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USE OF HABITAT ODOUR BY HOST-SEEKING INSECTS

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ABSTRACT

Locating suitable feeding or oviposition sites is essential for insect survival. Understanding how insects achieve this is crucial, not only for understanding ecology and evolution of insect-host interactions, but also for the development of sustainable pest control strategies that exploit insects' host-seeking behaviours. Volatile chemical cues are used by foraging insects to locate and recognise potential hosts but in nature these resources usually are patchily distributed, making chance encounters with host odour plumes rare over distances greater than 10s of meters. The majority of studies on insect host-seeking have focussed on short-range orientation to easily-detectable cues and it is only recently we have begun to understand how insects overcome this challenge. Recent advances show that insects from a wide range of feeding guilds make use of 'habitat cues', volatile chemical cues released over a relatively large area that indicate a locale where more specific host cues are most likely to be found. Habitat cues differ from host cues in that they tend to be released in larger quantities, are more easily detectable over longer distances, and may lack specificity, yet provide an effective way for insects to maximise their chances of subsequently encountering specific host cues. This review brings together recent advances in this area, discussing key examples and similarities in strategies used by haematophagous insects, soil-dwelling insects and insects that forage around plants. We also propose and provide evidence for a new theory that general and non-host plant volatiles can be used by foraging herbivores to locate patches of vegetation at a distance in the absence of more specific host-cues, explaining some of the many discrepancies between lab and field trials that attempt to make use of plant-derived repellents for controlling insect pests.

Key words: habitat cues, host location, olfaction, semiochemicals, insect-host interactions, insect behaviour.

CONTENTS

I. INTRODUCTION

Over the years considerable knowledge has accumulated on how insects use volatile chemical cues to locate and recognise their respective host species. These cues usually consist of specific-characteristic blends of volatile compounds or, in some cases, individual volatiles that are restricted to a narrow range of related host species (Bruce, Wadhams & Woodcock, 2005; Bruce & Pickett, 2011). These cues offer an effective means of locating a host at short range but, due to the physical properties of odour plumes, chance encounters with host odour at longer distances are rare. Volatiles emanating from an odour source in wind form an odour plume that meanders downwind. Molecular diffusion occurs at too small a scale to contribute significantly to plume structure and the distribution of odours within the plume's overall boundaries is mainly dictated by the forces of turbulence, which creates discrete filaments of relatively undiluted odour interspersed with clean air (Murlis, Elkinton & Cardé, 1992; Voskamp, Den Otter & Noorman, 1998; Koehl, 2006). Many insects are adept at following these plumes (Murlis *et al.,* 1992; Cardé & Willis, 2008; Bau & Cardé, 2015). Voskamp et al. (1998) showed that tsetse flies detected odour plumes up to 10-20 m downwind of an odour source in an open field and up to 60 m in woodland. At longer distances, however, odourant concentration can fall below insect detection thresholds (Murlis, Willis & Cardé, 2000; Koehl, 2006) and, together with increased intermittency of plume encounter (Koehl, 2006; Beyaert & Hilker, 2014), this means that insects' abilities to use host-originating odour to locate a feeding or oviposition site becomes increasingly difficult. This presents an enormous challenge to host-seeking insects. In areas with high plant species diversity, suitable hosts for phytophagous insects may be patchily distributed (Randlkofer *et al.*, 2010) and insects may not come close enough to detect odour plumes using random foraging movements alone, particularly if hosts are hidden within dense patches of non-host vegetation that may obstruct or adsorb odour (Beyaert & Hilker, 2014). Haematophagous insects face a similar problem since their animal hosts may occupy large home ranges with distances of up to many kilometres between individuals or groups of individuals (Potts & Lewis, 2014).

Where host odour cues are difficult to locate, the use of 'habitat odour cues' provides insects with a means of increasing their foraging success. As opposed to 'host cues' in the traditional sense, which are used to locate specific feeding or oviposition sites, habitat cues indicate a general area where such sites or associated cues are *most likely* to be found (Bell, 1990; Meiners, 2015). From a behavioural ecology perspective, habitat odour may be many things. Habitat odour may comprise the collective volatile emissions of all organisms inhabiting a potential foraging patch. Many of the volatile compounds that insects can detect are found ubiquitously across the plant or animal kingdoms, produced by host and non-host alike. For example, green leaf volatiles and other ubiquitous plant volatiles can indicate the presence of a patch of vegetation in a heterogeneous landscape. Respiratory CO_2 emissions produced by plant roots or animals can provide information on the presence of a patch of vegetation to below-ground feeding herbivores or a group of animals to blood-feeding insects. Alternatively, habitat odour may be associated with the collective secretions or excretions of host organisms, for example odours associated with dwellings or nests of animals that are hosts to blood-feeding insects. Since they tend to be produced over larger areas or by many different organisms within a habitat, habitat odour is generally emitted in greater quantities and detectable at greater distances than host odour, providing insects with information on a location to search for more host-specific volatile cues. The main ways in which habitat and host odour cues differ are summarised in Table 1.

Habitat cues may serve to increase foraging efficiency in a number of ways (Fig. 1). Insects may fly upwind in response to a habitat-odour plume before engaging in hierarchical plume switching (Beyaert& Hilker, 2014), abandoning the former long-range cue in favour of following the host plume to its source. Alternatively, habitat cues present as background odour may induce non-directional localised searching behaviours, for example through increases in rates of turning or changes in speed of movement in order to increase the probability of intercepting a host plume, after which movement upwind toward the host can occur. In these first two scenarios, habitat and host odour are encountered sequentially and encounter with host odour must override any behavioural response to the habitat odour cue. Habitat cues may also act in conjunction with host cues, reinforcing behavioural responses when detected by insects at the same time by providing important contextual information. Here, detection of habitat odour may sensitise insects to host volatiles, enhancing their responsiveness to these cues (Dekker, Geier & Cardé, 2005; Schröder & Hilker, 2008). This sensitization may work in conjunction with upwind flight and localised search behaviours in response to habitat cues (Fig 1a & b), ensuring that insects respond strongly to host cues once they are encountered.

Since habitat odour is generally released over a relatively large area and in large quantities, foraging insects will find themselves exposed to habitat odour cues for lengthy periods of time. Constant exposure to these background odourants can eventually result in olfactory adaptation and habituation (Schröder & Hilker, 2008), meaning insects may become less responsive to habitat cues over time. This may serve to allow insects to 'give up' on a resource patch after failing to locate any host cues within it. Alternatively, constant exposure to habitat odour could lead to sensitisation, reinforcing the behavioural response over time.

II. USE OF HABITAT CUES BY FORAGING INSECTS

The majority of studies on insect host location have focussed on easily detectable, short-range cues originating from the host and it is only recently that evidence of habitat cue use has emerged. Their use now appears to be exceptionally widespread, employed by insects from a diverse range of feeding guilds including soil-dwelling insects, haematophagous insects, predatory and parasitic insects, above-ground herbivores, and pollinators. The seemingly widespread use of these cues suggests they are a fundamental component of insect host location. Key examples from each of these insect feeding guilds are described below.

(1) Soil-dwelling insects

Herbivorous insects that dwell within the soil make use of exudates from roots to locate suitable feeding sites (Johnson & Gregory, 2006a; Johnson & Nielsen, 2012) and these often confer species-specific information on host identity (Soni & Finch, 1979; Rogers & Evans, 2013a). Respiratory CO_2 emissions have also been shown to elicit behavioural responses from a range of root-feeding insects, suggesting a role in host location (Johnson *et al.*, 2012).

Carbon dioxide is generally produced in much higher quantities than other root exudates and diffuses relatively rapidly through the soil (Payne & Gregory, 1988), making it detectable at greater distances (Johnson & Nielsen., 2012). Carbon dioxide emission from roots is ubiquitous, produced by all respiring tissue, and also exhibits strong vertical gradients between the upper soil and air and, in areas of high root density, horizontal concentration gradients may not always be perceptible to soil-dwelling insects (Johnson *et al.*, 2006b). This led Johnson and Gregory (2006a) to question the role of CO_2 in host location, particularly for specialist root herbivores for which CO_2 is unlikely to confer sufficient information. Instead, they proposed that CO_2 serves to inform as to the presence of a nearby patch of plants where more specific root exudates may subsequently be searched for, thus functioning as a 'search trigger' rather than a host cue. This hypothesis was supported by behavioural studies on the larvae of the root-feeding clover weevil, *Sitona lepidus* (Johnson *et al.*, 2006b). In behavioural experiments, no evidence of oriented movement towards point emissions of CO_2 was observed, regardless of emission rates. In the presence of constant CO_2 emissions, however, larvae made more tortuous and intensive searching movements compared to CO_2-free experiments, allowing insects to increase their chances of intercepting other root-derived chemical cues. Similar effects were observed for larvae of the wheat bulb fly, *Delia coarctata*, which displayed increased rates of turning and track length in elevated CO_2 but did not orientate toward point emissions in arena-based behavioural experiments (Rogers *et al.*, 2013b).

Reinecke *et al.* (2008) showed that exudates from undamaged roots of dandelion inhibited behavioural responses of the European cockchafer (*Melolontha melolontha*) to CO_2. This was interpreted as a possible plant defence strategy, with exudates serving to mask the attractiveness of the long range cue. An alternative hypothesis is that these exudates may be used by host-seeking larvae to switch off responses to CO_2 when close enough to a plant patch to be able to make use of more specific host cues or localised searching behaviours. Once host root exudates reach sufficient concentrations for inhibition of CO_2 detection, they are presumably also in sufficient concentrations to be used for host orientation, making CO_2 a redundant and, due to large horizontal gradients in CO_2 concentration close to dense plant patches, potentially disruptive signal at short range (Johnson & Gregory, 2006a). These two hypotheses are not mutually exclusive but further work is needed to validate either, preferably involving realistic plant densities that would be encountered by root-feeding larvae in field conditions. The use of host-originating CO_2 inhibitors may be a widespread phenomenon and future studies may uncover their use by other soil-dwelling insects.

(2) Haematophagous insects

Like root-feeding herbivores, haematophagous insects make use of a combination of CO_2 and more specific host volatiles, as well as heat, to locate a feeding site. The malaria mosquito, *Anopheles gambiae*, prefers to feed on human hosts and readily responds to human body odour in a wind tunnel by flying upwind but tends not to land on the source of emission unless it is heated (Spitzen *et al.*, 2013). We recently found that, when placed in a screen cage containing a source of human odour female *An. gambiae* did not land on the human odour source unless CO_2 was delivered through the side of the cage, instead preferring to rest on the walls and ceiling in the absence of CO_2 (Webster, Lacey & Cardé, 2015). Similar observations were made for the yellow fever mosquito, *Aedes aegypti,* which was found not to feed through a membrane when presented with human odour alone but nevertheless flew upwind upon detection of human odourants (Lacey, Ray & Cardé, 2014; McMeniman *et al.*, 2014). Anthropophilic mosquitoes such as *An. gambiae* search for blood meals in and around human dwellings. In our study we suggested that human odour, in the absence of carbon dioxide or heat, serves as a means for mosquitoes to locate a human dwelling since these continuously emit human odour even when its occupants are absent (Webster *et al.*, 2015).

On its own, human odour therefore likely serves as a habitat cue for these species, inducing location of and arrestment within a human dwelling, an ideal habitat within which to subsequently search for a blood meal. Once CO_2 or heat indicates a human is present, human odour acts together with these other cues to function as a host cue. This is an example of how the same volatile compounds can function as both habitat and host cue depending upon the context in which they are detected. Similar habitat cues are also used by mosquitoes feeding on non-human animals. The southern house mosquito, *Culex quinquefasciatus*, preferentially obtains blood meals from birds and is attracted by the odour of fresh chicken faeces, an effective cue indicating a physical location recently occupied by potential hosts (Cooperband *et al.*, 2008). Behavioural responses of haematophagous insects to urine and faecal odour are widespread and these offer effective and sometimes host-characteristic cues indicating a nest or general area regularly inhabited by potential hosts (Becker *et al.*, 1995; Baldacchino *et al.*, 2013; Nordéus *et al.*, 2014).

Exhaled CO_2 is generally considered a host cue for haematophagous insects (Cardé & Gibson, 2010) but recent studies have led us to question this hypothesis. Exhaled CO_2 offers little information regarding host species but is detectable over large distances (Zöllner *et al.*, 2004; Lorenz *et al.*, 2013) and, since many haematophagous insects tend to prefer social animals as hosts (Lehane, 2005), combined exhalations of a group of animals offers an effective long-range cue indicating a general area inhabited by potential hosts. Whilst CO_2 elicits upwind flight and plume following, haematophagous insects tend not to orient toward or land on the source at close range (Spitzen, Smallegange & Takken, 2008), instead initiating rapid 'zigzag' flight behaviour in the general vicinity of the CO_2 source (Spitzen *et al.*, 2008; Lacey *et al.*, 2014). This suggests a switch to localised searching behaviour in order to make contact with more specific host cues indicating potential feeding sites. Carbon dioxide therefore seems to function more as a 'habitat' cue by indicating a general area occupied by potential hosts where more specific host cues may be subsequently located, similar to the model proposed by Johnson and Gregory (Johnson *et al.*, 2006a) used by root-feeding herbivores. Support for this hypothesis comes from that fact that at close range CO_2 is almost completely ignored in favour of skin odour by most haematophagous insects. *Aedes aegypti* readily flies upwind along a plume of CO_2 but, when presented with a human foot-odour plume in parallel, the CO_2 plume was completely ignored (Lacey *et al.*, 2014). Similar observations were made for *Cx. quinquefasciatus*, which also seemed to orient at long range to CO_2 but only used human odour at close range for landing (Lacey & Cardé, 2011).

As with root-feeding herbivores, volatiles that inhibit detection of CO_2 by haematophagous insects have recently been uncovered (Tauxe *et al.*, 2013). These compounds were identified using *in-silico* screening to predict chemical structures likely to interact with the CO_2 receptor (Boyle, McInally & Ray, 2013), providing a large range of compounds only a few of which have been tested and so the possible ecological function of these CO_2 inhibitors remains unclear for now. As with root-feeding herbivores, inhibition of long-range habitat cue detection may represent a defensive strategy by the host or, alternatively, a mechanism employed by the insect to facilitate switching from habitat cues to host cues at shorter ranges. Further work is needed to test either hypothesis and could lead to novel strategies for controlling these important public health pests.

(3) Predatory and parasitic insects

Among the most widely-recognised examples of use of habitat cues comes from predatory and parasitic insects. This topic has already been reviewed extensively (Vet & Dicke, 1992; Hare, 2011; Heil, 2014; Pierik, BallarÉ & Dicke, 2014; Hilker & Fatouros, 2015; Meiners, 2015) and so is only discussed briefly here. When searching for prey or insect hosts for oviposition, predatory and parasitic insects can make use of volatiles directly emanating from their host's body or emitted as pheromones (Afsheen *et al.*, 2008). Due to the small size of such odour sources these are often emitted in minute quantities, however, and so use of such cues in long-range host location is difficult. The plant on which the host is feeding represents a larger and far more easily detectable target at long range. Predatory and parasitic insects use plant odours to locate their hosts' habitat at a distance and subsequently engage in more localised foraging behaviour once on the plant (Bukovinszky *et al.*, 2012; de Rijk, Dicke & Poelman, 2013). For example, the rove beetle *Aleochara bipustulata*, which feeds on and whose larvae parasitise the pupae of cabbage root flies, *Delia radicum*, uses volatiles emitted from fly-infested roots to locate a suitable area for foraging at a distance. Once in the vicinity of the root beetles can then make use of volatile cues from larval tracks and pupae (Goubert *et al.*, 2013). Plants can benefit from recruitment of natural enemies of their herbivores and consequently tend to produce elevated quantities of volatiles upon herbivory (Vet & Dicke, 1992; Heil, 2014) or in response to herbivore egg deposition (Hilker & Fatouros, 2015). Herbivore-induced volatile blends may also provide specific information reflecting infestation by specific herbivore species (De Moraes *et al.*, 1998; McCormick, Unsicker & Gershenzon, 2012), greatly facilitating eventual host-location by specialist parasitic insects. While herbivore-induced volatiles can substantially increase the detectability of prey at short-mid range, at distances of more than a few 10s of meters predatory and parasitic insects face the same challenge as other insects in that plume encounters may be too rare to provide an effective means of locating an infested plant. Larger-scale habitat cues that indicate an area of vegetation provide a solution to this problem, and evidence for use of such cues comes from pollinators and above-ground foraging herbivores.

(4) Pollinators and above-ground foraging herbivores

The use of habitat cues by insects foraging above-ground around plants remains largely unexplored but tantalizing indirect evidence exists for their use. Foliage offers a large source of odour but at greater distances downwind falls in concentration within odour plumes (Murlis *et al.*, 2000; Koehl, 2006) and increasing intermittency (Koehl, 2006; Beyaert & Hilker, 2014) means they may be difficult to detect. Host plants located within the middle of a patch may be even harder to detect due to obstruction or adsorbtion of volatiles onto surfaces of downwind vegetation (Beyaert & Hilker, 2014). In the absence of specific host plumes, orienting first toward a broad patch of vegetation could substantially increase the foraging insect's chances of subsequently encountering host cues. This is particularly true in areas where vegetation coverage is not complete and broken up by bodies of water, rocky areas, urban constructions etc. Even where vegetation coverage is fairly extensive, localised regions containing higher abundances/diversity of plants will generally offer the most promising locations to search for a suitable host. Many plant volatiles are ubiquitous, produced by a wide range of different plant species in large quantities and can indicate such areas of vegetation. Even non-host volatiles, normally avoided at short-range (Bruce *et al.*, 2005), may be used to indicate such areas at long range and may facilitate eventual host encounter.

(a) Green leaf volatiles

To be of any use, habitat cues should be detectable at relatively large distances and thus emitted from a larger area and/or in higher concentrations. Green leaf volatiles (GLVs) are C-6 fatty acid derivatives, produced ubiquitously throughout the plant kingdom, and may offer such a cue. Whilst GLVs are generally only produced in large quantities following herbivory or physical stress (Mwenda & Matsui, 2014), in nature such stresses are common and so most plant patches produce large quantities of GLVs. Strong behavioural responses of herbivorous insects to GLVs have been shown by a number of insect species, even for those which are generally thought to recognise their hosts using highly species-specific blends. The black bean aphid, *Aphis fabae*, is attracted to specific blends of volatiles emitted by its host *Vicia* *faba* (Webster *et al.*, 2008a; Webster *et al.*, 2008b) and the requirement for a blend is so pronounced that, when presented with individual host volatiles outside the context of the complete blend, aphids were repelled (Webster *et al.*, 2010a). Notable exceptions to this trend, however, were the GLVs (*Z*)-3-hexenol and 1-hexenol, both of which were attractive on their own at levels similar to those emitted by plants (Webster *et al.*, 2010b). Given the strict preference for host-specific blends by this aphid, and the total lack of host-specific information provided by GLVs, these responses are at first glance surprising. Such responses to GLVs are fairly widespread, however, with many insects responding positively to them despite normally showing preferences for highly host-specific volatile blends (Birkett *et al.*, 2004; Ruther & Mayer, 2005; Alagarmalai *et al.*, 2009; Li *et al.*, 2014). Their possible role as habitat cues, indicating general areas of vegetation worthy of closer inspection by host-seeking insects, may explain this pattern. GLVs may also be used to inform of habitat suitability once the insect is already within a habitat. (*Z*)-3-hexenol is not attractive to the leaf beetle *Cassida denticollis* but the presence of this volatile as background odour dramatically increased the speed at which beetles discriminate host tansy (*Tanacetum vulgare*) stems from non-odourous dummy stems (Muller & Hilker, 2000), possibly by informing on overall habitat quality.

(b) Non-host volatiles

Aside from GLVs, general vegetative odours from plants other than hosts may serve to inform as to the location of a plant patch. Unmated female cotton leafworm (*Spodoptera littoralis*) feed from nectar-rich lilac flowers and respond to their odour in a wind tunnel (Saveer *et al.*, 2012). Cotton plants, which are used for oviposition and are only attractive to mated females, were not landed on by unmated females but their odour elicited increased take-off flights compared to dummy plants (Saveer *et al.*, 2012). This suggests that 'non-host' odour may play a role in long-range orientation even if they do not induce orientation at short range. In fact, non-host volatiles that are repellent at short range may be attractive over longer distances when used as habitat cues. The idea that non-host volatiles may be used in host location is contrary to many long-held assumptions in insect behavioural ecology. A huge number of studies have demonstrated that insects will move away from non-host odours but the overwhelming majority of these studies used short-range olfactometers. Olfactometers are simple walking assays that are extremely efficient at screening short-range behavioural responses but do not account for long-range behavioural responses to volatile cues. Few studies have compared long- and short-range responses to the same odours but those that did showed surprising contradictions. Calatayud *et al.* (2014) showed that female cereal stem borers (*Busseola fusca*) avoided non-host Napier grass in preference of host maize in a Y-tube olfactometer (short-range orientation) but showed no such preference in a wind tunnel (longer-range orientation). Even more striking discrepancies come from *Drosophila* for which, in an olfactometer designed to assess walking behaviour, addition of CO_2 to vinegar odour decreased its attractiveness whereas in a free-flying cage assay the addition of CO_2 raised the attractiveness of vinegar (Faucher, Hilker & de Bruyne, 2013). It is impossible to draw broad conclusions from the few studies that use both short- and long-range behaviour assays but in these examples at least, odours which are avoided at short range at the host location stage may elicit different, or opposite, responses at long range during the habitat location stage.

Use of non-host odours in the field to deter insect pests have met with mixed results. Although there are numerous examples of non-host plants being extraordinarily effective at reducing pest numbers when planted alongside hosts (Pickett *et al.*, 2014), there are many more that fail to have any effect in the field or that deliver opposite than expected results. In an attempt to use a range of non-host plant odours to protect roses against Japanese beetle (*Popillia japonica*) it was found that addition of supposedly repellent non-host species actually increased numbers of invading beetles (Held, Gonsiska & Potter, 2003). Similar effects were observed when plants deterrent to the Colorado potato beetle (*Leptinotursa decemlineata*) were planted amidst potato plants, resulting in larger number of beetles than in untreated plots (Moreau, Warman & Hoyle, 2006). If non-host volatiles are avoided at short range but used to indicate the presence of a plant patch at longer ranges this may explain why attempts to incorporate non-host volatiles into integrated pest management strategies have often had opposite than expected outcomes in these, and other (Legaspi, Simmons & Legaspi, 2011; Moreno & Racelis, 2015) field experiments.

III. IMPLICATIONS

The use of habitat cues by foraging insects has now been demonstrated in a number of systems spanning several different feeding guilds. Despite this, use of habitat cues by insects is still widely overlooked. This is probably in part due to the difficulty of identifying such cues. Most studies use olfactometers that only record simple attractive/repellent behaviours used in short-range orientation. Some habitat cues may only operate at long range (thus walking assays may not always be appropriate) and may also elicit more complex non-directional searching behaviours that are difficult to detect without using advanced tracking techniques in suitably large arenas. Video-tracking technology has advanced considerably in recent years allowing detailed three-dimensional flight paths to be constructed for small insects both inside the lab and outdoors (Spitzen *et al*., 2013; Manoukis *et al*., 2014). Insect movement can also be tracked effectively using radar or by fitting insects with active transmitters and tracking using radio telemetry (Chapman, Drake & Reynolds, 2011; Kissling, Pattemore & Hagen, 2014). These techniques have been successfully employed to track insect movement in the field, often over large distances, and can provide important insights into how insects move within and between different habitats (Negro *et al*., 2008; Hagen, Wikelski & Kissling, 2011; Lihoreau *et al*., 2012). As use of these advanced tracking techniques becomes more widespread, we predict that identification of new habitat cues will accelerate.

Evidence for the use of habitat cues by above-ground-foraging herbivorous insects remains tentative with much work still to do. The possibility that non-host odours can function as habitat cues at long range deserves particular attention since this has obvious implications for the use of non-host volatiles as deterrents in integrated pest management strategies. The occurrence of habitat cue inhibitors in both soil-dwelling and haematophagous insects (Reinecke *et al.*, 2008; Turner & Ray, 2009; Tauxe *et al.*, 2013) suggests a widespread phenomenon and could fuel new pest management strategies. Attempts could be made to screen host volatiles for their ability to inhibit behavioural responses to known habitat cues when presented to insects simultaneously. Any identified habitat cue inhibitors could then potentially be used to disrupt habitat location at a distance if placed around the perimeter of an area to be protected, reducing the influx of pests. This would probably be more effective in situations where points of entry are limited, such as vents in a glasshouse or other man-made structure, where inhibitors can be most easily applied. A better understanding of habitat cues may also help with monitoring or mass-trapping strategies. For example, some haematophagous insects use CO_2 as a long-range habitat cue but may not orient towards it at close range, yet many modern CO_2 commercial traps still aim to trap insects at or very near to the point of CO_2 release (Vaidyanathan & Feldlaufer, 2013). More careful arrangement of habitat and host cues in such traps may lead to far more effective trapping rates (Spitzen *et al.*, 2008; Cooperband & Cardé, 2006).

Future studies on host-location behaviour should seek to better distinguish between habitat and host cues. This could easily be achieved by using a combination of long- (e.g. wind tunnel) and short- (e.g. olfactometer) range behavioural assays to observe differences in responses to volatiles at different spatial scales and also by precisely tracking movement paths of insects rather than simply recording their final destinations. Cues that elicit upwind orientation at long range but are ignored or avoided at short range are more likely to be habitat than host cues. Volatiles that elicit non-directional searching behaviour rather than directed movement to the point of emission are also more likely to be habitat cues, as are those whose presence as background odour enhances insects' responses to other host cues.

Whilst this review has focussed on examples from insects, use of habitat cues has also been recorded in vertebrates (e.g. use of dimethyl sulphide to indicate regions of biodiversity by procellariform seabirds (Nevitt, 2008)), suggesting they are a near-universal component of host location. An improved understanding of habitat cue use would greatly improve our understanding of insect foraging behaviour and ecology and may also lead to improved development of pest control strategies that aim to exploit or disrupt insect host-seeking behaviours.

IV. CONCLUSIONS

1. Habitat cues are used to improve chances of ultimately locating a host cue by inducing orientation toward, or triggering foraging behaviour within, a physical area that is likely to harbour hosts. Habitat cues differ from host cues in that they tend to be produced in larger quantities and detectable at greater distances than host cues and tend to provide less host-specific information.

2. Habitat odour comprises the collective volatile emissions of a habitat or physical region. These volatiles may originate collectively from the different organisms that inhabit the area. Many habitat cues are ubiquitous volatiles that, while offering little information on species identity, tend to be produced in large quantities from a wide range of plants/animals including host and non-host alike (e.g. GLVs from foliage, respiratory CO_2 emissions from roots or animals) and thus may be used to indicate patches of vegetation or groups of animals.

Alternatively, habitat cues may originate from excretions or secretions of host organisms, for example indicating a dwelling or nesting area of a group of animals.

3. In recent years evidence of habitat cue use by insects has accelerated. There are now numerous clear examples of their use by soil-dwelling herbivores, haematophagous insects, predatory and parasitic insects, above-ground foraging herbivorous insects, and even birds. Use of habitat cues is thus rapidly emerging as an essential component of host location for many host-seeking organisms.

4. Numerous field and laboratory experiments suggest that non-host plant volatiles may function as habitat cues for host-seeking herbivorous insects, used to indicate patches of vegetation where potential hosts may be searched for if host-specific cues are unavailable. Non-host volatiles that are repellent at short range can be attractive over larger distances, further evidencing their role as habitat cues with important implications for their use in integrated pest management.

5. Research into host-location behaviour has largely involved use of short-range olfactometer behavioural assays that, while possessing many advantages for rapid screening of behavioural responses and assessment of short-range orientation, are unsuitable for identification of habitat cues. This is likely a reason for the slow progress made in identifying new habitat cues and future research making use of state of the art tracking technologies will undoubtedly result in discoveries of new habitat cues.

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Figure 1. Use of habitat cues by foraging insects. Shown are three main ways in which habitat odour can used for eventual location of a suitable host for feeding/oviposition (green circle): a) The large red/green arrow represents odour from a potential resource patch. Insects follow this toward the patch before eventually encountering host odour (small green arrow). Encounter with host odour leads to a switch in behaviour, where insect ceases to move in

response to habitat odour and instead follows host odour to its source. b) Habitat odour (red/green area) present in the background induces non-directional localised searching behaviour in the form of increased rates of turning and changes in movement speed, increasing the probability of chance encounter with host odour. Once host odour is detected, the insect abandons this localised searching behaviour and instead follows the host odour toward its source. c) Habitat cues present as background odour gate behavioural responses to host cues. When relevant background odour is detected that indicates the insect is in a suitable habitat, insects become more responsive to host-odour cues. When this relevant background odour is lacking, host-odour cues that the insect detects are either responded to weakly or ignored.

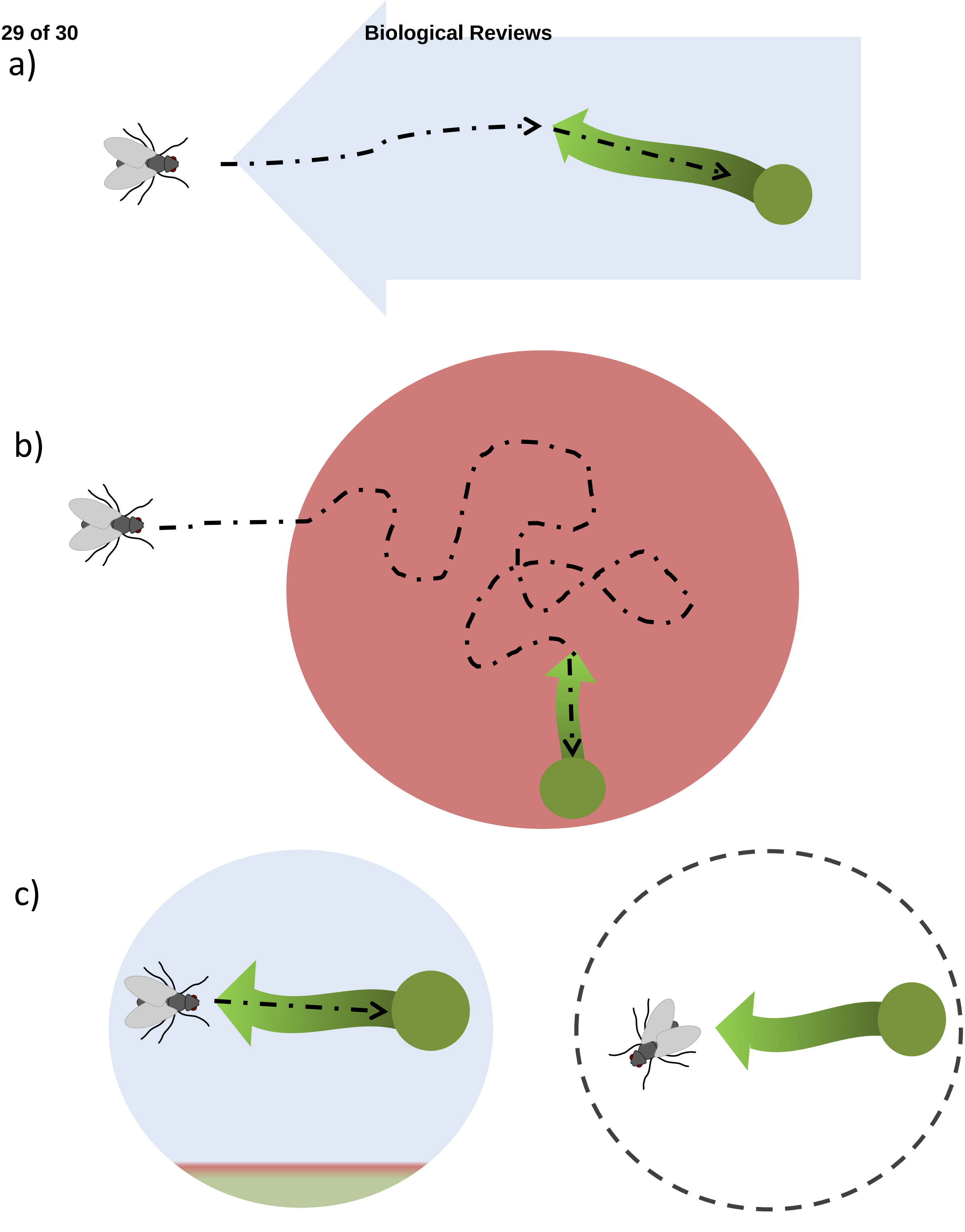
a)
b)
c)

Table 1 Host cues vs. habitat cues. 'Host cue' has become a general term used to refer to any cue used at any stage in host location. There is an important distinction, however, between cues used to locate a feeding/oviposition site (host cues) and cues used to inform of a general area where host cues may subsequently be found (habitat cues). We propose the following broad criteria to help distinguish between the two. This list is not intended to be overly prescriptive since there are undoubtedly exceptions to each and should instead serve only as a general guide to distinguish between the two.

Feature	Host cues	Habitat cues
Function	Indicate location of a feeding/oviposition site	Indicate a general area/location where host cues are most likely to be encountered
Source	Emitted from host	Emitted from host's habitat, which may include the host itself as well as non-hosts
Quantities emitted in	Lower quantities	Higher quantities
Detectability	Detectable at short distances	Detectable at longer distances
Specificity	Often host-specific	Not necessarily host-specific
Behaviours elicited	Directed movement towards odour source	General upwind movement, localised searching behaviour, or enhanced responses to host cues