Seasonal changes in mycophagous insect communities

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Key words. Agaricus bisporus, climate change, fungal host preference, host tracking, Diptera, Megaselia rufipes, Bradysia, United Kingdom

Abstract. The phenology of fungal fruiting has changed in the UK over the last 70 years, but whether the associated mycophagous insects are able to exploit 'out of season' fruit bodies is unknown. This study focused on whether fungal baits can be used as a proxy to examine changes in fungal fruiting on insect communities. Using Agaricus bisporus as a bait, mushrooms were placed into two separate woodlands monthly from November 2020 to July 2021. Megaselia rufipes (Phoridae) and Bradysia spp. (Sciaridae) were reared from both wild fungi and fungal baits at different times, making them appropriate species to consider for possible host tracking. Various factors affect an insect's ability to track a fungal host, these include host preference, season, period of fungal fruiting and age of mushroom. Increased fruiting of macrofungi in the future may benefit generalist mycophagous insects, by providing enhanced temporal and spatial resource opportunities. Using fungal baits as a proxy for the effects of climate change on fungal fruiting should be beneficial in uncovering the host preferences of mycophagous insects and may potentially indicate whether mycophagous insects can track fungal hosts across seasons.

1. INTRODUCTION

Many insects and fungi occupy the same or similar habitats, e.g., dead wood systems, the rhizosphere and areas containing rotting organic matter. Fungal and insect interactions are important as they reflect ecological processes such as phenology, competition, and succession and facilitation of population and community ecology (Hackman & Meinander, 1979; Väisänen, 1981; Hanski, 1989). Most research on fungal fruiting bodies and associated insects has occurred within boreal forests of Northern Europe, woodlands of the U.K. (Chandler, 1976; Jakovlev, 2011) and Japan (Tuno et al., 2019).

The close link between fungi and insects may be affected by climate change since growth and development of fungi and insects is highly dependent upon seasonality, temperature and humidity (Boddy et al., 2014; Cui et al., 2018). Climate change is strongly associated with changes in phenology of many fungi across the UK and more widely across Europe (Gange et al., 2007; Kauserud et al., 2008), as well as earlier flight times of insects and changes in range (Braschler & Hill, 2007). Much research on phytophagous insects shows a link between insect phenology and spatiotemporal availability of foodplants (Bridle et al., 2014), including the ability of insects to track their hosts and phenological mismatches between hosts and consumers (Zohner, 2018). However, whether such trends occur in mycophagous insects is unknown.

The majority of mycophagous insects are Coleoptera (Lawrence, 1989) and Diptera (Toda & Kimura, 1997), with the latter including species of Mycetophilidae, Phoridae, Sciaridae, Bolitophilidae, Muscidae and Heleomyzidae (Buxton, 1960) although rearing of insects has been strongly biased towards macro fruiting bodies of Agaricales. Physical characteristics such as size and shape of fruiting body (Thorn et al., 2015), chemical composition, and stage of decay are also important variables in host preference and composition of insect communities, with each of these factors likely to be impacted by climate change (Guevara et al., 2000; Leather et al., 2014).

Mushrooms are a patchy resource and their interactions with insects are dependent on insects’ abilities to find and exploit them. Since many insect species may utilise the same fruiting body, niche partitioning can occur either through differing spatial feeding preferences such as hyphae or sporocarps (Krivosheina, 2008), or temporally, with fruiting bodies utilised at different times (Guevara et al., 2000). Insect species that specialise on specific fungal hosts and are mono or oligophagous are more likely to suffer from the effects of climate change (Pureswaran et al., 2018). If certain mycophagous insects are expanding their range due to climate change then competitive interspecific interactions may cause insect species communities to change within fruiting bodies (Forrest, 2016). Conversely,
insect ranges may decrease with climate change, decreasing realised niche range (Halsch et al., 2021).

Although not uniform across all fungi, the effect of climate change may also impact mycophagous insects (Kauserud et al., 2008; Kauserud et al., 2010; Sato et al., 2012). Since many single season fungi now have two flowering seasons (Gange et al., 2007) shifts in temporal or spatial distribution of fungi (Gange et al., 2018) will require mycophagous insects to adapt, either through developing a broader host preference, as seen in the butterfly *Aricia agestis* (Bridle et al., 2014), through tracking the movement of the host from one area to another (Posledovitch et al., 2018), or temporally by adapting growth rates to match fungal host emergence times. Increased temperatures, humidity and precipitation may suit some mycophagous insects: many Diptera require wet, humid microclimates for pupation, whereas many Mycetophilidae may be negatively impacted as they prefer drier conditions (Hutson et al., 1980). Increased temperatures may also result in faster decaying of fruiting bodies especially in ephemeral fruiting bodies such as *Coprinus* spp., which deliquesce within hours of fruiting (Kües, 2000). Since fruit body appearance is unpredictable (Moore et al., 2008), a standardised method of examining whether mycophagous insects might be able to take advantage of the increased spatial and temporal fruiting (Gange et al., 2018) is needed.

In order to simulate different fungal fruiting times, this study used monthly placement of commercially cultured *Agaricus bisporus* as a bait, providing insects with a resource across seasons as a proxy for the effects of climate change on fungal fruiting to determine whether mycophagous insects can track their fungal hosts. This is the first-time such an approach has been used to investigate host tracking as a proxy for the effects of climate change, and as a preliminary study this may pave the way for future works considering a method to determine how mycophagous insects track fungal hosts in relation to climate change. Additionally, a native fungal bait over a long period provides a seasonal fungal presence and provides information about insect communities within the bait and a baseline comparison to insect communities of wild mushrooms.

Here, the first hypothesis was that baits would be a good proxy for changes in fungal fruiting on insect communities and that insects which can utilise the baits will be able to track them through time. Secondly, age of mushroom bait was hypothesised to be a key factor in choice preference for mycophagous insects following physical and chemical changes of the fruiting body as it ages and decays. The main aims of this experiment were to understand whether insects could track their fungal hosts phenology over a period of 9 months (November 2020 to July 2021), to see whether tracking of fungal hosts was consistent between two locations and to understand whether age of mushroom is a key factor in mycophagous host preference and subsequent shaping of insect communities.

2. MATERIALS AND METHODS

2.1. Field sites

Fungal baits were placed and collected from a mixed woodland in Heston, Hounslow, U.K. (51.48872, -0.363987) and a mixed woodland (Huntersdale) in Egham, Surrey, U.K. (51.41669, -0.57115). The mixed woodland in Heston is predominantly Ash (*Fraxinus excelsior*) with several mature Oaks (*Quercus robur*) and is situated between grassland and farmland, with a heavy clay-based soil. Huntersdale is dominated by Oak (*Q. robur*), Scots pine (*Pinus sylvestris*), Silver Birch (*Betula pendula*) and Beech (*Fagus sylvatica*) and situated on sandy soil along a slope (refer to Table S2 for tree species found in both locations). Locations were chosen since they are undisturbed, reducing the likelihood of samples being vandalised and containing mature woodland. The distance between the two locations is approximately 19 km, which reduces the chances of insects migrating from one area to the other as species such as *Musca domestica* have been found to fly up to 7 km (Nazmi et al., 2005).

2.2. Fungal baits

The cultivated mushrooms, *A. bisporus*, were supplied by Merrhills Mushrooms Ltd (Storrington, West Sussex, UK), grown in 17.5 × 17.5 × 17.5 cm containers on a medium of wheat straw, poultry and horse manure in a controlled-temperature room of 18°C, and took approximately three to four weeks to become viable for placement into the woodlands. Once mycelium was seen the mushrooms were watered (300 ml each spray) twice daily to maintain a wet, humid environment for efficient growth. Once mushrooms had formed and had begun to sporulate (determined visually), they were placed in the woodlands. To calculate a general approximation of fruiting body volume, the water displacement method was carried out with the use of 40 mushrooms (20 old and 20 young): each mushroom was weighed individually and measured, then plotted against weight (grams) of the fruiting body. Results from linear regressions were interpolated to predict volumes for collected cultivated samples based on weight, separately for young and old mushrooms. Weights of mushrooms used for water displacement ranged between 2.34 g to 128.56 g.

To test whether age was an important factor in insect oviposition preference, four batches of mushrooms were grown for each location, from March up until July. A two-week difference in growing time between young batches of mushrooms and old batches of mushrooms was maintained each month. Old mushrooms were typically shrunk and had lost a large amount of moisture as well as having a darker appearance. Overall, 739 cultivated mushrooms were placed into the woodland areas. Numbers of mushrooms collected per month along with total weight are given in Table S1.

2.3. Collection of wild mushrooms and fungal baits

Collection of wild fungi began on the 25th of October 2020 and continued monthly until July 2021. Fungal collections coincided with placement and removal of baits. The cultivated samples were left in the woodlands for five days to allow sufficient time for insects to locate them and for oviposition to occur (Icho et al., 2008). Wild fungi were collected from an approximately 100-m radius from the cultivated fungi samples, to determine whether insect communities reared from wild fungi were similar to those reared from cultivated samples. Host tracking of wild fungi by insects was not recorded or monitored as wild fungal fruiting did not occur each month and their emergence could not be accurately predicted.
Identification of wild fungi, to species level where possible, was based on physical observations (e.g., gills, sporocarp shape, substrate, spore prints), and verified by experts. Fungal collection consisted mostly of Agaricales, but Bracket fungi (Polypores) were also collected when possible.

2.4. Insect rearing methods

All mushrooms collected were broken from the mycelium and weighed before being placed into emergence traps (NHBS Ltd, Totnes, Devon, UK). Emergence traps (https://www.nhbs.com/insect-mosquito-breeder) contained single or multiple mushrooms dependent on the size of the mushroom. Irrespective of the number of mushrooms per emergence trap, the total approximate volume was known. As emergence traps contained varying numbers of mushrooms, count data of insects and which species was standardised against per unit volume (cm³) of mushroom. Approximately 6–7 cm of John Innes no. 3 compost was placed into each trap to provide larvae with a habitat in which to pupate and misted daily to maintain humidity. All mushrooms collected between October and December 2020 were kept in a Modular Cold Room (PORRKA, Watford, UK), a temperature-controlled incubation unit at 20 degrees centigrade with a day-night cycle of 12 h for each. From January 2021 onwards the collected mushrooms were then transferred to a polytunnel to reflect natural conditions with respect to light and temperature. Every two to three days the tubes were checked for insect emergence and lightly misted with a spray bottle with water when required.

All mushrooms collected from November to May were kept until mid-August 2021, as some eggs and larvae may have required a phase of diapause for extended periods of time before emerging. Mushrooms collected from June and July were kept until late September 2021. Insects were collected with the use of a pooter. Once emergence traps had been emptied of visible insects, the pooter was placed into the freezer for approximately 10 minutes to slow the movement of the insects substantially enough for straightforward placement into vials. They were then preserved in 70% ethanol for further analysis and identification.

2.5. Identification of Insects

References to Royal Entomological Society checklists, keys (Hutson et al., 1980; Chandler, 1998) and consultations with entomological experts (Peter Chandler, Henry Disney and members of the London Natural History Museum) aided insect identification. A combination of wing venation and examination of genitalia were used for identification (to species level where possible) to minimise error. Examination was performed with the use of compound and binocular microscopes followed by photography through a microscope lens with the use of a camera attachment and saved with computer software (Swift Imaging 3.0). Insect larvae which failed to pupate or become adults were not identified due to difficulties in accurate identification. Ten individuals of Collembola were collected but omitted from analysis.

2.6. Statistical analyses

All statistical analyses were conducted in R studio Version 1.4.1717. These included a Two-Way ANOVA (R’s Car package), a Negative Binomial GLM (R’s MASS package), Poisson GLM, Non-Metric Multidimensional Scaling graph (NMDS) (R’s vegan package), ANOSIM tests (R’s vegan package) and Linear Regression analysis. Two-Way ANOVA’s were implemented to compare means of insect abundances and means of insect species per unit volume of mushroom between months and location, months and age within the same location and between locations and age. When assumptions for a Two-Way ANOVA were not met, a GLM (Poisson or Negative Binomial) were implemented. Data used for NMDS were based on total abundances for each insect species per month for each location. NMDS were used to visually compare insect diversities between months, age of fruiting body, between locations and within locations (i.e., cultivated samples vs wild samples), and the Bray-Curtis dissimilarity method was used to test for dissimilarity between sites. ANOSIM tests indicated which factors were significant and contributed most to the spread of data in NMDS graphs. Linear regressions were implemented to understand whether there was a relationship between volume of mushrooms and insect abundance as well as number of insect species.

2.7. Weather data

Weather temperatures for Heston were collected from Freemeteo (https://freemeteo.co.uk) which uses data from the Heathrow Weather Station (London, U.K.). Weather temperatures from Egham were collected from data recorded from a Weather Station located in Silwood Park Campus from Imperial College London, Buckhurst Road, Ascot, Berkshire SL5 7PY, U.K. Average temperatures were calculated based on minimum and maximum daily temperatures.

3. RESULTS

In total, 13,612 insects were reared between October 25th, 2020, and July 22nd, 2021, from cultivated (12,516 individuals) and wild fungal fruiting bodies (1,096 individuals) across both locations of Heston and Huntersdale. Cultivated samples from Heston produced 24 insect species from 18 different families while wild fungi produced 33 insect species from 19 families. Cultivated samples collected from Huntersdale produced 26 insect species from 21 different families while wild fungi produced 15 insect species from 8 different families. Graphs showing months indicate the time in which fungal baits were placed into each woodland and the associated number of insects and insect species produced from them. They do not show when insects emerged, as the latter process took place over extended and variable time scales. To see the complete list of fungal and insect species found in both locations please refer to Tables S3, S4 and S5.

Insect abundance collected from young, cultivated mushrooms was considerably higher in the summer months (June and July) compared to winter months ($\chi^2 = 72.85, df = 5, p < 0.001$) (Fig. 1a), and this pattern was mirrored in numbers of insect species for both locations across the same period ($\chi^2 = 170.14, df = 5, p < 0.001$) (Fig. 1b). Insect abundance and the number of insect species from young mushrooms were similar between the two woodlands although in Huntersdale fungal baits produced no insects from December to March, whereas in Heston there were no insects produced from January to March. A similar trend was found for insect abundance and insect species in old, cultivated mushrooms, with there being a higher insect abundance in summer months for both locations ($\chi^2 = 42.18, df = 2, p < 0.001$) (Fig. 1c) and more species appearing in June and July in comparison to preceding months for Heston and Huntersdale ($F_{2,50} = 16.22, p < 0.001$). Additionally, it appeared that there were more insect species using old, cultivated mushrooms in Heston compared to insect species using old, cultivated mushrooms in Huntersdale ($F_{1,50} = 5.18, p < 0.05$) (Fig. 1d).
Fig. 1. a – Box and whisker plot displaying minimum, lower quartile, median, upper quartile and maximum numbers of mean insect abundance per unit volume of mushroom (cm³) collected from young cultivated mushrooms between November 2020 and July 2021 from Heston and Huntersdale. Outliers are indicated by black dots. b – Mean number of insect species per unit volume of mushroom (cm³) collected from young cultivated samples between November 2020 and July 2021 from Heston and Huntersdale. c – Mean insect abundance collected from old cultivated samples between March 2021 and July 2021 from Heston and Huntersdale. Note that no insects emerged from March and May baits. d – Mean number of insect species collected from old cultivated samples between March 2021 and July 2021 from Heston and Huntersdale.
When considering insect communities between each location, it appears that most insect families were present in both areas, irrespective of age of mushroom (Fig. 2a). Despite this finding, a small number of species were found in specific locations and age of sample, such as *Culicoides* spp. (Ceratopogonidae), *Pediciidae* (Diptera) and *Erotylidae* (Coleoptera) which were reared only from young, cultivated fruiting bodies in Huntersdale. *Chalcidoidea* (Hymenoptera) were only reared from old, cultivated samples collected from Huntersdale. Conversely, *Nemapogon cloacella* (Lepidoptera: Tineidae) was only reared from cultivated samples in Heston. Interestingly, these families were not reared from any wild fungal fruiting bodies (Table 1).

When considering season, it appears that insect communities change and differ considerably depending on the time of year (Stress = 0.09, R = 0.64, p < 0.001) (Fig. 2b). Insect communities did not differ between locations within each month. Insect communities were dissimilar between cultivated mushrooms and wild mushrooms (Stress = 0.11, R = 0.42, p < 0.001), but not between locations (Fig. 2c).

### Table 1. Complete list of all insect families (30 and Collembola) reared from both cultivated and wild samples from both locations along with their abundance (n).

| Order   | Family                  | Total n | Huntersdale | Heston | Huntersdale | Heston |
|---------|-------------------------|---------|-------------|--------|-------------|--------|
|         |                         |         | Cultivated  | Cultivated | Wild       | Wild   |
| Diptera | Phoridae                | 4280    | 1172        | 2707    | –           | 401    |
| Diptera | Sciaridae               | 2022    | 550         | 1462    | –           | 10     |
| Diptera | Drosophilidae           | 1721    | 367         | 1186    | 1           | 167    |
| Hymenoptera | Braconidae            | 1524    | 363         | 1047    | –           | 114    |
| Diptera | Cecidomyiidae          | 1260    | 504         | 756     | –           | –      |
| Coleoptera | Leiodidae              | 911     | 474         | 436     | –           | 1      |
| Diptera | Sphaeroceridae          | 493     | 111         | 373     | 3           | 6      |
| Diptera | Mycetophilidae         | 255     | 1           | 23      | 81          | 150    |
| Diptera | Muscidae                | 248     | 21          | 226     | –           | 1      |
| Diptera | Fanniidae               | 244     | 139         | 99      | –           | 6      |
| Diptera | Psychodidae             | 177     | 143         | 34      | –           | –      |
| Diptera | Helemyzidae             | 138     | 8           | 95      | 5           | 30     |
| Diptera | Chloropidae             | 115     | 19          | 31      | –           | 65     |
| Coleoptera | Morphospecies 1     | 52      | 39          | 13      | –           | –      |
| Lepidoptera | Tineidae              | 47      | –           | 47      | –           | –      |
| Diptera | Ceratopogonidae         | 20      | 20          | –       | –           | –      |
| Diptera | Bolbitophiliidae        | 16      | –           | –       | –           | 16     |
| Coleoptera | Erotylidae             | 15      | 15          | –       | –           | –      |
| Diptera | Astiidae                | 13      | –           | 6       | –           | 7      |
| Diptera | Pediciidae              | 11      | 11          | –       | –           | –      |
| Colembola* | Entomobryidae          | 10      | –           | –       | 6           | 4      |
| Hymenoptera | Chalcidoidea          | 9       | 9           | –       | –           | –      |
| Hymenoptera | Diapriidae             | 8       | –           | –       | 8           | –      |
| Coleoptera | Trichocerida           | 5       | –           | 1       | 1           | 3      |
| Coleoptera | Latridiidae            | 5       | 4           | 1       | –           | –      |
| Hymenoptera | Proctotrupidae         | 4       | –           | –       | 3           | 1      |
| Diptera | Chironomidae            | 2       | –           | –       | –           | 2      |
| Psocoptera | Ectopsocidae           | 2       | 2           | –       | –           | 2      |
| Hymenoptera | Formicidae             | 2       | –           | –       | –           | 2      |
| Hymenoptera | Oxytorinae             | 2       | –           | –       | 2           | –      |
| Coleoptera | Staphyliniida          | 1       | 1           | –       | –           | –      |
| Total    |                        | 13612   | 3973        | 8543    | 102         | 994    |

* Ten individuals of Collembola were collected but omitted from analysis as focus was strictly upon insect communities.

### Table 2. Total abundance per month of *M. ruﬁpes* and *Bradysia* spp. collected from cultivated and wild fungal samples.

| Date     | *M. ruﬁpes* | *Bradysia* | *M. ruﬁpes* | *Bradysia* |
|----------|--------------|------------|--------------|------------|
|          | Cultivated   | Wild       | Cultivated   | Wild       |
| Nov-20   | 23           | 22         | 53           | 0          |
| Apr-21   | 943          | 0          | 5            | 0          |
| May-21   | 0            | 172        | 0            | 9          |
| Jun-21   | 2746         | 15         | 766          | 0          |
| Jul-21   | 167          | 191        | 1188         | 0          |

4. DISCUSSION

This is the first study in which fungal baits have been used over an extended period as a proxy for the potential effects of climate change on fungal fruiting and changes in associated mycophagous insect communities. It was not possible to find conclusive evidence of host tracking by any of the insects reared from wild or cultivated fungal samples. Instead it was found that insect communities of *A. bisporus* were distinctly different to wild mushrooms in both locations, with many Mycetophilidae being collected from wild fungi (e.g., *Exechia fusca* and *Mycetophila fungorum*) and species of various insect families (e.g., Drosophilidae, Fanniidae and Muscidae) being collected from fungal baits (supplementary data Table S4 and

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of the sampling period, namely reared from wild fungi and fungal baits at differing times communities. Despite this, two species were successfully a proxy for monitoring changes in fungal fruiting on insect seems 2003; Jakovlev, 2011; Põldmaa et al., 2015), but overall it host speci fi S5), agreeing with studies that suggest there is a degree of Fig. 2. a – NMDS portraying similarities between insect diversity collected from young and old cultivated samples in Heston and Huntersdale. Circles represent Heston and Triangles represent Huntersdale. b – NMDS portraying similarities of insect diversity of cultivated samples between Heston and Huntersdale collected from November 2020 to July 2021. c – NMDS portraying similarities of insect diversity between cultivated samples and wild samples collected from Heston and Huntersdale from October 2020 to July 2021. SS), agreeing with studies that suggest there is a degree of host specificity amongst mycophagous insects (Komonen, 2003; Jakovlev, 2011; Põldmaa et al., 2015), but overall it seems Agaricus bisporus is not a suitable bait for acting as a proxy for monitoring changes in fungal fruiting on insect communities. Despite this, two species were successfully reared from wild fungi and fungal baits at differing times of the sampling period, namely M. rufipes (Phoridae) and Bradysia spp. (Sciaridae). Suggestions of possible host tracking could be attributed to M. rufipes which appeared in high numbers from fungal baits during April 2021 when there were very few wild fungal fruiting bodies in each location.

In terms of fungal age and host preference, it appears that there could be an element of resource partitioning between M. rufipes and Bradysia spp. Despite using the same fungal host, M. rufipes was not found in old fruiting bodies and was mostly reared from young specimens. Results for Bradysia spp. show presence in both old and young specimens, which suggests a successional component to fruiting bodies and the insect communities related to them.

The low abundance of insects and insect species from cultivated samples between the months of November and December 2020 in both locations is perhaps a result of the cold weather, which is detrimental to many insects as they can struggle to maintain core temperatures and mobility is heavily reduced (Teets & Denlinger, 2013). From wild fungi there were a few insect species that were able to emerge during the winter, namely T. fenestralis, Trichocera spp. and Boliophila spp. Trichocera spp., are commonly known as Winter Crane flies and appear to be adapted for cold conditions (Hågvar & Krzeminska, 2007; Ci & Kang, 2021). As fungal fruiting predominantly occurs in the autumn it would be expected that the highest insect numbers would occur during this time, however the data suggests the opposite, the answer is likely due to the food preferenc es of the insects reared as there may be more insect species in summer which can exploit several food resources other than fungal material. This is evident in various species of Drosophilidae and Phoridae as well as other Dipteran families (Brown, 2001). High average temperatures during the months of June and July in comparison to previous months could have accelerated the rates of decomposition of organic matter in woodlands (Song et al., 2014), increasing the possible food resources available to a range of insect species; this is reflected in the insect species and insect abundance reared from June and July samples, mainly consisting of Drosophilidae, Sphaeroceridae, Muscidae and Fanniidae, all of which contain species that are known to feed on a wide variety of decaying materials (Brown, 2001). Being able to consume fungal spores as well as rotting fungal material provides a perfect habitat for larvae of insects with such adaptability. M. rufipes is a saprophage and can exploit a broad range of decaying materials including human cadavers (Disney, 2005), which suggests that their use of the fungal baits is likely to be opportunistic behaviour. M. rufipes may be active at times which typically occur outside of fungal fruiting periods and can overwinter both as an adult and as a pupa (Eisenschmidt, 1958; Herbert & Braun, 1958), which may explain why it was absent in baits between January and early March.

In the case of M. rufipes, specimens were reared from six species of wild fungi (Agaricus sylvaticus, Agaricus campestris, Calocybe gambosa, Coprinus micaceus, Macrolepiota rhacodes and Russula spp.) across this study. The use of Coprinus spp. (the ‘ink-caps’) was surprising as they usually deliquesce within a matter of hours, which suggests that this species can locate suitable food sources extremely quickly (Disney, 2005). Bradysia spp. were reared from fungal baits and three wild species (Auricularia auricula-judae, C. gambosa and Psathyrella spadiceogrisea). It is possible that many mycophagous insects are in the soil feeding on fungal mycelium and hyphae, and so it would be beneficial to collect soil samples in future studies during periods of low insect abundance (Sawahata et al., 2002). It may be that ‘generalist’ insects which can utilise multiple
fungal hosts are more likely to be capable of host tracking compared to ‘specialist’ insects, as suggested by our findings with *M. rufipes* and *Bradysia* spp.

Collection of wild fruiting bodies provided useful insights into how insect communities change over time. Mycetophilidae dominated in Huntersdale’s wild mushrooms and yet they were rarely reared from fungal baits; this highlights the importance of fungal host preference for mycophagous insects. It has been suggested that fungal chemical compounds play a role in insects host preference (Jakovlev, 2012; Leather et al., 2014) but season is also an important factor as a majority fungi tend to fruit at specific times, typically autumn and for fewer fungi, spring. The fact that fungal baits and wild mushrooms were present at the same time and produced differing insect communities suggests that fungal properties also shape associated mycophagous insect communities (Thorn et al., 2015).

Overall, this study has highlighted that fungal baits are useful for attracting and rearing a range of mycophagous insects which differ to wild fungi, but was inconclusive for whether mycophagous insects can track fungal hosts. Host tracking will likely be dependent on several different factors such as season, host preference, fungal fruiting period and age of fruiting body. The fact that *M. rufipes* and *Bradysia* spp. were collected from wild fungi and fungal baits at differing times is interesting and requires further investigation. Although this study is inconclusive about whether insects track fungal hosts, it is important to note that it cannot be ruled out. Furthermore, the two species mentioned seem to display behaviours and preferences

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**Fig. 3.** a – Total abundance per month of *M. rufipes* and *Bradysia* spp. reared from cultivated samples and wild samples. b – The average air temperatures (degrees) in Heston and Egham during the entire sampling period from November 2020 until the end of July 2021.
which would be suitable for host tracking (e.g., generalist saprophones which can exploit numerous food resources). The lack of Mycetophilids, which are strongly associated with fungi and thought to be predominantly mycophagous, collected from cultivated samples in this study, simply suggests that A. hisporus is unsuitable as a fungal host for this family, and so severely limited the number of insects that we could monitor and analyse for possible host tracking. We suggest that collaboration is needed between mycologists and entomologists to analyse the insect communities that occur in wild mushrooms fruiting “out of season”, to determine whether host tracking can occur. Periods of low insect activity also require attention as more information is needed on the general ecology of mycophagous insects. Fungal fruiting bodies should be seen as complex and diverse habitats for a range of organisms: their role as a food source is essential to many insects and is likely to play an important role in food webs especially in forest/woodland habitats. We suggest that naturally occurring, common species are used instead of A. hisporus. A. hisporus as a bait is simple and easy to grow but uncommon in woodland environments and seems to be avoided by most Mycetophilidae. Therefore, to test whether Mycetophilidae can track fungal hosts, an alternative cultivated fungus should be used which is naturally occurring and common in the wild, Pleurotus ostreatus may be a more suitable alternative for future studies.

AUTHOR CONTRIBUTIONS. The study was designed by all authors and executed and analysed by R.B. All authors contributed to writing of the manuscript.

ACKNOWLEDGEMENTS. Many thanks to C. Haokip, N. Morley and R. Prouse for their technical assistance. We are grateful to H. Disney, P. Chandler, B. Ferry, A. Polaszek and D. Sivell for their help with insect and fungal identifications and Merryhill Mushrooms Ltd for provision of mushroom growing kits.

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Table S1. Number of cultivated mushrooms collected and total weight from each location per month.

| Age | Date     | Huntersdale mushrooms | Heston mushrooms | Huntersdale weight (g) | Heston weight (g) |
|-----|----------|------------------------|------------------|------------------------|------------------|
| Young | 06/11/2020 | 6 | 6 | 364.57 | 440.53 |
| Young | 16/11/2020 | 29 | 25 | 827.84 | 850.65 |
| Young | 23/12/2020 | 30 | 33 | 795.76 | 769.65 |
| Young | 17/01/2021 | 39 | 30 | 883.21 | 988.32 |
| Young | 17/02/2021 | 36 | 22 | 915.34 | 887.32 |
| Young | 21/03/2021 | 26 | 22 | 604.69 | 539.56 |
| Old | 21/03/2021 | 30 | 28 | 701.24 | 621.65 |
| Young | 20/04/2021 | 41 | 33 | 740.53 | 849.63 |
| Old | 20/04/2021 | 32 | 24 | 211.05 | 217.87 |
| Young | 24/05/2021 | 22 | 13 | 670.68 | 640.2 |
| Old | 24/05/2021 | 31 | 27 | 234.43 | 213.28 |
| Young | 21/06/2021 | 13 | 14 | 620.04 | 564.75 |
| Old | 21/06/2021 | 26 | 14 | 247.74 | 195.63 |
| Young | 22/07/2021 | 31 | 19 | 223.71 | 235.98 |
| Old | 22/07/2021 | 11 | 26 | 131.67 | 142.46 |
| Total | 403 | 336 | 7672.47 | 7749.47 |

Table S2. Tree genera found in each location, ranked based on the most dominant trees in terms of abundance (most dominant = 1).

| Location | Tree | Tree genus | Rank |
|----------|------|------------|------|
| Huntersdale | Oak | Quercus | 1 |
| | Scots Pine | Pinus | 2 |
| | Birch | Betula | 3 |
| | Beech | Fagus | 4 |
| | Maple | Acer | 5 |
| | Holly | Ilex | 6 |
| Heston | Ash | Fraxinus | 1 |
| | Oak | Quercus | 2 |
| | Hawthorn | Crataegus | 3 |
| | Elm | Ulmus | 4 |
| | Birch | Betula | 5 |
| | Blackthorn | Prunus | 6 |
| | Holly | Ilex | 7 |
Fig. S1. a – Comparison of mean insect abundance between young and old cultivated samples between March 2021 and July 2021 from Heston. b – Comparison of mean insect abundance between young and old cultivated samples between March 2021 and July 2021 from Huntersdale. c – Comparison of mean number of insect species between young and old cultivated samples from March 2021 to July 2021 in Heston. d – Comparison of mean number of insect species between young and old cultivated samples from March 2021 to July 2021 in Huntersdale.
Table S3. Fungal genera and the abundance of fruiting bodies collected from both locations. For simplicity only the genus has been provided.

| Location  | Fungal genus | Abundance |
|-----------|--------------|-----------|
| Heston    | Mycena       | 59        |
|           | Coprinus     | 28        |
|           | Flammulina   | 28        |
|           | Auricularia  | 24        |
|           | Agaricus     | 21        |
|           | Rhodotus     | 16        |
|           | Clitocybe    | 15        |
|           | Lactarius    | 14        |
|           | Psathyrella  | 10        |
|           | Calocybe     | 10        |
|           | Parasola     | 8         |
|           | Marasmius    | 8         |
|           | Macrolepiota | 6         |
|           | Lepista      | 6         |
|           | Pleurotus    | 6         |
|           | Hypholoma    | 6         |
|           | Collybia     | 5         |
|           | Armillaria   | 5         |
|           | Cortinarius  | 5         |
|           | Tubaria      | 5         |
|           | Panaeolus    | 4         |
|           | Laccaria     | 4         |
|           | Volvariella  | 3         |
|           | Lentinellus  | 3         |
|           | Hygrocybe    | 3         |
|           | Tremella     | 3         |
|           | Lycoperdon   | 3         |
|           | Russula      | 3         |
|           | Polyporus    | 2         |
|           | Boletitus    | 2         |
|           | Geastrum     | 2         |
|           | Stropharia   | 1         |
|           | Postia       | 1         |
|           | Total        | 319       |
| Huntersdale| Tubaria      | 48        |
|           | Mycena       | 14        |
|           | Helbeloma    | 12        |
|           | Laccaria     | 10        |
|           | Lyophyllum   | 6         |
|           | Tricholoma   | 4         |
|           | Leptota      | 4         |
|           | Coprinus     | 3         |
|           | Hypholoma    | 3         |
|           | Clitocybe    | 3         |
|           | Parasola     | 3         |
|           | Russula      | 2         |
|           | Lycoperdon   | 2         |
|           | Phaeolepiota | 1         |
|           | Hygrophorus  | 1         |
|           | Macrolepiota | 1         |
|           | Formitopsis  | 1         |
|           | Total        | 128       |

Table S4. Insect species reared from wild fungi collected from Heston. Instances of absences of species name of both fungi and insects was due to an inability to identify the specimens to species level.

| Fungal species  | Insect family   | Insect species |
|-----------------|-----------------|----------------|
| Agaricus        | Braconidae      | Dinotrema spp. |
| Armillaria mellea| Mycetophilidae  | Exechia fusca  |
| Aureicularia auricula-judae| Mycetophilidae  | Brady sia spp. |
| Bolbitius titubans| Mycetophilidae | Allodia spp.   |
| Calocybe gambosa | A steiidae       | Leia bimaculata|
| Clitocybe       | Mycetophilidae  | Mycetophila fungorum |
| Collybia        | Bolitophilidae  | Bolitophila spp. |
| Coprinus micaceus| Phorinidae      | Megaselia ru pes |
| Cortinarius     | Mycetophilidae  | Exechia fusca   |
| Flammulina velutipes| Mycetophilidae | Tamania fenestralis |
| Hygrocybe nivea | Mycetophilidae  | Allodia spp.    |
| Lactarius       | Mycetophilidae  | Allodops rustic a |
| Leptista nuda   | Bolitophilidae  | Bolitophila spp. |
| Macrolepiota maccodes | Diapriidae  | Aclista spp.    |
| Mycena          | Mycetophilidae  | Exechia fusca   |
| Panaeolus acuminatus| Chironomidae   | Allodia spp.    |
| Parasola conopli s | Chironomidae    | Allodia spp.    |
| Pleurotus       | Sphaeroceridae  | Copromyza equina|
| Polyporus durus | Mycetophilidae  | Mycetophila fungorum |
| Psathyrella     | Mycetophilidae  | Exechia fusca   |
| Psathyrella     | Psudehexia chia trivittata |
| Psathyrella     | Psudehexia chia trisignata |
| Psathyrella     | Psudehexia chia spp.   |
| Psathyrella     | Allodia spp.      |
| Psathyrella     | M ycetophila fungorum |
| Rhodotus palmatus| Mycetophilidae   | Tamania fenestralis |
| Russula         | Mycetophilidae   | Mycetophila fungorum |
| Russula         | Phorinidae       | Megaselia ru pes |
| Russula         | Sphaeroceridae   | Spelobia spp.   |
Table S5. Insect species reared from wild fungi collected from Huntersdale. Instances of absences of species name of both fungi and insects was due to an inability to identify the specimens to species level.

| Fungal genus | Insect family | Insect species                          |
|--------------|---------------|----------------------------------------|
| Clitocybe    | Ichneumonidae | Oxytorinae                             |
| Collybia     | Mycetophilidae| *Exechia fusca*                         |
| Hebeloma     | Proctotrupidae|                                        |
| Laccaria     | Heleomyzidae  | *Suillia variegata*                     |
|              | Ichneumonidae | Oxytorinae                             |
|              | Mycetophilidae| *Exechia dorsalis*                      |
| Lepiota      | Mycetophilidae| *Exechia fusca*                         |
|              |               | *Allodia spp.*                         |
|              |               | *Docosia gilvipes*                      |
| Lyophyllum   | Heleomyzidae  | *Suillia spp.*                         |
|              | Drosophilidae | *Drosophila spp.*                      |
|              | Trichoceridae | *Trichocera spp.*                      |
| Mycenaria    | Mycetophilidae| *Exechia fusca*                         |
| Parasola     | Heleomyzidae  | *Suillia variegata*                     |
| Russula      | Mycetophilidae| *Mycetophila fungorum*                  |
|              |               | *Exechia fusca*                         |
|              |               | *Allodia lugens*                       |
|              |               | *Allodia spp.*                         |
|              |               | *Tarnania fenestralis*                  |
| Tubaria      | Mycetophilidae| *Mycetophila fungorum*                  |
|              |               | *Mycetophila ruficollis*                |