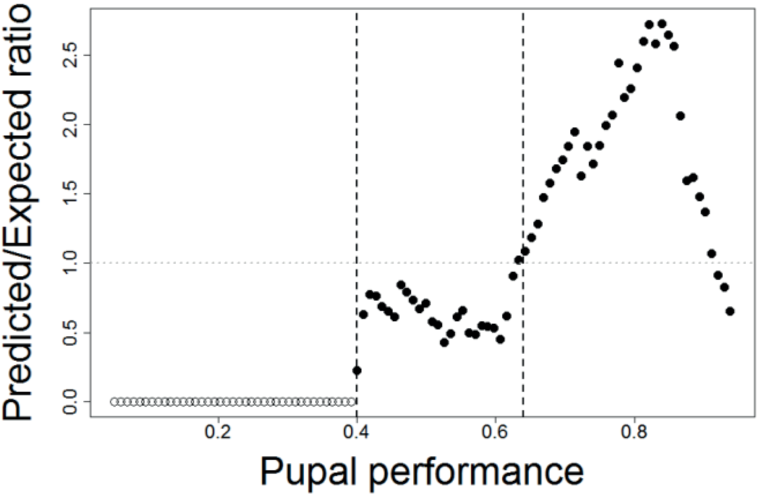


*Figure 2. Predictive ability of the small hive beetle (*Aethina tumida*) pupal performance model. Predictive ability is measured by the ratio of the frequency of actually predicted performance values for the evaluation points over the expected frequency based on the predictions for the entire considered area, in this case for all evaluation areas (see Fig. 3) combined (see Hirzel et al., 2006). Horizontal grey dotted line indicates a ratio of one. Above this line, pupal performance is predicted more often than expected by chance. Areas with performance values above the corresponding threshold of 0.64 (right dashed vertical line) are considered as highly suitable for pupation. Below a threshold of 0.4 (left dashed vertical line) no established populations were reported and areas with such predicted performance values are considered as unsuitable. The intermediate range is considered as marginally suitable.*

The observed drop in the predicted/expected ratio at predicted pupal performance higher than 0.9 might be caused by a systematic bias in the observations (too few observations in the "best" areas) or caused by other environmental or biotic conditions apart from climate. We further observed SHB at predicted pupal performance values between 0.64 and 0.40, but at lower frequencies than expected by chance. Conditions in such areas are considered as marginal. No observations have been made in areas of predicted pupal performance values lower than 0.40 and are thus considered as unsuitable.

Predictions of pupal performance of SHB under current climatic conditions indicated high climatic suitability in its native range in sub-Saharan Africa and generally in the Southern hemisphere (Figure 3). Here, all (sub-)alpine areas, almost all of New Zealand and the southern-most part of South America, below 45° South latitude, are unsuitable for pupation (Figure 3). Climatic constraints currently limit the distribution of SHB for large parts of the Northern hemisphere. Marginal to optimal conditions for pupation occur in vegetated areas in Asia, North Africa, Southern Europe and North and Central America up to 57° North latitude, with some exceptions up to 60° North latitude (Figure 3).

Under a moderate warming scenario (RCP2.6), large areas in the Northern hemisphere are projected to become highly suitable by 2060 (28.5% gain; Supplemental Table S5) but remaining rather constant until 2080 (28.8% gain; Figure 4a - b). The northern boundary for marginal pupation performance is projected to shift to 67° N in 2080. In particular, large areas in the Russian Federation, Canada and Europe could become suitable for SHBs to pupate and thus establish populations. Moreover, in areas where SHB pupal performance under current conditions could be considered marginal, conditions are likely to shift to optimal.

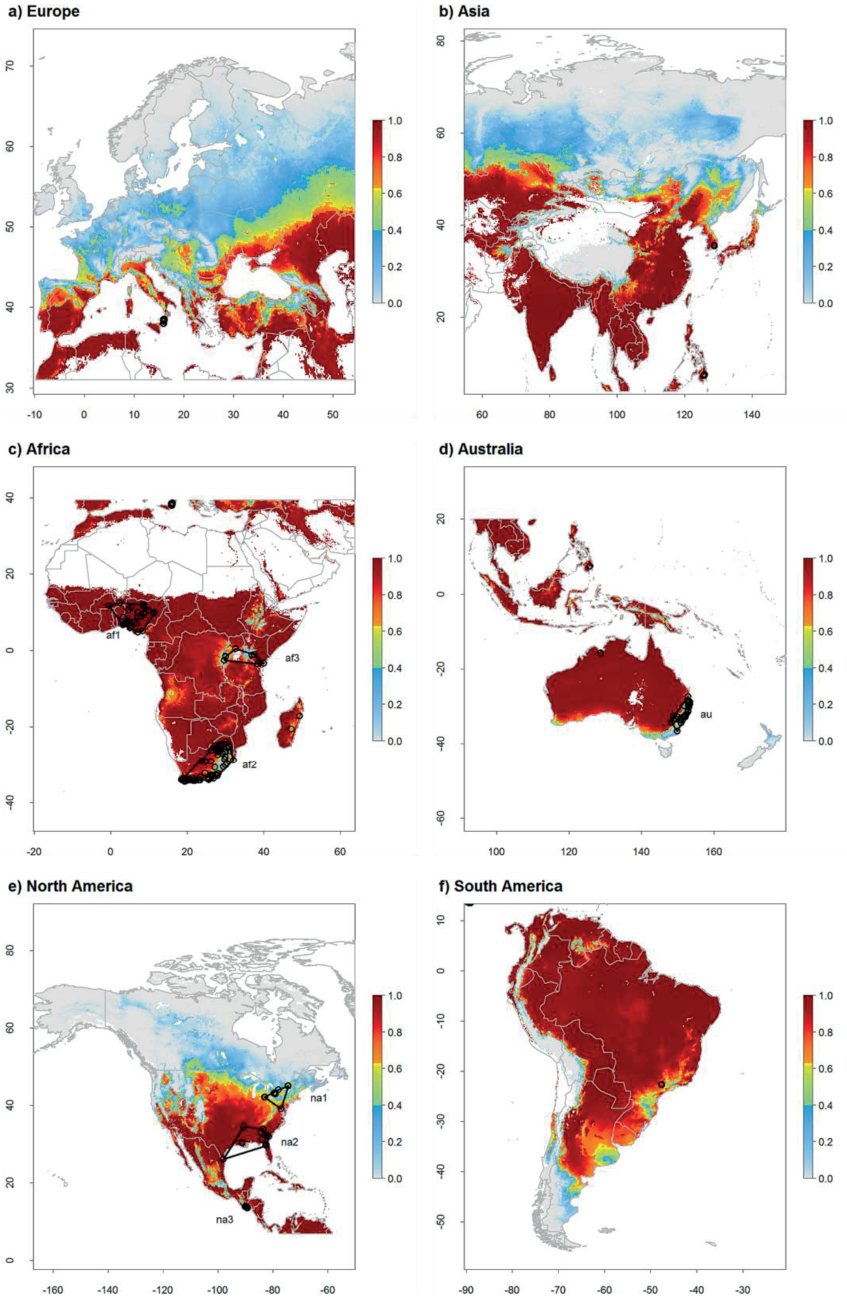
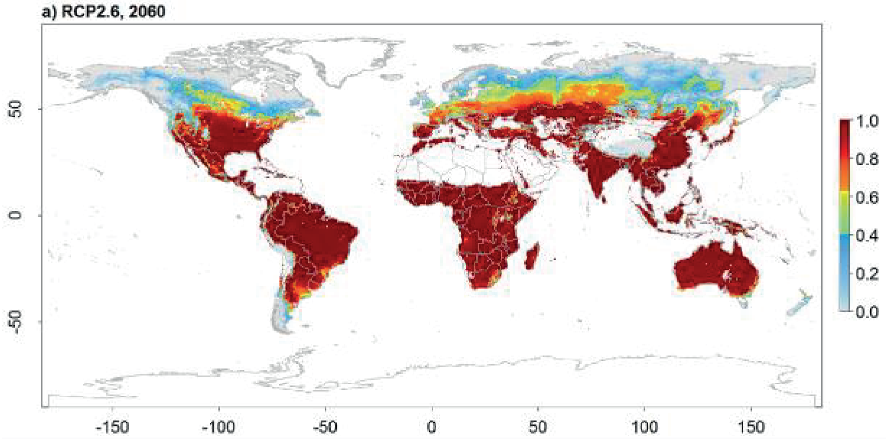


*Figure 3. Global predicted pupal performance of small hive beetle (*Aethina tumida*). Pupal performance is based on a composite index combing pupal survival rate and development time (equation 3.1) and ranges between zero (no*

figure 3 continued
performance) and one (maximum Legend performance). According to thresholds obtained from model validation (see fig. 2), continuous pupal performance values were classified into conditions of high climatic suitability (values higher than 0.64; red to orange colours), marginally suitable (values between 0.4 and 0.64; yellow to green) and unsuitable climatic conditions (values below 0.4; blue to grey colours). The X-axis shows latitude degrees and the Y-axis shows longitude degrees. Non-vegetated areas are masked in white. Open circles show locations with georeferenced occurrences of SHB. Black polygons depict areas used to determine expected frequency distribution of performance values used to assess predictive ability with the Boyce index (see Table 1).

No major changes in pupation performance are projected for the southern hemisphere (Supplemental Table S5), with a slight boundary shift southward from 45°S to 46°S. Under the more severe warming scenario (RCP8.5), projected increases in pupal performance are drastic for the Northern hemisphere (2060: 48.2% gain, 2080: 84% gain; Figure 4c - d). By 2060, the northern boundary for marginal pupal performance shifted to 69°N and to 71°N by 2080, projecting the possibility of marginal SHB pupal performance on the South Island of the Novaya Zemlya archipelago. At the Southern hemisphere, changes are still less pronounced (Supplemental Table S5). A maximum southward shift of marginal to optimal performance to 49°S is projected for 2080. Furthermore, large areas of New Zealand and Tasmania will become suitable for SHB pupation.



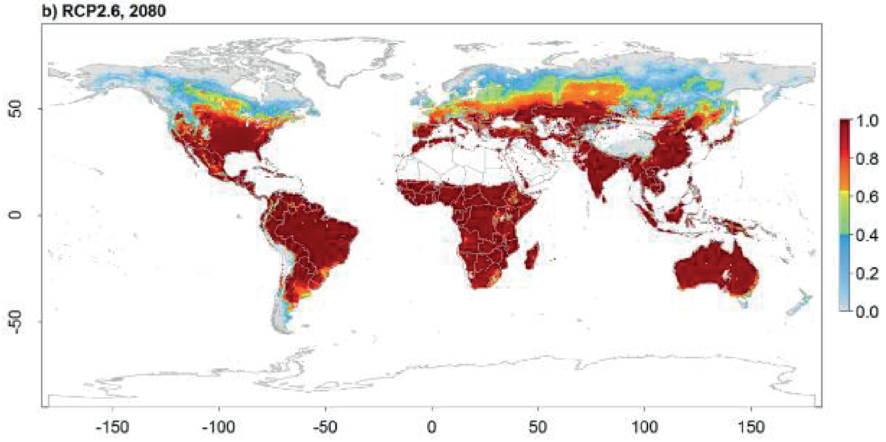
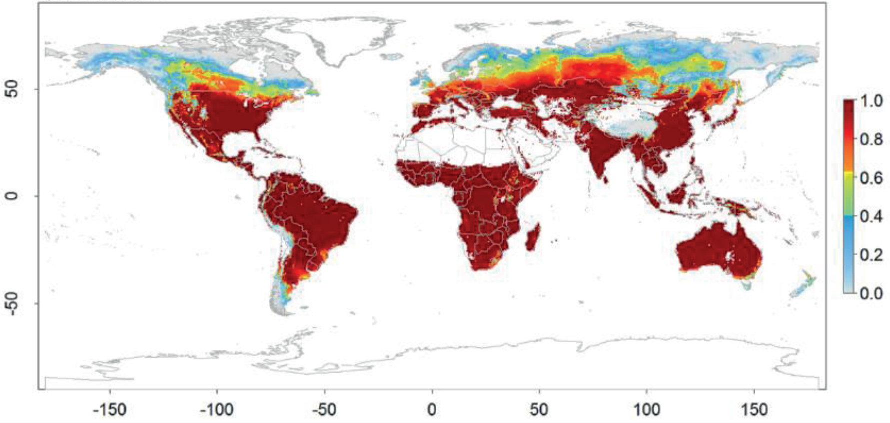


Figure 4 continued

c) RCP8.5, 2060



d) RCP8.5, 2080

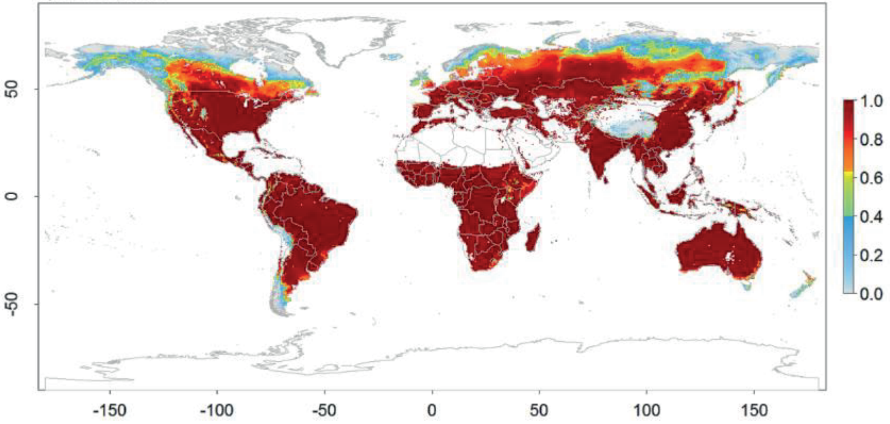


*Figure 4 Pupal performance of small hive beetle (*Aethina tumida*) projected to the representative concentration pathways (RCPs) 2.6 (a,b) and 8.5 (c,d) for the years 2060 (a,c) and 2080 (b,d). Pupal performance is based on a composite index combing pupal survival rate and development time (equation 3.1) and ranges between zero (no performance) and one (maximum performance). According to thresholds obtained from model validation (see Fig. 2), continuous pupal performance values were classified into conditions of high climatic suitability (values higher than 0.64; red to orange colours), marginally suitable (values between 0.4 and 0.64; yellow to green) and unsuitable climatic conditions (values below 0.4; blue to grey colours). The X-axis shows latitude degrees and the Y-axis shows longitude degrees. Non-vegetated areas are masked in white.*

Discussion

Here we present the first study to assess the impact of global warming on an invasive honey bee pest on a global scale. Our results show a high invasion risk across the globe with potential dire consequences for its hosts. Moreover, the risk increases considerably with increasing temperatures in the future. By categorising pupal performance from unsuitable to marginal to optimal, we show that SHB can potentially colonize an area much larger than is currently the case, confirming earlier concerns (Neumann et al., 2016). With our mechanistic approach, combining impacts of soil temperature and moisture on pupal survival and development time into a composite thermal performance curve for pupation, we can go beyond a mere expression of survival and define its importance in relation to habitat suitability (Kearney & Porter, 2009) and moreover also in terms of severity in the impact on bees. For instance, development time is a limiting factor for SHB performance under temperate climatic conditions, since short growing seasons can prevent the completion of metamorphosis (Bernier et al., 2014). Under warmer climatic conditions, developmental time is not a limiting factor for pupal survival, but it can foremost be a predictor of the number of life cycles that can be completed during a growing season, which is indicative of the population build-up and moreover on the impact on bees (Ellis et al., 2004; Neumann et al., 2016; Spiewok et al., 2007).

Performance curves of ectotherms, most often measured as thermal response curves, generally show a slowly increasing slope to optimal performance and a sharp decrease thereafter (Dowd et al., 2015; Kingsolver et al., 2011), partially due to structural constraints (Huey & Kingsolver, 1989). The performance curve of SHB pupal survival could be considered an unusual shark fin shape, with a sharp increase in survival from the lower thermal limit over a short temperature interval and a gradual decrease in survival towards the upper thermal limit. However, a similar survival curve has been shown for other beetle species pupating in the soil (*Entomoscelis americana*, Lamb & Gerber, 1985) and other ectotherms like amphibians (Bachmann, 1969). Our results indicate that SHB is highly sensitive to small changes at the lower end of its temperature niche, which is similar to other beetle species pupating in soil such as *E. americana* pupae exposed to constant low temperatures in the laboratory (Lamb & Gerber, 1985). However, under field conditions temperatures are fluctuating and may lead to a different result. For instance, *E. americana* larvae and pupae were able to survive lower temperatures and also used thermo-regulation to partially overcome fluctuations and constraining temperatures (Lamb & Gerber, 1985). As a consequence, insect species may survive in cooler temperatures under natural conditions than explained by their thermal niche modelled on the basis of constant laboratory conditions. Further, temperatures can also spatially vary within the grid cells we used for modelling. While our models predict general patterns of pupal performance at global scales, specific local microclimatic conditions, e.g. heat island effects of cities in cooler areas, might, in some cases, allow for sustainable local populations even in areas currently marked as unsuitable. However, the reproductive potential of such populations might be significantly reduced because of the limited time available for completing one or more generation cycles.

Overall, predicted SHB pupal performance fitted very well with observed presence data of SHB. Occurrences in New South Wales, Australia and Florida, USA and even latitudinal outliers like Ontario, Canada are well explained (see Figure 3D - E and Table 1). Nevertheless, invasions beyond the currently predicted limits of SHBs might also be possible if the species manages to adapt to novel conditions (Atwater et al., 2016; et al., 2017; Krehenwinkel, et al., 2015). Since SHBs naturally occur from the Kalahari to equatorial rainforests of sub-Saharan Africa (Neumann & Elzen, 2004), it is inevitable that the native range holds different ecotypes (Neumann et al., 2016) likely leading to a high adaptive potential of this species after invasions.

Combining models of pupal survival and development time led to a shift of optimal temperature from 19°C for survival only to 28°C for overall pupal performance. This shift is predictable given that only a marginal difference in the survival rate between the thermal optimum (19°C) and high temperatures up to 30°C can be observed while developmental time exponentially decreases with increasing temperatures (Figure 1). The strong increase of SHB pupal survival rate in response to small changes at lower temperatures (Figure 1a) is also reflected by a high sensitivity of overall pupal performance (Figure 1d). This high sensitivity is the likely cause of the considerable increases of climatically suitable areas even under a moderate warming scenario (RCP2.6). The rather tropical nature of the thermal niche profile, compared to a temperate one (Deutsch et al., 2008; Huey et al., 2012), on the other hand, can explain why no areas were projected to become unsuitable towards the upper limit of the thermal tolerance of SHB even under the most severe scenario. Further, areas where currently only marginal pupal performance is predicted are likely to facilitate optimal performance with increasing temperatures. For future projections of SHB pupal performance, we could not include scenarios of changing soil moisture (see Material and Methods), but since SHB has a very broad tolerance level for soil moisture conditions and is extremely insensitive to dry soils (Supplemental Table S1a - S1c), we believe that projected soil moisture anomalies would not have impacted our general results appreciably.

SHB can occur in particularly dry, sparsely vegetated semi-desert areas such as the Kalahari in Namibia and Botswana when hosts are present (Ellis & Munn, 2005; Phokedi, 1985). Nonetheless, such extreme environments pose a challenge for beetles to survive. In particular soil physics might prevent SHB to successfully complete the life cycle. Under laboratory conditions, SHB has been shown to successfully pupate below 3 cm (Meikle & Diaz, 2012) and it has been found at depths up to 20cm in the field (Pettis & Shimanuki, 2000), but a minimum depth rather than a preferred depth is likely (Meikle & Diaz, 2012). In our study we assumed a depth of 10cm to estimate pupal performance. In (semi-) desert top soils moisture levels could fluctuate and even dry out completely. This could affect the chances to complete the life cycle and thus establishment of populations of SHBs under these extreme conditions.

SHBs can survive temporarily unsuitable environmental conditions due to the thermoregulatory capacity of honey bee colonies (Schäfer et al., 2011). SHBs have been shown to survive in honey bee winter clusters for several months. However, SHBs cannot maintain populations close to the temperate climatic limits of beekeeping (Neumann et al., 2016) as we found these areas to be unsuitable for pupation. Therefore, within the predicted current and future range expansion of SHB presented here, honey bee colonies will inevitably be present and thus potentially exposed to this pest species.

Moreover, SHBs infest colonies of other social bees as well (Cervancia et al., 2016; Greco et al., 2010; Spiewok & Neumann, 2006) and may also use solitary bees as hosts (Gonthier et al. 2019). Even though the impact and its magnitude are unknown for many social bees, the predicted invasion risk could have a broad impact on this important group of pollinators and the ecosystem service they provide. While it is well known that European honey bee subspecies are more susceptible to SHB infestations compared to African ones, probably due to quantitative differences in a range of defence behaviours (Ellis & Hepburn, 2006; Neumann & Elzen, 2004), there are no data available for other honey bee or other bee species. This notion vastly expands the impact of SHB as an invasive species, having potential consequences for species already declining, such as certain bumble bees (Meeus et al., 2011; Potts et al., 2010).

With our mechanistic niche models, we assessed critical dimensions of the fundamental niche of SHBs, which allowed a quantification of the direct impacts of ambient climate on pupal performance outside the host colony. This provides a first basis for risk assessment at the global scale, but local realisations might still be modified to some extent by factors impacting other life stages, e.g. by dispersal limitation, different factors affecting establishment and epidemiology or local soil physics. The first step of successful invasion is the introduction (Richardson et al., 2000), which can be by natural spread of the beetle alone or via the bees, once it arrives in a new area, or by anthropogenic movement of colonies or bee products (Lounsberry et al., 2010; Neumann et al., 2016). Given the well-documented role of global trade and movement of bees and bee products for the spread of SHBs (Neumann et al., 2016; Idrissou et al., 2019), introduction seems not to be a limiting factor for the invasion success of SHB.

For successful establishment and further spread, the presence and density of known and new hosts as primary and alternative food sources can be important (Schäfer et al., 2019; Gonthier et al., 2019). In the native range of sub-Saharan Africa, feral honey bee populations show higher densities compared to other regions in the world (Jaffé et al. 2010). However, in Asia, Europe and the United States managed honey bee populations are much more prolific and colonies are concentrated in apiaries, probably affecting invasion dynamics of SHBs accordingly (Spiewok et al., 2008; Neumann et al., 2010). Especially when considering the good flight ability of adult SHBs (Neumann and Elzen, 2004), such high host densities likely benefit the establishment and further spread of SHB in addition to movement of colonies or bee products (reviewed by Neumann et al. 2016), in particular in the absence of early detection systems (Schäfer et al, 2019). Furthermore, the host could also be affected by climatic conditions, indirectly altering the conditions for SHB in different life stages than the pupa. The effect of climate change on honey bees, however has not been well studied, but are likely to include effects on phenology and survival (Le Conte & Navajas, 2008). For instance, elevated temperatures can expedite the onset of brood rearing in honey bee colonies in temperate climate zones (Nürnberger et al. 2018). Furthermore, the availability of food sources and the opportunity to forage could change (Le Conte & Navajas, 2008), thereby affecting brood rearing cycles. SHB follows honey bee phenology (Lundie, 1940) and likely seizes to reproduce in winter, as only adult are found in winter clusters (Schäffer et al, 2011). The availability of brood in winter could trigger the onset of reproduction in SHB, but seasonality of SHB reproduction in temperate climatic zones and the relation to its host has not yet been studied (Neumann et al, 2016). While our study provides a first identification of suitable areas and potential severity of SHB under current and future conditions, which can be used for precautionary management plans, local realisations, e.g. by identifying the relative importance of soil conditions, dispersal and anthropogenic translocations or indirect impacts of global change on the host species, still warrant further investigations.

Generally, our study further highlights the urgent need for slowing down the global spread of SHBs (Schäfer et al., 2019), until better mitigation options are available. The results provide a science-based approach in support of strategic management of this invasive species as measures can be taken where they are deemed fit for now or for the future. Past invasions of SHB have resulted in an economic deficit to the beekeeping industry (Neumann & Elzen, 2004; Rhodes & McCorkell, 2007). The focus of management strategies should therefore firmly be on detection in the early stages of invasion (Hulme,

2009) by focussing on the global trade in bees and bee products (Neumann et al., 2016; Idrissou et al., 2019). Options for which are provided by improving the international trade regulations (Lecocq et al., 2016).

In conclusion, our study shows for the first time an increased global invasion risk of a honey bee pest due to global warming. As managed honey bee populations and many wild bee species are either suffering from unsustainably high colony losses (Neumann & Carreck, 2010) or decline (Potts et al. 2010), it appears prudent to further investigate the interplay between climate change and biological invasions in the context of bee health. Furthermore, our study merits further investigation of the potential risks posed by other alien, invasive honey bee pests like *Varroa destructor* and *Tropilaelaps* sp. under climate change scenarios. But even native pests and pathogens should not be excluded from such analysis (Le Conte & Navajas, 2008). In more general terms, we need a better understanding of the impact of climate change on biological invasions and the impacts on ecosystem services (Knight et al., 2018; Phillips et al., 2018).

Acknowledgements

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Supplemental files

Supplemental Method M1 *Methods description of complementary experiments to fill critical gaps for pupal performance measures of Aethina tumida. (See Supplemental Table S1a-S1c)*

In September 2015, adult SHBs (N=50) were collected from naturally infested *Apis mellifera scutellata* colonies at the experimental farm of the University of Pretoria, Gauteng, South Africa [25°44'45.1"S, 28°15'31.1"E] using aspirators (Neumann et al., 2013). The beetles were kept at 25°C in plastic 1 litre boxes, fed with a diet consisting of protein supplement (Dadant & Sons, AP23®) and honey (3:1 weight ratio) *ad libitum*. An initial amount of 50g diet consisting of protein supplement (Dadant & Sons) and honey (3:1 weight ratio) was added on which adults were able to oviposit (Neumann et al., 2013). To keep moisture levels sufficiently high for eggs to hatch, a wet paper tissue was put in the container, which was replaced daily (Neumann et al., 2013). After 3 – 6 days, the adults were removed and the boxes were checked for the presence of eggs and larvae. Larvae were fed *ad libitum* with the protein/honey diet and placed in clean boxes once a week. After approximately 14 days, the larvae reached the post-feeding wandering stage (Neumann, Pettis, & Schäfer, 2016) and were used for the pupation experiment. A sandy soil (density 1552kg/m^3) was used for the entire experiment, which was collected at the experimental farm. Before use, the soil was dried and subsequently autoclaved to exclude any biotic factors potentially interfering with pupation success (e.g. nematodes Ellis et al., 2010; entomopathogenic fungi Murrle & Neumann, 2004). Different amounts of sterilized water were added to the soil in order to get specific levels of soil water content (SWC, see Supplemental Table S1c)). Considering the physical properties of the sandy soil, a SWC of 0.16mm^3/mm^3 dry soil can be interpreted as a moderately wet soil, while 0.04mm^3/mm^3 dry soil represents the soil leading to wilting of plants under field conditions (Locher & De Bakker, 1990).

One litre plastic containers (Experiment 1,108mmx105mmx105mm) or three litre plastic bottles (Experiment 2, width: 142mm, H: 220mm, purchased at Plastilon, Pretoria, RSA) were filled with 750ml of the prepared soil. Wandering larvae were placed on the soil and left to dig their way down for an hour.

Between 10 to 30 beetle larvae were used per container because previous observations suggest that this is a suitable number for the provided soil volume (Murrle & Neumann, 2004). The containers were then lid sealed, although not airtight. This allowed some water to evaporate (~0.2ml/day at 30°C). Containers were placed in a temperature controlled room or incubator at 20°C, 30°C and 40°C in experiment 1 and 30°C and 37°C in a second experiment. For up to 50 days after the start of the experiment, the containers were checked daily for emerging adult beetles, which were collected.

Statistics

A Generalized Linear Mixed Model was used to analyse pupation success and development time (targets). Fixed factors were soil water content, temperature and the interaction between the two. The random effect of experiments or batches was tested as a block effect as batch plus (to cover batches from both experiment 1 and 2) with Variance Components or Scaled Identity as covariance type with the best fit. Significance of the model was realised at 95% confidence levels. Pairwise comparison of the fixed factor effects on the target was used to estimate mean values. Pearson residuals were normally distributed for all scaled data (development time). Treatments with no emergence were left out of the analysis. All analyses were conducted using the program SPSS 23(IBM).

Supplemental Tables S1a- S1c *Data on pupal performance of Aethina tumida from literature and additional laboratory experiments used to parameterise performance curves. Tables containing survival rate (a), development time (b) and conversion data (c) for Aethina tumida survival in soils, obtained from various published studies and additional laboratory experiments used to parameterise pupal performance curves.*

S1a Survival rate

Soil moisture [mm^3/mm^3]	Soil temperature [°C]	Survival rate	Study	Reference
0.37	16	0.147	LAB	Bernier et al., 2014
0.56	16	0.229	LAB	Bernier et al., 2014
0.73	16	0.125	LAB	Bernier et al., 2014
0.37	18	0.903	LAB	Bernier et al., 2014
0.56	18	0.89	LAB	Bernier et al., 2014
0.73	18	0.416	LAB	Bernier et al., 2014
0.37	20	0.976	LAB	Bernier et al., 2014
0.56	20	0.978	LAB	Bernier et al., 2014
0.73	20	0.383	LAB	Bernier et al., 2014
0.37	13,1	0	model	Bernier et al., 2014[1]
0.56	10,1	0	model	Bernier et al., 2014[1]
0.73	11,3	0	model	Bernier et al., 2014[1]
0.161	24,6	0.962	LAB	Ellis et al., 2004
0.001	24,6	0	LAB	Ellis et al., 2004
0.161	24,6	0.918	LAB	Ellis et al., 2004
0.161	24,6	0.976	LAB	Ellis et al., 2004
0.161	24,6	0.72	LAB	Ellis et al., 2004
0.161	24,6	0.96	LAB	Ellis et al., 2004
0.161	24,6	0.956	LAB	Ellis et al., 2004
0.126	28	0.7	LAB	Meikle & Diaz 2012[2]
0.126	32	0.8	LAB	Meikle & Diaz 2012[2]
0.126	35	0.4	LAB	Meikle & Diaz 2012[2]
0.116	21	0.95	LAB	Meikle & Patt 2011[3]
0.116	25	0.9	LAB	Meikle & Patt 2011[3]
0.116	28	0.9	LAB	Meikle & Patt 2011[3]
0.116	32	0.86	LAB	Meikle & Patt 2011[3]
0.116	35	0.35	LAB	Meikle & Patt 2011
0.116	9,9	0	model	Meikle & Patt 2011
0.001	20	0	LAB	Cornelissen[4]
0.02	20	0	LAB	Cornelissen[4]
0.04	20	0.96	LAB	Cornelissen[4]
0.08	20	0.96	LAB	Cornelissen[4]
0.16	20	0.99	LAB	Cornelissen[4]
0.001	30	0	LAB	Cornelissen[4]
0.02	30	0	LAB	Cornelissen[4]
0.04	30	0.91	LAB	Cornelissen[4]
0.08	30	0.87	LAB	Cornelissen[4]
0.16	30	0.7	LAB	Cornelissen[4]
0.001	40	0	LAB	Cornelissen[4]
0.02	40	0	LAB	Cornelissen[4]
0.04	40	0	LAB	Cornelissen[4]

Supplemental Table S1a continued

Soil moisture [mm^3/mm^3]	Soil temperature [°C]	Survival rate	Study	Reference
0.08	40	0	LAB	Cornelissen[4]
0.16	40	0	LAB	Cornelissen[4]
0.04	30	0.984	LAB	Cornelissen[4]
0.04	30	0.984	LAB	Cornelissen[4]
0.04	37	0.415	LAB	Cornelissen[4]
0.08	30	0.969	LAB	Cornelissen[4]
0.08	37	0.408	LAB	Cornelissen[4]
0.16	30	0.984	LAB	Cornelissen[4]
0.16	37	0.278	LAB	Cornelissen[4]

[1] data extracted by O. SCHWEIGER

[2] visually extracted by O. SCHWEIGER from Fig. 2 as average of 4 and 8 cm

[3] visually extracted by O. SCHWEIGER from Fig. 4A

[4] complementary laboratory experiment, see supplemental method 1

- For Ellis et al., 2004: Moisture given as water by weight ratio. In order to calculate the fraction water per dry soil (m^3/m^3), soil density estimation based on texture characteristics using Poelman & van Egmond, (1979) and Krabbenborg et al (1983) in Locher & Bakker
- For Meikle & Diaz, 2012: Moisture given as water by weight ratio. In order to calculate the fraction water per dry soil (m^3/m^3), soil density estimation based on texture characteristics using Poelman & van Egmond, (1979) and Krabbenborg et al (1983) in Locher & Bakker (1990). (See S1c)
- For Meikle & Platt, 2011: Moisture given as water by weight ratio. In order to calculate the fraction water per dry soil (m^3/m^3), soil density estimation based on texture characteristics using Poelman & van Egmond, (1979) and Krabbenborg et al (1983) in Locher & Bakker (1990). Soils were assumed to be sand (density:1650kg/m^3). (See S1c)
- Visual data extraction was based on published figures, if original data have not been provided in the publication, using WebPlotDigitizer v4.2, https://automeris.io/WebPlotDigitizer. With this tool, we defined the scales of the axes in the respective plot and subsequently extracted the respective values.

S1b Development time

Soil moisture [mm^3/mm^3]	Soil temperature [°C]	Development time [days]	Study	Reference
0.37	16	78.1	LAB	Bernier et al., 2014
0.56	16	69.1	LAB	Bernier et al., 2014
0.73	16	71.6	LAB	Bernier et al., 2014
0.37	18	54.4	LAB	Bernier et al., 2014
0.56	18	48.9	LAB	Bernier et al., 2014
0.73	18	47.6	LAB	Bernier et al., 2014
0.37	20	38.3	LAB	Bernier et al., 2014
0.56	20	36.8	LAB	Bernier et al., 2014
0.73	20	39	LAB	Bernier et al., 2014
0.161	246	23	LAB	Ellis et al., 2004
0.161	24.6	23.9	LAB	Ellis et al., 2004
0.161	24.6	23.4	LAB	Ellis et al., 2004
0.161	24.6	22.8	LAB	Ellis et al., 2004
0.161	24.6	22.9	LAB	Ellis et al., 2004
0.161	24.6	22.5	LAB	Ellis et al., 2004

Supplemental Table S1b continued

Soil moisture [mm³/mm³]	Soil temperature [°C]	Development time [days]	Study	Reference
0.116	21	32.7	LAB	Meikle & Patt 2011[1]
0.116	25	25.8	LAB	Meikle & Patt 2011[1]
0.116	28	16.8	LAB	Meikle & Patt 2011[1]
0.116	32	15.3	LAB	Meikle & Patt 2011[1]
0.116	35	14.8	LAB	Meikle & Patt 2011
0.116	15	70	model	Meikle & Patt 2011
0.116	12	174	model	Meikle & Patt 2011
0.04	20	35.65	LAB	Cornelissen[2]
0.08	20	33.65	LAB	Cornelissen[2]
0.16	20	33.32	LAB	Cornelissen[2]
0.04	30	15.67	LAB	Cornelissen[2]
0.08	30	14.5	LAB	Cornelissen[2]
0.16	30	13.68	LAB	Cornelissen[2]
0.04	30	14.864	LAB	Cornelissen[2]
0.04	37	14.361	LAB	Cornelissen[2]
0.08	30	14.661	LAB	Cornelissen[2]
0.08	37	14.189	LAB	Cornelissen[2]
0.16	30	14.2	LAB	Cornelissen[2]
0.16	37	13.615	LAB	Cornelissen[2]

[1] visually extracted by O. Schweiger from Fig. 4A

[2] complementary laboratory experiment, see supplemental method 1

- For Ellis et al., 2004: Moisture given as water by weight ratio. In order to calculate the fraction water per dry soil (m³/m³), soil density estimation based on texture characteristics using Poelman & van Egmond, (1979) and Krabbenborg et al (1983) in Locher & Bakker
- For Meikle & Diaz, 2012: Moisture given as water by weight ratio. In order to calculate the fraction water per dry soil (m³/m³), soil density estimation based on texture characteristics using Poelman & van Egmond, (1979) and Krabbenborg et al (1983) in Locher & Bakker (1990). (See S1c)
- For Meikle & Platt, 2011: Moisture given as water by weight ratio. In order to calculate the fraction water per dry soil (m³/m³), soil density estimation based on texture characteristics using Poelman & van Egmond, (1979) and Krabbenborg et al (1983) in Locher & Bakker (1990). Soils were assumed to be sand (density:1650kg/m³). (See S1c)
- Visual data extraction was based on published figures, if original data have not been provided in the publication, using WebPlotDigitizer v4.2, https://automeris.io/WebPlotDigitizer. With this tool, we defined the scales of the axes in the respective plot and subsequently extracted the respective values.

S1c Conversion table

				conversion	
from Locher & Bakker 1990	soil density	1.6	gram/cm³	1600	kg/m³
known values Ellis et al., 2004	volume	1000	cm³	0.001	m³
	water	10%		10%	
Calculated values	assumed soil weight	1400	gram	1.4	kg
	assumed weight water	155.56	gram	0.16	kg
	assumed total weight	1555.56	gram	1.56	kg
	volume water	155.56	ml	0.16	liters
	volume water	155.56	cm³	0.00	m³
	fraction water	0.16	cm³/cm³	0.16	m³/m³

from Locher & Bakker 1990	soil density	1.65	gram/cm³	1650	kg/m³
Known values meikle & Diaz 2012	volume	80	cm³	0.00008	m³
	water	6%		6%	
Calculated values	assumed soil weight	132	gram	0.132	kg
	assumed weight water	8.61	gram	0.01	kg
	assumed total weight	140.61	gram	0.14	kg
	volume water	8.61	ml	0.01	liters
	volume water	8.61	cm³	0.00	m³
	fraction water	0.11	cm³/cm³	0.11	m³/m³

from Locher & Bakker 1990	soil density	1.65	gram/cm³	1650	kg/m³
Known values meikle & Diaz 2012	volume	80	cm³	0.00008	m³
	water	8%		8%	
Calculated values	assumed soil weight	132	gram	0.132	kg
	assumed weight water	11.48	gram	0.01	kg
	assumed total weight	143.48	gram	0.14	kg
	volume water	11.48	ml	0.01	liters
	volume water	11.48	cm³	0.00	m³
	fraction water	0.14	cm³/cm³	0.14	m³/m³

from Locher & Bakker 1990	soil density	1.63	gram/cm³	1630	kg/m³
Known values meikle & Diaz 2012	volume	120	cm³	0.00012	m³
	water	5%		5%	
Calculated values	assumed soil weight	195.6	gram	0.1956	kg
	assumed weight water	10.63	gram	0.01	kg
	assumed total weight	206.23	gram	0.21	kg
	volume water	10.63	ml	0.01	liters
	volume water	10.63	cm³	0.00	m³
	fraction water	0.09	cm³/cm³	0.09	m³/m³

from Locher & Bakker 1990	soil density	1.63	gram/cm³	1630	kg/m³
Known values meikle & Diaz 2012	volume	120	cm³	0.00012	m³
	water	8%		8%	
Calculated values	assumed soil weight	195.6	gram	0.1956	kg
	assumed weight water	17.01	gram	0.02	kg
	assumed total weight	212.61	gram	0.21	kg
	volume water	17.01	ml	0.02	liters
	volume water	17.01	cm³	0.00	m³
	fraction water	0.14	cm³/cm³	0.14	m³/m³

***Supplemental Tables S2a – S2b** AIC values for model selection.*

S2a AIC values for models relating pupal survival rate of *Aethina tumida* to soil moisture according to equation 1.2 in the main text with exponents of the parabolic function increasing from 2 to 20 in steps of two (to avoid flipping the sign for negative values when the exponent is uneven). Exponent with lowest AIC (10) is marked in bold.

Exponent	AIC	Delta AIC
2	-69.20	11.04
4	-76.18	4.06
6	-79.13	1.11
8	-80.12	0.11
10	**-80.23**	**0.00**
12	-79.57	0.66
14	-80.18	0.06
16	-80.16	0.08
18	-80.14	0.09
20	-80.13	0.10

S2b AIC values for model comparisons relating pupal survival rate and development time of *Aethina tumida* to all possible combinations of temperature and soil moisture functions covering single, additive and interactive effects. Models are ordered with increasing AIC.

Response	Model	AIC	Delta AIC
Survival rate	Temperature * Moisture	-80.12	0.00
	Temperature + Moisture	-79.82	0.30
	Temperature	-35.19	44.93
	Moisture	9.59	89.71
Development time	Temperature	197.02	0.00
	Temperature * Moisture	268.23	71.21
	Temperature + Moisture	312.80	115.78
	Moisture	330.99	133.97

Supplemental Table S3 *Georeferenced observations of* Aethina tumida *used for assessing predictive ability of modelled pupal performance.*

Country	Administrative unit	City	Lat	Lon	Reference
Australia	NA	Kununura	-15.77361	128.738611	1
Australia	Queensland	Highvale	-27.37900	152.812	1
South Africa	NA	Ixopo	-30.15722	30.064722	1
Philippines	Davao	Panabo	7.30833	125.683333	2
Philippines	Davao	Tagum	7.44821	125.80942	2
Italy	Calabria	Candidoni	38.50000	16.083333	3
Italy	Calabria	Cinquefrondi	38.41667	16.1	3
Italy	Calabria	Cittanova	38.35000	16.083333	3
Italy	Calabria	Galatro	38.46667	16.116667	3
Italy	Calabria	Gioia Tauro	38.43333	15.9	3
Italy	Calabria	Melicucco	38.43333	16.05	3
Italy	Calabria	Oppido Mamertina	38.30000	15.983333	3
Italy	Calabria	Polistena	38.40000	16.066667	3
Italy	Calabria	Rizziconi	38.41667	15.966667	3
Italy	Calabria	Rosarno	38.50000	15.983333	3
Italy	Calabria	San Ferdinando	38.48333	15.916667	3
Italy	Calabria	Santa Cristina D.Aspromonte	38.25000	15.966667	3
Italy	Calabria	Serrata	38.53333	16.083333	3
Italy	Calabria	Taurianova	38.35000	16.016667	3
Italy	Calabria	Varapodio	38.31667	15.983333	3
South Africa	Gauteng Province	Meyerton	-26.55830	28.0197	4
South Africa	Gauteng Province	Pretoria (Brooklyn)	-25.77012	28.23657	4
South Africa	Gauteng Province	Pretoria (Hatfield)	-25.74800	28.238	4
South Africa	Gauteng Province	Randfontein	-26.17972	27.704167	4
South Africa	North West Province	Rustenburg	-25.66667	27.25	4
Burkina Faso	Garango	Lergo	11.70004	-0.55001	5
Niger	Tillabéri Region	Moli-Haoussa	12.53735	2.31841	6
Nigeria	Rivers	University of Port Harcourt	4.82417	7.033611	7
Uganda	Kampala	Makerere University Agricultural Research Institute Kabanyolo	0.46368	32.60956	8
Madagascar	Amoron	Ambositra	-20.53300	47.24500	9
Madagascar	Analanjirofo	Fenerive	-17.16667	49.3	9
El salvador	Ahuachapan	San Francisco Menendez	13.85000	-90.016667	10
El salvador	La Libertad	Colon	13.71667	-89.366667	10
El salvador	La Libertad	Jayaque	13.66667	-89.433333	10
El salvador	La Libertad	La Libertad	13.48833	-89.322222	10
El salvador	La Libertad	San Juan Opico	13.88333	-89.35	10
El salvador	La Paz	EL ROSARIO	13.50000	-89.033333	10
El salvador	Sonsonate	Caluco	13.71667	-89.666667	10
Kenya	Coast province	Chawia-Taita	-3.47519	38.33831	11
Kenya	Coast province	Watamu	-3.30675	40.01789	11
Kenya	Coast province	Watamu	-3.30806	39.99894	11
Kenya	Eastern Province	Ndalani	-1.08508	37.47031	11
Kenya	Nairobi	Kamiti	-1.19044	36.89308	11
Kenya	Nairobi	Kasarani	-1.22369	36.89700	11
Italy	NA	Lutrò di San Carlo Condofuri	37.95980	15.88440	12
Italy	NA	Spropoli Palizzi	37.96667	15.98333	12
South Africa	NA	Adelaide	-32.70000	26.3	12
South Africa	NA	Butterworth	-32.33333	28.15	12
South Africa	NA	Ezemvelo	-25.70404	28.93964	12
South Africa	NA	Fort Beaufort	-32.78333	26.633333	12
South Africa	NA	Grahamstown	-33.31000	26.526667	12
South Africa	NA	Graskop	-24.93167	30.841667	12
South Africa	NA	Heilbron	-27.28361	27.970833	12

Supplemental Table S3 continued

Country	Administrative unit	City	Lat	Lon	Reference
South Africa	NA	Heilbron	-27.28361	27.970833	12
South Africa	NA	Hermanus	-34.41667	19.25	12
South Africa	NA	Jacobsdal	-29.12833	24.775	12
South Africa	NA	Kokstad	-30.55389	29.426944	12
South Africa	NA	Ladysmith	-28.55972	29.780556	12
South Africa	NA	Settlers	-24.95008	28.53436	12
South Africa	NA	Standerton	-26.95000	29.25	12
South Africa	NA	Stutterheim	-32.56667	27.416667	12
South Africa	NA	Uitenhage	-33.76667	25.4	12
South Africa	NA	Worcester	-33.64500	19.443611	12
Swaziland	NA	Mbabane	-26.31667	31.133333	12
United states	Maryland	Beltsville	39.02810	-76.93158	12
Nigeria	Abuja FCT	Kwali	8.84698	7.06060	13
Nigeria	Bauchi	Gubi/Durum	10.44285	9.80970	13
Nigeria	Bauchi	Tilden Fulani	10.03715	8.99377	13
Nigeria	Cross River	Odukpani	5.08124	8.34992	13
Nigeria	Delta	Itagbere	6.05000	5.13333	13
Nigeria	Delta	Opuame	5.88333	5.06667	13
Nigeria	Edo	Agenebode	7.10660	6.68787	13
Nigeria	Edo	Lampese	7.45128	6.10544	13
Nigeria	Gombe	Kaltungo	9.81667	11.30000	13
Nigeria	Gombe	Kumo	10.04208	11.21814	13
Nigeria	Kano	Bebeji	12.00209	8.53460	13
Nigeria	Kano	Rano	11.56101	8.58172	13
Nigeria	Nasarawa	Keffi	8.84712	7.87760	13
Nigeria	Niger	Agwarra	10.70728	4.58050	13
Nigeria	Niger	Mokwa	8.84712	7.87760	13
Nigeria	Niger	Rijau	11.09911	5.25277	13
Nigeria	Niger	Sarkin-Pawa	10.01829	7.12786	13
Nigeria	Ogun	Ifo	6.81924	3.19303	13
Nigeria	Ogun	Logbara	6.89283	3.57747	13
Nigeria	Ogun	Ota	6.67488	3.19805	13
Nigeria	Ondo	Akure	7.25713	5.20579	13
Nigeria	Ondo	Okeigbo	7.17102	4.72258	13
Nigeria	Osun State	Iwo	7.62921	4.18722	13
Nigeria	Osun State	Osogbo	7.78267	4.54181	13
Nigeria	Oyo	Jericho. Ibadan	7.39447	3.86044	13
Nigeria	Oyo	Shaki	8.67256	3.39433	13
Nigeria	Plateau	Kwongo-Hoss	9.58333	8.70000	13
Australia	New South Wales	Anna Bay	-32.75142	152.10750	14
Australia	New South Wales	Bega	-36.66667	149.833333	14
Australia	New South Wales	Casino	-28.86667	153.05	14
Australia	New South Wales	Coffs Harbour	-30.30222	153.118889	14
Australia	New South Wales	Glenn Innes	-29.75000	151.736111	14
Australia	New South Wales	Grafton	-29.68333	152.933333	14
Australia	New South Wales	Kempsey	-31.08333	152.833333	14
Australia	New South Wales	Lismore	-28.81667	153.283333	14
Australia	New South Wales	Mudgee	-32.60000	149.583333	14
Australia	New South Wales	Nabiac	-32.10000	152.383333	14
Australia	New South Wales	Newcastle	-32.91667	151.75	14
Australia	New South Wales	Port Macquarie	-31.43333	152.9	14
Australia	New South Wales	Rylstone	-32.77142	149.97856	14
Australia	New South Wales	Tocal	-32.63333	151.60000	14
Australia	New South Wales	Wellington	-32.55000	148.93333	14
Australia	New South Wales	Wollogong	-34.43306	150.88306	14
Australia	New South Wales	Woodstock	-33.75000	148.85000	14

Supplemental Table S3 continued

Country	Administrative unit	City	Lat	Lon	Reference
Australia	New South Wales	Yamba	-29.43333	153.35000	14
Australia	New South Wales	Young	-34.30000	148.30000	14
South Africa	NA	Albertinia	-34.20000	21.583333	15
South Africa	NA	Bela Bela	-24.88333	28.283333	15
South Africa	NA	Bellville	-33.90000	18.633333	15
South Africa	NA	Benoni	-26.18833	28.320556	15
South Africa	NA	Boksburg	-26.21250	28.2625	15
South Africa	NA	Bonnievale	-33.92750	20.100556	15
South Africa	NA	Brakpan	-26.23528	28.37	15
South Africa	NA	Caledon	-34.23000	19.428333	15
South Africa	NA	Delmas	-26.15000	28.683333	15
South Africa	NA	Durbanville	-33.83250	18.6475	15
South Africa	NA	East London	-32.98333	27.866667	15
South Africa	NA	Ermelo	-26.53333	29.983333	15
South Africa	NA	Graaf Reinet	-32.25222	24.540556	15
South Africa	Cape	Heidelberg	-34.08333	20.95	15
South Africa	Gauteng province	Heidelberg	-26.50056	28.358333	15
South Africa	Kwazulu Natal	Hilton	-29.55000	30.3	15
South Africa	NA	Kempton Park	-26.10000	28.233333	15
South Africa	NA	Knysna	-34.03556	23.048889	15
South Africa	NA	Ladismith	-33.48333	21.266667	15
South Africa	NA	Lady grey	-30.70972	27.214444	15
South Africa	NA	Lichtenburg	-26.15000	26.166667	15
South Africa	NA	McGregor	-33.95000	19.833333	15
South Africa	Mpumalanga	Middelburg	-25.76842	29.478333	15
South Africa	NA	Midrand	-25.99917	28.126389	15
South Africa	NA	Modimole	-24.70000	28.406111	15
South Africa	NA	Mookgophong	-24.51667	28.716667	15
South Africa	NA	NewCastle	-27.74639	29.932778	15
South Africa	NA	Plettenberg bay	-34.05000	23.366667	15
South Africa	NA	Plumstead	-34.02222	18.472222	15
South Africa	NA	Port Alfred	-33.59167	26.8875	15
South Africa	NA	Randburg	-26.09361	28.006389	15
South Africa	NA	Richards bay	-28.80000	32.1	15
South Africa	NA	Robertson	-33.80000	19.883333	15
South Africa	NA	Springs	-26.25472	28.442778	15
South Africa	NA	Stanford	-34.44167	19.455556	15
South Africa	NA	Swartklip	-24.94500	27.163	15
South Africa	NA	Tzaneen	-23.83333	30.166667	15
South Africa	NA	White river	-25.31667	31.016667	15
South Africa	NA	Willowmore	-33.28333	23.483333	15
Canada	Ontario	Colborne	44.00527	-77.88828	16
Canada	Ontario	Fort Erie	42.91667	-79.016667	16
Canada	Ontario	Lincoln	43.13333	-79.43333	16
Canada	Ontario	Niagara on the lakes	43.25528	-79.071667	16
Canada	Ontario	Pelham	43.03333	-79.333333	16
Canada	Ontario	St. Catherines	43.18333	-79.233333	16
Canada	Ontario	Thorold	43.11667	-79.2	16
United states	Florida	Gainesville	29.62705	-82.35674	17
United states	Florida	High Springs	29.81117	-82.54504	17
United states	Mississippi	Lula	34.45361	-90.478056	18
United states	Florida	La Crosse	29.85000	-82.4	19
Canada	Ontario	Amhurst	45.00390	-74.44930	20
Canada	Ontario	Andrew	45.00070	-74.36260	20
Canada	Ontario	Garnet	42.11460	-82.86860	20

Supplemental Table S3 continued

Country	Administrative unit	City	Lat	Lon	Reference
Canada	Ontario	Sheply	42.12080	-82.83970	20
Canada	Ontario	Smith	42.10530	-82.94660	20
United states	Louisiana	St. Gabriel	30.25361	-91.101389	21
United states	Louisiana	Baton Rouge	30.36182	-91.18641	22
United states	Georgia	Watkinsville	33.88265	-83.41883	23
United states	Texas	Weslaco	26.15917	-97.9875	24
Australia	New South Wales	Richmond, NSW	-33.61207	150.74484	25
Australia	New South Wales	Richmond, NSW	-33.58781	150.72569	25
United states	Georgia	Claxton	32.16083	-81.908611	26
United states	Georgia	Moultrie	31.16667	-83.783333	26
United states	Georgia	Mt Vernon	32.18139	-82.593889	26
United states	Georgia	Richmond Hill	31.93806	-81.313611	26
United states	Georgia	The Rock	32.96389	-84.241111	26
Australia	New South Wales	Colo	-33.41667	150.8	27
Australia	New South Wales	Kurrajong	-33.55000	150.666667	27
Australia	New South Wales	Windsor	-33.61500	150.817222	27
Rwanda	NA	Byumba	-1.57611	30.0675	28
Rwanda	NA	Huye	-2.58562	29.68825	28
Rwanda	NA	Nyande	-1.62031	30.05750	28
Rwanda	NA	Shangasha	-1.52561	30.06850	28
South Korea	NA	Miryang-Si	35.49416	128.78769	29
Brazil	NA	Piracicaba	-22.71515	-47.62981	30
South Africa	NA	Port Elizabeth	-33.95806	25.6	1, 12
Australia	New South Wales	Bathurst	-33.42000	149.577778	1, 14
Australia	New South Wales	Richmond	-33.60000	150.75000	1, 14
Australia	New South Wales	Mona Vale	-33.67756	151.30456	12, 14
South Africa	NA	Bloemfontein	-29.11667	26.216667	12, 15
South Africa	NA	Douglas	-29.05000	23.766667	12, 15
South Africa	NA	King Williams Town	-32.88333	27.4	12, 15
South Africa	NA	Mosselbaai	-34.18333	22.133333	12, 15
South Africa	NA	Paarl	-33.72417	18.955833	12, 15
South Africa	NA	Pietermaritzburg	-29.61667	30.383333	12, 15
South Africa	NA	stellenbosch	-33.92000	18.86	12, 15
South Africa	NA	Swellendam	-34.03333	20.433333	12, 15
South Africa	NA	George	-33.96667	22.45	12, 15
South Africa	NA	Hartbeespoort	-25.74417	27.899444	12, 15
South Africa	Gauteng province	Krugersdorp	-26.10000	27.766667	4, 15

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Supplemental Tables S4a – S4b *Model parameters for pupal survival and developments time according to equations 1.1 - 1.3 and 2.1.*

S4a Model parameters for pupal survival of *A. tumida* according to equations 1.1 - 1.3 in the main text.

Parameter	Estimate	Standard error	P-value
T_{opt}	18.880	0.342	< 0.001
T_{max}	40.900	0.502	< 0.001
⍰$_{P\,T}$	0.877	0.158	< 0.001
M_{opt}	0.040	0.051	0.44
M_{max}	0.768	0.010	< 0.001
⍰$_{P\,M}$	194.200	504.800	0.702
z	1.034	0.025	< 0.001

S4b Model parameters for pupal development time of *A. tumida* according to equation 2.1 in the main text.

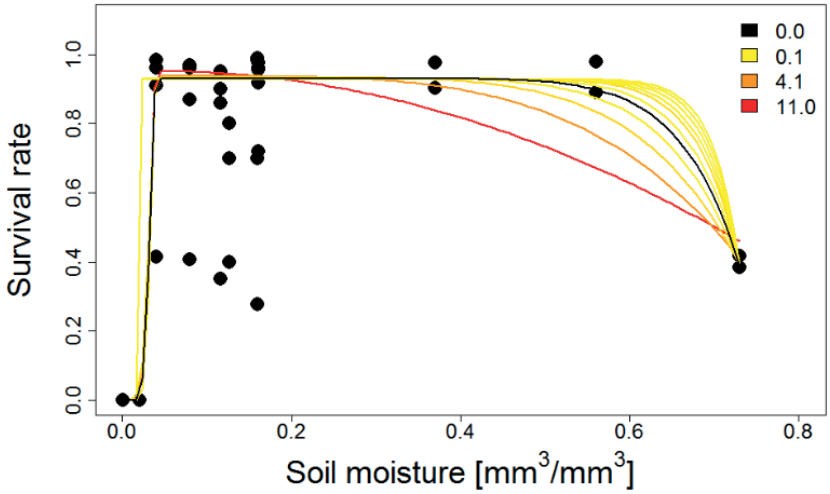
Parameter	Estimate	Standard error	P-value
a	14.982	1.160	< 0.001
R0	3252.332	416.057	< 0.001
c	-1.373	0.038	< 0.001

Supplemental Table S5 *Predicted changes in percentage climatically highly suitable for* Aethina tumida *pupal performance relative to overall vegetated land according to current conditions and four climate change scenarios*

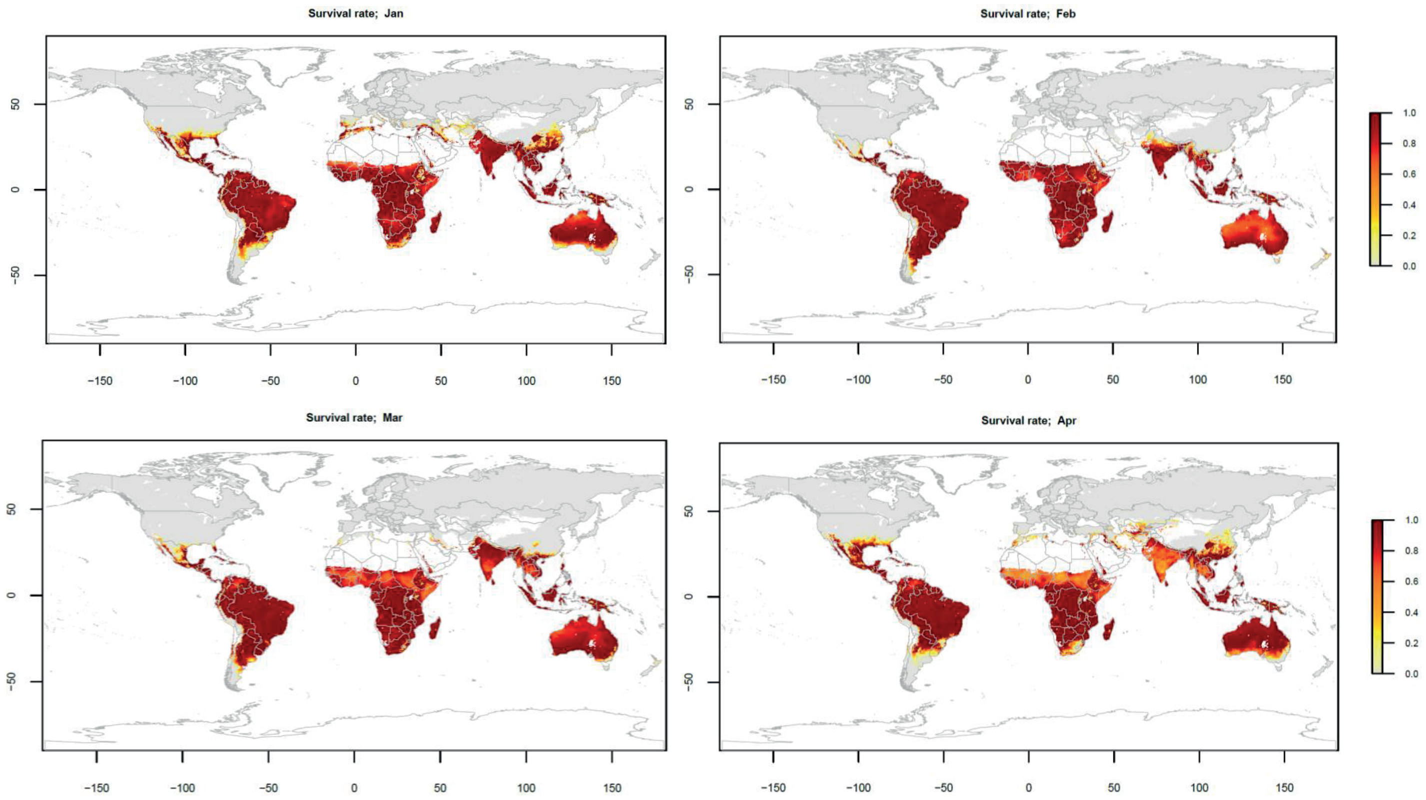
Hemisphere	Current	RCP2.6 2060	RCP2.6 2080	RCP8.5 2060	RCP8.5 2080
North	36.8%	47.3%	47.4%	54.5%	68.0%
South	87.4%	90.4%	90.5%	91.7%	92.9%
Global	48.5%	57.2%	57.4%	63.1%	73.8%

Supplemental Figure S1 *Performance curves of* Aethina tumida *pupal survival rate in response to soil moisture calculated with varying exponents of the parabolic function.*

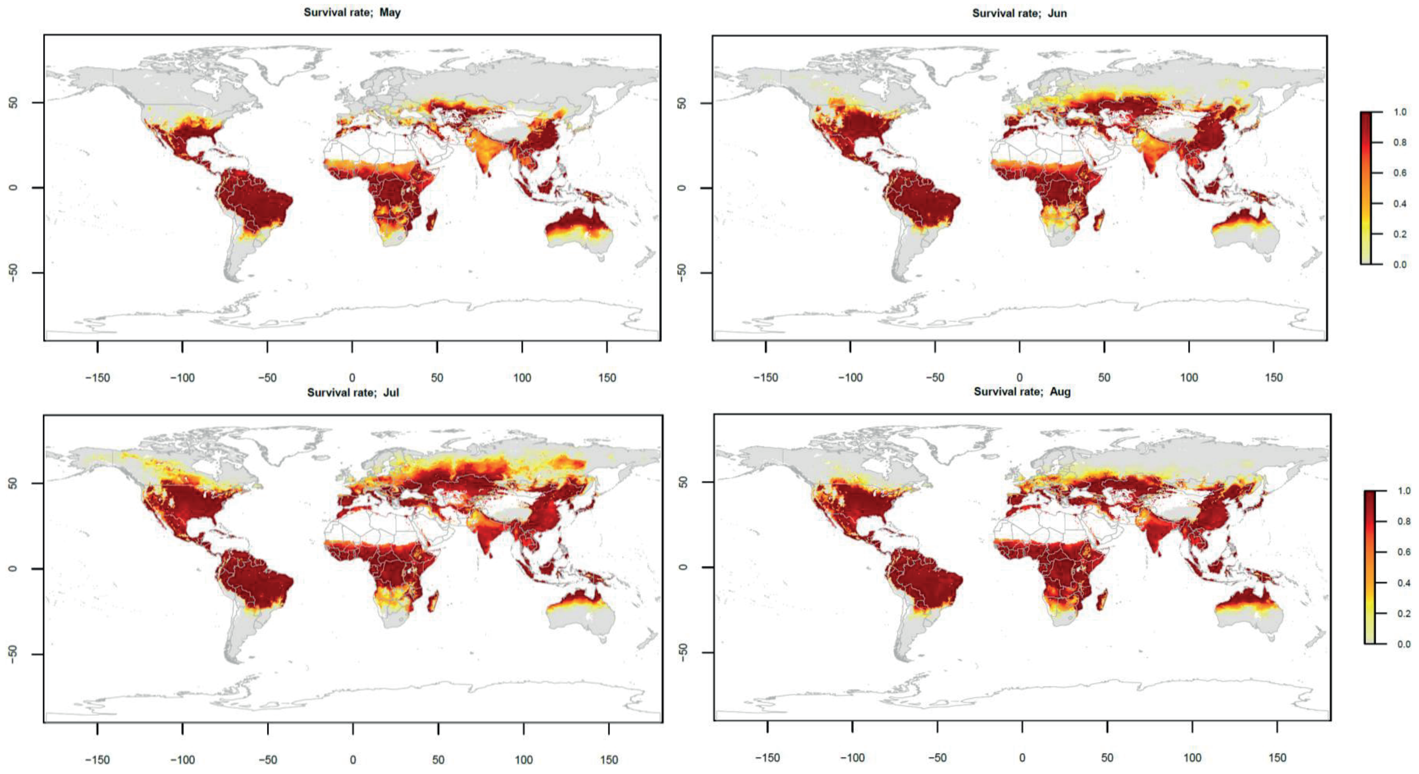
Performance curves of SHB pupal survival rate in response to soil moisture calculated according to equation 1.2 with exponents of the parabolic function increasing from 2 to 20 in steps of two (to avoid flipping the sign for negative values when the exponent is uneven). Model fit was assessed with AIC which was lowest for an exponent of 10 (see Supplemental Table S2a – S2b). Colour codes represent delta AIC.



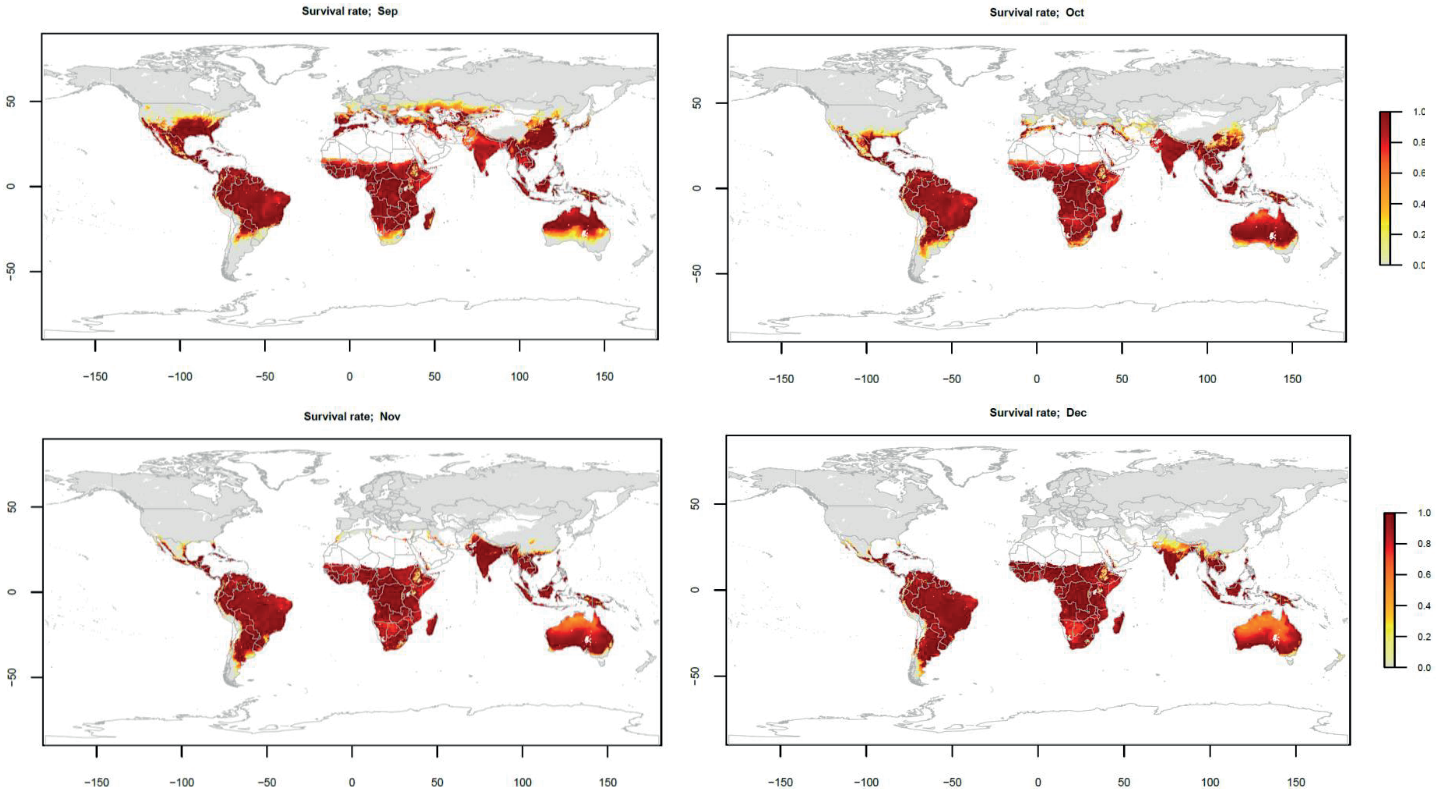
Supplemental Figure S2 *Global maps of monthly survival rate of* Aethina tumida *under current climatic conditions. Non-vegetated areas are masked in white.*



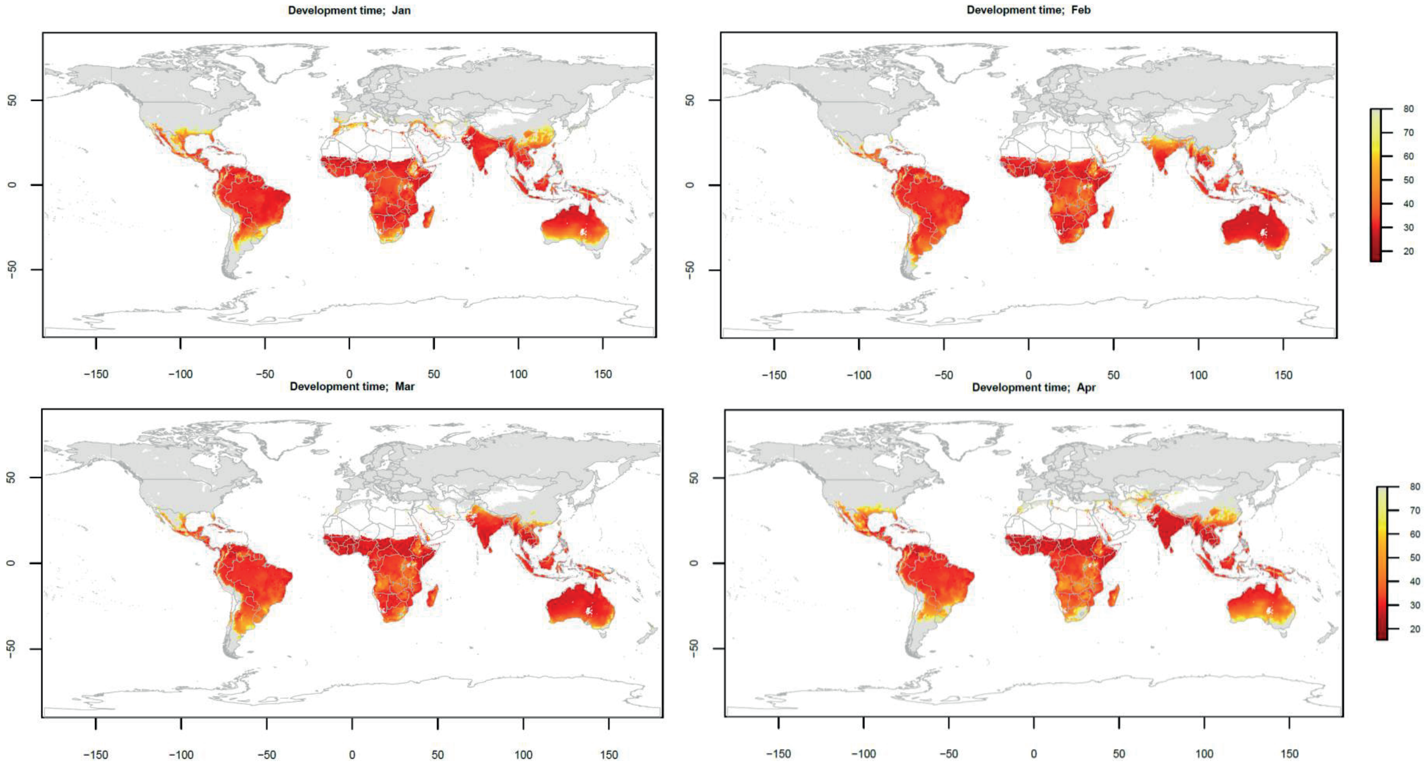
Supplemental Figure S2 continued



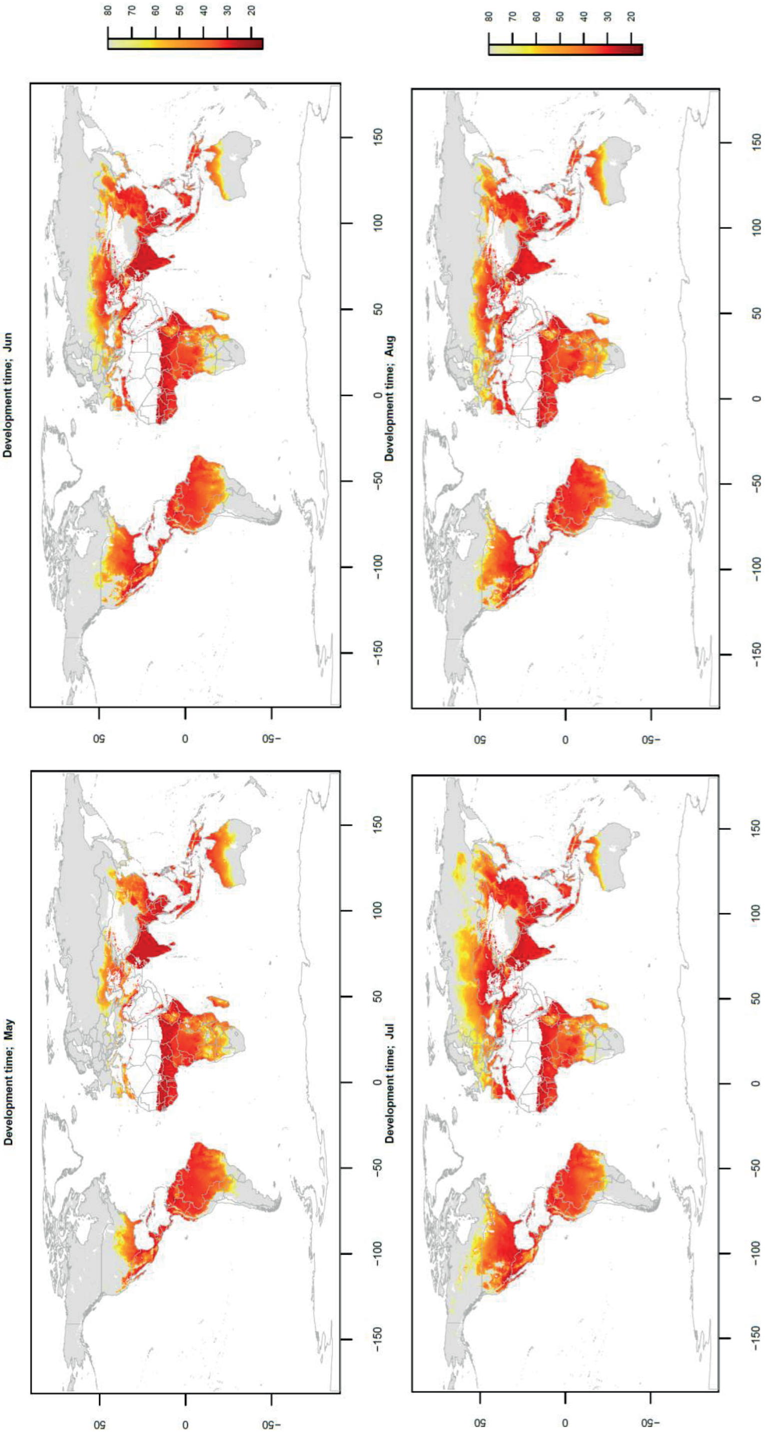
Supplemental Figure S2 continued



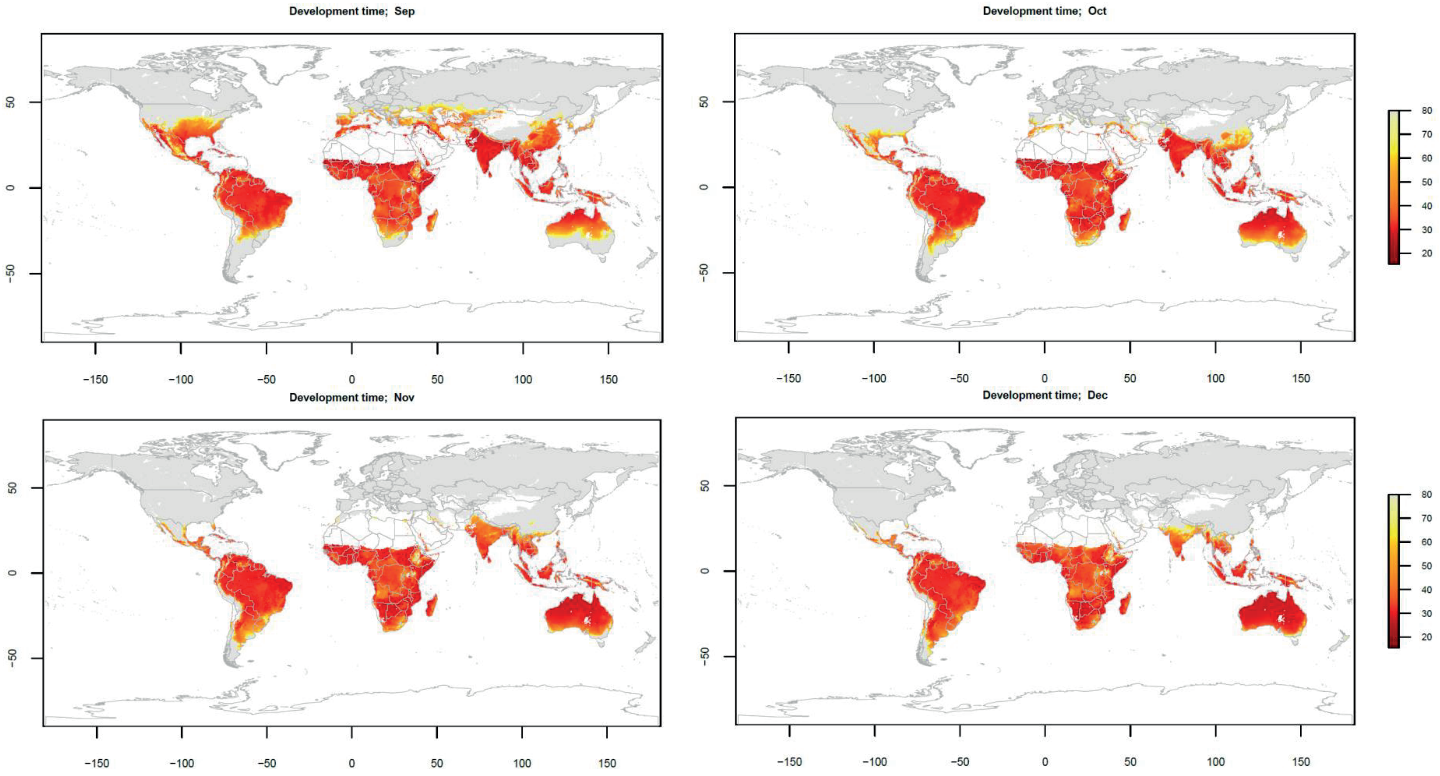
Supplemental Figure S3 *Global maps of monthly development time of* Aethina tumida *under current climatic conditions. Areas with development times longer than 80 days correspond to soil temperatures lower than 15°C and pupal survival rates close to zero (see Fig. 1) and are thus considered irrelevant and set to grey. Non-vegetated areas are masked in white.*



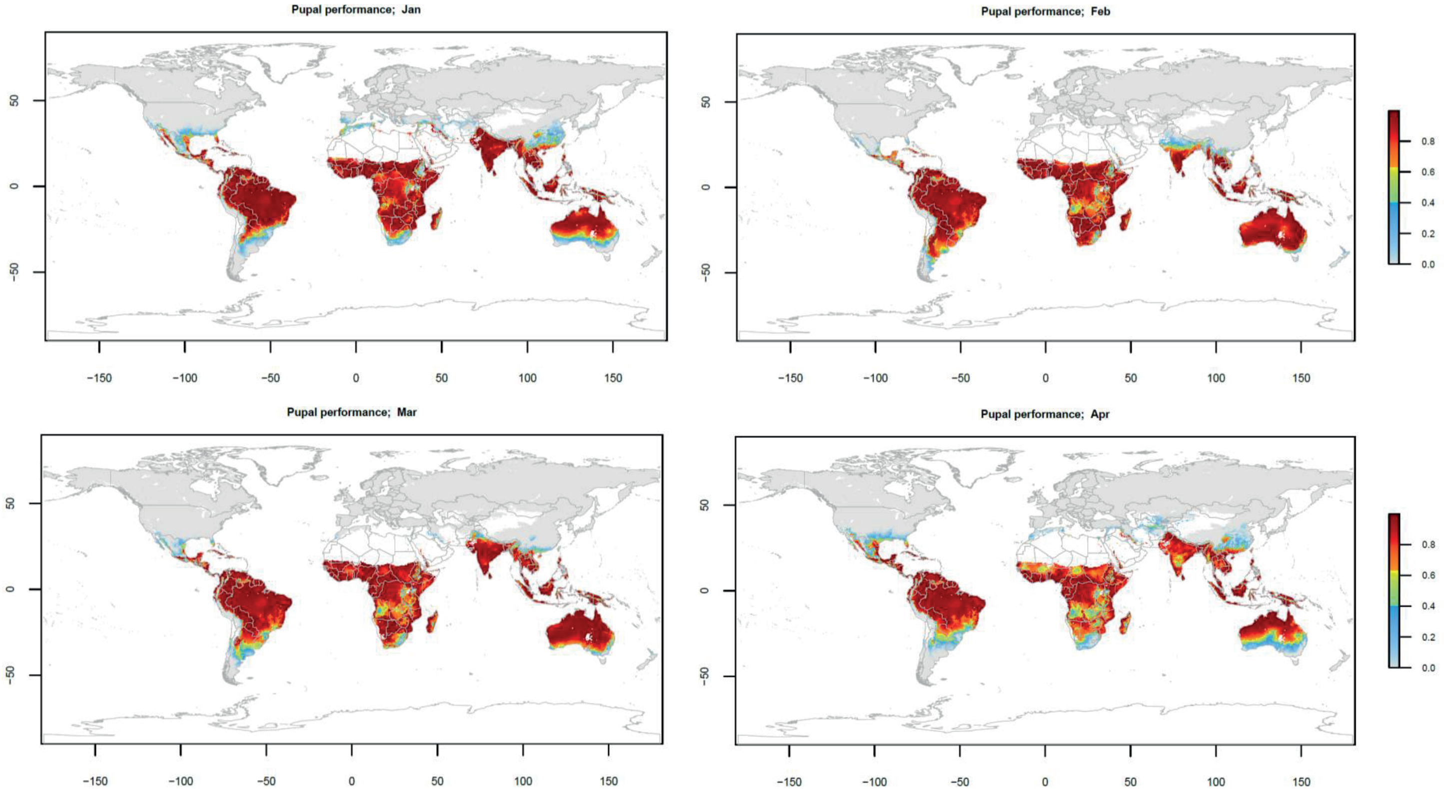
Supplemental Figure S3 continued



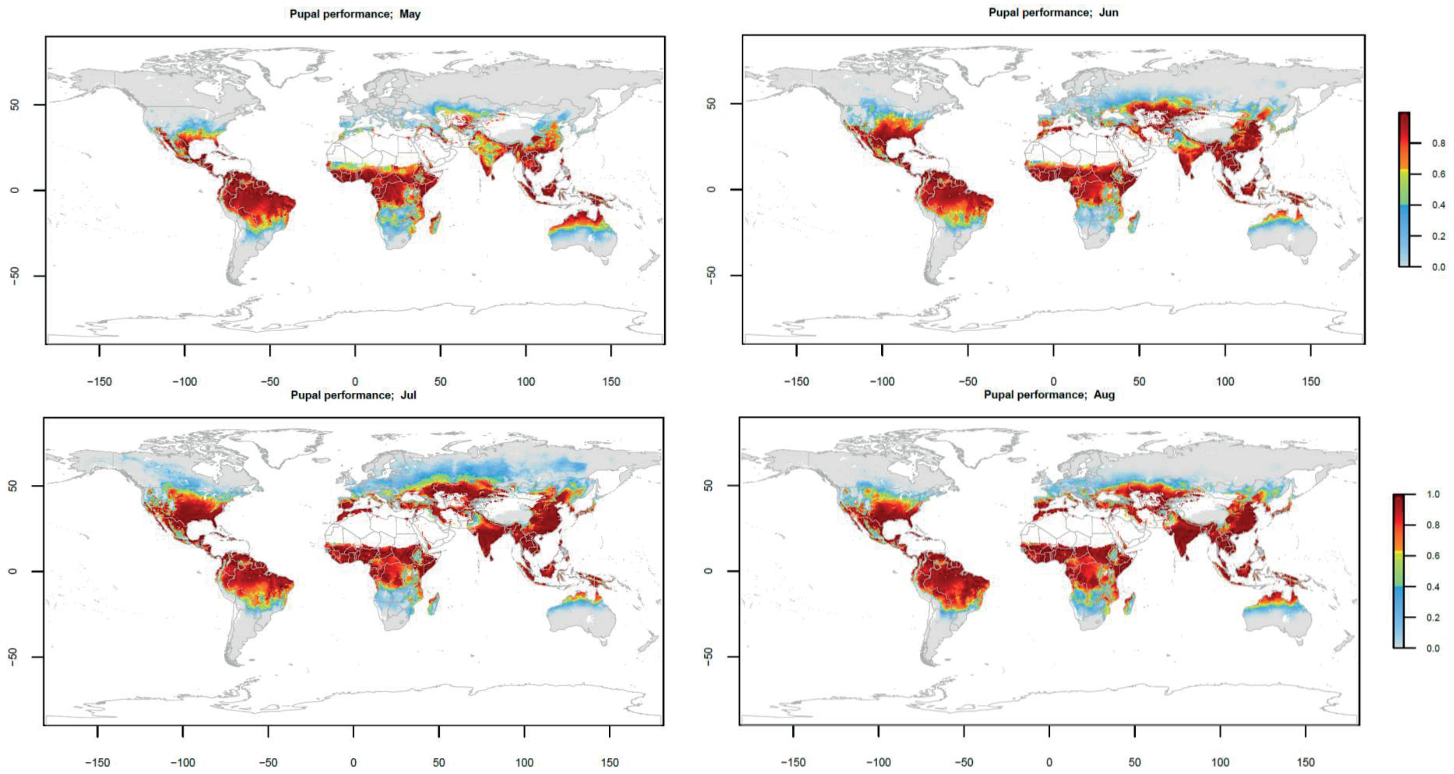
Supplemental Figure S3 continued



Supplemental Figure S4 *Global maps of monthly pupal performance of* Aethina tumida *under current climatic conditions. Red to orange colours indicate highly suitable areas, yellow to green colours indicate marginally suitable areas and blue to grey colours indicate unsuitable areas. Non-vegetated areas are masked in white.*



Supplemental Figure S4 continued



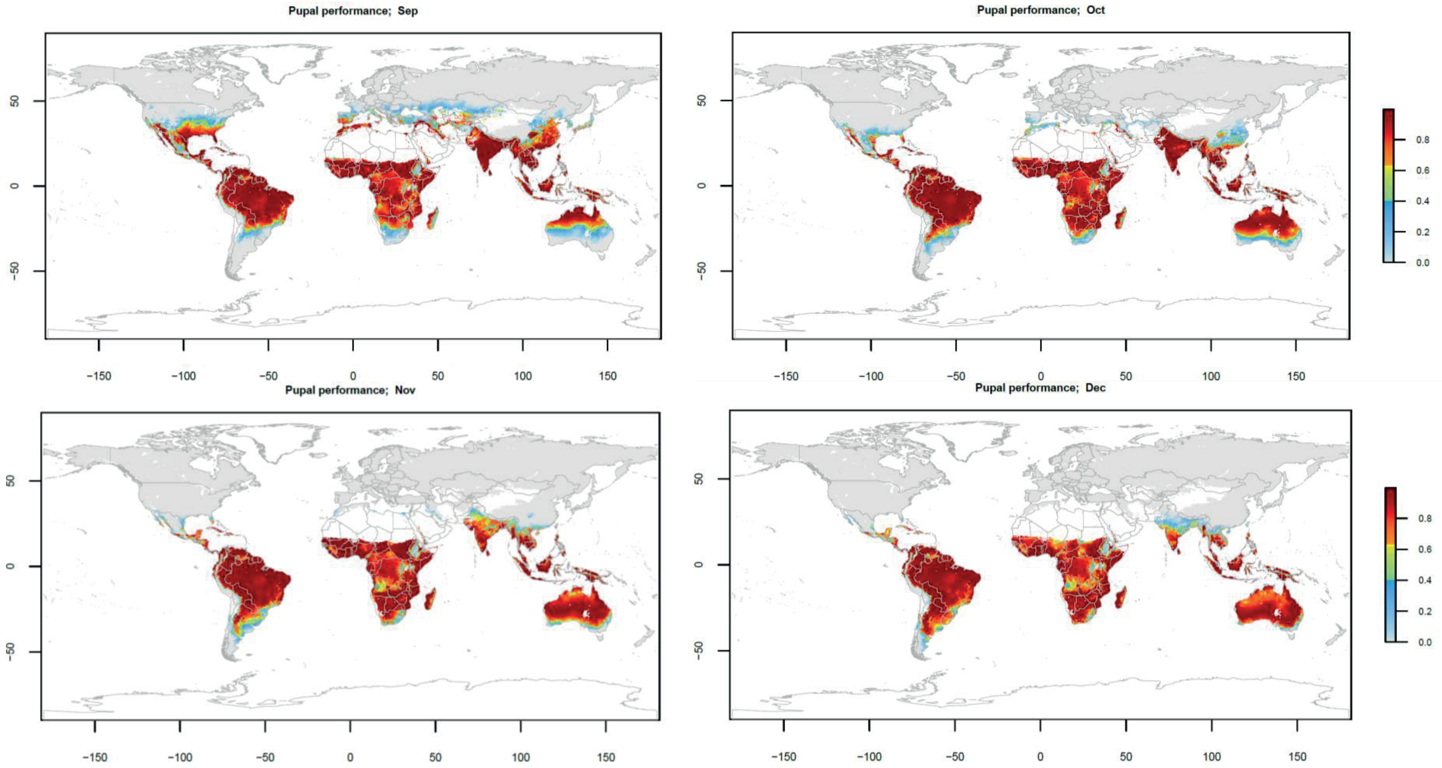
Pupal performance; May

Pupal performance; Jun

Pupal performance; Jul

Pupal performance; Aug

Supplemental Figure S4 continued



Chapter 7

General discussion

In 1996, the small hive beetle (SHB, *Aethina tumida* Murray) crossed the natural boundaries that once restrained its presence and impact in the world. The opening of this proverbial flood gate sparked the need for a better understanding of the species' biology and ecology. Up to 1996, only a handful of scientific publications, often in the form of very sound reports (Lundie, 1940; Schmolke, 1974) were available. The need for knowledge then rapidly led to giant scientific leaps forward (Ellis & Hepburn, 2006; Neumann et al., 2016). However, our understanding of the SHB as an invasive alien species, has mostly stayed within the boundaries of the research fields of life history, honey bee health and biological control. Only in recent years has the scope broadened to include the perspective of invasion ecology. With this thesis, I have sought to answer questions relevant to SHB ecology and ultimately the way in which the knowledge gained, can contribute to a science-based approach for dealing with the SHB as an invasive species. More specifically, the aim of this thesis was to investigate and understand the dispersal of the SHB by flight and the factors that are of influence on this important aspect of SHB invasion ecology (**Chapters 3 and 4**). Furthermore, over time and space, the realm of SHB occupation expands and the notion of such, requires a deeper knowledge of stabile and changing conditions that determine its impact on honey bees and beekeeping, which I investigated in **Chapters 5 and 6**. I will then highlight how the findings contribute to combating the SHB as an invasive species as proposed in **Chapter 2** and end with suggestions for future research.

Before I delve into the research findings, I wish to present a bigger picture in an attempt to explain how SHB could become an invasive alien species. In particular, I wish to highlight the concept of invasion ecology in relation to the primary host of the SHB, the western honey bee (*Apis mellifera* L. subspecies).

A plethora of species is associated with *A. mellifera*, and these utilize this social insect's nest and/or what's in it. Those that can endure the hardship that nest conditions and worker behaviour bestow on them (Seeley, 2019), find an opportunity to evade possibly even more challenging conditions outside. Some are passers-by like the kleptoparasitic death's head hawkmoth (*Acherontia atropos* L.), that uses chemical camouflage and sounds to enter and exit the honey bees' nest to feed on honey stores (Kitching, 2006). True symbionts are rare in honey bees compared to other social insects (Ellis & Hepburn, 2006), still some reside within the nest. For instance, *Braulio coeca* Nitzschz (Diptera: Braulidae) , also known as the bee louse, is a dipteran wingless commensal that lives solely in the nest. Here, it elicits royal jelly from worker bees feeding the queen (Imms, 1942). These examples highlight how the honey bee colony functions as a (partial) habitat for those that have evolved means to counter honey bee defensive strategies. Increasingly, however, invasive species inhabit honey bee colonies and do not have the evolutionary legacy that natural symbionts have. At least seven invasive species are known to be associated with *A. mellifera* (*Vespa velutina* Lepeletier: Arca et al., 2015; *Vespa orientalis* Linnaeus: Werenkraut et al., 2021; *Vespa mandarinia* Smith: Alaniz et al., 2021; *Tropilaelaps spp.* Delfinado & Baker: COLOSS, 2023; *Nosema ceranae* Fries: Fries, 2010; *Aethina tumida* Murray: Neumann & Elzen, 2004; *Varroa destructor* Anderson and Trueman: Rosenkranz et al., 2010). The past decades have revealed the vulnerability of *A. mellifera* to invasive species, in particular subspecies of European origin in temperate climates. Most noteworthy, the varroa mite (*Varroa destructor*), has been implicated as an important factor explaining mass winter mortality of honey bee colonies

(Brodschneider et al., 2018; Dooremalen et al., 2012; Schüler et al., 2023). But what are the drivers for the biological invasions that have impacted *A. mellifera* so much?

The global presence of honey bees facilitates the invaders

The transport of *A. mellifera* across biogeographic boundaries by humans has led to the establishment of managed and feral populations of *A. mellifera* on all continents, except Antarctica, whilst the native range covers large areas of Africa, Europe and Asia (Moritz et al., 2005). In fact, of the top ten of honey producing countries by volume, in four countries (Argentina, Brazil, Mexico and the United States of America) *A. mellifera* is not endemic (FAO, 2021). The consequences from the perspective of invasion ecology are multiple. First of all, the global distribution of *A. mellifera* populations has expanded the invasion network and thus increased the chance of introductions by functioning as bridgehead populations for further range expansion of invasive species (Renault et al., 2018). For instance, the first SHB invasion occurred in the United States of America, where *A. mellifera* is not endemic. From here, through trade (Idrissou et al., 2019), introductions of SHBs occurred in Europe (Portugal, 2007, eliminated), Korea (2017) and countries in the western hemisphere (see Figure 3, Chapter 1). Likewise, *V. destructor* spread through queen and colony trade from Europe to the Americas and Africa (Rosenkranz et al., 2010) and further on. In short, the trade and transport of honey bees and related commodities in itself is an important driver for the associated biological invasions, but coupled with the global presence of *A. mellifera,* the effect is enhanced significantly. This concept shows many similarities with livestock, where the introduction of for instance cattle has brought with it the introduction of associated pest species on a global scale (Biguezoton et al., 2016; Pimentel et al., 2000).

Intertwined with the global distribution of *A. mellifera* is the notion that the relative adaptation of *A. mellifera*-associated invasive species to the host is high, compared to more generalist invasive species. The cause of this is that non-sympatric parasites and pathogens associated with African *A. mellifera* subspecies and Asian honey bee species (*Apis sp.*) have shifted to European *A. mellifera* subspecies as a novel host and *vice versa*, due to range overlap and the aforementioned trade and transport. The comparatively similar biology of honey bee species, allows symbiont species that lived exclusively in colonies of Asian honey bee species e.g. the parasitic mites *V. destructor* and *Tropilaelaps sp.* to complete the lifecycle in *A. mellifera* colonies. But while Asian honey bee species have adapted resistance traits through long-lasting host-parasite relations, *A. mellifera* has not, and is thus vulnerable to these honey bee parasites (Panziera et al., 2022). These parasitic species are also excessively dependent on humans to allow them to become invasive, as they can only be transported with the live host. This is different for species with a lower level of adaptation and dependency on the natural host. For instance, because they are more mobile, have alternative food sources, and at least partially can complete the life cycle outside of honey bee colonies if environmental conditions favour survival. This allows them to complete at least some stages of invasion relatively independent from the host. For instance , *V. orientalis* and *V. velutina,* vespid species highly specialized on the predation of honey bee colonies (but not exclusively, see Stainton et al., 2023), can be transported in commodities entirely independent of honey bees or related commodities. The introduction of *V. velutina* in Europe for example, was caused by the transport of a single hibernating, mated queen in pottery from China (Arca et al., 2015). Even so, dietary preferences and the attractiveness to honey bee odours and cues suggests that the global presence of honey bee colonies increases the probability of invasion success even for species that are not dependent on honey bees. If environmental conditions are limiting the invaders, this could also prevent them from becoming established. This is, however, unlikely in the presence of honey bees, as the environmental conditions that sustain *A. mellifera* largely overlap with that of the invader (**Chapter 6**) and because honey bee colonies can provide a niche habitat for species that at least partially use the nest during their life cycle (Imms, 1942; Kitching, 2006; Lundie, 1940).

In conclusion, the global presence of *A. mellifera* exacerbates the effect of trade and transport of related commodities on the biological invasion of honey bee-associated invasive species. Moreover, the range overlap of subspecies and species of honey bees has allowed symbiont species to shift to European subspecies of *A. mellifera* as a novel host. Invasive species have profited from the apparent similarity in attractiveness and the lack of behavioural and defensive traits of European subspecies of *A. mellifera* against these invasive species compared to their natural hosts, ultimately increasing their invasion success. This is true even for species that are not entirely dependent on *A. mellifera* as a host. Lastly, it is highly likely that environmental requirements attributed to *A. mellifera* survival, are similar to those of associated invasive species.

This conclusion fits well with the status of the SHB. Ellis & Hepburn (2006) highlighted the unique position of the SHB as a symbiont of the honey bee. They can be considered inquiline endoparasites of the honey bee 'superorganism' (Ellis & Hepburn, 2006), apart from the fact that they do not complete the entire life cycle in the host colony (Lundie, 1940). In most cases the SHB acts more as a commensal, that inhabits the colony, with little harm caused. The SHB has adapted to life in the honey bee nest, which provides it access to protein-rich food sources (Montag et al., 2022), yet the honey bee nest does not accommodate pupation due to the lack of a suitable substrate. Hence, the SHB needs to exit the host colony, and like other Nitidulidae species, pupates in the soil. It also means that once the SHB emerges from the soil as an adult, it requires movement in order to find a host. It is within this critical phase in the life cycle of the SHB, from leaving the hive as a wandering larva and pupating underground, to flying in search of a host that this thesis took shape.

SHB dispersal capacity as an adaptation to the host

Given the well-developed wings of both male and female SHBs, flight seems the logical *modus operandi* for SHBs to disperse, although adults are able to walk in and out of colonies (Annand, 2011). SHBs can occur in absence of honey bee colonies and thus dispersal can be initiated independently of its foremost host (Arbogast et al., 2009). However, dispersal in SHB does seem an adaptation to honey bees, based on the findings in **Chapter 3 and 4**. In a natural habitat with only wild feral host colonies, the SHB mostly acts as a commensal, causing no significant damage to the host (Ellis & Hepburn, 2006). This is also expressed by the low cryptic reproduction (Spiewok & Neumann, 2006). It is likely that under these conditions, most, but not all SHBs emerging from the soil, will re-enter the host colony they originated from as larvae. This is supported by the results obtained in **Chapter 3**, which showed that the recapture rate of SHBs is highest close to the release point and diminishes with distance and time. This implies that flight in these circumstances can be avoided, due to the close proximity of the host to the place of emergence. Furthermore, in accordance with the results presented in **Chapter 4**, SHBs are prone to remain in a host colony. However, when the host colony dies or absconds, either due to mass reproduction of SHB or other causes, emerging and resident adult SHBs need to venture outwards in order to find another host or accompany the absconding swarm (Ellis et al., 2003; Neumann et al., 2018). Under natural conditions in sub-Saharan Africa, where SHBs and *A. mellifera* colonies occur as sympatric species, hosts are not concentrated in one location (as with managed colonies) and the density of honey bee colonies varies between 5.7 to 10.2 colonies per km^2 (Jaffé et al., 2010). Hence, long-range flight is needed to cover the distance between the site of emergence and the host, offering an explanation for the physical capacity of the SHB to fly. However, the potential distance to a suitable host itself in the natural setting, does not entirely explain the flight capacity of the SHB. In fact, based on the findings in **Chapter 3**, the flight capacity of the SHB exceeds the need, as individuals were found to cover distances far greater than those between host colonies in natural conditions. An explanation can be found in seasonal and spatial variation in resource availability for honey bees in the tropical natural area of distribution, which can force honey bee colonies to migrate by flight over distances of more than 10 km and over 100 km over the course of a week (Hepburn & Radloff, 1998; Otis et al., 1981; Schneider & McNally, 1992; Vijayan & Somanathan, 2023). It seems that SHBs would need to follow suit, in order to survive and has thus adapted the flight capacity to do so. Some evidence points in this direction, besides anecdotal reports. SHBs have been observed absconding with and recaptured from within absconding honey bee colonies (Ellis et al., 2003), although observations of migration in accordance with host colonies has not been studied.

Physiological aspects of SHB dispersal

Although host migration offers an explanation for the flight capacity of the SHB, it does not explain why in **Chapter 3**, some individuals seemingly ignore close-by hosts and end up in colonies over 10 km away from the release point. A possible explanation could be variation in dispersal in relation to the physiological development of individual SHBs. For instance, unmated, recently emerged dispersing individuals could initially be unreceptive to host cues and fly outward over several kilometres, before becoming responsive to chemical stimuli. This has been shown for many insect species with bark beetles as a well-studied example (Byers, 2004; Meurisse & Pawson, 2017). This would favour outward spread. It is currently unknown if this is the case in SHB. From the perspective of host migration, it seems more likely that mature, mated and reproductive SHBs originating from the host colony migrate. In this case, dispersing SHBs have to shift strategies from reproduction to dispersal, which means a costly reallocation of energy resources (Candy et al., 1997; Chapman, 2012). This would at least be true for females, although I did not find any clear differences in flight distance between the sexes. However, females did use wind to navigate towards host colonies, while this was not the case for males. This could point to some level of sexual dimorphic dispersal in response to the host, which is in line with a recent study (Montag et al., 2022). David et al. (2015) showed that in the long-lived monomorphic beetle *Monochamus galloprovincialis* a lack of trade-off between reproduction and dispersal was evident and no negative effect of reproduction was observed on flight capacity. A similar situation may be the case in the SHB, which is long-lived, monomorphic and can be considered an income breeder. Moreover, as shown in **Chapter 3**, SHB is capable of long-range flights. This level of plasticity undermines any specific reasoning behind long-range dispersal in the presence of a host, in which case heterogeneity due to individual's response to its environment is likely to offer an explanation (Bonte & Dahirel, 2017). Intraspecific variation in dispersal behaviour is not uncommon in insects (Stevens et al., 2010). More research is needed to study the relationship between dispersal and individual traits of SHBs, for which recommendations for future research are made further on.

Genetics and SHB dispersal

As a heretic trait (Bonte & Dahirel, 2017), dispersal and flight are prone to be affected by the genetic background of SHB populations. The natural area of distribution is vast and although migration of honey bee subspecies of the host are well described, some subspecies do not migrate (Hepburn & Radloff, 1998). This condition would limit the need for dispersal of the SHB, which could potentially result in reduced flight capacity over time. Furthermore, invasive populations are mostly the result of single introductions from specific locations in the natural range, which show genetically distinct characteristics (Bai et al., 2022). Hence, the dispersal capacity could vary between invasive populations as well, which can have consequences for the spread of SHB as an invasive species (Tobin & Robinet, 2022).

Host and conspecific cues

No effect of conspecifics could be found on free-flying SHBs in the presence of honey bee colonies (**Chapter 4**). SHBs are able to detect conspecifics and much attention has been given to aggregation pheromones and tactile behaviour in relation to reproduction. Laboratory studies show that the attraction of SHBs to aggregation pheromones is age-dependent and that SHBs use chemical and tactile cues in relation to mating (Mustafa et al., 2015). In the absence of a colony, the presence of SHBs did attract more SHBs than the presence of only a food source. This does indicate that SHBs are able to detect conspecific cues. However, this effect was not shown in the presence of a colony, the stimuli of which probably overrule the odour emitted by the SHBs (Papach et al., 2021). It is likely that SHBs use conspecific cues within the colony, but mostly use host odours for orientation outside of colonies. In **Chapter 4**, I showed that the majority of SHBs that were released inside of colonies were found to remain there, irrespective of colony strength. This suggests that when SHBs have found a host, they are most likely to stay. It is unclear what could trigger dispersal in such a case, but it is likely that resource depletion is an important factor, similar to the situation which occurs when a host migrates or absconds in the natural range (Neumann et al., 2018).

Effects of weather and climate on dispersal

The results found in **Chapters 3 and 4** again underline the intricate relation of the SHB with honey bees as is clear from other studies on different life history traits (Lundie, 1940; Neumann et al., 2016). However, for dispersal an important factor lies outside of the host colony in the space and time where the SHB flies. Mobility of SHBs is greatly affected by temperature within a set range (**Chapter 3**). As an ectotherm, SHBs are not able to annul the effect of temperature once outside the host nest, which therefore can play a role in the spread of SHB as an invasive species. Moreover, seasonality sets the time window in which SHBs can disperse. This is of particular importance in temperate climates, but less so for (sub)tropical climates, although dry versus wet seasons affect other aspects of the SHB life cycle (Akinwande & Neumann, 2018). Detailed studies of temperature dependence of flight activity are needed to further understand how this affects SHB dispersal. Lastly, it should be noted that changes in climatic conditions as shown for other aspects of SHB ecology (**Chapter 6**) can alter impact and importance of SHB dispersal. For instance, the availability of alternative food sources in relation to climatic conditions could alter the dependency on honey bee colonies, possibly leading to changes in dispersal strategies (Arbogast et al., 2009).

Pupation as a measure for establishment

Whilst conditions for dispersal can partially be linked to the host, pupation is fully subject to other environmental conditions and in particular, edaphic conditions (Bernier et al., 2014; Ellis et al., 2004). Pupation is considered a crucial part of the life cycle with regard to survival and ultimately establishment in invasive populations (Bernier et al., 2014). As shown in **Chapter 6**, it acts as a precursor for the potential impact of SHB in the world. In **Chapter 5**, I showed that even in the absence of natural substrates, SHBs are able to pupate. In fact, the findings showed that pupation in greenhouse substrates was comparable to natural substrates in terms of development time and survival. This highlights a certain level of plasticity of the SHB in response to its environment. When fully grown, wandering SHB larvae leave host colonies in search of suitable substrates for pupation (Lundie, 1940). Theoretically, they can crawl a distance of over 1 km to find a suitable substrate (Stief et al., 2020), although this has not been shown under field conditions. The breadth of substrates suitable for pupation increases the possibilities of SHBs to pupate, and subsequently the chances of establishment during invasion. Furthermore, the relation with greenhouse conditions can allow for a disconnection from adverse climatic conditions that might prevail outside of the greenhouse environment. This could effectively extend the season and temporarily overrule the effect of the environment on SHBs. Similar events have been described for other invasive insects in the greenhouse environment (Cao et al., 2019; Messelink et al., 2021). Such an event is highly correlated to host presence, which in the case of greenhouses can be honey bee colonies, but bumble bees or stingless bees too. Although the effect could be significant in places with large greenhouse production areas such as the Netherlands, the effect on a global scale is probably limited.

Global and future impact based on pupation performance

This is in contrast to global climatic conditions as shown in **Chapter 6**. Based on pupation performance, I found that many areas in which the SHB is not yet found are suitable for SHB populations to become established. Although moisture and temperature effects limit SHB expansion and impact under current climate conditions, the future outlook suggest that even with conservative climate change scenarios, the area suitable for SHBs to survive will increase significantly. Due to the global scale of the study, the resolution is quite crude and locally other factors can come into play. In terms of climate and weather patterns, future predictions are difficult to make (Kyselý & Beranová, 2009). Moreover, biotic factors are similarly important and require further study. In particular, natural enemies should be considered, and as mentioned for dispersal, it is realistic to assume that the genetic background of invasive SHB populations could be relevant (Bai et al., 2022).

SHB dispersal and pupation in the context of invasion ecology

Although there is a clear framework for the process of biological invasion (Figure 1, Chapter 1), the context in which it occurs is subject to a vast variety of conditions (Lockwood et al., 2013), leading to many potential outcomes of the invasion process. Therefore, it is difficult to make general assumptions on how the study subjects of this thesis are applicable to SHB invasion in practice. However, it is worthwhile to portray the way in which the gained knowledge of dispersal and pupation is an essential element in the process of SHB invasion.

As an invader, SHBs can utilize dispersal by flight in several ways. First, newly introduced adult SHBs are likely to fly from a point of entry to a host colony. Secondly, from an incipient population, emerging adults can spread outward to invade new areas. Whilst normal colonies seem to be preferred over weak ones (depleted from adult workers), it is likely that SHBs opportunistically fly to the first colony they detect. Potentially this colony can be found at a distance of over 10 km from the point of introduction, however, there is a higher chance that colonies closest to the point of introduction are invaded. Moreover, once SHBs have invaded a suitable host colony, they are more likely to stay within a colony or possibly move to adjacent colonies when they are congregated, as in an apiary, than to disperse outward. Furthermore, it is unclear if there are differences in flight performance and dispersal between freshly emerged, unmated and unexperienced adults, compared with experienced adults in the reproductive phase of development. The former are mostly implicated when the incipient population is spreading outward, the latter upon introduction as well. Dispersal is, however, subject to environmental conditions, and as such low temperatures can prevent the onset of flight. It is therefore unlikely that SHBs will fly below 11°C as found in **Chapter 3.** It is not clear if there is an optimum flight temperature. Until established otherwise, flight performance within the temperature range is presumed to be relatively stable (Taylor, 1963). However, it should be taken into account that (sub)tropical climates favour outward spread of the SHB, due to more stable temperatures. Moreover, it is likely that due to the lack of seasonal limiting conditions and abundant food sources, these areas could harbour SHBs in low density populations in the absence of honey bees (Arbogast et al., 2009). Within the temperate climate range alternative hosts and food sources are available, however, no records exist of field observations, besides experimental ones, that have shown such interactions to take place. Based on the findings in **Chapter 4**, the presence of SHBs and the cues they emit only play a minor role in SHB dispersal. It is unlikely to affect outward spread in incipient populations, but it is also questionable that it will increase the likelihood of SHBs to find a host colony.

Within the invasion framework, pupation is of particular relevance to the establishment of SHB, though not exclusively. As there is evidence for the association of bee wax as a pathway for introduction (Idrissou et al., 2019), SHBs could invade in various life stages, including the larval stage. If wandering larvae find a suitable substrate for pupation, this could then surpass the contribution of adults in the invasion process and could potentially lead directly to the establishment of an incipient population. Nonetheless, the edaphic conditions suitable for pupation have to meet the SHB's criteria in particular with regard to temperature and moisture. Also, conditions in temperate climates might be suitable for pupation, but seasonality could prevent pupation from being completed, for instance because of a drop in soil temperature or inundation. This can greatly affect survival of SHB, but also its impact when the number of reproductive cycles is limited. Conversely, conditions in (sub)tropical climates could support up to 6 generations per year and there the impact could therefore be much higher. The outlook with future global warming in mind has some uncertainty, but it is expected that the impact of SHB as an invasive alien species will increase due to increased pupal performance.

Bringing the knowledge into practice

Beside contributing to our general understanding of SHB ecology, the results can be used to improve awareness and policy making decisions on how to deal with SHB invasions. In **Chapter 2,** a plan is described on how to effectively slow down the spread of SHB as an invasive species. The research in this thesis has contributed to several aspects of this plan as well as providing partial answers to the knowledge gaps that were identified. Early detection is identified as a key element in dealing with SHB introductions. In order to achieve this, it is recommended to use sentinel colonies to monitor SHB. This entails the use of honey bee colonies around high risk areas prone to introductions, such as international ports or honey and wax processing facilities, to 'catch' SHBs that arrive. These colonies are then subject to visual inspection at regular intervals. Based on the research in this thesis, strong colonies are preferred over weakened ones and since SHBs tend to stay in the colony they enter, intervals between inspections do not need to be less than a week in between. Furthermore, it seems unlikely that SHB-derived cues will enhance the chances of interception. The closer colonies are placed to the potential source of entry, the higher the chance of catching SHBs. Also, the higher the density of colonies, the higher the chance of catching SHBs. It should be noted that this could increase the work load, which in case of visual inspection could undermine the success of monitoring efforts. This is due to the relatively low success rate of visual inspection (Cornelissen & Neumann, 2018). However, novel approaches are being developed. In particular, the use of eDNA to detect traces of SHBs in honey bee colonies is now tested and shows promise for accurate, fast and low cost screening (van Gent-Pelzer & Cornelissen, 2021). Given the fact that SHBs are capable of long distance flights, other options could incorporate stakeholder involvement, in which SHBs are screened in honey bee colonies of local beekeepers. This, however, has several downsides, which undermine successful monitoring. Engagement of beekeepers should be strong, but is in practice often undermined by a lack of incentive. However, it is worthwhile to investigate this option based on a SWOT-analysis including the financial consequences of several scenarios based on the above. Additionally, the development of an effective trap should be considered, although it would only prove an alternative if it outperforms honey bee colonies as the preferred option for introduced SHBs. Traps have been shown to attract SHBs in field experiments (Arbogast et al., 2007), but the lack of data in conditions relevant to introduction scenarios (e.g., low number of individuals) make these unsuitable with the state-of-the-art in mind. Furthermore, it should be pointed out that feral colonies are generally not subject to monitoring routines and that these can harbour incipient SHB populations. Moreover, it is unclear to what extent alternative resources are of importance for introductions of SHBs. It might well be that incipient populations develop in wax processing plants. Personal observations of SHB larvae on crude wax residues in the United States lead me to believe that this is a feasible option (Figure 1).

The knowledge gained on dispersal is also relevant to contingency plans with regard to outbreaks. An outbreak in this case can be considered a veterinary analogy of an introduction or established incipient population. Current plans involve travel restrictions and visual inspection of apiaries in a fixed radius around an outbreak. Given the flight range of SHBs, it should be considered that measures are needed for an area with a radius of at least 10 km around the outbreak. It is important to note that improvement of our understanding of the effect of temperature on SHB mobility, would increase an accurate account of possible scenarios and could be used to make science-based adjustments to the scope of a contingency zone.

In terms of spread of SHB, more attention should be given to use insights gained **in Chapter 6**, to attribute the proper degree of impact of SHB and the potential growth of populations over time in relation to the local climatic conditions. From field observations and experimental data (Spiewok et al., 2007) it is apparent that in temperate climates, SHB population growth is slow, compared to subtropical or tropical climates. Realistically though, current contingency plans should incorporate scenarios to anticipate possible climatic changes in the future. In most scenarios, this leads to the conclusion that the impact of SHB will increase. Moreover, one should consider the possible impact not only from the perspective of the honey bee, which is an important native pollinator in Europe, but also a commercially relevant one in many areas in the world, but also the potential impact on other pollinator species, including but not exclusively *Apis* species in Asia, and bumble bees (**Chapter 2**).

An important element in combatting SHB as an invasive species involves awareness of stakeholders (**Chapter 2**). Beekeepers, but also veterinarians involved in policy making and application should be made aware of the potential impact of SHB on beekeeping, honey bees and other possible host species. The findings in this thesis underline the relationship between SHBs and honey bees and as such, beekeepers have a crucial part to play. Most importantly, beekeepers should be aware of the role they play in containing or preventing introductions and the spread of SHB. This means beekeepers can do two things, which actually are true for many other honey bee-associated invasive species.

1. Keep beekeeping local. Do not move colonies around and do not import queens, colonies and materials, period.
2. Know how to identify SHBs and what they are capable of, including dispersal and pupation as essential aspects of the lives of these beetles.

Recommendations for future research

The challenge is now to build upon the results obtained in this thesis. To start with aspects of dispersal, a deeper understanding of SHB dispersal is warranted. While the results in this thesis improve our understanding of SHB dispersal, no clear understanding of the mechanisms which drive individuals was gained. An additional approach could be performing tethered flight studies (Jones et al., 2015; Robinet et al., 2019) in which variables can be applied to study their effect on SHB flight performance. Preliminary investigations (Cornelissen, unpublished data) show that using tethered flight to study SHBs is a feasible option. Additionally, laboratory assays could be developed to study aspects of dispersal and flight in detail.

Since SHBs can be reared individually (Neumann et al., 2013), the life history of SHBs, for instance the feeding habits of larvae or mating, can be manipulated and controlled. In combination with variable conditions to be set during flight performance or dispersal experiments, this leads to a multitude of research opportunities. However, it seems wise to first and foremost explore the following.

- Effect of temperature on dispersal and flight performance.
  This can be achieved in several ways, of which two are highlighted here. First, cage experiments using SHBs of known origin can be performed under different temperature regimes to explore an accurate threshold for the onset of flight. Secondly, tethered flight experiments could be used to investigate flight performance (e.g., duration, distance and speed) under various temperature conditions. Additional variables to consider are sex, genotype, phenotype (e.g. weight and size of fat and carbohydrate stores).
- Effect of life history of the SHB on flight performance.
  It seems prudent to develop a better understanding of the effect of life history on SHB flight and dispersal using tethered flight. It is questionable whether or not for instance SHBs which actively reproduce, are capable of flight and how their performance compares to SHBs that have not yet reproduced. Similar questions can be asked about the effect of mating status. Also, such an experiment could shed light on the level of plasticity of individual SHBs.
  One should consider sex, phenotype and genotype and nutrient stores as important variables. Additionally, egg production as a measure of reproduction both prior to and after flight could be taken into consideration as additional variables.
- Furthermore, recent studies have combined genetic studies with flight mill experiments (Jones et al., 2015; Renault et al., 2018). It seems feasible therefore to further investigate genetic diversity between and within native and invasive populations in relation to flight performance. This could help understand if dispersal is subject to selective pressure either due to host life history or genetic carry-over effects of invasion. Possibly, some invasive populations are less likely to spread, based on their genetic background.

Whilst experimental studies are important for a deeper understanding of dispersal and flight, one should consider knowledge gaps related to the natural history of SHBs as well. In particular, it seems important to further investigate dispersal in relation to the natural host, the honey bees of sub-Saharan Africa. Although migration of honey bees has been well studied (Schneider & McNally, 1992; Vijayan & Somanathan, 2023), there is limited knowledge available on how this affects associated species such as the SHB (Neumann et al., 2018). Field studies in feral populations could shed light on this matter. Marking techniques developed and used in this thesis (in particular scar-marking) could be applied to track the whereabouts of SHBs in connection with their hosts.

Many studies have been done to understand pupation of the SHB and it seems that there are only a limited number of questions spilling over from **Chapters 5 and 6.** However, some attention to substrate composition is warranted. During experiments in South Africa, it was observed that while burrowing into the ground, wandering larvae were unable to enter dry sandy soils as the funnels these larvae created repeatedly collapsed. This suggests that even if subsoil conditions could sustain SHB pupation, topsoil condition might prevent them from reaching it. Most studies on SHB pupation have focussed on edaphic conditions for pupation itself such as soil type, moisture and temperature, however, little is known about other factors related to soil structure. Furthermore, during pupation, SHBs are likely exposed to many naturally present soil dwellers, which could act as natural enemies. So far, no studies have been published to show the relative contribution of predation and pathogens on SHB pupae under natural conditions.

Lastly, as the SHB inevitably will expand its range beyond its current distribution, new areas are invaded, which add information and data on the existing impact and expansion models at hand. Such is the case with the model presented in **Chapter 6.** This of course is also relevant in relation to climatic changes. The current consensus in fact is that the scenarios used in **Chapter 6** could be considered conservative by today's standard. Additionally, a further exploration of explanatory modelling of the impact of the SHB, could include dispersal as a factor. This would integrate the results of this thesis into such modelling exercises.

Final words

With this thesis I have answered pivotal questions regarding SHB ecology, relevant not only for natural history, but also and more importantly for our understanding of the SHB as an invasive species. We now know more about the dispersal of the SHB by flight and the impact it can have as an invader, through successful pupation and ultimately establishment. This thesis therefore contributes to the design of containment and mitigation strategies of the SHB as an invader, a fate which will continue to befall the world.

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Summary

A biological invasion is an event that occurs when a species crosses the natural borders of its biogeographical distribution. The rate of biological invasion has increased exponentially over the past decades, caused by increased global trade and transport. When species invade new geographic regions, they can cause detrimental effects to nature and society and such species are referred to as invasive alien species. The process of biological invasion entails a set of stages, from transport, to survival of an incipient population, to spread. Other species can facilitate the establishment of invasive ones. This is the case with the Western honey bee (*Apis mellifera* L. subspecies), which is a host for several invasive parasites. Some of these parasites are completely dependent on their host for their survival. Others however, need to venture outside of the colony to complete their life cycle.

The small hive beetle (SHB; *Aethina tumida* Murray; Coleoptera: Nitidulidae) parasitizes honey bees by residing in colonies where the beetle larvae feed on hive constituents. Pupation does not take place in the hive, but in soil substrates. Adult SHBs spend time outside of a host colony when they emerge from the soil. They will then locate a host colony using chemical cues, and in most cases use flight to reach a host. Dispersal can also take place from one host colony to another. The temporary detachment from the host creates a vulnerable condition for the SHB as they are exposed to unfavourable environmental conditions, such as cold, and predation. Pupation and dispersal are also key elements in the process of biological invasion of the SHB. Pupation is required for establishing an incipient population and dispersal is initiated upon arrival after transport or when an incipient population is spreading naturally. A better understanding of dispersal and pupation is crucial for understanding the invasiveness of the SHB. In this thesis, I aim at increasing our knowledge of the SHBs' flight capacity and of the environmental factors influencing its dispersal. A further exploration is aimed at understanding the role of edaphic factors in the establishment and impact of the SHB as an invasive alien species under different and changing environmental conditions. This constitutes basic biology, but also lessons for applying better policy for preventive and mitigative measures when biological invasions of the SHB occur.

Chapter 2 offers a concise science-based policy framework for the biological invasion of the SHB. This framework extends from preventive actions to avoid introductions, to mitigative measures needed to limit the spread of the SHB invasions. A first line of defence includes import inspections, raising stakeholder awareness, increased stakeholder education and monitoring by using sentinel apiaries. This will increase the likelihood of early detection, but also increase the chance of eradication and containment of outbreaks during later stages of invasion if introductions cannot be prevented. The need for increased knowledge of basic biology, relevant for the SHB as an invasive alien species is identified. This includes a better understanding of host finding mechanisms.

An important question is how effective the use of sentinel apiaries is for monitoring SHB introductions and spread. An underlying mechanism is the dispersal capacity of the SHB by flight. First, a mark-release-recapture experiment was conducted (**Chapter 3**) in which laboratory-reared, dye-marked SHBs were released from a central point. Honey bee colonies were used to attract flying small hive beetles at fixed spatial intervals from the central release point. SHBs were recaptured at a maximum distance of 3.2 km after 24 hours and 12 km after one week. Most SHBs were collected closest to the release point at 0 m (76%, replicate A) and 50 m (52%, replicates B to F) suggesting that the SHB prefers nearby host colonies, thereby limiting flight investment. Temperature and wind deviation had significant effects on dispersal, with more SHBs being recaptured when temperatures were high and confirming the role of wind for odour-modulated dispersal of flying insects and the SHB in particular.

As honey bee colonies vary in size, so could their attractiveness to SHBs. This is relevant for monitoring strategies aimed at the SHB. In a similar fashion, aggregation pheromones are used by SHB to detect conspecifics inside a colony. Cues from both hosts and conspecifics could play a role in the spread and dispersal behaviour of the SHB as an invasive species. Therefore, an experiment was performed to assess the relative importance of host colony strength, conspecifics and cues from alternative food on dispersal (**Chapter 4**). SHBs were released in an apiary consisting of identical hives with either unmanipulated or weakened honey bee colonies or alternative food (ripe bananas). Prior to release, marked SHBs were introduced into half of the colonies and banana boxes to *a priori* provide conspecific cues. The data show that significantly more SHBs were found in unmanipulated colonies compared to weakened ones. No significant effect was observed for conspecific cues, except for alternative food which had more incoming SHBs when conspecifics were present. The number of SHBs leaving a colony was not significant different between unmanipulated and weakened colonies. The majority of SHBs remained in the colonies into which they had been released. The results show SHB dispersal is governed by host colony cues rather than conspecific ones with strong colonies being preferred. Alternative food sources were not attractive. SHBs appear to show host colony fidelity despite their flight ability.

A crucial factor for the establishment of invasive populations of the SHB is the ability to complete its life cycle. Pupation can act as a constraining factor as it occurs in soil substrates, where edaphic conditions can differ in invaded areas compared to the native range. In most cases though, it offers an opportunity and the plasticity of SHB to utilize pupation substrates is shown in **Chapter 5**. In a laboratory experiment, wandering SHB larvae were introduced into containers with coconut fiber, perlite, a mixture of both and stone wool substrates to investigate pupation success and development time. Sand was used as control substrate. In all but one substrate (perlite), SHBs developed into adults equally well as they did in the sand. Development time ranged between 23 and 37 days and was not different from that of the control. We showed that SHBs can pupate in greenhouse substrates. This could constitute a problem for greenhouse pollination and it could facilitate SHB survival in areas which otherwise would be deemed unsuitable or marginal environments for the SHB to become established.

The small hive beetle has now established populations on all continents except Antarctica, but it is unclear what the impact will be. In **Chapter 6**, SHB invasion risk was investigated globally under current and future climate scenarios. Survival and development time of the pupal stage (= pupal performance) was modelled in response to soil temperature and soil moisture using published and novel experimental data and presence data on SHB distribution were used for model validation. This model was then linked with global soil data in order to classify areas (resolution: 10 arcmin; i.e. 18.6 km at the equator) as unsuitable, marginal and suitable for SHB pupation performance. Under current climate conditions, the results show that many areas globally yet uninvaded are actually suitable, suggesting considerable SHB invasion risk. Future scenarios of global warming project a vehement increase in climatic suitability for the SHB and corresponding potential for invasion, especially in the temperate regions of the Northern hemisphere, thereby creating demand for enhanced and adapted mitigation and management. The analysis shows for the first time effects of global warming on a honey bee pest and will help areas at risk to prepare adequately. In conclusion, this is a clear case of global warming promoting biological invasion of a pest species with severe potential to harm important pollinator species globally.

With this thesis, two important aspects of SHB biology, dispersal and pupation, have been investigated in light of the recent expansion of this beetle's territory across biogeographic boundaries. The results increase our understanding of SHB biology, and can be used to improve mitigation and prevention policies to deal with this invasive pest now, and in the future.

Future research should focus on a further understanding of dispersal behaviour by focussing on environmental factors, but also on genetics and life history. Although pupation is well understood, improvement of impact models can be realized by adding other factors relevant for impact assessment of SHB invasion.

Samenvatting

Een biologische invasie is een gebeurtenis die plaatsvindt wanneer een soort de natuurlijke grenzen van zijn biogeografische verspreiding overschrijdt. Het aantal biologische invasies is de afgelopen decennia als gevolg van wereldwijde handel en transport exponentieel toegenomen. Als soorten nieuwe geografische regio's bereiken, kunnen ze schadelijke gevolgen hebben voor de natuur en de samenleving. Deze soorten worden aangeduid als invasieve exoten. Het proces van biologische invasie omvat een aantal stadia, van transport tot overleving van een beginnende populatie tot verspreiding. Andere soorten kunnen de vestiging van invasieve soorten vergemakkelijken. De westerse honingbij (*Apis mellifera* L.; Hymenoptera: Apidae) is zo'n soort omdat deze als gastheer dient voor verschillende invasieve parasieten. Sommige van deze parasieten zijn voor hun overleving volledig afhankelijk van hun gastheer. Anderen moeten zich echter buiten de kolonie wagen om hun levenscyclus te voltooien, zoals de kleine bijenkastkever (KBK; *Aethina tumida* Murray; Coleoptera: Nitidulidae).

De KBK komt van nature voor in Afrika, bezuiden de Sahara, maar wordt op alle continenten, behalve Antarctica als invasieve exoot aangetroffen. De KBK verblijft in bijenvolken en de keverlarven voeden zich met honing, broed en stuifmeel. De verpopping vindt echter niet plaats in het bijenvolk, maar in bodemsubstraten en als de verpopping voltooid is, moeten de uitgekropen kevers op zoek naar een gastheervolk, dat ze lokaliseren door chemische signalen van het volk waar te nemen. In de meeste gevallen zullen ze zich zelfstandig verplaatsen door te vliegen. Verspreiding kan ook plaatsvinden van het ene gastheervolk naar het andere. Buiten het volk zijn KBKs kwetsbaar, omdat ze worden blootgesteld aan mogelijk ongunstige omgevingsfactoren, zoals koude en predatie. Verpopping en verspreiding zijn sleutelelementen in het proces van invasie van de KBK. Verpopping is noodzakelijk voor de vestiging van een beginnende populatie. Verspreiding is nodig na introductie via transport, als een gastheer gezocht moet worden en als de invasieve populatie groeit. Door meer te weten te komen over de verspreiding en verpopping van de KBK, is het mogelijk om de invasiviteit van de soort beter te begrijpen. In dit proefschrift richt ik me op het vergroten van de kennis over de vliegcapaciteit van de KBK en over de omgevingsfactoren die van invloed zijn op verspreiding. Daarnaast onderzoek ik de rol van bodemfactoren op de verpopping van de KBK en hoe verpopping de kans op de vestiging en de invasiviteit van de KBK, nu en in de toekomst, beïnvloedt. Deze studies vergroten de kennis over de biologie van de KBK. Bovendien kan deze kennis gebruikt worden om preventieve en mitigerende maatregelen te ontwikkelen, gericht op het indammen van uitbraken van de KBK.

In **Hoofdstuk 2** wordt een handelingsperspectief geboden dat voor de biologische invasie van de KBK wordt gebruikt. Dit perspectief is breed opgesteld met het oog op het implementeren van de juiste beleidsmaatregelen. Dit behelst de hele breedte van preventieve maatregelen die nodig zijn om een introductie te voorkomen tot mitigerende maatregelen om verspreiding van de KBK na vestiging te limiteren. De eerste lijn van verdediging bestaat onder meer uit import inspecties, het vergroten van het bewustzijn en het kennisniveau van belanghebbenden en monitoring door het gebruik van bijenvolken. Dit vergroot de kans op vroege detectie, maar ook de kans op het uitroeien en beheersen van uitbraken als een introductie reeds eerder heeft plaatsgevonden. De behoefte aan basiskennis over de biologie van de KBK als invasieve exoot en kennishiaten worden in het hoofdstuk geïdentificeerd. Dit betreft onder andere het gebrek aan kennis over de gastheerherkenning en verspreiding van de KBK. Een belangrijke vraag is hoe effectief het gebruik van bijenvolken is ten behoeve van het monitoren op de KBK. Dit is relevant voor het monitoren van de verspreiding na transport en om de verspreiding vanuit een beginnende invasieve populatie te volgen. Een onderliggend mechanisme is de verspreidingscapaciteit van de KBK door middel van vliegen. Het is bekend dat KBKs goed kunnen vliegen, maar over welke afstand, en welke factoren daarbij een rol spelen is grotendeels onbekend. Om dit te onderzoeken zijn twee experimenten uitgevoerd. Ten eerste is een zogenaamd 'mark-release-recapture' experiment uitgevoerd (**Hoofdstuk 3**). Gekweekte KBKs werden gemarkeerd met een kleurstof en vervolgens op een centraal loslaatpunt vrijgelaten. Bijenvolken werden gebruikt om de KBKs aan te trekken en terug te vangen op vaste afstanden van het loslaatpunt. KBKs werden na 24 uur op een maximale afstand van 3,2 km gevangen. Twee kevers werden een week na het loslaten op 12 kilometer afstand aangetroffen. De meeste gevangen KBKs werden op korte afstand van het loslaatpunt aangetroffen. Bij de eerste herhaling (replicate A, 0 meter van het loslaatpunt) betrof dit 76% van de gevangen KBKs en voor de overige herhalingen (replicates B – F, 50 meter van het loslaatpunt) was dit 52% van de gevangen KBKs. Dit resultaat suggereert dat de KBK een voorkeur heeft voor een dichtbij zijnde gastheer, om zo de investering die nodig is om te vliegen te minimaliseren. Temperatuur en de windrichting hadden een significant effect op verspreiding. Er werden meer KBKs gevangen bij warme weesomstandigheden in vergelijking met koudere omstandigheden en de rol van wind bij geur-gemoduleerde verspreiding van vliegende insecten en de KBK in het bijzonder werd bevestigd.

De variatie in grootte van bijenvolken kan leiden tot een verschil in de aantrekkingskracht op KBKs. Dit is relevant voor monitoringsstrategieën gericht op het opsporen van KBKs met bijenvolken. Daarnaast gebruikt de KBK aggregatieferomonen om soortgenoten te vinden in bijenvolken. Signalen van de gastheer en van soortgenoten kunnen een rol spelen bij de verspreiding van de KBK als invasieve exoot. Om dit verder te onderzoeken, werd een experiment uitgevoerd om het relatieve belang van de sterkte van het gastheervolk, soorteigen signalen en signalen van alternatieve voedselbronnen op de verspreiding van de KBK te onderzoeken (**Hoofdstuk 4**). KBKs werden losgelaten op een bijenstand met identieke bijenkasten waar normale en verzwakte volken, en een alternatieve voedselbron in de vorm van een rijpe banaan werd geplaatst. Voordat kevers werden losgelaten op de bijenstand lieten we in de helft van de volken en kasten met bananen kevers inlopen om *a priori* soorteigen signaalstoffen toe te voegen. De resultaten wezen op een significant effect van de volksgrootte waarbij normale volken meer KBKs van buitenaf aantrokken dan verzwakte volken. Er werd geen effect aangetoond van soorteigen signaalstoffen op het aantrekken van KBKs, behalve in de vergelijking tussen rijpe bananen zonder en rijpe bananen met kevers. Het aantal KBKs dat een volk verliet verschilde niet tussen normale en verzwakte gastheervolken. Bovendien bleef de meerderheid van de KBKs in de volken waarin ze werden losgelaten. De resultaten laten zien dat met name de signalen van de gastheer van belang zijn bij de verspreiding van de KBK en dat soorteigen signaalstoffen buiten het volk geen rol spelen.

Alternatieve voedselbronnen zijn niet aantrekkelijk voor zich verspreidende KBKs en als KBKs eenmaal een volk zijn binnengetreden, is de kans groot dat ze daar ook blijven.

De vestiging van een invasieve populatie kan alleen plaatsvinden als de KBK de levenscyclus kan voltooien. Verpopping vindt plaats in de bodem en het is in deze fase dat de KBK is blootgesteld aan omstandigheden die mogelijk afwijken van de situatie in het natuurlijke verspreidingsgebied. Bodemfactoren zoals temperatuur en vocht kunnen de overleving van poppen negatief beïnvloeden. In veel gevallen is dit echter geen belemmering en door de hoge mate van plasticiteit van de KBK ten aanzien van zijn omgeving kan de KBK op vele plekken overleven. Dit blijkt ook uit het onderzoek dat in **Hoofdstuk 5** is beschreven.

In een laboratorium opstelling werd de verpopping van de KBK onderzocht in substraten die in de glastuinbouw worden gebruikt. De overleving van poppen en de duur van de verpopping in kokosvezels, perliet, een mix van deze twee substraten en steenwol werd onderzocht. Als controle substraat werd zand gebruikt. In alle substraten behalve in perliet ontwikkelden de KBKs zich tot volwassen kevers in dezelfde mate als in het controle substraat zand. De ontwikkelingsduur varieerde van 23 tot 37 dagen en was niet verschillend ten opzichte van de ontwikkelingsduur in zand. Dit experiment laat zien dat KBKs in deze substraten kunnen verpoppen. Dit kan mogelijk een probleem vormen voor de bestuiving in de glastuinbouw en maakt het mogelijk dat de KBK kan overleven op plekken die normaal gesproken niet geschikt zijn voor het voltooien van de levenscyclus.

Op dit moment zijn er gevestigde invasieve populaties van de KBK op alle continenten, behalve Antarctica, maar het is onduidelijk welke gevolgen dit heeft. In **Hoofdstuk 6** is onderzocht wat het risico is van de wereldwijde invasie van de KBK in relatie tot het huidige en toekomstige klimaat. De overleving en ontwikkelingstijd van het popstadium als maat voor de verpopping, werd gemodelleerd als respons op de bodemtemperatuur en -vocht. Hiervoor werden zowel gepubliceerde gegevens alsook nieuwe data van eigen experimenten gebruikt. Daarnaast werden beschikbare en geverifieerde waarnemings-data gebruikt om het model te valideren. Dit model werd vervolgens gekoppeld aan bodemdata. Dit maakte het mogelijk om op wereldschaal gebieden te classificeren als ongeschikt, marginaal of geschikt voor de verpopping van de KBK. De resultaten laten zien dat bij de huidige heersende klimatologische omstandigheden, grote gebieden aan te wijzen zijn als geschikt voor de verpopping en dus de vestiging van de KBK. Bij een toekomstige toename van de temperatuur wereldwijd, is de verwachting dat er een grote toename is van het voor de KBK geschikte leefgebied, met name op het noordelijk halfrond. Dit toekomstbeeld schept de verwachting dat er meer aandacht voor mitigatie en beheersing van invasieve populaties nodig zal zijn. Deze analyse laat voor het eerst een effect zien van de opwarming van de aarde op een plaag van de honingbij en maakt het mogelijk om risicogevoelige gebieden beter voor te bereiden op de komst van de KBK.

Met dit proefschrift zijn twee aspecten van de biologie van de KBK onderzocht die belangrijke factoren zijn bij de verspreiding van deze kever als invasieve exoot. De resultaten dragen bij aan de kennis over de biologie van de KBK en kunnen gebruikt worden voor de verbetering van het beleid om deze invasieve exoot nu en in de toekomst te kunnen aanpakken. Toekomstig onderzoek zou gericht moeten zijn op een verdere verdieping van inzicht in het verspreidingsgedrag in relatie tot omgevingsfactoren, maar ook de genetische achtergrond en biologie van de KBK.

Hoewel de verpopping van de KBK goed is onderzocht, kunnen modellen verbeterd worden door het toevoegen van relevante factoren. Dit kan leiden tot een beter inzicht in de gevolgen van de KBK als invasieve exoot.

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