The potential of bird feeders to be used for ecological monitoring of vectors for *Phytophthora ramorum* in garden and woodland environments

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Matthew William Tosdevin

Abstract

Bird feeders were investigated for their use in determining whether birds are vectoring the tree disease *Phytophthora ramorum*, and whether feeders are acting as nodes of spore transfer. This was done by studying feeders at residences in Keele University and in Olderwood and Denham Woods, Devon. Keele borders a *P. ramorum* managed woodland and the Devon sites had past confirmations of the disease and ongoing control management. The feeders were tested for *P. ramorum* using swabs in combination with rapid diagnostic test kits, and no positive results were detected. Surveying the households produced data showing the potential use of feeders for ecological monitoring, including avian *Phytophthora* vectors. Questions included frequency of cleaning the feeders and 48% reported less than once per year. The range of birds reported were predominantly common passerine species and 76% reported regular use by Grey Squirrels. Observations and camera traps were used to study feeders in woodland environments. A total of 14 bird species were observed and 4 species of mammal. Based on visitation numbers, feeders took between 1.5-3 weeks to become established in these woodlands. Preferences for hanging feeders were found for Great Tit, Coal Tit, Blue Tit and Siskin, whereas Marsh/Willow Tit, Nuthatch and Robin showed preference for ground feeders. All the species seen on the feeders were resident species, although migrants were observed in the same environment. Few correlations were found between feeder visits and environmental variables, and unmeasured factors may have determined the differences observed in visitation rates across the study period. Camera traps recorded behaviour not captured by observations, indicating time preferences by species. Conversely observations provided information not gained through camera traps, displaying merit in a joint approach to monitoring feeders. The data collected is used to create recommendations for future study using feeders and avian *Phytophthora* dispersal.

Acknowledgements

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I am also grateful to Forestry England, especially to Ben Robinson who assisted in the identification of the two woodland study sites in Devon and supported my fieldwork on these sites, liaising with different stakeholders to ensure I could conduct my research. Ben also supplied background information on the sites along with Ben Phillips, and gave me use of one of their wildlife camera traps.

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1. Introduction

1.1 *Phytophthora Ramorum*

Phytophthora ramorum is a species in the class Oomycetes which causes foliar blights, shoot tip dieback, and bleeding cankers in different species of tree (Beakes & Sekimoto, 2009; Webber, 2008). The tree pathogen was formally named in 2001, but had already been introduced into the USA and Europe by the 1990's (Frankel & Palmieri, 2014; Kliejunas, 2010). The disease was named 'sudden oak death' in the US after killing large numbers of Oaks in California, but in the United Kingdom and Europe it has become better known for causing high mortality in Larch (*Larix*) species since 2009 (Brasier & Webber, 2010; Kliejunas, 2010). The disease is mainly prevalent on the West coast of the United Kingdom, where the weather is warmer and wetter, and has particularly devastated Devon and Cornwall in England where the disease is thought to originate in Great Britain (Forestry Commission, 2018a). The policy of removing infected Larch and Rhododendron in the UK has had limited effects of reducing the presence of the pathogen, however, new hosts are becoming more significant for its propagation such as Sweet Chestnut (*Castanea sativa*) (Harris & Webber, 2016). Prior to the spread of *Phytophthora* to Larch, the species occupied an estimated 154,000 hectares of woodland in Great Britain, and Sweet Chestnut a further 29,000 hectares (Forest Research, 2020). With the greater spread to new sporting hosts and the threat of climate change increasing the warmer, wetter winter conditions in which *P. ramorum* thrives, there is greater concern for ecologically important species. Sweet Chestnut themselves have many veteran trees, and there is concern for Bilberry (*Vaccinium myrtillus*) and Heather (*Calluna vulgaris*) which currently only have limited infections besides the concern for the spread to previously unaffected trees (Forestry Commission, 2018a).

Phytophthora ramorum have three types of spore, as do the other species in the genus. They have zoospores, capable of taxis to find new hosts, as well as two types of long-lived spore, the chlamydospore and the oospore (Ribeiro, 2013). Although the zoospores are short-lived, they are dispersed in large quantities in rainfall and can travel large distances in watercourses (Johnson, 2010; Davidson et al, 2005). Rainsplash can directly lead the spore to new hosts, and their flagella enable taxis for short distances of a matter of centimetres (Hayden *et al,* 2013). For *Phytophthora ramorum*, the main sporting host in *Rhododendron* in the UK and Europe, with large quantities of zoospores produced in the leaves (Harris & Webber, 2016). Infected Larch produces larger quantities of these spores, but the infection eventually leads to mortality in these trees as opposed to *Rhododendron* which survives infection. The chlamydospore and oospore are resting spores capable of surviving much longer than the zoospores as well as through adverse conditions, the main difference between the two being that chlamydospores are asexual and oospores are sexual (Crone *et al*, 2013; Hayden *et al*, 2013).

1.2 Avian Vectors

Apart from abiotic aerial and water dispersal, there are many biotic methods of *Phytophthora* spore dispersal theorised and researched. This includes human vectors, transporting infected material on boots and vehicles as well as through the trade in wood, soil, and nursery raised trees (Brasier, 2008; Cushman *et al*, 2008; Hansen *et al*, 2000). Insects have been found to be able to transport *Phytophthora* spores on their exoskeleton and even *P. ramorum* spores through their excrement (Hyder *et al*, 2009; Konam & Guest, 2004). There is less evidence of vertebrate vectors, although there is some for wild pigs (*Sus scrofa*), but it has been often theorised (Li *et al*, 2014). This is true for avian species, which are often seen as some of the most effective vectors of diseases due in part to the large distances that they can cover (Viana *et al*, 2016). Birds have been implicated in spore movement in the same way as humans, by transporting infected material, as well as through excrement for some *Phytophthora* species and through collecting spores from rain splash on their feathers before transporting them elsewhere, but there is still a limited body of evidence to determine the role of birds in the dispersal of *P. ramorum* or other *Phytophthora* species (Dadam *et al*, 2012; Coleman & Clark, 2010).

1.3 Supplementary Bird Feeders

If birds are a possible vector of *Phytophthora ramorum*, then a non-invasive way to determine this would be to investigate the places which they frequent for spore deposition or transfer. In the UK, the Winter Garden Bird Feeding Survey of winter 2016/17 recorded over 230,000 individual birds from 75 different species in just 241 gardens (Boothby, 2017). This suggests that bird feeders could be used to study the possibility that birds are transporting phytopathogens due to the large numbers and the diversity of species that visit these sites; approximately 48% of British households provide supplementary food for birds, and so the potential area for feeders to act as nodes of spread is significant (Davies *et al.*, 2009). Furthermore, bird feeders have been implicated in the transmission of avian diseases, such as the bacterium *Mycoplasma gallisepticum* (Adelman *et al*, 2015). These figures of garden feeder use also indicate how they could be used as a widespread ecological monitoring tool, and they are already monitored to some degree by residents.

1.4 Aims and Objectives

This study and thesis sets out to explore how supplementary wild bird feeders may be used in both residential environments and natural woodlands to investigate whether birds may be vectoring *Phytophthora ramorum* and transferring spores. The aims and objectives for the study are set out below, with how each is to be achieved denoted by roman numeral;

1. To examine whether supplementary bird feeders used in natural or semi-natural environments can be used to study woodland bird populations
    a. Identify which species will use feeders in woodland environments
        i. Regular observations
        ii. Identification of which type feeder is visited, ground or hanging
    b. Assess how long it takes for feeders placed in woodland environments to establish
        i. Regular observations
        ii. Regular measurements on how much food is being eaten
    c. Assess how frequently feeders are used by birds in woodland environments
        i. Regular observations
        ii. Regular measurements of how much food is being eaten
    d. Identify any other animals that might be using the feeders (i.e. mammals)
        i. Regular observations
        ii. Camera traps
    e. Identify reasons for variations in objectives 1a to 1d
        i. Stem maps to compare environment differences
        ii. Digital photographs to compare canopy cover differences
        iii. Identify weather events that could cause differences
    f. Compare bird species seen in woodland environments to those in garden environments
        i. Residents survey
        ii. Regular observation of woodland feeders
2. Assess whether birds could be transferring spores of the tree pathogen *Phytophthora ramorum* on bird feeders
    a. Test residents bird feeders proximate to woodland with potentially infected material
        i. Door knocking residents of Keele University campus and taking swab samples
        ii. Residents survey to gain information on how much time spores would have to build up (before the feeder is cleaned)

b. Test feeders in a *P. ramorum* site for proof of concept
    i. Establish feeders in *P. ramorum* affected woodland
    ii. Test feeders after allowing time for spore build up using swab samples

1.5 Study Locations

A residential site in Keele, Staffordshire, near to a *P. ramorum* managed woodland was investigated using a door to door survey and with swab samples taken from their feeders in combination with rapid diagnostic *Phytophthora* test kits. Two woodland sites were then chosen in Devon to investigate the use of supplementary feeders in natural woodlands; Denham Woods and Olderwood. These sites are both under active management to control *Phytophthora ramorum*. Bird feeders were placed and maintained over a period of 9 weeks between March to May 2019, with swabs taken from the feeders after week 5 and week 9 in combination with rapid diagnostic *Phytophthora* tests, as well as using these tests on the leaf litter in week 9.

Before investigating the bird feeders of Keele residences, a preliminary study of *Phytophthora* in the woodlands at Keele University was conducted. Although an actively managed site for *P. ramorum* since 2013, using pocket diagnostic test kits on *Rhododendron* and leaf litter from the most affected zones produced no positive results. Other sites in North Staffordshire proximate to Stoke-on-Trent that have also had infected *Rhododendron* and Larch were investigated but similarly had no positive results. However, Keele was still determined to be a suitable site for studying residential bird feeders. Denham woods and Olderwood in Devon were chosen to install bird feeders after a desktop investigation found them to be suitable sites which had confirmed *Phytophthora ramorum* within 18 months of the study. Both areas are owned and managed by the Forestry Commission but are different in character and with different stakeholders.

2 - Literature Review

2.1 Introduction

Phytophthora is a genus in the class Oomycetes, which are fungus-like heterotrophs that are saprophytic or parasitic to a diverse range of organisms (Beakes & Sekimoto, 2009). *Phytophthora,* like many Oomycetes, are the cause behind numerous diseases in plants, indicated by the Greek translation of *Phytophthora* meaning 'plant destroyer' (Ribeiro, 2013). In keeping with this reputation, the genus has a notorious reputation in global agriculture and forestry, as it can seriously damage crops and trees; *Phytophthora sojae* alone has been estimated to cause US$1-2billion worth of loses to soybean (*Glycine max*) crops annually (Tyler, 2007). The effects of Phytophthora depend on the species, but may include root rot, bleeding cankers, foliar blight, and often mortality (Hayden et al, 2013).

Phytophthora are similar to fungi in many ways, and when the genus was discovered, it was not identified as a separate kingdom. There are distinct differences that are reflected by different classifications, although between the Phylum and the Class, it is sometimes sub grouped as a Pseudofungi (Beakes & Sekimoto, 2009). The taxonomy is shown below (Kliejunas, 2010):

Domain: Eukaryota
Kingdom: Stramenopila (Chromista)
Phylum: Heterokontophyta
Class: Oomycetes
Order: Peronosporales
Family: Pythiaceae
Genus: Phytophthora

The discovery and study of *Phytophthora* was an important turning point for plant pathology, as pre 19th century it was not believed that fungus caused plant disease, but was instead purely symptomatic (Ribeiro, 2013; Turner, 2005). Its discovery was catalysed by the infamous Irish potato famine of 1845-1846 that led to the loss of a quarter of Ireland's population through death and immigration (Ribeiro, 2013). The cause of this was Potato Blight, which is an infestation of *Phytophthora infestans* (originally named *Botrytis infestans*) discovered and first described by Reverend Berkeley in the mid 19th century. The genus was given its current name in 1876, and since then new *Phytophthora* species have been discovered on an almost yearly basis (Hayden *et al,* 2013; Turner, 2005). However, *P. infestans* remains a prevalent scourge of potato crops (*Solanum tuberosum*) as well as tomatoes (*Solanum lycopersicum*), with estimated costs of losses and control measures varying in the region of US$3-6 billion every year (Nowicki *et al*, 2013; van West *et al*, 2003).

2.2 The Impact & Distribution of *Phytophthora*

2.2.1 *Phytophthora* in Agriculture

Over 100 species are now known, but it has been estimated that there could be over 340 left to be discovered, with a maximum estimate of over 500 more (Brasier, 2009). This means that, with the known species so far, the number of host-pathogen associations for *Phytophthora* is estimated to be in the region of 4,400 (Scott *et al*, 2013). Many of these are of great concern to agriculture, such as *P. sojae* aforementioned, as well as *Phytophthora nicotianae* and *Phytophthora capsici* to name just a few. *Phytophthora nicotianae* infects a large number of species, despite its tobacco related name which alludes to its discovery on the plant. It is considered one of the most devastating worldwide species, as the number of hosts it can infect is estimated to be over 255 and can be found across five continents; it is therefore sometimes known as *Phytophthora parasitica* (Panabieres *et al*, 2016). *Phytophthora capsici* is a pathogen that is becoming more severe, and infects a variety of vegetables, such as all cucurbits, peppers (*Capsicum* spp.), and lima beans (*Phaseolus lunatus*) on continents all over the world, similar to *P. nicotianae* (Lamour *et al*, 2012). Plantations are also affected by *Phytophthora* species, with, for example, black pod disease of cocoa (*Theobroma cacao*). This disease is thought to be responsible for global yield losses of 20-30%, as well as 10% mortality from further infestation, costing hundreds of millions US dollars worldwide every year (Guest, 2007; Bowers *et al*, 2001). Black pod disease is caused by a number of *Phytophthora* species but most notably *Phytophthora megakarya* and *Phytophthora palmivora*, the latter of which is also a significant pathogen for numerous other plantation crops such as rubber (*Hevea brasiliensis*), coconut (*Cocos nucifera*), and continually leads to outbreaks in oil palms (*Elaeis* spp.) (Torres *et al*, 2016; Guest, 2007).

2.2.2 *Phytophthora* in Forestry

Hayden *et al* (2013) identify the *Phytophthora* species *cinnamomi, ramorum, alni, cactorum, cambivora, citricola, kernoviae, lateralis, palmivora, pinifolia* as some of the most notable to forestry. These species have various distributions across the globe, and affect an extensive variety of trees (Hayden *et al,* 2013). *P. cinnamomi* serves as a good example of the damage *Phytophthora* can do to forests. It is commonly a root rot pathogen with a large host list numbering over 900, and has severe impacts on American Chestnut (*Castanea dentata*), Sweet Chestnut (*Castanea sativa*), European Oak (*Quercus robur*), Avocado (*Persea americana*) and Walnut (*Juglans* spp.) in South America (Sena *et al*, 2018; van West *et al*, 2003). In Australia it has caused severe damage to *Eucalyptus* forests, in particular the Jarrah species (*Eucalyptus marginata*) (van West *et al*, 2003). It is estimated to be infesting over 1 million ha in Victoria and Tasmania alone, with tens of thousands more in South Australia (Commonwealth of Australia, 2014). Jarrah showed 52% cumulative mortality where the disease was present, and as this Eucalyptus can represent 68% of these forests, this has had a significant impact on the ecosystem (Sena *et al*, 2018). Additionally, *Banksia* species have shown 92% mortality in some areas (Sena *et al*, 2018).

2.2.3 *Phytophthora* and UK Woodlands

Of particular interest to UK forestry and trees, as identified by the Forestry Commission (2018a), are *Phytophthora alni*, *Phytophthora austrocedri, Phytophthora lateralis, Phytophthora kernoviae,* and *Phytophthora ramorum*. *Phytophthora alni* is a species estimated to infect 20% of Alders (*Alnus glutinosa*) in the UK, impacting damp woodlands where Alder is prevalent since the mid 1990s (Forest Research, 2018). *Phytophthora austrocedri* is an even more recent introduction. First found in the UK in 2011, it is infesting the UK's native Junipers (*Juniperus communis*), a particularly vulnerable tree that has already been in decline (Forestry Commission, 2018b). *Phytophthora lateralis* is predominantly a concern for ornamental gardens, infecting Lawson's Cypress (*Chamaecyparis lawsoniana*) thus far mainly in Scotland and Northern Ireland in Britain. However, as another recent introduction into the UK, it could further cause problems to forestry if the so far limited infection of Western Red Cedar (*Thuja plicata*) expands (Forestry Commission, 2018c).

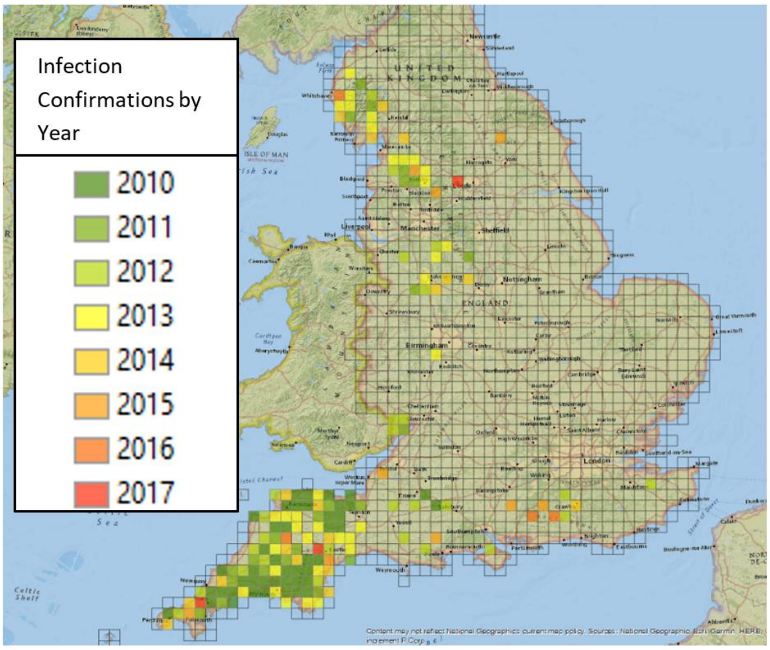
2.2.4 *Phytophthora ramorum* & *kernoviae*

Phytophthora ramorum and *Phytophthora kernoviae* are both pathogens that cause foliar blights, shoot tip dieback and bleeding cankers, sharing numerous hosts and symptoms (Webber, 2008). Hosts afflicted by foliar and shoot tip dieback do not typically result in mortality, but these are the plants in which *Phytophthora* produces reproductive spores (Denman *et al*, 2008). For *P. ramorum* and *P. kernoviae*, the most significant foliar hosts are *Rhododendron* species, in particular *Rhododendron ponticum*. Hosts that produce bleeding cankers, such as Beech (*Fagus sylvatica*) typically succumb to mortality as these infections lead to phloem necrosis and blockage of the xylem from abundance of tyloses, reducing sap and water flow (Collins *et al*, 2009; Denman *et al*, 2008). No spores are produced in the cankers, and foliar symptoms are not typical of tree species that are infected in this way, although as leaves are restricted of water and nutrients, they die along with infected branches (Webber *et al*, 2010; Collins *et al*, 2009). There are some exceptions to this, such as Japanese Larch (*Larix kaempferi*), which show bleeding sap, twig and branch necrosis, and eventual death, but are also known to produce large quantities of spores (Harris & Webber, 2016; Webber *et al*, 2010).

2.2.5 Distribution in the UK

Phytophthora ramorum was first detected in the UK in 2002, shortly after the discovery of the pathogen in Europe after attention from sudden oak death in the United States of America (Kliejunas, 2010; Lane *et al*, 2003). *Phytophthora kernoviae* was subsequently identified in 2003 at a site suspected of being infested by *P. ramorum* (Brasier *et al*, 2005). This was the first time *P. kernoviae* had been described, but it has since been found in New Zealand (Webber, 2008). Both of these species were introduced in the South West of England, yet *P. kernoviae* has not spread to a great extent outside of this region in the UK as shown in figures 2.1a and 2.1b (DEFRA, 2014). From figure 2.1a however, it can be seen that *P. ramorum* has spread throughout England, as well as the rest of the Britain, primarily on the Western coast where the climate tends to be warmer and wetter which optimally suits infection (Forestry Commission, 2018a; DEFRA, 2014). Under the changing climatic conditions that are predicted in the UK, as well as globally, over the next 50-100 years, there could be implications for *Phytophthora* distribution. For example, with milder winters that are predicted, *Phytophthora* species including *P. ramorum* and *P. kernoviae* could increase their range, perhaps encroaching eastward. With projected increases in rainfall, sporation might increase too, as wet conditions can stimulate sporulation (Rey *et al*, 2010; Davidson *et al*, 2005).

a



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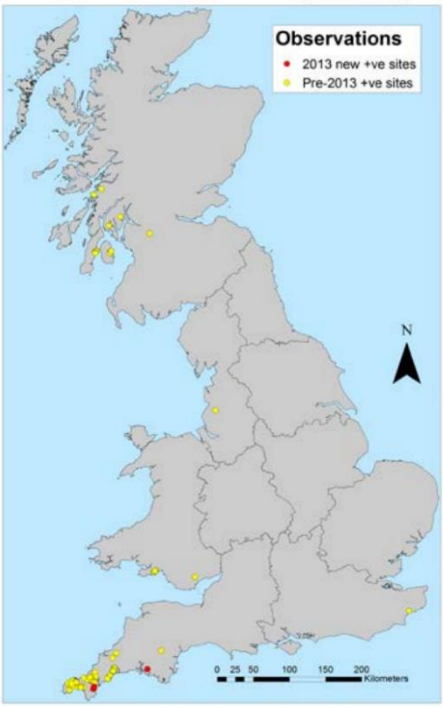


Figure 2.1a: Map of confirmed *P. ramorum* infections in England, also showing the year in which the pathogen was found (Forestry Commission, 2018d).

Figure 2.1b: Map of *P. kernoviae* observations taken as of 2014, showing pre 2013 infection sites in yellow and 2013-2014 infection sites in red (Defra, 2014).

When the pathogen was first detected in the United Kingdom, it was observed predominantly on ornamental shrubs such as on *Rhododendron* and *Viburnum* species but was not found to be a serious concern to wild trees or forestry (Harris & Webber, 2016; Lane *et al*, 2003). Most native or naturalised diseased trees were found in close proximity to infected *Rhododendron*, which produce a large number of spores and are consequently considered target species for containing the pathogen in the UK (Coleman & Clark, 2010). However, in 2009 *P. ramorum* was discovered on Japanese Larch, a tree grown across the UK for timber. This was the first time *P. ramorum* was found infecting a commercial species anywhere in the world, as well as the first widespread infection of a coniferous species by the pathogen (Brasier & Webber, 2010). European Larch and Hybrid Larch (*Larix decidua, L. x eurolepis*) are also very susceptible. Before the outbreak in 2009, Larch was estimated to occupy 154,000 hectares in Great Britain, or 5% of the total woodland area, and since then most of this has been lost and the species is no longer planted for timber (Forest research, 2020; WoodlandTrust, undated). In addition, all of these species found to produce spores in greater quantities than other hosts including *Rhododendron ponticum* and Bay Laurel (*Laurus nobilis*), the latter being the primary sporating host propagating sudden oak death in Western USA (Harris & Webber, 2016). Many of the outbreaks in Larch have been atypical in that they have not been in proximity to *Rhododendron*, and so there is the possibility that the spread has been enabled by a vector, although there is much uncertainty about this (Coleman & Clark, 2010). These new sporating hosts increased the risk (and observation) of the pathogen spreading to other tree species, such as Beech, Birch (*Betula pendula* & *B. pubescens*), Douglas Fir (*Pseudotsuga menziesii*), and Sweet Chestnut, leading to a policy of felling Larch from infected and high risk areas (DEFRA, 2014; Brasier & Webber, 2010).

2.2.6 Impact of *Phytophthora ramorum*

In 2014, Larch represented 4.5% of the UK's woodlands, with an estimated social and environmental value of £80 million, and a commercial value of £60 million per year to the UK (DEFRA, 2014). This demonstrates the magnitude of the social and economic consequences of *Phytophthora ramorum*. The policy of Larch and *Rhododendron* removal has had limited effects on eliminating the pathogen’s presence however, as new hosts are becoming more significant for the propagation of the pathogen; for example, Sweet Chestnut trees where they are dominant (Harris & Webber, 2016). Sweet Chestnut occupies about 29,000 hectares of woodland in the country, but unlike Larch includes many veteran trees with higher ecological and social value (Forest Research 2020). The impact and loss of these trees has implications for wildlife, but an even greater concern for the wider environment is the infection of Bilberry (*Vaccinium myrtillus*) and Heather (*Calluna vulgaris*) in heathland which, though currently limited, could become a more serious threat to conservation as they are integral to their respective ecosystems (Forestry Commission, 2018a). Drake & Jones (2017) estimate the total public value at risk from the spread of *P. ramorum* and *P. kernoviae* to be £1.446 billion per year in the UK, and the pathogens remain a threat to UK wildlife and ecosystems (Brasier & Webber, 2010). However, *P. ramorum* has led to a strategy of removing the invasive *Rhododendron* from areas that are at risk of, or already have, the pathogen. This could have some benefits, as *Rhododendron* species left unmanaged can outcompete and shade out native plants while offering little in return to wildlife due to their inedibility to UK fauna. Whatsmore, *Rhododendron* can display allelopathy, further damaging native plants and ecosystems. Their removal from woodlands could lead to recovery of natural species and increase biodiversity (Maclean *et al*, 2017). This could only occur if these areas are given an extended period of time to recover after invasion, as the seed banks of woodlands with *Rhododendron* are reduced in diversity and are still susceptible to reinvasion (Maclean *et al*, 2018).

Phytophthora ramorum is also having large economic consequences in the United States. Some of these are indirect, for example it is expected to reduce the value of property in California by US$135 million in total between 2010-2020 from the presence of dying oak trees (Kovacs *et al*, 2011). There are also some impacts in the USA that are not presently a great concern in the UK, such as increased fire risk. This is because of more dead wood and litter providing fuel in an already fire prone region (Frankel & Palmieri, 2014). With UK temperatures set to increase under climate change, it is predicted that drier summer weather patterns are likely to increase the risk of forest fires, and *P. ramorum* could further compound this as it has done in the Western USA (Rey *et al*, 2010). In California and Oregon, over 5 million trees were killed by the pathogen by 2014 and the infection has spread further since then, despite ongoing control measures (Frankel & Palmieri, 2014). Although the effects of *P. ramorum* were first felt in the USA, the pathogen shows distinct genetic differences, indicating that they were introduced to Europe and America separately from an exotic origin (Van Poucke *et al*, 2012; Kliejunas, 2010). Four lineages have been identified; NA1 and NA2 named for their discovery and prevalence in the USA, and EU1 and EU2 in Europe. Identification of the different lineages has been useful in tracking the pathogen's spread; for example, EU1 has now been found in North American nurseries, likely introduced since the outbreak in Europe. It also shows different origins of the pathogen, whereby each lineage comes from a different but near country, thought to be in the Far East (Van Poucke *et al*, 2012). These lineages can behave differently, with EU1, the strain found in the UK in 2002, described as the most aggressive and best at propagating (Brasier*, et al*, 2006). EU2 was the latest to be discovered, and is less aggressive than EU1, only being prevalent in Scotland and Northern Ireland (Van Poucke *et al*, 2012).

2.3 *Phytophthora* Biology

2.3.1 Structural Biology

Phytophthora is an Oomycete, and displays a similar filament structure as fungi, with hyphae (filament-like structures) collectively forming a mycelium (Ribeiro, 2013; Hayden *et al,* 2013; Ristaino & Gumpertz, 2000; Latijnhouwers *et al*, 2003). These similarities lead to their being thought of as the same when first discovered, however, in other aspects they differ greatly; comparing these similarities and differences to fungi remains useful in describing their physiology. Firstly, the hypha cell walls in fungi are largely made of chitin, whereas in oomycetes the main component is cellulose. This means the phylogeny of oomycetes has them more closely related to plants, unlike fungi that share a closer ancestor to animals (Hayden *et al,* 2013; Rossman & Palm, 2006; Latijnhouwers *et al*, 2003). In addition, the mycelium of oomycetes, including *Phytophthora*, tend to be coenocytic, which means that there are no cross walls between the hyphae cells (Rossman & Palm, 2006; Latijnhouwers & Govers, 2003). Furthermore, oomycetes have diploid nuclei, whereas fungi are usually haploid (Rossman & Palm, 2006; Latijnhouwers *et al*, 2003).

2.3.2 Sporangia

Phytophthora, other oomycetes and fungi produce spores for reproduction, although there are large differences between them. Compared with fungi, *Phytophthora's* survival as a saprophyte is limited, meaning *Phytophthora* produces reproductive spores as the plant declines in health (Judelson & Blanco, 2005). *Phytophthora* forms sporangia, zoospores, chlamydospores, and oospores for reproduction, shown in figure 2.2, and are important structures to understand the pathology of the oomycete. Sporangia are multinucleate bodies produced on specialised hyphae called sporangiophores and in certain species (known as caducous), sporangia can readily detach in wind or rain and can be carried in water (Judelson & Blanco, 2005; Latijnhouwers & Govers, 2003; Davidson *et al*, 2002). From sporangia, unicellular zoospores are formed through the process of cytoplasmic cleavage and are released through sporangial papilla by turgor pressure, illustrated in figure 2.3 (Gisi *et al*, 1979). These zoospores are able to find and infect new hosts, or they can be transported abiotically to hosts as unicellular bodies or as sporangia ready to be released.

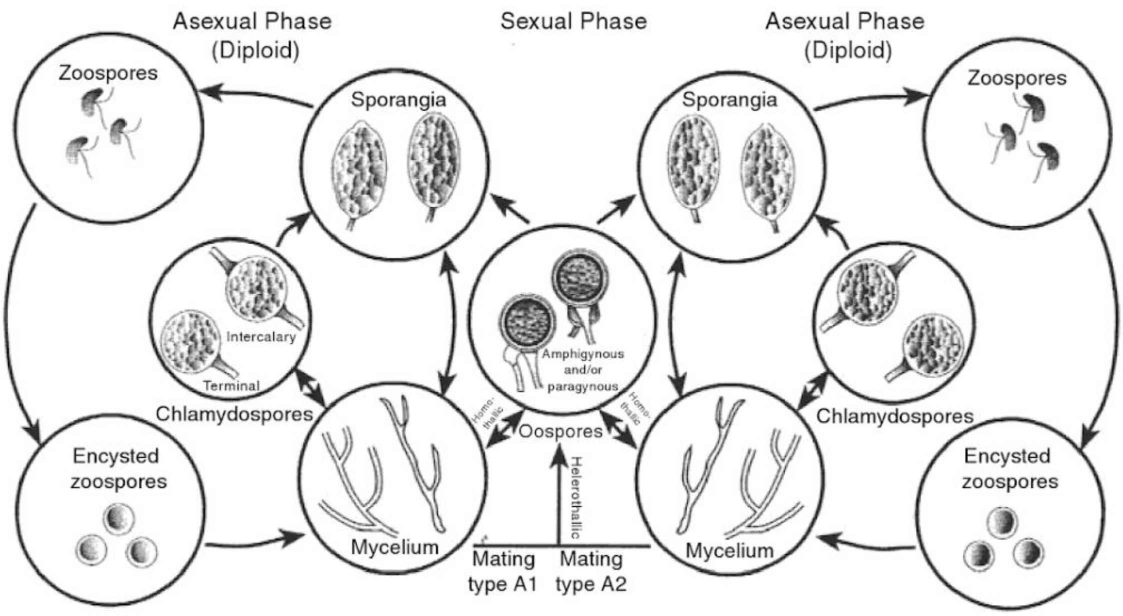


Figure 2.2: The typical life cycle of *Phytophthora* species, showing the asexual pathway of chlamydospores and sporangia, as well as the sexual pathway producing oospores for heterothallic or homothallic species. Different gameate structures are shown in the oospore illustration (Ribeiro, 2013).

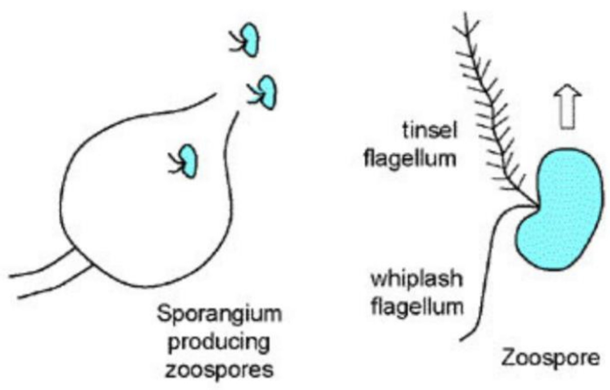


Figure 2.3: Representation of sporangia releasing zoospores, and the basic structure of a zoospore (Rossman Palm, 2006).

2.3.3 Zoospores

Zoospores are motile, unicellular, asexual spores. They are short-lived with no cell wall (only a membrane) and have two flagella that are used to move to find new hosts, as shown in figure 2.3 (Latijnhouwers *et al*, 2003; Hardham, 2001). In comparison, fungal spores are mostly dispersed by the wind and the few that produce motile spores do so with only one flagellum (Hayden *et al,* 2013; Rossman & Palm, 2006; Hardham, 2001). The tinsel flagellum of *Phytophthora* is covered in tiny hair-like structures and is used for pulling the cell, and the other posterior, whiplash flagellum is used for steering (Walker & van West, 2007; Judelson & Blanco, 2005). They can only move short distances of millimeters to centimeters with these flagella, but they can be carried in water for many kilometres (Hayden *et al,* 2013; Kinal *et al*, 1993). An essential factor is the presence of water, which is not only an important distributor of spores but also a requirement for zoospores to move in and to survive (Walker & van West, 2007; Judelson & Blanco, 2005). There are several theories as to how zoospores find their host, but it is thought they use a combination of chemotaxis, electrotaxis, and autotaxis or autoaggregation (Walker & van West, 2007; Judelson & Blanco, 2005; Hardham, 2001). Taxis is an important consideration for the dispersal of the disease, as it could mean that spores do not necessarily have to be transported directly onto a weak point of the plant to cause infection.

2.3.4 Taxis

Chemotaxis is significant once the zoospores are released from sporangia, whereby they can follow concentration gradients of a range of chemicals in the soil to new hosts (Khew & Zentmyer, 1973). This can be non-host specific, such as any amino acid, but there are many examples where *Phytophthora* species might find their specific host (Judelson & Blanco, 2005). An example of this is *P. sojae* which can follow chemicals such as isoflavones produced by their soybean host plant (Hosseini *et al*, 2014; Morris & Ward, 1992). These chemicals are naturally exuded by plants; in the case of legumes such as soybean, some of these chemoattractants are produced to attract the rhizobia with which it forms a symbiosis. *Phytophthoras* that have a specific host relationship with legumes, such as *P. sojae*, are able to utilise this chemical attractant, displaying how certain *Phytophthora* species have evolved to find the specific host that they survive on (Hosseini *et al*, 2014; Hassan & Mathesius, 2012). Electrotaxis, on the other hand, operates on the attraction from ion exchange around the root surface creating an electrical field, with some evidence that this overrides chemotaxis as zoospores near the root (Judelson & Blanco, 2005; Van West *et al*, 2002). These electrical and chemical attractants are able to not only direct zoospores towards hosts, but also settle on favourable locations. For example, chemotaxis can lead zoospores to anticlinal wall junctions (where two cells meet that are perpendicular to the surface) of roots, which is thought to aid in successful infection (Hardham, 2001). Electrotaxis appears to have more relevance for root infection pathways as this is the location of ion exchange, but chemotaxis could play an important role for directing zoospores to optimal sites for infecting bark and stomata. However, there is some evidence of chemical attractants leading to *Phytophthora cinnamomi* zoospore taxis towards regions of auxiliary shoot emergence and of thin or discontinuous periderm too (O'gara *et al*, 2015).

Zoospores can also locate potential hosts through the process of autotaxis, or autoaggregation, whereby zoospores have some level of attraction to each other which aids them in finding hosts (van de Mortel *et al*, 2009; Judelson & Blanco, 2005). Calcium is an element thought to be involved in this, perhaps being secreted by zoospores upon encystment, and pH is also believed to have an influence (von Broembsen & Deacon, 1996). Autotaxis seems to have some level of species specificity with *P. palmivora* not attracting zoospores of *Pythium* species (a similar oomycete) (Reid *et al*, 1995).

2.3.5 Encystment and Germination

Once zoospores or sporangia reach a new host, chemical or physical stimulus causes encystment. This is a process by which the zoospores lose their flagella, form a cell wall, and an adhesive material is discharged from vesicles within the zoospores (Hardham, 2001; Hardham, & Hyde, 1997; Sing & Bartnicki-Garcia, 1975). This settlement and encystment is extremely rapid, and can take place within minutes (van West *et al*, 2003). Figure 2.2 outlines these steps of infection from sporangia production, to zoospore genesis, to encystment. Encystment is thought to be stimulated by pressure, electrochemical charge, and chemical signalling, including Ca2+ and phosphatidic acid (Hayden *et al*, 2013; Walker & van West, 2007). Unlike cysts of numerous non-oomycete organisms, these do not function as survival structures, but germinate almost immediately, as quickly as within 20-30 minutes (Judelson & Blanco, 2005; Harham, 2001).

Germination and subsequent infection can be achieved directly through the growth of a germ tube, that can enter through openings such as the stomata, or from the use of an appressorium that creates an opening for *Phytophthora* to infect the host, as shown in figure 2.4 (Hayden *et al*, 2013; Judelson & Blanco, 2005; Harham, 2001). For root infection pathways, the orientation of the zoospore upon encystment have been shown to be of importance to the emergence of the germ tube and subsequently successful infiltration of the host. This demonstrates how the taxis mechanisms are of vital importance to the propagation of *Phytophthora* (Hardham & Gubler, 1990). An appressorium, or appressorium-like swelling, from *Phytophthora* is required to penetrate periclinal walls of root epidermal cells, cuticle-covered leaf or stem epidermal cells (Harham, 2001). This is done by mechanical pressure, with penetration pegs, or cell wall degrading enzymes (Lebeda *et al*, 2008; Latijnhouwers *et al*, 2003). This can take place within an hour of encystment, requiring only a matter of hours from initial encystment to penetrate the host, as little as two hours in some cases (Kebdani *et al*, 2010; Harham, 2001). Once the host has been penetrated, *Phytophthora* can develop hypha in the living plant tissue creating a mycelium mesh using haustoria to acquire the host's nutrients (van West *et al*, 2003; Hohl & Suter, 1976). This is also shown in figure 2.4, as well as the necrosis of the plant from the point of infection as the *Phytophthora's* haustoria kill the cells by depleting their nutrients. From the mycelium, sporangia can then be produced as quickly as 48 hours after initial infection, starting the infection cycle again (van West *et al*, 2003).

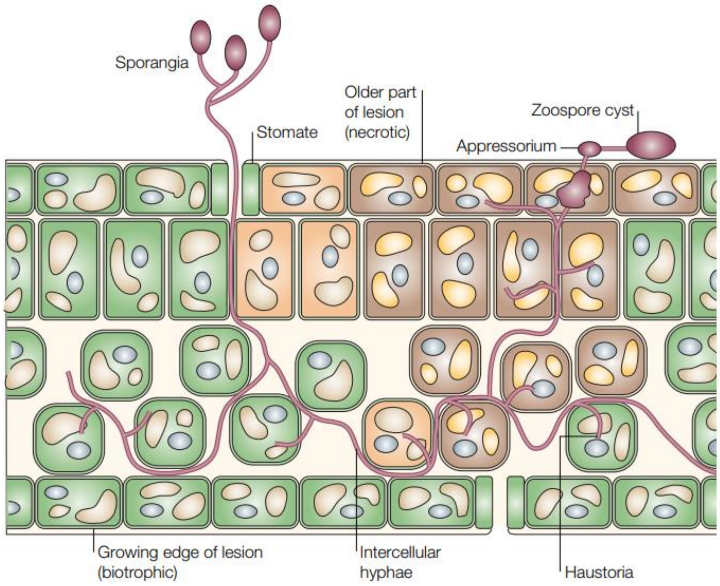


Figure 2.4: Infection course of *Phytophthora infestans*, showing how an aerial species infects a host, draws nutrients from the plant cells causing necrosis, and produces new reproductive sporangia on the outside of the host (Judelson & Blanco, 2005).

2.3.6 Resting Spores

Chlamydospores and oospores are both resting spores that do not move with thick cell walls (Hayden *et al*, 2013; McCarren *et al*, 2005). They have some structural differences, but the main differentiation is that chlamydospores are asexual and oospores are sexual, shown in figure 2.2 as different pathways (Hayden *et al*, 2013). Chlamydospores are produced in the mycelium within the infected host, unlike sporangia which tend to be produced outside of the host to be dispersed, illustrated in figure 2.4 (Kliejunas, 2010; Judelson & Blanco, 2005). Chlamydospores' thick cell walls that help them survive unfavourable conditions, including host death, functioning as survival structures ready to infect new hosts when conditions improve (Puértolas, *et al*, 2018; Kamoun *et al*, 2015; Mircetich & Zentmyer, 1967). For example, *P. cinnamomi* chlamydospores can survive the dry, hot summers in the Mediterranean that could kill the pathogen and the host until plant life recovers for infection (Crone *et al*, 2013). The consensus on how long chlamydospores can survive is varied both within and between species, but is usually in the region of months, although up to a year and beyond has been recorded, unlike zoospores which can generally only survive a matter of days (Puértolas, *et al*, 2018; Collins *et al*, 2012; Shishkoff, 2007; Weste & Vithanage, 1979; Hwang & Ko, 1978). However, saprotrophically surviving mycelium in the soil in combination with chlamydospores may be able to survive in the soil for several years (Hayden *et al*, 2013; Zentmyer & Mircetich, 1966).

2.3.7 Sexual Reproduction

Oospores are similar to chlamydospores as survival structures, but often have thicker walls, meaning an even greater ability to persist in the soil (Hayden *et al*, 2013). In addition and aforementioned, oospores are sexual. Oospore production is a result of the combination of gametes, whereby the male gamete, antheridium, empties some of its contents into the female oogonium, whereupon the DNA combines and subsequently the oospore is developed (Judelson & Blanco, 2005; van West *et al*, 2003). Figure 2.2 contains illustrations of the combined gametes, and also shows ‘heterothallic’ and ‘homothallic’ pathways. Heterothallic species are those which have two different mating types, both of which are required for sexual reproduction. Homothallic species, accounting for about half of all currently known, do not require different mating types, and selfing, or self-fertilization, is possible (Crone *et al*, 2013; Judelson & Blanco, 2005). From chlamydospores and oospores, hyphae and sporangia can be produced upon germination to then infect new hosts (Shishkoff, 2007; Judelson & Blanco, 2005). *Phytophthora ramorum* is a heterothallic species, meaning there are two mating types known as A1 and A2. However, in *P. ramorum* lineages, presence of both mating types has been rare; for example, with the EU1 lineage, the majority of the pathogen’s population seems to be A1 Mating type (Van Poucke *et al*, 2012; Kliejunas, 2010). This means that the species largely seems to rely on asexual reproduction, and sexual oospores are rare to the extent that none have been found in nature (Kliejunas, 2010).

2.4 Dispersal of *Phytophthora*

2.4.1 Abiotic Dispersal

Phytophthora generally spreads either aerially or through ground and surface water. Aerial dispersed *Phytophthora* species generally infect new hosts through leaves or the stem, while ground/surface water dispersed species generally infect the roots (Parke & Lewis, 2007; Davidson et al, 2005; Madden et al, 1992). Root infection occurs through soil water, from zoospores and chlamydospores (to a lesser extent) dispersed through flowing surface water, ground water, or rain (Hayden *et al*, 2013). Precipitation can move spores from above ground portions of plants into groundwater and surface water, whereby this root infection pathway can take effect. In addition to transporting these spores, rain can cause the release of zoospores in quantities as high as 5,200 spores per leaf in the case of *P. ramorum* and Bay Laurel host, with rainwater moving these spores via splash as far as 10m (Davidson *et al*, 2005). Once in surface water, spores can be transported great distances. Spores have been found up to 7 kilometres away from the source in stream water, and as well as the possibility of infection of flora in immediate proximity to the water flow, it is thought that this could spread the disease through human interaction/use; for example through nurseries irrigating their plants directly with surface water (Johnson, 2010; Tjosvold *et al*, 2008). Some *Phytophthora* have been found in flowing subsurface water too, with *Phytophthora cinnamomi* being recovered from over 2m depths in soil water (Kinal *et al*, 1993). Stream water, flowing subsurface water, and soil water can directly cause infection by bringing spores into contact with roots, as well as from zoospores moving through this water to find hosts. *P. cinnamomi* and *P. lateralis* are two examples of forest *Phytophthora* species that spread in such a manner (Jules *et al*, 2002; Weste & Taylor, 1971).

Species which disperse their spores aerially and infect plants above the ground are usually caducous with short pedicles, meaning the sporangia are easily detached from the mycelium, and they usually enter plants through the leaves, the stem, or sometimes through wounds. (Robin *et al*, 2011; Ristaino & Gumpertz, 2000). This is well documented for oomycete species that are significant within agriculture, such as *P. infestans* and *Peronospora tabacina,* but is less studied in forests (Hansen *et al*, 2008; Aylor *et al*, 2001). *P. ramorum* is a caducous species that infects trees above ground, with spatial correlations between new understory infections and overstory mortality providing some evidence to suggest it is spread through aerial dispersion (Peterson *et al*, 2014). Hansen *et al* (2008) likewise suggests that the spatial patterns of new infections ranging over 100m from known inoculum sources indicates aerial dispersal, whereby spores are lofted above the canopy in turbulent air and deposited in rainfall. However, for *P. ramorum*, rain splash is considered the most common cause of local dispersal between individual trees and between shrub canopy to tree stems to overstory (Peterson *et al*, 2014; Kliejunas, 2010). Rain can cause the release of spores in great quantities as already described, and these rainfall events do not have to be lengthy to lead to successful infection, with one study finding that subsequent infections of *P. cactorum* can increase from 1% after two minutes of rain, to 26% after sixteen minutes (Madden *et al*, 1992). Moreover, in a similar way to dispersal through turbulent air, the water droplets in fog or mist could contain inoculum and be moved by wind (Davidson *et al*, 2002). Likewise, wind-blown rain can explain some infection patterns of *P. ramorum* that have been seen in some of the forests in Western USA, whereby droplets with inoculum are transported possibly up to 15m (Johnson, 2010). Despite these water transportation routes for *P. ramorum*, there is some debate as to the extent of transmission to new sites in running surface water. Peterson *et al* (2014) and Hansen *et al* (2008) found that infection patterns did not correlate to waterways and/or roads, but viable inoculum has been found kilometres away from the nearest source, suggesting waterways could be transporting the disease (Johnson, 2010). There have also been studies showing that although *P. ramorum* is a caducous species, it can infect Rhododendron species at the roots, although not causing root rot as other species that utilise this pathway do (Fichtner *et al*, 2011; Parke & Lewis, 2007). This suggests that spores in the soil can cause direct infection through the roots, alongside the possibility of soil inoculum reaching and infecting leaves from rain splash (Fichtner *et al*, 2009).

2.4.2 Anthropogenic Dispersal

Phytophthoras' ability to survive for extended periods of time as spores has implications for the spread of the disease by movement of the soil by people. The trade of plants is likely the largest contributor to the long-distance movement of *Phytophthora*, and one of the ways it may have done so is through potting medium (Brasier, 2008; Parke & Lewis, 2007). However, the most direct way the nursery trade has spread *Phytophthora* is from supplying infected plants. It is through trading infected plants that *P. ramorum* is thought to have spread into Europe and the USA, and perhaps not surprisingly, as one study has found, that the infestation rate of forest nursery stands and forest plantings was 80%, despite most plants not displaying any symptoms (Jung *et al*, 2016; Brasier, 2008).

Aside from forestry and plant trade activities causing the spread of *Phytophthora*s, leisure pursuits in woodlands may also be spreading the disease. For example, Cushman & Meentemeyer (2008) found that *P. ramorum* was more commonly found in public forests as opposed to privately owned, and that spores were more often on hiking trails than areas off trail. Moreover, Cushman *et al* (2008) found on a forested recreational area that hikers could carry spores a distance of 60-100m, with 5-10% of visitors bringing spores with them in soil on boots or tyres and 20-30% of visitors leaving with spore infested soil. Another study found this figure to be higher at their site, recording 33-50% of hikers tested to be carrying spores (Davidson *et al*, 2005). Likewise, 28% of new infection sites identified by Jules *et al* (2002) for *P. lateralis* were attributed to hikers spreading infected soil. The extent of hikers and mountain bikers may be limited by a relatively short time window for spore viability estimated at 24-72 hours, but this could still have implications for the pathogens' dispersal, particularly on local scales (Cushman *et al*, 2008). Soil containing viable *Phytophthora* spores has also been found to be spread by vehicles on roads in a similar way. This was an important pathway for the spread of *P. cinnamomi* in Australia, where roads and road-making activities for the mining industry is thought to have been one of the main ways in which the disease spread across Jarrah forests (Weste & Taylor, 1971). Likewise, mud on tyres and frequency of road use has been the principal pathway by which *P. lateralis* has spread between watersheds in the West Coast of North America, as the roadways are infested with the pathogen (Hansen *et al*, 2000).

2.4.3 Invertebrate vectors

Humans can undoubtedly carry *Phytophthora* spores further than most animals could, through globalised trade of plants and organic material as well as travel, but it follows that spore infested soil can be moved by animals, especially on a local scale. In forest pathology, there has been a large focus on insects as vectors of diseases. For example, insect species from the suborder Auchenorrhyncha have been found to be vital for the propagation of destructive *Phytoplasmas*, a genus of plant infecting bacteria causing diseases such as Ash yellow (*Phytoplasma fraxini*) (Weintraub & Beanland, 2006; Castello *et al*, 1995). There are numerous examples of insects vectoring fungal pathogens too. This includes *Orsillus maculatus* transmitting Cypress canker fungus *Seiridium cardinale*, and Bark beetles vectoring the Dutch Elm disease fungus *Ophiostoma ulmi* (La Porta *et al*, 2008; McLeod *et al,* 2005). For the Oak wilt pathogen *Ceratocystis fagacearum*, the mechanism has been more specifically identified as spores on the exoskeleton of sap beetles (family Nitidulidae) (Cease & Juzwik, 2001).

There have additionally been studies focusing on invertebrates as vectors of *Phytophthora* species. Beetles have been observed to colonise lesions of cocoa pods caused by *P. palmivora*, and carry viable spores on their body which can spread the disease between pods (Konam & Guest, 2004). Snails and ants have also been found to carry spores of *P. citricola,* and other invertebrates have been implicated such as weevils and flies for

Phytophthora species (El-Hamalawi & Menge, 1996). Aside from the transportation of spores on the invertebrates' surface, there have also been studies that have found viable spores in excrement. This includes snails for *P. ramorum*, and fungal gnats for several Oomycete species in addition to *P. ramorum* (Hyder *et al*, 2009). This could have further implications if the spores are able to survive an additional digestive process should the spore carrying insect be eaten by a predator. However, the viability of spores passing through even the relatively simple invertebrate digestive tracts is severely reduced, and so it seems unlikely that they would be able to survive the additional digestion of the predator (Hyder *et al*, 2009; Parke *et al*, 2008).

2.4.4 Vertebrate Vectors

Insects seemingly have seen a larger amount of research as vectors of fungal and fungal-like diseases than other animals, but vertebrates also have the capacity to transport spores, propagules, and infected material. This is well documented for the transportation of plant seeds, for example in Europe, Wood-mice (*Apodemus sylvaticus*) transport and store acorn seeds with the potential to germinate (Ouden *et al*, 2005; Chambers & MacMahon, 1994). Fungal propagules have also been found to be transported by mammals, predominantly as spores in faeces whereby the spores have been able to survive the digestive process (Reddell *et al*, 1997). This has been documented for small mammals such as marmots (*Marmota*), as well as large herbivores transporting spores on a scale as to affect the entire landscape (Baker *et al*, 2016; Cázares & Trappe, 1994).

Although the viability of *Phytophthora* spores is significantly reduced after insect digestive tracts, one study has shown that spores of *P. cinnamomi* are able to survive that of wild pigs (*Sus scrofa*) by surviving on indigestible plant material (Li *et al*, 2014). For pine plug feed (de-barked pine stem), the recovery rate was as high as 94%, although most was much lower. Despite the high recovery rates from this study, it still seems unlikely that spores could survive multiple digestive tracts when considering the case of insectivores, as it is on the plant material that the spores survive. Wild pigs have not only been implicated in the transferral of *Phytophthora* pathogens from their excretions but also by transferring soil. This could be the case for other mammals too, but wild pigs may be more likely than most as their foraging habits means they disturb large quantities of soil in which spores could be present (Krull *et al*, 2013). Additionally, Cardillo *et al* (2018) used topographical and distribution information of new infection sites of *P. cinnamomi* to provide some further evidence of animal vectors. Beyond these studies, there is not a great amount of evidence collected on vertebrate vectors of *Phytophthora*, although it is often theorised for many *Phytophthora* species (Cushman & Meentemeyer, 2008; Webber & Rose, 2008; Hansen *et al*, 2000). One of the animals thought to play a role in *Phytophthora* spread, including *ramorum*, is the Grey Squirrel (*Sciurus carolinensis*), which often creates wounds in trees that in theory could lead to infection through spores in their claws; however, the forestry commission considers this a highly unlikely scenario, at least for *P. ramorum* (Mayle & Webber, 2012).

2.4.5 Bird Vectors

Beyond ground dwelling vertebrates, birds also could be playing a role in *Phytophthora* disease transmission. Indeed, birds are considered by some as the most abundant and effective vertebrate dispersal vectors generally (Viana *et al*, 2016). Birds transmitting plants, fungi, viruses, and bryophytes has often been theorised and in many cases proven (Viana *et al*, 2016). For example, the spread of the Western Nile Virus on the East coast of the USA was linked to the migration of birds acting as hosts to the disease (Reed *et al*, 2003). But perhaps an example closer to *Phytophthora* is the spread of the Chestnut Blight fungus (*Cryphonectria parasitica*) which was found to be spread by birds picking up spores from diseased trees (Anagnostakis, 1987). The precise mechanism was not outlined in this study, with no detail as to whether it was via feathers or via feet that these birds were transporting spores, but it does provide some proof of concept. What's more, and alluded to in the Western Nile Virus case, there is the potential for dispersal at great distances by migratory birds, which have been vectoring seeds and bryophytes, possibly up to hundreds of kilometres if not over a thousand (Viana *et al*, 2016; Lewis *et al*, 2014).

When considering whether birds could be vectoring *Phytophthora* specifically, there are few studies that provide evidence of this. Birds could be acting as a vector through eating and excreting spores, and although considered unlikely, evidence of this has been found for some bird species and *P. cinnamomi* in Australia (Coleman & Clark, 2010; Keast & Walsh, 1979). However, the potential of vectoring via movement of spores on feathers and/or feet is perhaps more significant. It is plausible that rain splash could introduce spores onto the birds feathers which are then carried elsewhere to be washed out by rain (Coleman & Clark, 2010). It is also possible that birds could transport soil material on their claws in the same way as humans' and wild pigs' feet. A study by Dadam *et al* (2012) aimed at providing evidence of birds as vectors in the ways outlined, found that only ten samples out of 2,017 were confirmed for *P. ramorum*, but nonetheless, this does show the possibility. The implications of birds being able to carry spores is great, as birds could be a mechanism of how, on a local scale, infested soil can infect aerial parts of trees, as well as migratory birds being able to transport soil material on large scales (Coleman & Clark, 2010). Indeed, there is some correlation between the migration paths of certain species and the main concentrations of *P. ramorum* in the UK, although without further study this is inconclusive (Coleman & Clark, 2010).

2.5 Bird Tables and Feeders

If the possibility exists of birds transporting spores on their claws or feathers, then it could be the case that there are concentrations where spores are either collected or perhaps even transferred between individuals. Bird feeders could be such a hotspot, as they can be visited by numerous birds of various species. During the BTO (British Trust for Ornithology) Winter Garden Bird Feeding Survey of winter 2016/17, over 230,000 individuals were recorded covering 75 different species in just 241 gardens (Boothby, 2017). In addition, bird feeders can act as reservoirs of other disease types if they're not kept clean. In particular, salmonellosis is a disease that can lead to illness and mortality in birds frequenting feeders, but other diseases include trichomoniasis, coccidiosis, aspergillosis, avian pox, and avian mange (Brittingham & Temple,1988). Trichomoniasis has been a recent problem in the UK, with some links to bird feeders, causing a 35% reduction of the Greenfinch population and 20% reduction in Chaffinch population in the area of highest disease report frequency during the first year of the epidemic in 2006 (Lawson *et al*, 2018). Adelman *et al* (2015) directly linked bird feeders with disease transmission, with time spent on feeders the largest contributing factor for increasing the likelihood of becoming infected by the bacterium *Mycoplasma gallisepticum*. However, it is not clear whether birds could be transferring spores on bird feeders, either for diseases that affect them or ones that impact the wider environment such as *Phytophthoras*.

Bird feeding in the UK has grown in popularity since the 1970s, to the current extent that approximately 48% of households are providing supplementary food for birds and double the amount of feed used in the 1990s (Boothby, 2017; Davies *et al*, 2009; Burton, 2003). However, there are many variables that determine when a feeder receives a visitor, and what visitors are attracted. Most obviously, different types of feeder, such as table or hanging, attract birds that are adapted to feeding in different ways; a hanging feeder will attract light, agile birds that are used to feeding from the ends of branches such as the Blue Tit (*Cyanistes caeruleus*), whereas ground feeders are better adapted for ground foraging birds that are too heavy for a hanging feeder, such as Mistle Thrush (*Turdus viscivorus*) (Burton, 2003). There are also preferences for food types by species (Burton, 2003). However, there are further complicated interactions, an example of which is the preference birds have displayed for the colour of feeders; different species have different preferences;

however, silver is the most preferred generally (Rothery *et al*, 2017). Birds will also visit feeders more regularly at different times of year; in late winter (February-March) when food availability is lowest, and during the nesting season in late spring/summer when demand is highest (Burton, 2003). There is some evidence that there is a positive relationship between the frequency of feeder visits from birds and the distance from wooded areas, which is perhaps caused by decreasing competition, from squirrels for example, or as there are less natural food sources available at greater distances from wooded areas (Hanmer *et al*, 2018). In contrast, Lee *et al* (2005) found that in rural settings, birds prefer proximity to woodlands over exposed environments. This could be because they are at greater risk of predation in these settings, especially from raptors, or it could be that the increased exposure deters visitors as greater energy would be expended to keep warm (Lee *et al*, 2005). Bird feeders may be visited by other animals too, most notably the Grey Squirrel in the UK. Hanmer *et al* (2018) found that over 40% of bird feeder visits in an urban environment were from these squirrels, with greater frequency closer to wooded habitats and vice versa. This could have further implications for spore transfer at feeders, as it has already been noted that squirrels could theoretically transport *Phytophthora* spores, and there are numerous other diseases that they are known carriers of (Hanmer *et al*, 2018; Mayle & Webber, 2012). This means that squirrel visitors to bird feeders may also contribute to the possibility of spores being exchanged and vectored from bird feeders.

3. Methodology

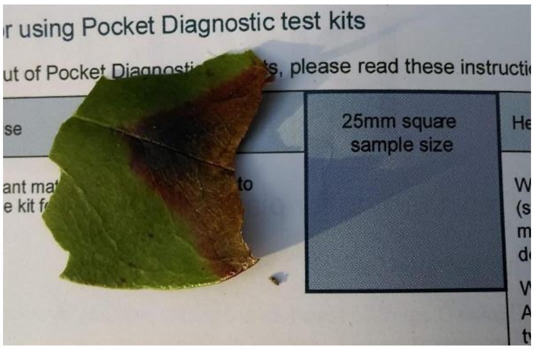
In order to test whether birds could be transferring spores of the pathogen from infected material to local bird feeders, bird feeders were established in two woodland sites which were both found to have the disease by Forestry England (B. Robinson, *pers comm*, 2019), and have active management measures in place. Although fellings have been conducted to control the pathogen, both sites potentially still contain *P. ramorum* living saprotrophically in deadwood and leaf litter, as well as through asymptomatic infections of living trees. These feeders do not accurately represent bird feeders used in residential gardens as there is a large difference in natural food abundance and the suitability of habitat for different species due to environmental factors. Therefore to extend the study, data was also collected from residential bird feeders from members of the public living within Keele University grounds, where there are houses located near to woodlands on a campus that has had confirmed infection of *P. ramorum* within the past 5 years (Keele University, undated).

3.1 Preliminary Site Exploration

Before data was collected to investigate the aims of this study, a preliminary investigation was conducted to ascertain suitable sites. Firstly, Keele University grounds were investigated, as it was known to have had recent outbreaks of *Phytophthora ramorum,* and as University held land, the stakeholders are open to studies taking place. This means that as well as providing an area large enough to research the first aim, the site could be used to investigate the phytopathogen and its vectors, in order to explore aim 2. An important factor that made Keele University an ideal site to investigate was that as well as having these woodlands, it has residents on site that are not students, providing households that could be investigated for objectives 1f and 2a.

Phytophthora ramorum was found in Keele woods in 2013, and despite best management practices to prevent the spread of the disease and minimise its presence, it was still expected that the disease might be found within the woodlands (J. Barker, *pers comm*, 2019). Therefore, the hypothesis of the preliminary study was that Keele woods still contained spores of *Phytophthora ramorum*. Initially, Pocket Diagnostic test kits (described in section 3.3.2) were used on *Rhododendron ponticum* leaves that were suspected of being infected and producing spores, an example of which is shown in Figure 3.1. Approximately 20 *Rhododendron* leaves were tested for the presence of the disease, with the locations shown in Figure 3.2, and *Rhododendron* that displayed symptoms most consistent with *P. ramorum* were tested multiple times to reduce the chance of a false negative result leading to infestation being missed. However, as no positive results were found from the *Rhododendron* leaves, leaf litter was also tested in the Terrace woodland, which was identified as a focal point of infection in Keele woods (A. Bethell, *pers comm*, 2019). Approximately 6 tests were used on leaf litter in the Terrace woodland, all of which produced negative results.

a



b

Figure 3.1a: A leaf sample prepared for testing, with the border of necrotic region and healthy leaf being selected for testing.

Figure 3.1b: An example of *Rhododendron* with leaves showing necrosis similar to symptoms expected from *P. ramorum* infestation.

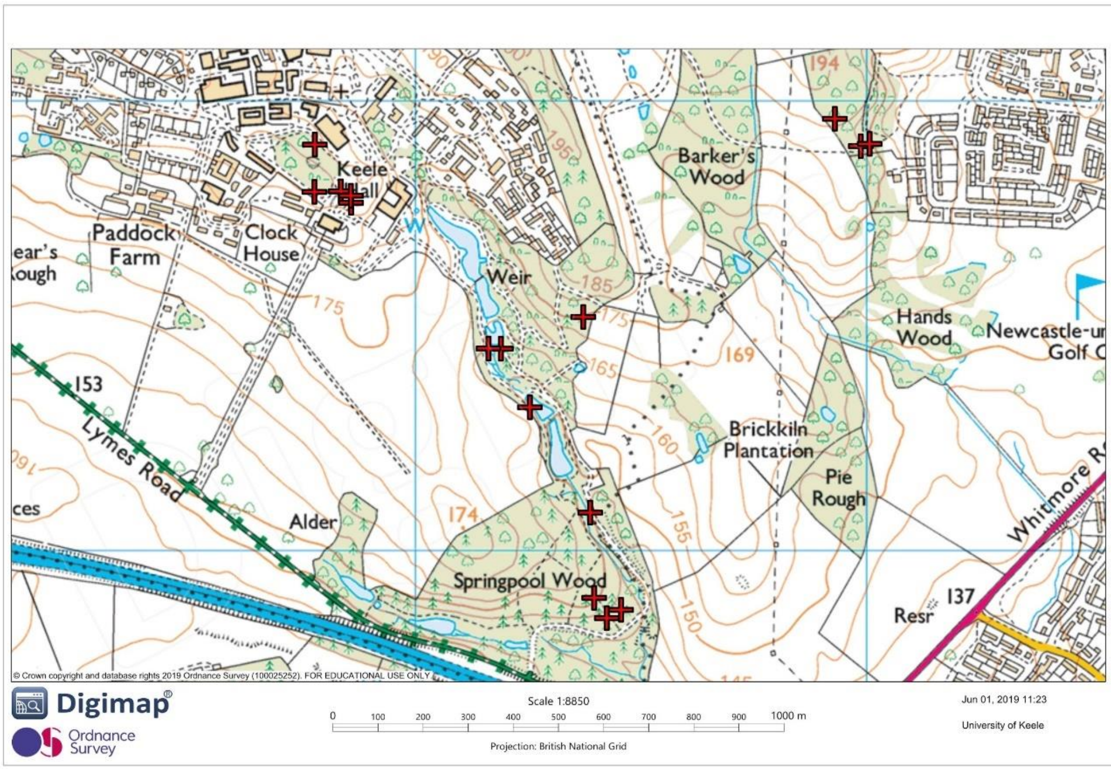


Figure 3.2: The approximate locations of the *Rhododendron* plants tested for *P. ramorum*, shown as red crosses, in Keele woodlands and the proximate Flagstaff plantation in the North East, unlabelled at this map scale (edited in Edina Digimap, 2019a).

Numerous other sites in North Staffordshire received statutory plant health notices due to *Phytophthora ramorum* at similar dates to Keele woodlands, and therefore presented other possible sites where bird feeders could be established to test whether birds could transfer spores of the disease. Trentham is one of these sites, also proximate to Stoke-on-Trent as shown in Figure 3.3, and receiving a notice in 2013 due to *P. ramorum* infestation of *Rhododendron pontificum* (A. Bethell, *pers comm*, 2019). Therefore, the site was also visited to test *Rhododendron* regrowth and leaf litter. However, there were far fewer *Rhododendron* individuals on the site than in Keele woods, and no positive results were found. This led to an exploration of sites that have had *Rhododendron* and *Phytophthora* more recently, as it was thought this would be more likely to produce positive results for the presence of the pathogen in the environment.

Figure 3.3: the locations of Keele Woods and the woods on the Trentham estate, west and south of Stoke-on-Trent respectively.

Phytophthora ramorum was first found in the UK in the South West of England, and new infestation sites continue to be found in the region due to the optimal climate (Lane *et al*, 2003; Forestry Commission, 2018d). A desktop investigation found the sites Olderwood and Denham Woods located in West Devon, shown in Figure 3.4, to be suitable sites for field study, as both have had outbreaks of the disease and more recently than those sites investigated in Staffordshire. Unlike the Keele and Trentham sites, Sweet Chestnut was the primary tree species of concern in these woods, as opposed to *Rhododendron*. Consequently, a thorough preliminary survey of the sites was not possible, as Sweet Chestnuts are deciduous and so did not have leaves which could display symptoms before the start of the study. However, both woodlands have certain features that made them suitable for achieving the aims of the study, and both sites have had confirmed presence of *P. ramorum* within 18 months of the start of fieldwork. The disease presence was found by the Forestry Commission, who manage both sites, and it is suspected that *P. ramorum* remains present (B. Robinson, *pers comm*, 2019).

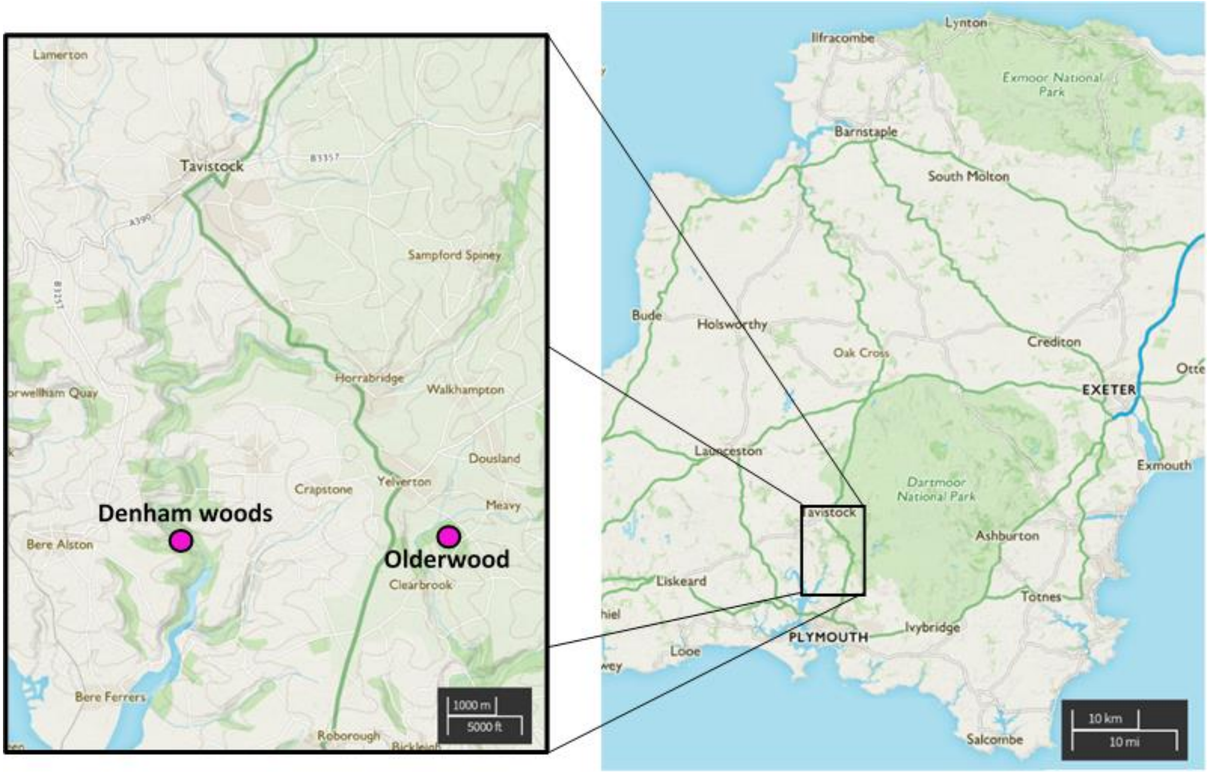


Figure 3.4: Map showing the locations of the study sites indicated by pink circles, Denham Woods on the Tavy and Olderwood on the river Meavy (edited from Ordnance Survey, 2019). Olderwood; 50°28'51.80"N, 4° 4'25.55"W. Denham; 50°28'42.59"N, 4° 9'17.49"W.

3.2 Garden Bird Feeder Survey

3.2.1 Site description

Although *Phytophthora* was not found in the preliminary site in Keele woodlands, it was still determined to be a suitable site for objectives 1f and 2a. In addition, surveying households in a location adjacent to a relatively large woodland area provides information on their value as a study site by what was found on these feeders, and how the feeders are used in terms of how often they were cleaned shows their value in *Phytophthora* studies. If birds are transporting spores of *P. ramorum* and carrying them to bird feeders, it would be reasonable to assume spores are more likely to be found on feeders closer to woodlands that have had confirmed infestation, as there is a higher chance that the birds visiting the feeders have been in contact with infected material. Therefore, it appears beneficial to test this hypothesis on the feeders in the gardens of the Keele residents, as nearly 50ha of wooded area on campus has been managed for infection of *P. ramorum*; and so it is thought likely the disease still has some presence despite the negative results found in the exploration (calculated using Google Earth, 2019). This is in addition to confirmed cases in nearby woods, with Trentham (the other site explored for suitability) only being approximately 6 kilometres away in addition to other proximate sites that were known to have the disease within 5 years of the study (A. Bethell, *pers comm*, 2019).

The majority of the wooded areas on Keele campus are shown in Figure 3.5, with the zone number referring to the timeline of *P. ramorum* management. Stacy Plantation, Rosemary Hill Wood and Roadside Plantation (also on Keele grounds) are not indicated on this map, as the disease was absent in these areas. Springpool Woods received a statutory plant health notice in 2013 for infected Larch in the Southern part of the woodlands, requiring infected individuals and all Larch within 100m of them to be felled (Keele University, undated). Once this was completed, the Forestry Commission advised Keele University to remove all major host species from infected woodland, which included *Rhododendron*, across all of the wooded areas on campus (*R. pontificum* in particular) and any remaining Larch in 2014. Larch represented 20% of Springpool Woods, and *Rhododendron* was present in all woodlands as the dominant understory, meaning this was an intensive strategy that has significantly changed the face of the woodlands at Keele (Keele University, undated).

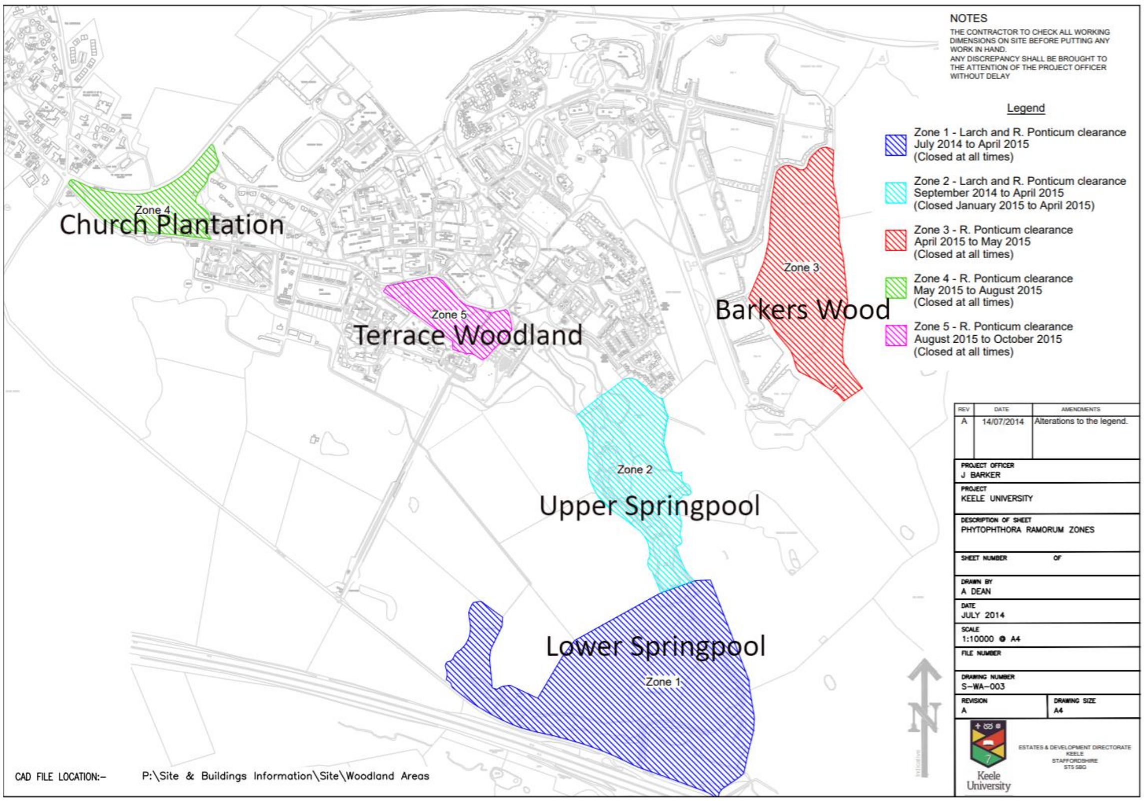


Figure 3.5: Map of Keele grounds showing areas of Larch and *Rhododendron* removal and the planned time frame for the works (edited from Dean, 2014, in Keele, undated).

3.2.2 Swab Sampling

Swabbing is a commonly used method to collect samples when testing for substances, organisms, or compounds on surfaces. However, little evidence was found in the literature of their use in studies involving birds, other than the use of cloacal swabs. However, Shriner *et al* (2016) utilised external swabs on birds' feet, feathers, and bills to collect data on avian influenza. The swabbing of eggshells has also proven to be a viable, non-intrusive technique to collect DNA data from numerous bird species (Martin *et al*, 2011; Schmaltz *et al*, 2006). It therefore follows that swabs could be an effective method to collect samples of *Phytophthora*. Furthermore, swab samples of the environment, including bird cages, have been used in numerous studies on zoonotic pathogens (Indriani *et al*, 2010; Wang *et al*, 2006). This suggests that swabs could be used on objects in the environment to test for phytopathogens, such as bird feeders and *P. ramorum*, although no precedent was found in the literature. This formed the basis for using swab sampling to collect samples from bird feeders in combination with lateral flow devices. In addition, the rapidity, minimal expense, and ease of use makes this methodology ideal for combining with citizen engagement in collecting data from household feeders; this could be replicated in other areas and very feasibly be scaled up in future studies.

The method of sample collection from bird feeders via swabs was kept the same for those placed in Devon woodlands and those collected from Keele University resident's feeders. The cotton bud was dampened with tap water if the feeder was dry and rubbed against the perches of the hanging feeders, rotating the bud to ensure contact with all of the cotton. On the square ground feeders, the cotton bud was rubbed the full extent of one side, then rotated 90 degrees and repeated on all four sides of the feeder. Cotton buds were used while wearing disposable vinyl gloves that were changed after collecting each sample; this was to ensure there was no human-induced cross-contamination of *Phytophthora* or other avian pathogens between feeders. The swab samples were then tested for the presence of *Phytophthora* by using lateral flow devices commercially available from Pocket Diagnostic ®. The head of the cotton bud was cut off into the buffering solution with ball bearings used to prepare the sample. Following communication with Pocket Diagnostics, who confirmed some uncertainty about this collection technique, a decision was made to shake the solution for 30 seconds, as although cotton is a soft material (usually requiring a 20 second shake), the sensitivity of using a cotton bud was uncertain; however, the tests have been found to be sensitive to leaf tissue with less than 1% infection (Lane *et al*, 2007). Two or three drops of the solution were then tested using the lateral flow device, picture in Figure 3.6. The device can be read after four minutes, whereby the presence of the control (C) demonstrates the device is working, and the presence of the second (T) line indicating a positive result for *Phytophthora*.

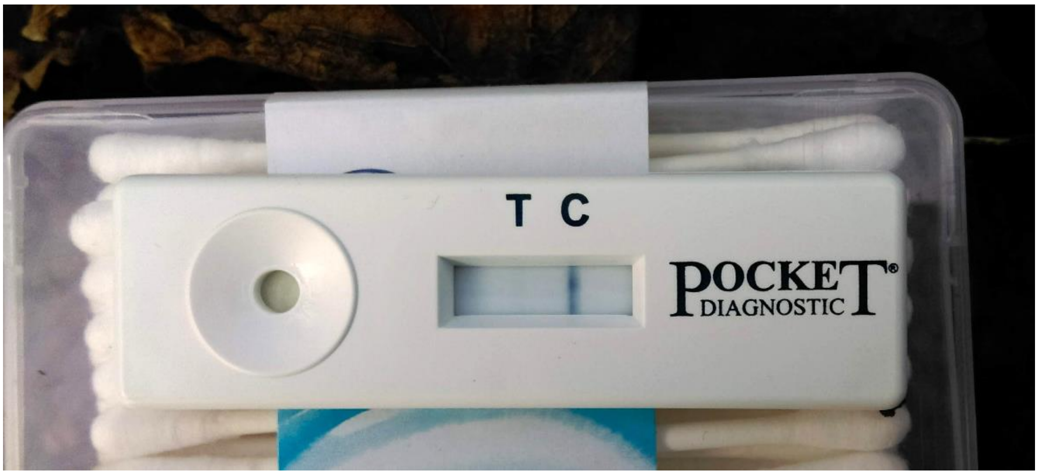


Figure 3.6: An example of a lateral flow device, showing the presence of the control line (c), meaning the device is operating correctly, but with no test line (T) present, indicating a negative result for *Phytophthora*.

Precedent for this sampling methodology was not found in the literature, and similar use of swab collection was rare, and so the methodology was constructed to be standardised and replicable. The test kits used are designed to produce positive results for all *Phytophthora* species, which has been confirmed for 13 species including *P. ramorum, P. kernoviae P. lateralis, P. cinnamomi,* and *P. cactorum* (Abingdon Health, 2018; Lane *et al*, 2007). One evaluation of the test kits found false positives for 11% of occasions, which was considered in the data interpretation (Lane *et al*, 2007).

3.2.3 Keele University Residents Data

Data was collected from residents at Keele University, who are all either current or previous employees of the University and live in houses on the campus, of which there are approximately 125 with private gardens. This involved going door to door asking questions in person which can be viewed in Appendix i. As the questions were asked verbally, in some instances the discretion of the researcher was used in choosing whether to ask the full set of questions when the resident expressed time constraints or other limitations, meaning some questions have fewer responses than the total number of respondents. Answers were recorded anonymously and consisted of information about bird feeder use habits, such as which food was used, how frequently they were cleaned, and what animals were seen using the feeders. Following these questions, swab samples were taken from bird feeders of residents who agreed, which were likewise recorded anonymously. The swab samples were taken from the perches of seed feeders, the bars of nut/suet feeders, and the wooden/metal edges of ground feeders, up to a maximum of three per residence to maximise sample size with limited test kits. These swab samples were then tested for the presence of *Phytophthora* in the method previously detailed, giving an immediate result. This information was collected for several reasons. As this data has been gathered from feeders used by members of the public in residential areas, it provides a more suitable comparison to the rest of the country than with the data collected in woodlands in Devon. It also enables comparisons with data taken from the feeders placed in the woodlands, for example the birds that were seen in residential areas compared to the birds seen using feeders in the woodlands in Devon.

3.3 Woodland Bird Feeders

3.3.1 Pilot study

Olderwood and Denham Woods in West Devon were chosen as sites for establishing bird feeders after the preliminary site exploration. As discussed, both woods had confirmed *Phytophthora ramorum* within the last 18 months, and so presented sites that seemed suitable to test the hypothesis that birds can transfer spores onto bird feeders for objective 2b at the same time as being ideal sites for aim 1. However, before establishing bird feeder stations on site in Devon, a small pilot was conducted in Barkers Wood on Keele grounds, the location shown in Figure 3.5. This was to test the planned methodology of setting feeder stations consisting of a hanging and ground feeder and observing them for bird visitations, as well as the stem data that was also collected for objective 1e. It was conducted in a different woodland so if any changes were made, this would not affect the continuity in the study sites. Primary aims of the pilot study were to assess if bird visits could be recorded accurately during the period, whether the presence of the observer with some cover at over 10 meters would prevent birds from using the feeder, and whether birds would be observed within the limited timeframe of 30 minutes. It would also test whether the observer’s concentration could be adequately maintained to provide accurate recordings over this period of time. The results of the pilot are presented and discussed only to a minimal extent, as the period was not considered significant enough to make any conclusions with regards to answering the aims of this study. However, as a result of the pilot study, the following changes were made to the methodology; estimated tree height was not recorded in the stem maps in the Devon sites, use of photographs and the software ‘imageJ’ was incorporated into the Devon study. Despite the aim of the pilot study being to create a consistent methodology, two further changes were made during the first week of observations of feeder stations in Olderwood and Denham Woods; specifying whether visits were to the ground or hanging feeder, and the method of gathering data on seed being consumed. These are described below.

3.3.2 Woodland Bird Feeder Methodology

Twelve locations were chosen from the two Devon woodlands to test whether birds are bringing spores to bird feeders. One hanging feeder and one ground feeder were placed approximately 0.5 - 1.5 meters apart at each location, the pair being labelled as a feeder station. Six stations were placed in each woodland, with an example shown in Figure 3.7 and the locations discussed in section 3.3.4 and shown in Figures 3.10 and 3.12. These were all supplied with mixed seed food containing cereals and grains, kibbled corn maize, kibbled sunflower hearts, flaked naked oats, pinhead oats, kibbled peanuts, and suet pellets produced by 'Happy Beaks'. This was chosen to attract the broadest range of species and to prevent the introduction of viable plant seeds to the woodlands (Burton, 2003).

Figure 3.7: An example of a Feeder Station with a hanging feeder and ground feeder within 1.5m. This example is from the pilot study discussed in section 3.3.3, and less food was placed in the ground feeders used in Olderwood and Denham Woods.

Olderwood and Denham Woods were each visited 5 times a fortnight, and on each visit observations of each bird feeder were taken over half hour periods at different times of the day over the 9 week study period. Before starting the observation period, five minutes of settling was allowed for, as arrival at the site could cause disturbance to any birds or squirrels present. Observations of what species were visiting the feeder, how many individuals, and how many times they visited were made from approximately 10-20 metres away using 8x40 binoculars. This was to provide data on which birds or other animals visited the feeders, and the number of visits each species made was recorded to give an indication of effort. A visit was defined as arriving then leaving the perch of the hanging feeder or the ground feeder entirely. The exception to this was if the bird had left one perch of the hanging feeder to immediately occupy the second perch; for example, Blue Tits were frequently observed to hop from one perch to the hanging pole to the second perch, and these cases were recorded as one visit.

Before observations, each feeder station was checked for damage, refilled with seed if necessary and weighed. Refilling was planned to maintain continuity of seed for objectives 1b and 1c whereby the consumption of seed mix would be measured to gauge feeder use, which could not happen if there were breaks in supply due to being depleted or damaged. This was done before the observations to ensure there was a food supply to attract birds in order to investigate what species were using the feeders. The stations were filled in a corresponding order to ensure there was approximately 60 minutes between refilling the feeder and returning for observations. The hanging feeders were weighed to the nearest 5 grams, and if filled a second weight was taken. This was so that the amount of seed being eaten could be assessed, giving further evidence of feeder activity, although it does not provide species specific data. The same method would be impractical for ground feeders, and so only the weight of added food was taken. This was done by weighing the feed, placed in a container in a cloth bag, before and after supplying each ground feeder so that the weight of feed added may be calculated. Each feeder was swabbed and tested for spores in the described methodology in section 3.2.1 (page 1) twice over the 9 week period; during the course of week 4 and week 9. In addition to the swab samples, a sample of leaf litter was also directly tested using the lateral flow devices over the course of week 9 of observation.

At the end of the study, measurements to create stem plot maps were taken. This was done by measuring the distance of each tree stem within a 10 meter radius of the hanging feeder, and recording the angle to the feeder using a compass. Diameter at breast height (1.3m) was measured by taking a circumference and dividing by π for stems with diameter above 5cm, and those stems with a smaller diameter but above 1 meter in height were labelled as saplings. Three exceptions to this methodology were necessary due to the high number in the environments, those being feeder stations 1, 3 and 4 in Denham Woods, where the same detailing of saplings was impractical beyond utility. Other features were also recorded, such as banks and significant deadwood. In addition, photographs were taken of the canopy from 5 meters North, East, South, and West from the hanging feeder at each station as well as from the hanging feeder itself. This was done at the end of the field data collection period, between May 17th and May the 20th, to ensure all the trees were in full leaf. These were used to create canopy cover estimates at each feeder station, by using the software 'ImageJ' (Schindelin *et al*, 2012). Images were converted to binary, whereupon the proportion of black pixels to white can be processed and analysed to give percentages of open sky; each of the five image results are given in section 4.2.4 alongside an average, thereby giving a canopy cover estimate.

Camera traps were used to provide supplementary information to the study. Objective 1d was created during the fieldwork, whereupon it was suspected from observations that damage to feeders and the rapidity of uptake of ground feeder seed may be due to mammals that were not being observed as a result of the presence of the observer. Therefore, the purpose of using camera traps was to gain a more complete picture of what was using the feeders, and although the information was not as consistent as the observations, the traps provided some data that can be analysed to examine the utility of supplementary bird feeders in natural/semi-natural environments for research. The camera traps were placed by feeders which had experienced damage to gain an idea of what could be the cause; as camera traps could not be used by every feeder, it cannot be certain whether the cause of damage to each feeder was the same. Due to the sensitivity of the camera traps, the feeders were moved up to 2 meters to position them in an area closer to the traps. This was not thought to affect visitation of the feeders. An 'x-lounger' model was used in Olderwood over the course of three weeks, and was moved between feeders 2, 5, and 6b (see Figure 12 in section 3.4.4) to assess whether there would be different species visiting them and whether the cause of damage to feeders was the same at each station. In Denham Woods, a 'little acorn' model camera trap was used during the last week of the study, as damage to feeders became a regular occurrence. The trap was used at feeder station 3 (see figure 9 in section 3.4.3) but was not moved to maximise the chance of capturing the cause of feeder damage.

3.3.3 Denham Woods Site Description

Denham Woods is located in West Devon near to the settlement of Bere Alston, shown in Figure 3.8. Denham Woods is the colloquial name given to the entire forest area south of Denham Bridge, and the name used for the forest block by the Forestry Commission, although Figure 3.8 shows that this is actually made up of several, separately named woods. For the purposes of this study, the local reference of Denham Woods referring to the entire forest block is used hereafter, and this covers 98.8 hectares next to the Tavy river, a major tributary of the river Tamar (Robinson, 2016). The region is part of an Area of Outstanding

Natural Beauty (AONB) designation, recognising the importance of the natural environment as well as the cultural heritage value of the Tamar Valley. Denham Woods sits on top of the Tavy slate formation, as shown in Figure 3.8, and consists of Upland Brown Earth soils (Robinson, 2016; Edina Digimaps, 2019). The area receives 1200.1-1400mm average annual rainfall and has an elevation approximately between 5 to 125 mASL (Robinson, 2016; NRFA, 2019a). The woods is bordered by improved grasslands, most of which is used for grazing of sheep (Edina, 2019d).

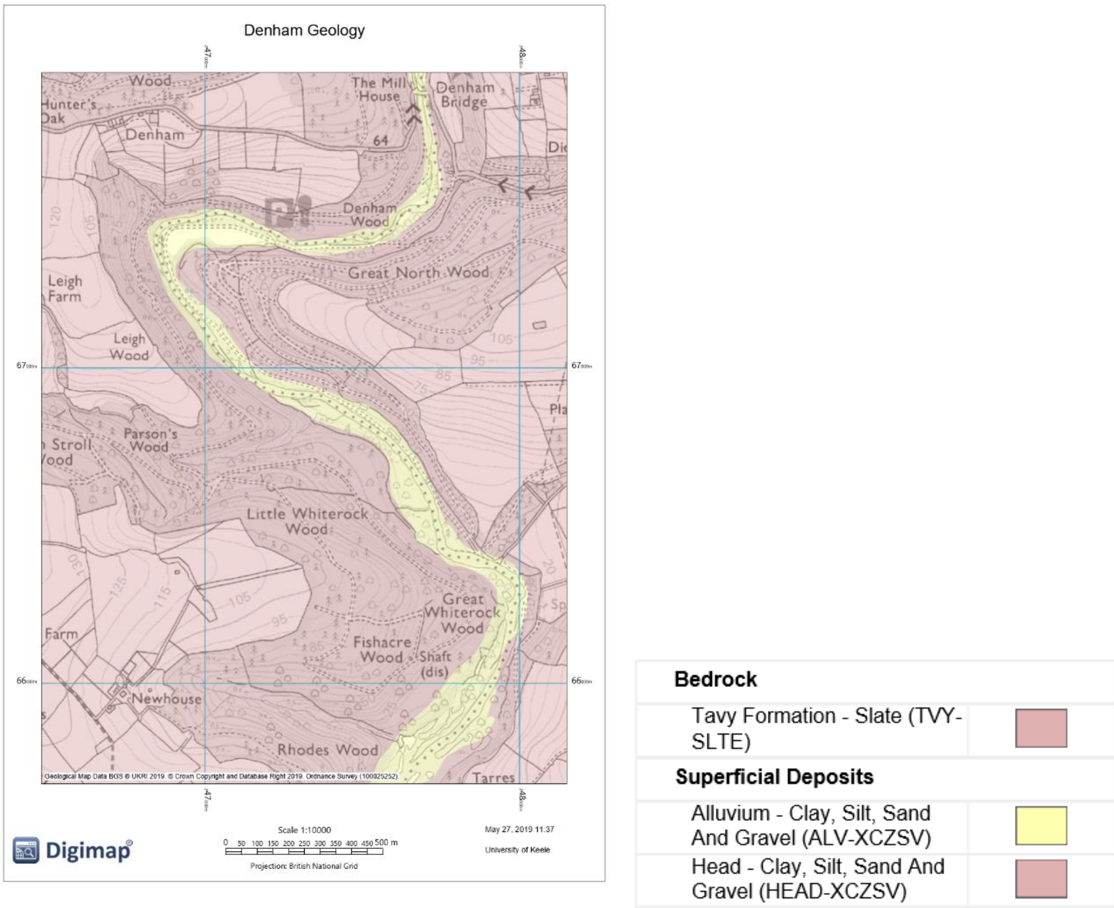


Figure 3.8: Map showing the geology of Denham Woods (Edina Digimaps, 2019b).

Denham Woods is a mixture of deciduous broadleaf and evergreen coniferous trees, as shown in Figure 3.9. Most of the area is designated Ancient Semi-Natural Woodland (ASNW) or Plantation on Ancient Woodland Site (PAWS), as reflected in the tree composition and forest management. Douglas Fir forms the majority of planted conifer species, with Norway Spruce and Western Hemlock being supplementary (Robinson, 2016). Oak, Sweet Chestnut and Beech are the most numerous mature broadleaf trees, but with Ash, Birch, and Sycamore present as pioneer species within some areas of the woodland. The conifers of Denham were predominantly planted in the 1960s, leading to an even-aged stand considered resilience poor, although current management strategies are aimed at creating greater diversity in age and structure of the crops (Robinson, 2016). This includes selective thinning as opposed to clear felling for timber production, as this allows for the development of the understory as well as being preferable under PAWS, ANOB, and for providing sustainable timber yields. Animals that are found in Denham Woods which influence management include Otters, Dormice, and Goshawk (Robinson, 2016). Goshawk could have particular relevance to this study, as the feeder station locations, as further discussed in the next paragraph and shown in Figure 3.10, are near to the 'impact zone' surrounding their nesting site and in which activities are restricted. All feeder stations were placed outside this zone in Denham Woods, but the possibility existed of Goshawks predating on animals drawn to the feeders.

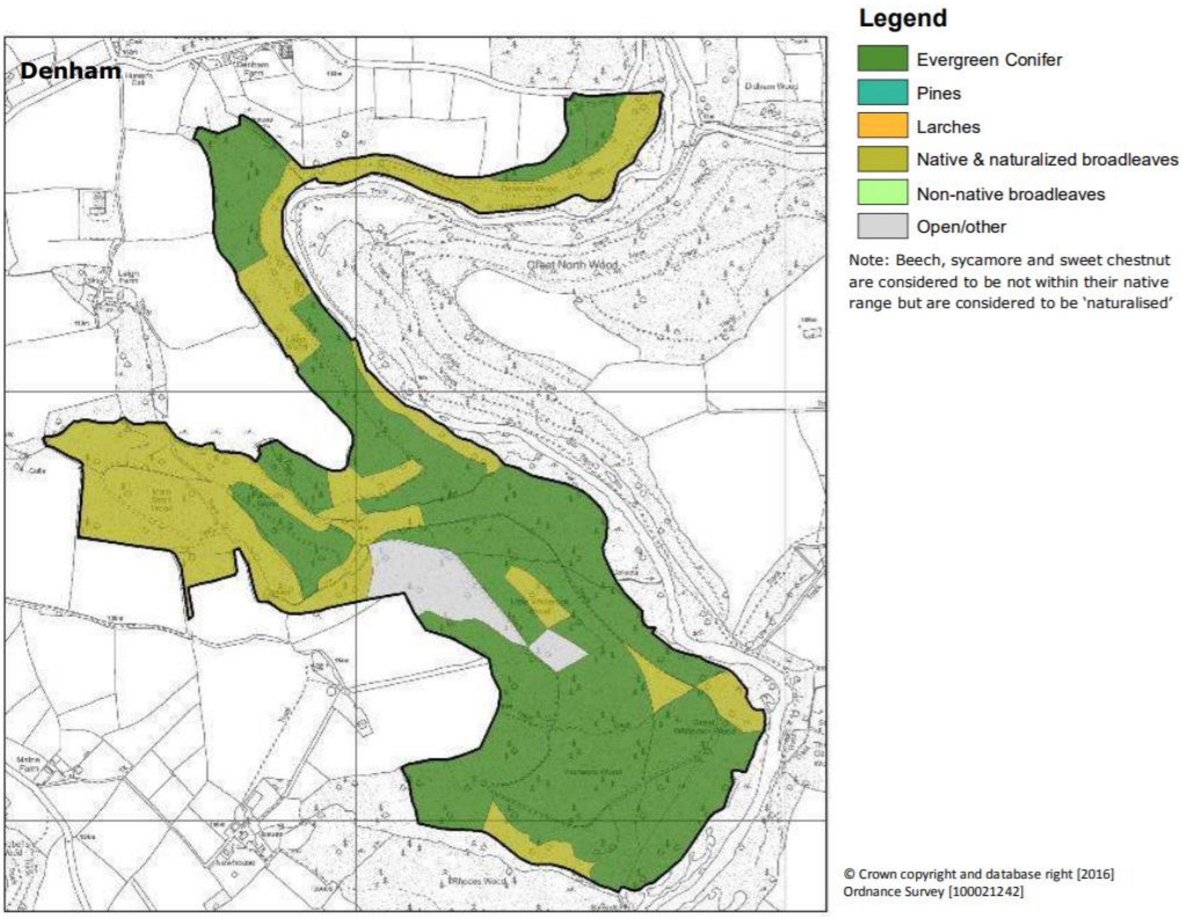


Figure 3.9: Map showing the composition of Denham Woods (Robinson, 2016).

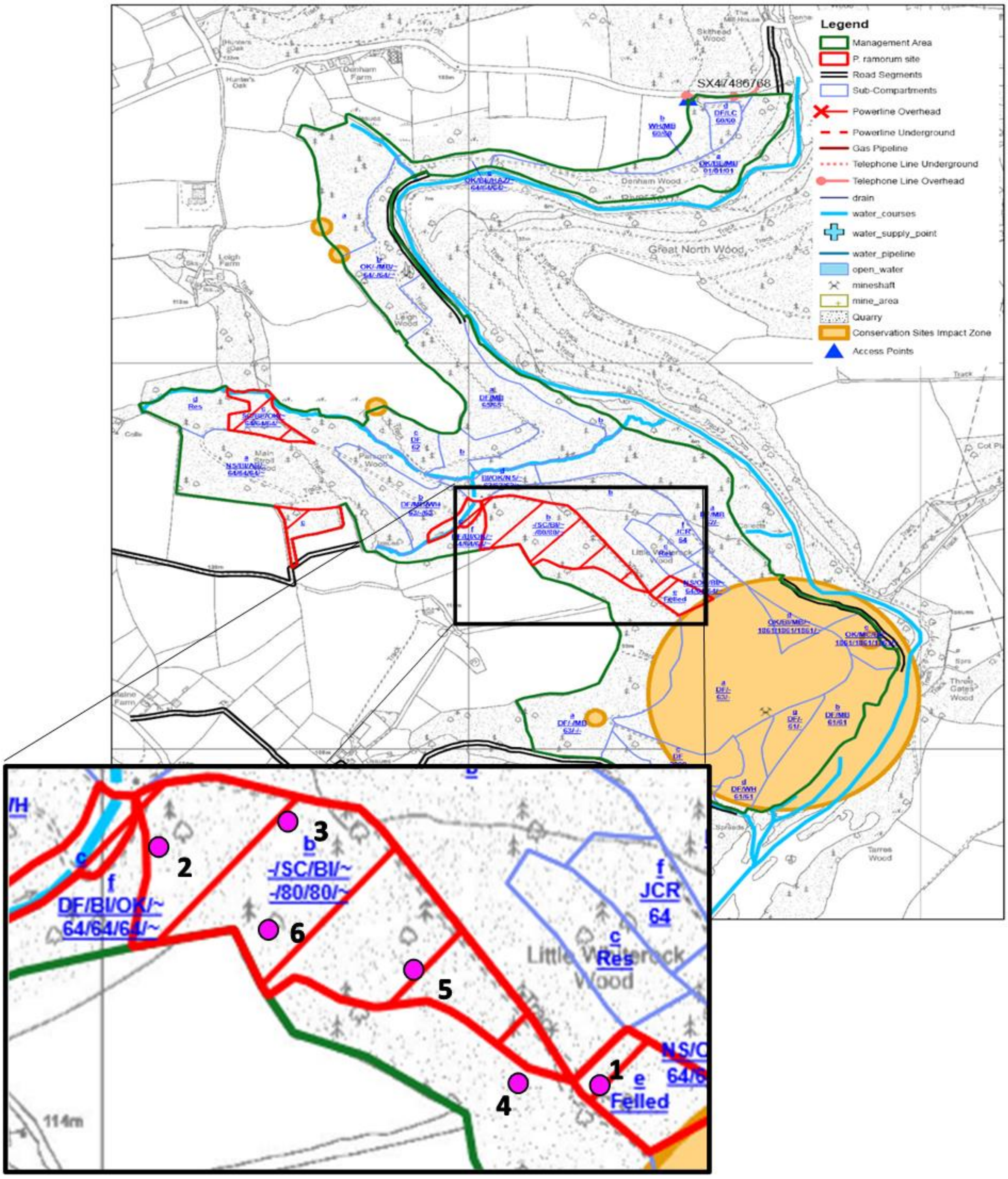


Figure 3.10: Map of Denham Woods, showing the locations of the feeder stations in pink with the respective numbers indicated in black, as well as showing significant features (edited from Forestry Commission, 2019a). This includes the Conservation Sites Impact Zones, the largest of which is present to protect nesting Goshawks.

The land is leased by the National Trust to the Forestry Commission as part of the Buckland Abbey estate, and this agreement has led to 'de facto' access of the forest by the public, although there are no rights of way on the site (Robinson, 2016). Therefore, the site receives walkers and cyclists in small numbers, as well as minimal horse riders in the southern area. These visitors are largely reliant on a well-maintained access road, and due to the often thick vegetation and difficult terrain, it is extremely rare that any of the walkers would enter the woodlands off the track.

Phytophthora ramorum was found in Denham Woods in 2014, infecting Sweet Chestnut and European Larch (Robinson, 2016). Management has focused on felling all Larch, and other symptomatic trees. Figure 9 shows the location of the feeder stations in Denham Woods, as well as showing the areas where *Phytophthora ramorum* has been found and managed. The stations were positioned according to multiple priorities, which also applied to the placement of stations around Olderwood. The first priority was to be in or adjacent to the specific areas in which *P. ramorum* has been found, as the distances over which birds might be able to transport spores of the disease is uncertain and not an aim of this study. Instead, by placing all of the feeder stations in close proximity to areas which have had the disease, the likelihood of answering whether birds are transferring spores on feeders is maximised. The second priority of feeder placement was to maximize the distance between the feeders, and the third was accessibility, both of which were considered equally. Undergrowth and stem density would make some positions otherwise ideal for maximising distance between feeders unusable, as well as some of the terrain being difficult to transverse. The fourth and last priority was to choose positions that create some diversity to more accurately represent the forest pens being utilised. Feeder station 4 was placed in the adjacent Douglas Fir pen so as to assess whether there is a large contrast between visits and bird species as different environments. This is a habitat suitable for different bird species yet close enough to the *Phytophthora*-managed, broadleaf area as to be within the territorial range of any birds that would forage in both broadleaf and coniferous woodlands. Feeder 1 was placed as a contrast to feeder 4 since this pen had been recently clear felled, so the canopy was entirely open with young pioneer species dominating, with the exception of some coppiced Sweet Chestnut that were not felled. The fact that this area was clear felled alludes to a secondary reason for placing a feeder here; this was the site of infected Larch. As Larch is the greatest spore producer of *P. ramorum* in the UK, there remained a possibility of survival of the disease in deadwood, as well as Larch saplings being found. Feeders 2, 3, 5 and 6 were placed in the broadleaf zone demarcated in figure 9 as a *P. ramorum* site, and the composition of trees was similar for all these stations; mostly Beech with some Birch and mature Sweet Chestnut. In this area, infected Sweet Chestnut trees have been managed by removing those showing symptoms. In some cases where there is a shared rootstock, only the symptomatic stem is felled, leaving the other intact. This means there is a possibility that *P. ramorum* is still present, and furthermore individual trees are still being found with the disease on occasion, with at least one testing positive in Denham within the last 18 months (Robinson, *per com*, 2019).

3.3.4 Olderwood Site Description

The second site used for this study was Olderwood, also located in West Devon. Olderwood is near to the settlement of Yelverton within the boundaries of Dartmoor National Park and on the river Meavy, a tributary of the Plym. It is much smaller than Denham Woods however, covering a total 28 hectares. The plantation sits on a similar slate formation to that of Denham Woods, with some granite intrusion, as shown in Figure 3.11 (Edina Digimap, 2019b). Average annual rainfall is also similar in Olderwood to Denham, but borders from 1200.1-1400mm and 1400.1-1600mm (NRFA, 2019b). This is perhaps reflective of its higher average elevation, as Olderwood ranges from 100-200 mASL, and proximity to Dartmoor (NRFA, 2019c). Olderwood, like Denham Woods, is surrounded by improved grassland, predominantly used for sheep grazing (Edina Digimap, 2019d).

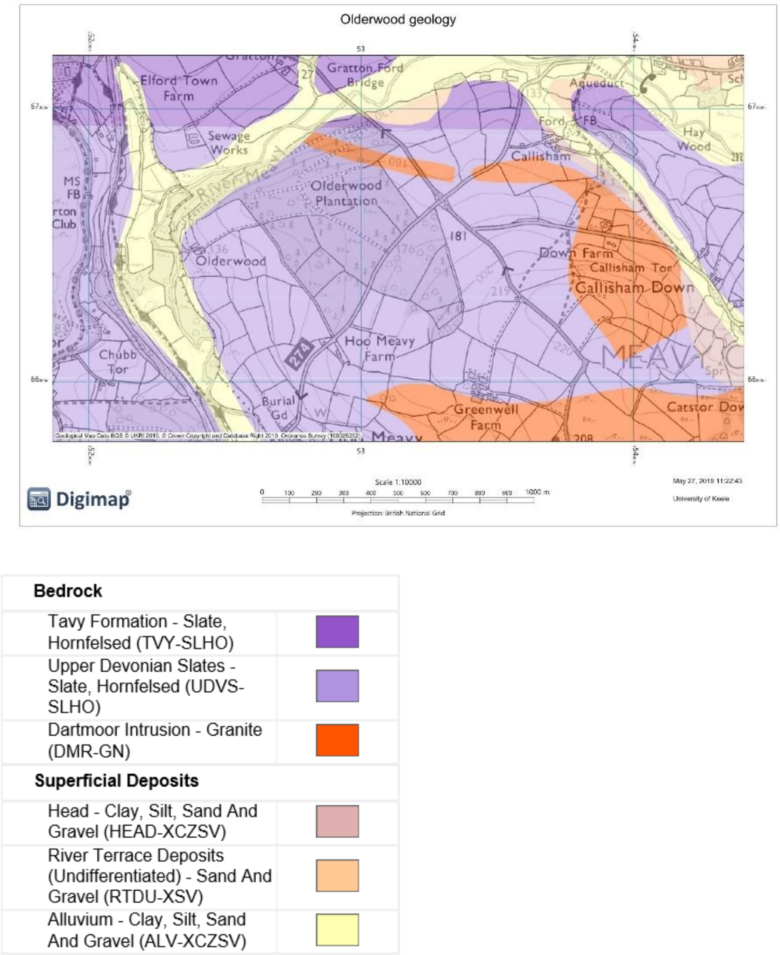


Figure 3.11: Geology of Olderwood plantation (Edina Digimap, 2019c).

Olderwood is a mixture of broadleaf and coniferous plantation. Unlike Denham, Olderwood is being managed for clear fell forestry, though only small portions are being felled at a time for more consistent timber yield and to create diversity of age between stands (Forestry

Commission, 2014). Currently, most of the conifers within the Plym valley forest management plan are 50-70 years old, with little diversity in age structure, and this is reflected in the coniferous coups in Olderwood (Forestry Commission, 2014). Sitka Spruce and Douglas Fir account for the majority of the coniferous trees in Olderwood, but with the recently restocked areas being planted with less common Serbian Spruce (B. Robinson *per comm*, 2019). Within the broadleaf areas of Olderwood, Beech dominates, but other species such as Sweet Chestnut, Birch and Oak are present. Goshawk are also present in Olderwood and are likewise marked in Figure 3.12 as a Conservation Impact Zone in which activities are limited.

Olderwood is managed by the Forestry Commission under leasehold and it is closed off to the public, so does not receive visitors as Denham Woods does. However, a shoot with a Pheasant release pen operates in the grounds (Forestry Commission, 2014). Outside of the Pheasant shooting season, Grey Squirrels are irregularly shot (B. Robinson *per comm*, 2019). This provides a contrast between the two sites, where Grey Squirrel populations aren't controlled in Denham Woods, as opposed to Olderwood where there is some level of population control, as well as a Pheasant release pen in Olderwood perhaps being indicative that there would be a greater number of this bird here compared to Denham.

Phytophthora ramorum was found in Plym Valley in 2010 in Larch and was found in Sweet Chestnut trees in Olderwood in 2017 (Forestry Commission, 2014; B. Robinson *per comm*, 2019). Management has been similar to Denham Woods, whereby symptomatic trees were felled. This had been done as recently as 2018, and because the felling commenced as the Goshawk entered nesting season, the timber was unable to be taken off site (B. Robinson *per comm*, 2019). Therefore, it is possible that *P. ramorum* has been able to survive (saprotrophically or as long-lived spores) in the deadwood from felled Sweet Chestnut, as well as the possibility of asymptomatic infection of the remaining stock. Figure 11 shows the *P. ramorum* management area, but Sweet Chestnut is the prime concern, with it being present as a broadleaf element even in the mostly coniferous coups. Figure 11 also shows the locations of the feeder stations in Olderwood. The priorities of feeder station position were the same as in Denham Woods; firstly, within or proximate to *P. ramorum* affected areas, secondly to maximise distance between the stations, thirdly to ensure that they were accessible, and lastly to cover some of the diversity within the woods. However, undergrowth was extremely light in Olderwood, and so accessibility was not so great a concern as the terrain was much safer to traverse. Another consideration here was avoiding the Pheasant release pen. Indicated on the map, there is an additional feeder station, 6b. This is a result of moving feeder station 6 due to consecutive damage to the feeder, meaning that gaining results contributing to answering the aims of this study were not possible. Therefore, over the course of week 5 of the study, station 6 was replaced by station 6b. Stations 6 and 6b were placed outside of the *P. ramorum* area to see whether this affected the results of finding spores on bird feeders; however, as aforementioned in section 3.3.4 (p.12), distance was not being tested, and these feeders would still be well within the range of many birds inside the *P. ramorum* zone.

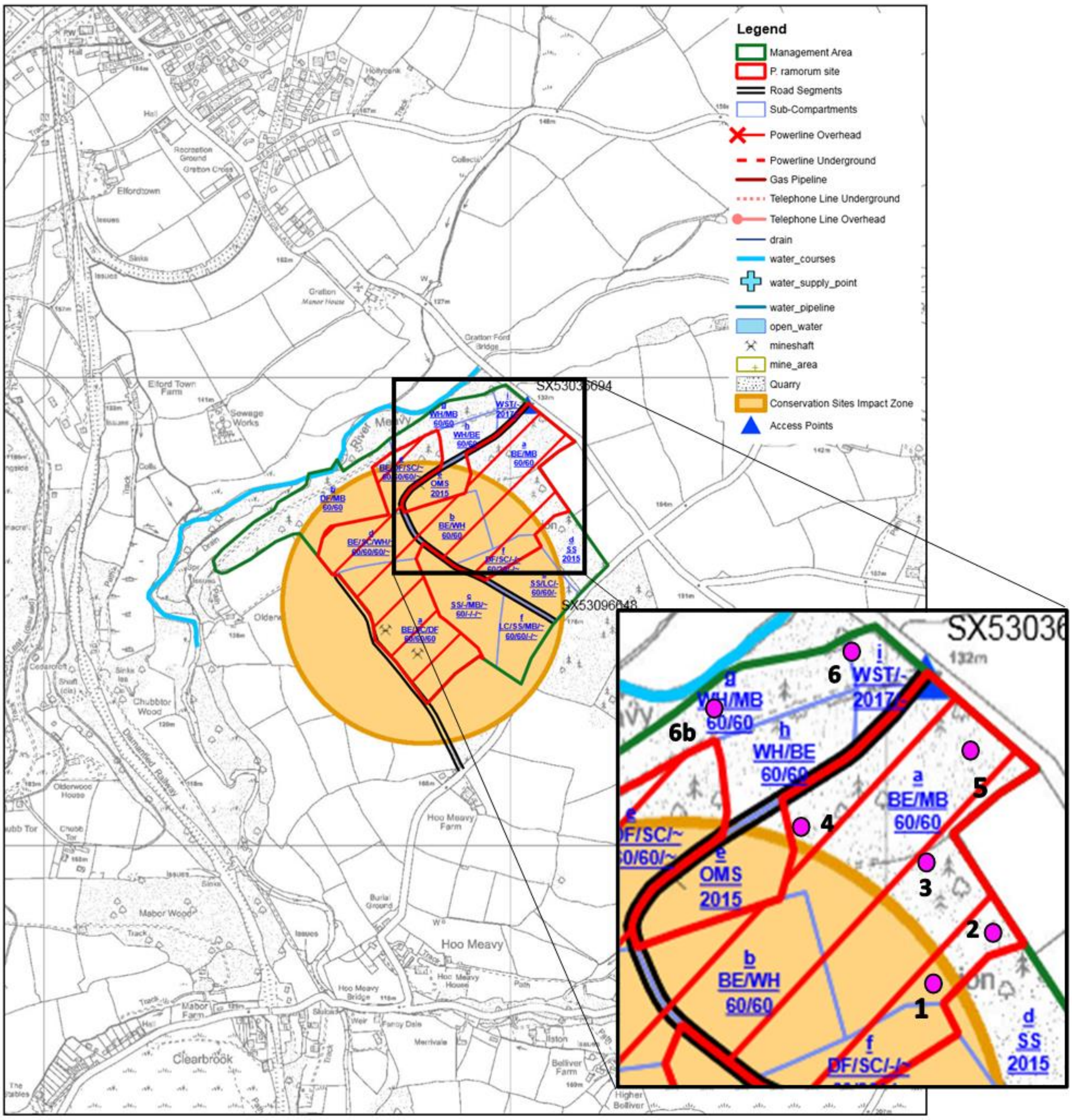


Figure 3.12: Approximate locations of feeder stations in the Olderwood site shown in pink (edited from Forestry Commission, 2019b). Other features are also labelled, including the Conservation Sites Impact Zone.

3.4 Weather Data

For the purpose of additional background data into the site and the conditions during which the study was undertaken, weather data was used from a private monitoring station in Crownhill, Plymouth. The approximate location is shown in Figure 3.13, approximately 7.2km away from the Olderwood site and 6.4km from the Denham site. In addition, correlations between bird visits and key weather variables were tested to ascertain whether the weather caused differences in bird visitation behaviour;; air temperature, rainfall, wind speed, sunshine hours, and dew point temperature. These relate to chill and balancing energy required from the cold with being vulnerable to predation whilst foraging, with wind speed, rainfall, and dew point temperature influencing the calories necessary to keep warm. Sunshine hours can influence this too, affecting visibility and therefore how easy it is to find the feeders as well as the risk factor from avoiding predators (how well the birds can hide from and see predators). This data is made freely available online, and was used with permission from the weather station owners in this study (bearsbythesea, 2020).

Figure 3.13: The location of the weather station at Crownhill, Plymouth, relative to the study sites indicated by pink circles (edited from Ordnance Survey, 2019).

3.5 Data Analysis

Basic descriptive analysis was used for the data collected from the residence survey. Count data, averages and percentages are used to give an understanding of the feeders and the birds that use them to highlight their use in ecological monitoring and to explore the implications should *Phytophthora* be found to transfer at these sites. Some of the data can be extrapolated to make inferences about the complete residential population at Keele from the population surveyed.

Count data and averages collected on the woodland feeder's species visits, observations, and the camera trap data have been arranged by different variables to explore patterns. These include time of day and by feeder type to indicate preference. Throughout the data analysis, chi-square tests are used to determine whether there are significant differences within the data between woodlands and to verify some trends in bird visits and observations. This is done to a 95% confidence level. There are some limitations to the use of chi-square testing, including that it cannot be used accurately when the expected values are less than one, and if more than 20% of values are less than 5 (Boslaugh, 2012). Because of this, there were some data sets where it could not be used to determine whether the difference in values was significant for every species. Error bars displaying standard error have been used on appropriate figures that display averages. These are not of uniform value within the figures, reflecting the different data samples and their variation. Standard error can be used to indicate the reliability of the mean, indicating whether the mean reflects that of the true population.

To analyse the environmental data, measures of abundance, diversity and richness were used. Differences in abundance were ascertained using chi-square tests as described, as an initial step towards exploring differences in the feeder visitation results. Simpson's Index (SI) was used as a measure of diversity between the feeder locations using the formula below, where n is the total number of organisms of a particular species and N is the total number of organisms of all species;

$$SI = \frac{\Sigma n(n-1)}{N(N-1)}$$

This gives values between 0 and 1, whereby 0 is maximum diversity and 1 is no diversity. Simpson's Index was used for tree species around each feeder location, and was broken down into mature species diversity and sapling diversity as well as the total. Mature individual trees were considered as those with diameter at breast height (DBH) of greater than 5cm, whereas saplings were those with DBH of less than 5cm.

Pearson's correlation coefficient was used to determine whether variations in bird visits and observations were influenced significantly by environmental factors over the study period. This could determine the optimum environment or weather to conduct future investigations using bird feeders. As some of the data sets were not found to be normally distributed, all of the data was converted using natural logarithms, with the formula ln(x), before the correlation tests were conducted. The outcome gives an indication of strength and direction of linear relationships in the value of r, which can range between -1 to 1. The P-value calculated in this test gives an indication of significance; in this study, a 95% confidence level was used to determine significant relationships, meaning a P-value of less than 0.05 indicating significant results.

4 - Results

4.1 Residents survey

The residents survey consisted of 7 questions, which are shown in full in Appendix i. All houses in Keele University that were identified as having a garden to place a feeder were grouped by location which were labelled A, B, C, D, E, and were visited across two weeks. In these location groups, there were a total 125 residences, of which 61 were occupied at the time of the residents survey. Of these, 25 owned at least one feeder, and 35 did not own any. This gives a feeder ownership rate of nearly 40% for Keele University as a total. Figure 4.1 shows the rate of ownership as percentage per housing group, with D having the highest rate of 70% ownership and A the lowest with 23%.

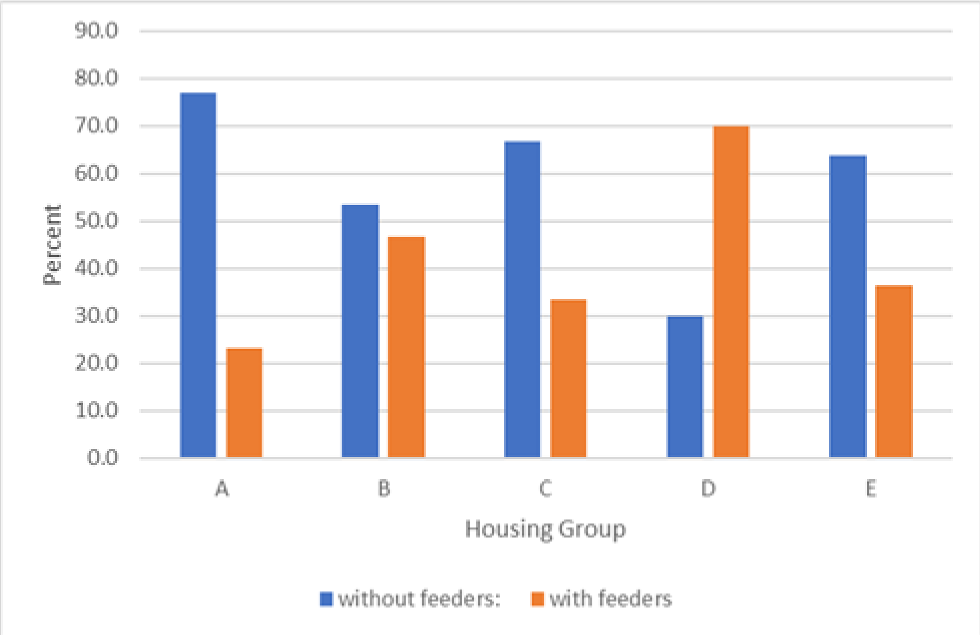


Figure 4.1: Responses to question 1 showing feeder ownership as a percentage, per housing group A-E based on location.

The first question asked of residents was 'how long have you had a bird feeder at this address?'. This was asked to 24 residents and was recorded in years. The results had a large range as shown in Figure 4.2, with the maximum length of time being 40 years and the minimum being two weeks (0.04 years). The mean was 13 years, but the most common response was 5 years.

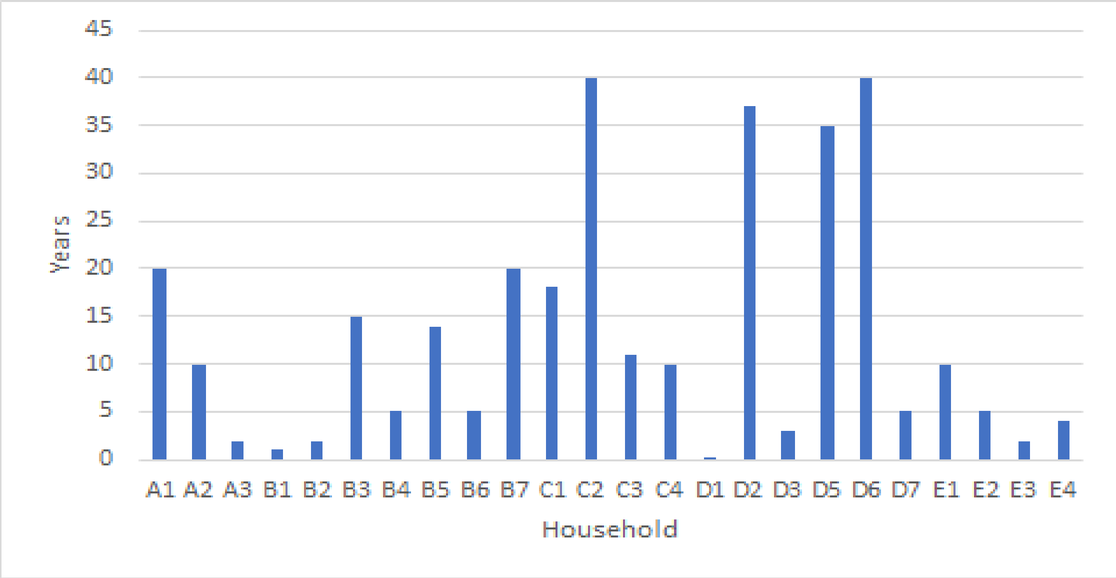


Figure 4.2: Results from question 2 of the residents survey on length of time feeders have been owned in years at that address. The household is given in code to preserve anonymity.

The results for question 2 show how many bird feeders are owned by the respondents, and Figure 4.3 shows the number of respondents with each number of feeders. Respondents with 1 or 2 feeders accounted for 50% of the total, but the maximum was 7. The total number of feeders owned by the 24 responses was 72, giving a mean average of 3 per household. When accounting for the number of respondents without a feeder, 35, this becomes an average of 1.2 per household; scaled up to the total number of houses, there is predicted to be approximately 150 feeders owned by the residents of Keele campus.

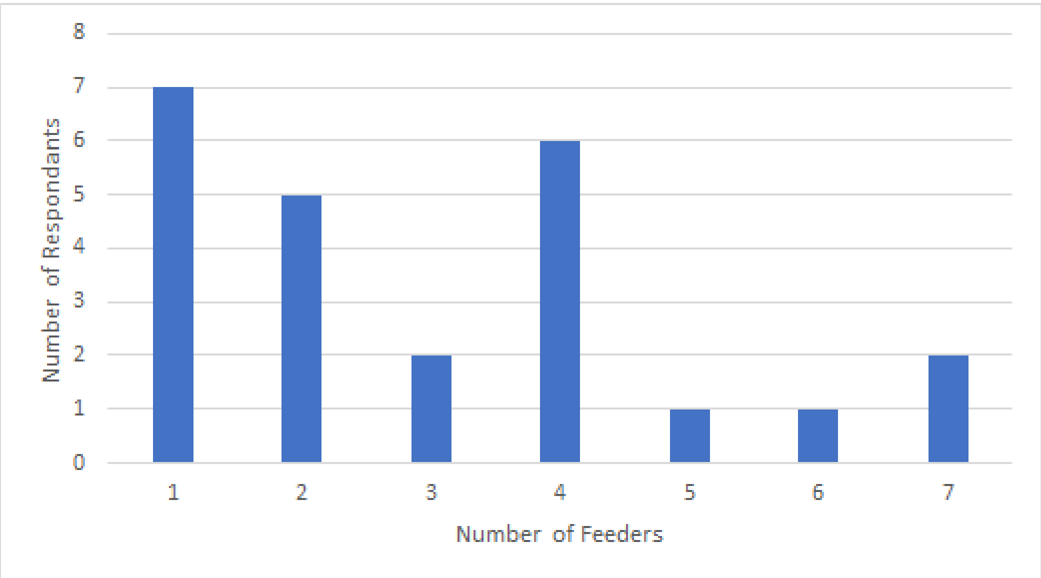


Figure 4.3: The number of feeders owned by respondents.

Questions 3 and 4 regarded the type of feeder and what food were used. The majority of feeders were hanging at 60%, whereas ground feeders and hanging bowls were only owned by one respondent each, shown in table 1. However, hanging feeders can be extremely varied, and so the results for question 4, shown in Figure 4.4, give a more detailed representation of which feeders might be being used; fatballs are mostly used in hanging cages with large gaps, whereas nyjer seed requires a specialised hanging tube feeder with perches. Fatball and mix seed foods are the two most commonly used food source and account for 50% of responses. The number of responses for food types used is larger than the number of feeder types as residents would change what food was used in the feeders.

Table 4.1: Feeder types owned by respondents.

hanging	21
hanging w. cage	4
table	4
window	2
ground	1
hanging bowl	1
coconut	2

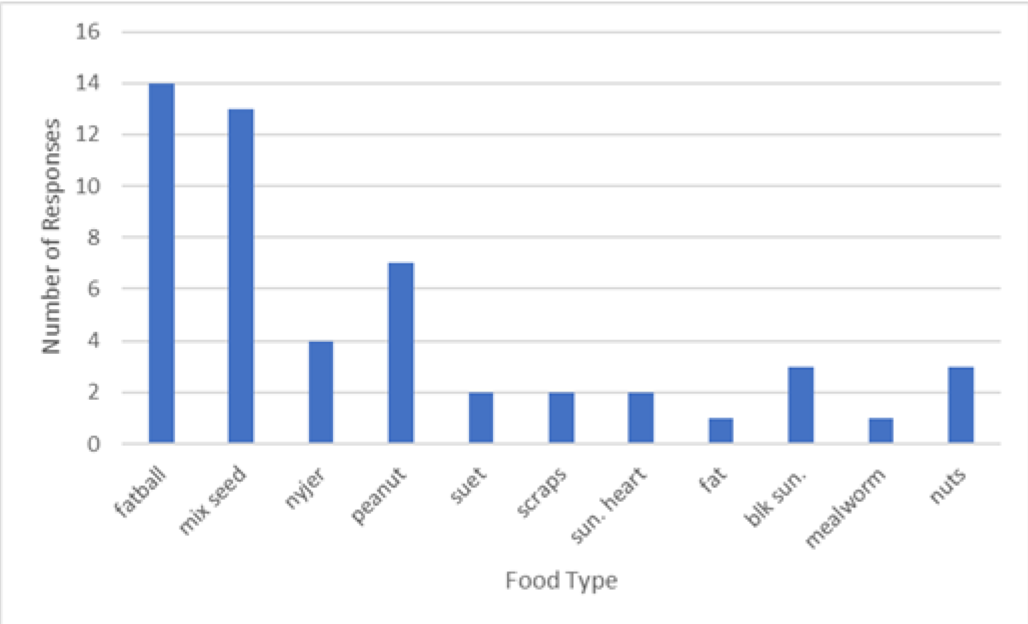


Figure 4.4: The number of responses for each food type.

Question 5 asked which birds were seen by residents at their feeder. This does not represent a comprehensive list as most residents did not keep any records of what they saw and could forget. In addition, it should be noted that mistakes could have been made with identification with hard to distinguish birds as the experience and attention paid to the bird feeders seemed to differ greatly between respondents judged from conversations. The results are shown in Figure 4.5, and BTO bird codes were used when responses were given to a species level. These bird codes can be viewed in Appendix ii. The bird which was seen most was the Blue Tit (BT), followed by Robin (R.) and Wood Pigeon (WP). The responses that were given the least were Black Cap (BC), Collared Dove (CD), Hawfinch (HF), and House Sparrow (HS) all with one response each. However, with regards to the House Sparrow, 2 responses of non-species specific sparrow were also given. The only bird of prey reported was Sparrow Hawk (SH), and from further detail given by respondents, there seems to be at least a male and female pair that are seen consistently, with other individuals also probable.

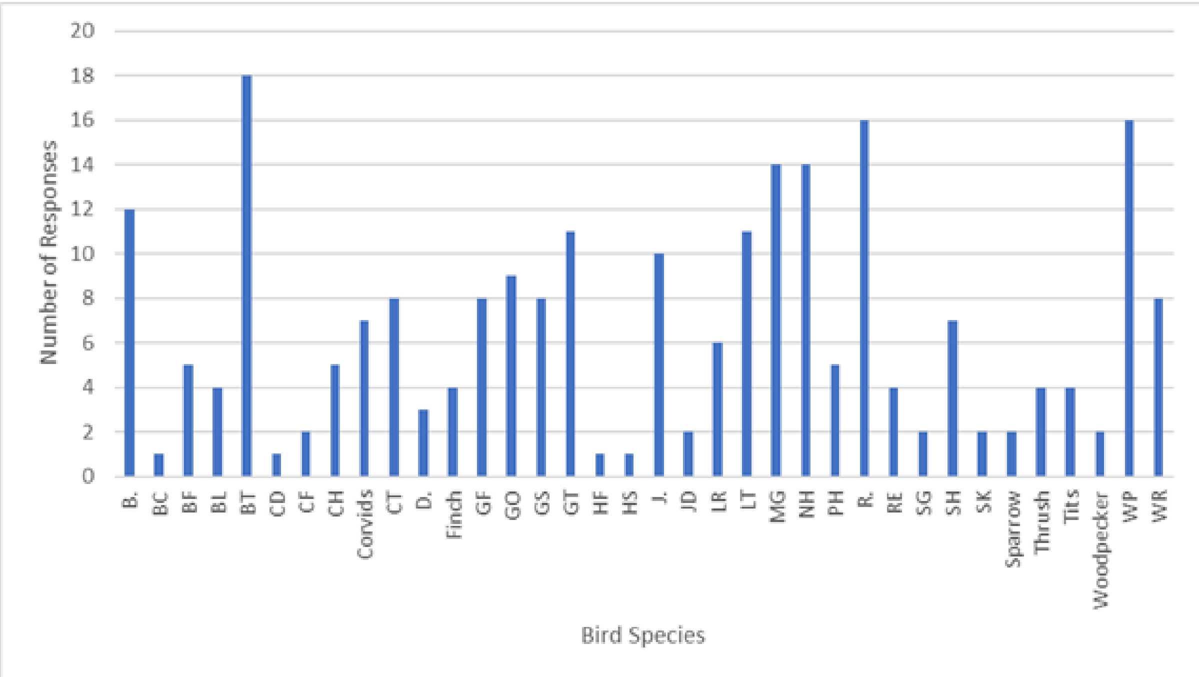


Figure 4.5: The number of respondents seeing different bird species using the residents' feeders. The BTO bird codes can be found in Appendix ii.

Question 6 aimed to gain information on how often squirrels were seen feeding from residents' feeders. Three options were given: a) not at all b) regularly or c) occasionally. Most respondents reported that this was a regular occurrence, with a total of 76% choosing option b). No residents reported not seeing squirrels feeding at all, leaving 24% who reported occasional feeding.

Question 7 asked for an estimate of how often the respondents cleaned their feeders, and the results for each of the 5 response choices are shown in Figure 4.6. The categories c, d, and e represent nearly a quarter of responses each (24%).

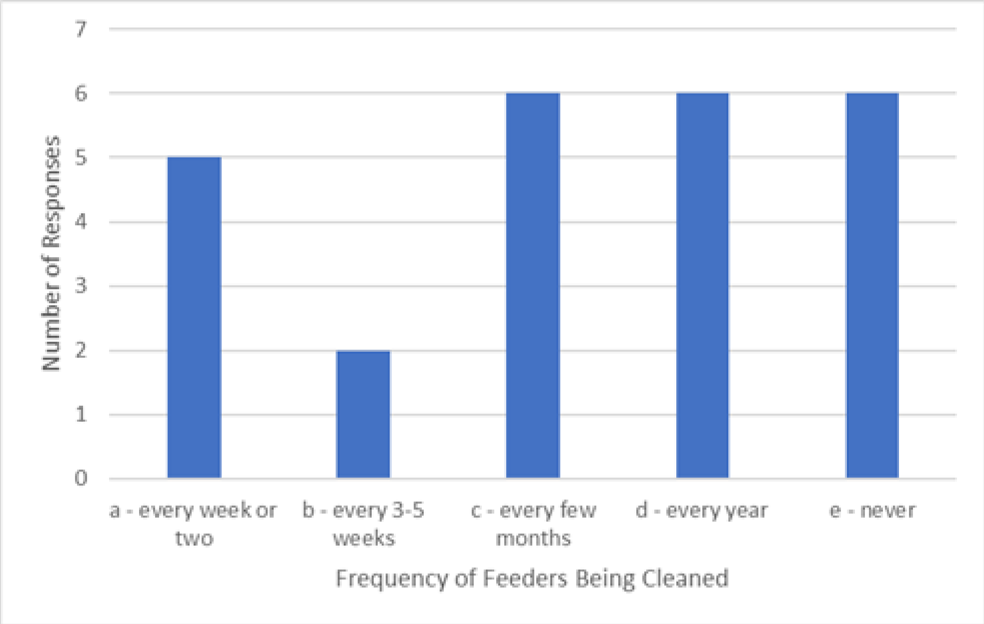


Figure 4.6: The frequency respondents reported cleaning their feeder.

For residents who gave permission, swab samples were then tested with rapid diagnostic test kits for the presence of *Phytophthora*. The results for this are shown in Table 4.2. A total of 14 properties agreed, and the number of samples taken from each varied according to how many feeders the resident owned (up to a maximum of three). A total of 21 tests were taken, and none of these gave a positive result. Three samples were marked as void as the control line did not appear, with the other 18 giving a negative result.

Table 4.2: The outcome of *Phytophthora* swab sample testing with rapid diagnostic test kits for each housing group.

Housing Group	**No. of houses**	**No. of swabs**	**Negative**	**Positive**	**Void**
A	2	4	3	0	1
B	5	8	6	0	2
C	4	6	6	0	0
D	3	3	3	0	0
E	0	0	0	0	0
Total	14	21	18	0	3

4.2 Woodland Feeders

Throughout the results, graphs and tables are colour coded to denote the woodland to which they refer, with Denham Woods being displayed in green and Olderwood being displayed in yellow. Scientific names of all bird, mammal and tree species recorded in the study can be found in Appendix iii. To conserve space within the graphs and figures, BTO bird codes consisting of two characters have been used, and the full list is given in Appendix ii. The exception to this is 'M/WT' which has been used as Marsh and Willow Tit could not be distinguished in full confidence. In addition to the BTO bird codes, mammals have been coded in a similar way but using two uppercase and one lowercase character; Grey Squirrel is referred to as GSq and Roe Deer as RoD. Similarly to Marsh/Willow Tit, the differences between Field Mouse and Bank Vole could not be determined in observations due to distance. It was noted from photographs taken that both species were present at the feeders in both woodlands, but they cannot be separated out by species in the results. Therefore, in the graphs and tables, the code FMs refers to a combination of Field Mouse and Bank Vole. The list of species codes used in the graphs and figures is given in Table 4.3.

Table 4.3: Species codes used in graphs and figures throughout the results to conserve space, separated by birds and mammals.

Birds				Mammals	
BT	Blue Tit	**J.**	Jay	**FMs**	Fieldmouse/Bankvole
CH	Chaffinch	**M/WT**	Marsh/Willow Tit	**FOx**	Fox
CT	Coal Tit	**MP**	Magpie	**GSq**	Grey Squirrel
D.	Dunnok	**NH**	Nuthatch	**RoD**	Roe Deer
GR	Greenfinch	**PH**	Pheasant	**SHp**	Sheep
GS	Greater Spotter Woodpecker	**R.**	Robin		
GT	Great Tit	**SK**	Siskin		

4.2.1 Lateral Flow Tests

Lateral flow devices were used on the feeders and leaf litter in Denham Woods and Olderwood following the methodology described in sections 3.2 and 3.3, and the results are shown in Table 4.4. The lateral flow devices were used in combination with swabs to test the ground and hanging feeders at each station, whereas the leaf litter was tested by direct addition of a sample to the buffer solution. The swab test taken after week five of the study and the swabs taken at the end of the study period, in week 9, all came back negative for both woodlands. The leaf litter tests also came back negative.

Table 4.4: Lateral flow device test results for *Phytophthora* species including *P. ramorum* for each feeder station comprised of the hanging feeder (hf) and groundfeeder (gf).

	Woods	Denham				
	Date	11/04		17/05		
	Feeder	gf	hf	gf	hf	Leaf litter
Station	1	N	N	N	N	N
	2	N	N	N	N	N
	3	N	N	N	N	N
	4	N	N	N	N	N
	5	N	N	N	N	N
	6	N	N	N	N	N
	Woods	Olderwood				
	Date	12/04		17/05		
	Feeder	gf	hf	gf	hf	Leaf litter
Station	1	N	N	N	N	N
	2	N	N	N	N	N
	3	N	N	N	N	N
	4	N	N	N	N	N
	5	N	N	N	N	N
	6	N	N	-	-	-
	6b	-	-	N	N	N

4.2.2 Observation Results

The total number of visits observed of birds using the woodland feeders for Denham Woods and Olderwood per date are displayed in Figures 4.7 and 4.8 respectively. For Olderwood (Figure 4.8), feeder stations 6 and 6b have been excluded, as they did not run for the same length of time as the other feeders, with O6 (Olderwood feeder station 6) running from the 19th of March to the 12th of April, and O6b running from the 16th of April to the 17th of May; that is 23 days and 31 days respectively, as opposed to the 58 day running time of the other feeder stations in Olderwood. However, the number of visits still exceeds those of Denham in many instances. In both woodlands, the number of bird visits fluctuates by date, although this is much more pronounced in Olderwood. In Denham Woods, it can be seen that the overall trend follows an increasing number of visits as the feeders become established through to the 28th of March, whereupon the trend becomes more consistent. In Olderwood, there is a larger variance between the values, and so a date by which the feeders have established is harder to identify. The larger variance in Olderwood is reflected in the mean number of visits, which is shown for both woodlands in Figures 4.7 and 4.8 respectively. Although Olderwood often receives more visits, the means for both woodlands are similar at 63 for Denham and 62 for Olderwood. The lower deviation is also shown, at 46 for Denham Woods and 51 for Olderwood.

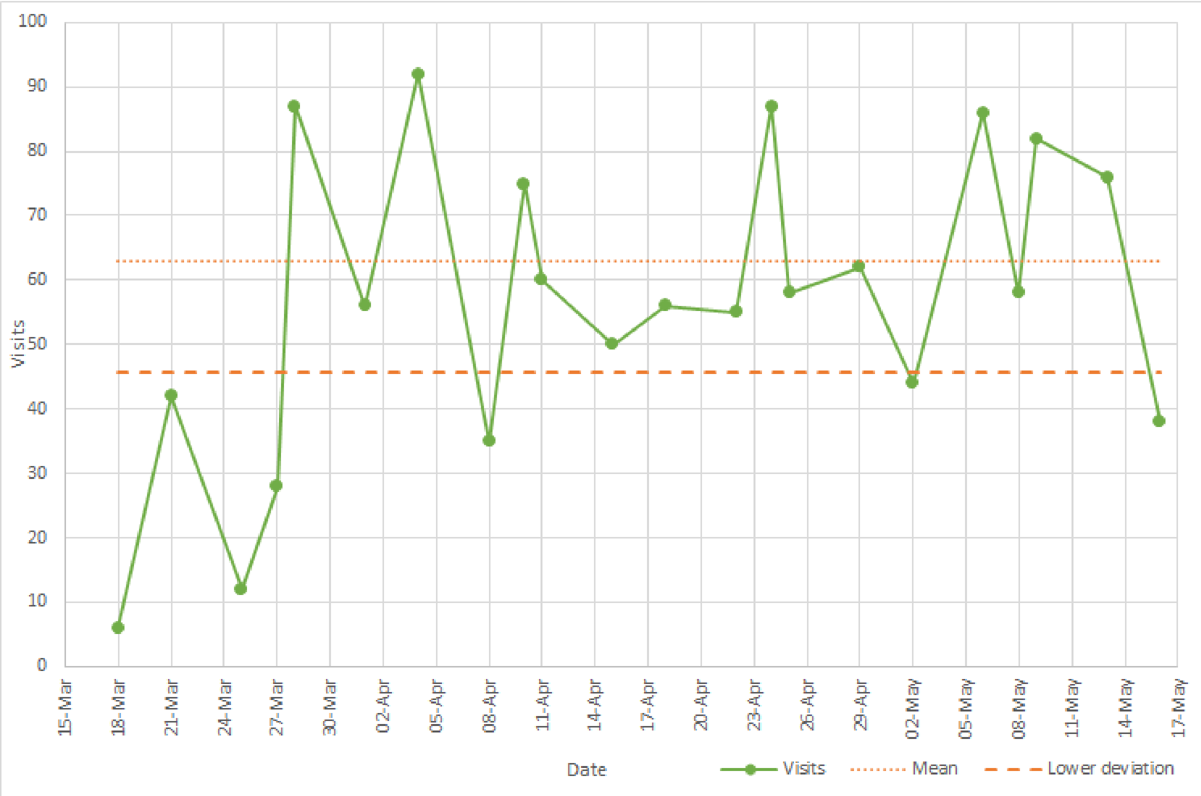


Figure 4.7: The total number of bird visits to the woodland feeders observed in Denham woods per date for all species.

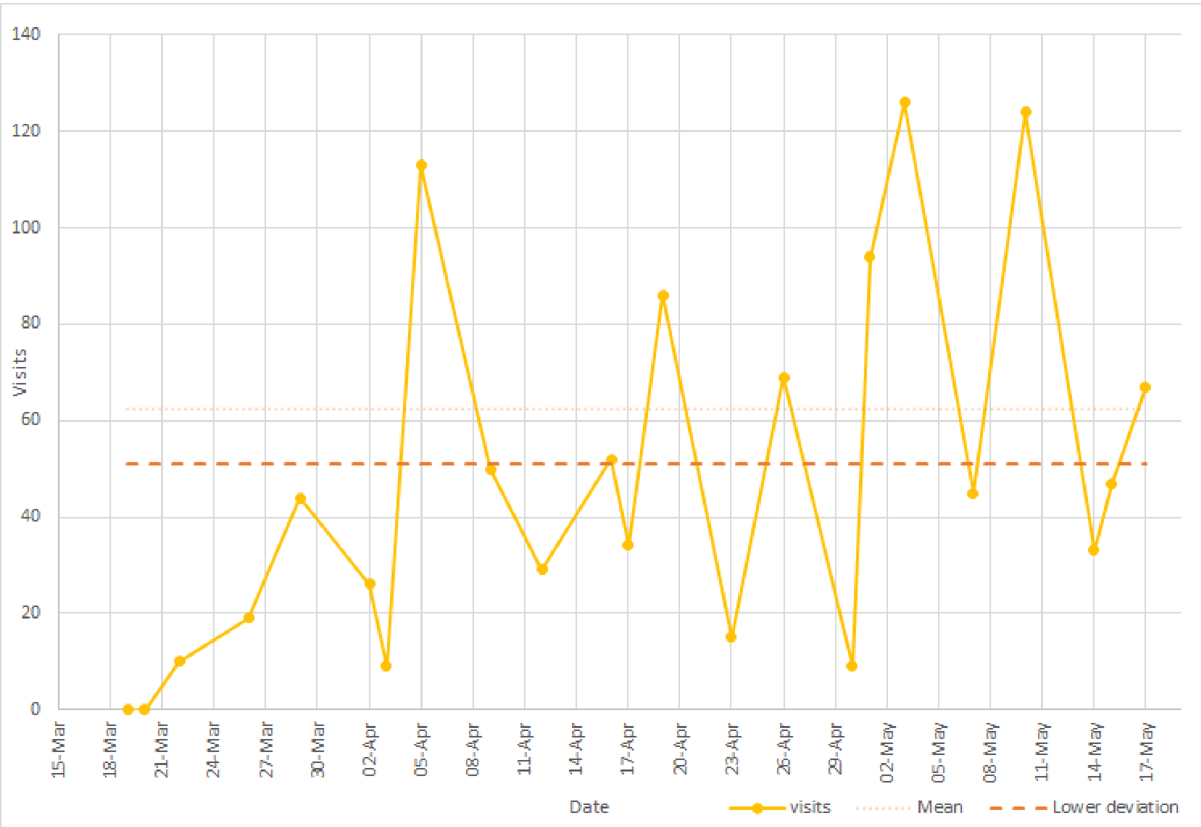


Figure 4.8: The total number of bird visits observed to feeders in Olderwood by date.

Figure 4.9 displays the mean number of total visits across bird species by time of day for Denham Woods. Peaks in activity seem to be visible between the hours of 9-10, 13-14, and 18-19. Dips in activity are suggested at 08:00 and 14:00. Figure 4.10 displays the same data for Olderwood, but with average visits per time category being higher before 12pm, and dips at 13:00 and 16:00. The peaks visible in Denham Woods are not observed in Olderwood. No observations were made in Olderwood within the hours of 18-19. Mammals were excluded from these figures, and are considered separately in Figure 4.12 (p. x)

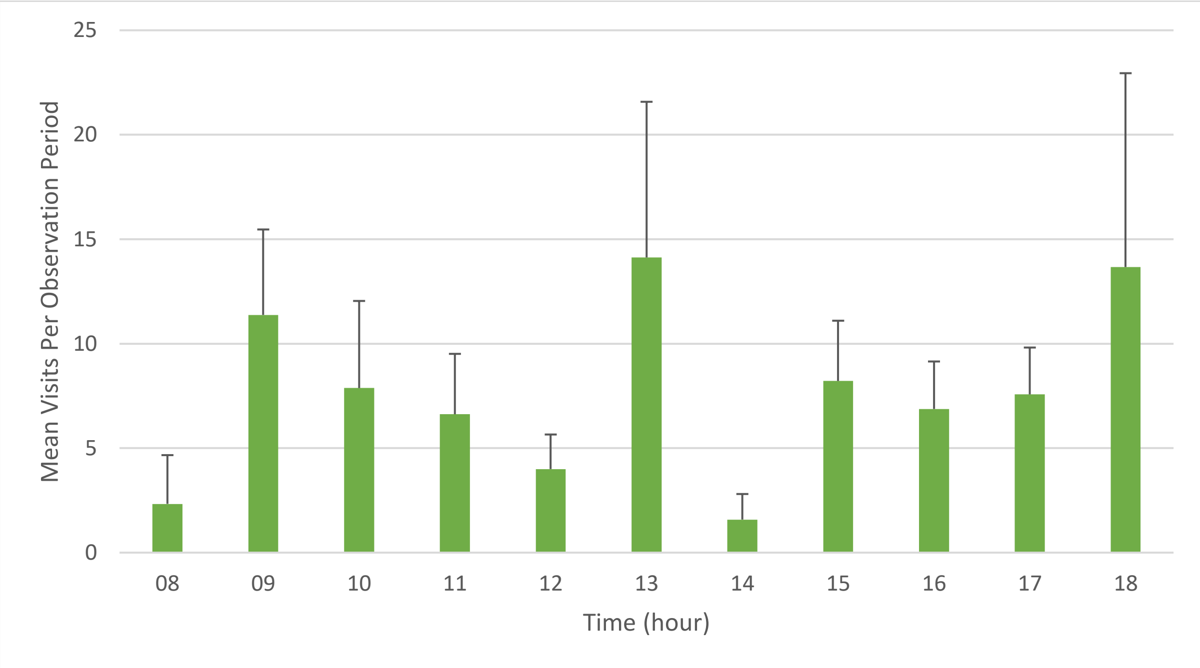


Figure 4.9: The mean number of total bird visits per observation period by time of day (24-hour clock) for Denham feeders. Each hour indicates when the observation period started, so that 08 is every observation period beginning from 07:46 (so the majority of the observation period was in the 08 hour) until observation periods beginning at 08:45. Standard error is shown above the bars.

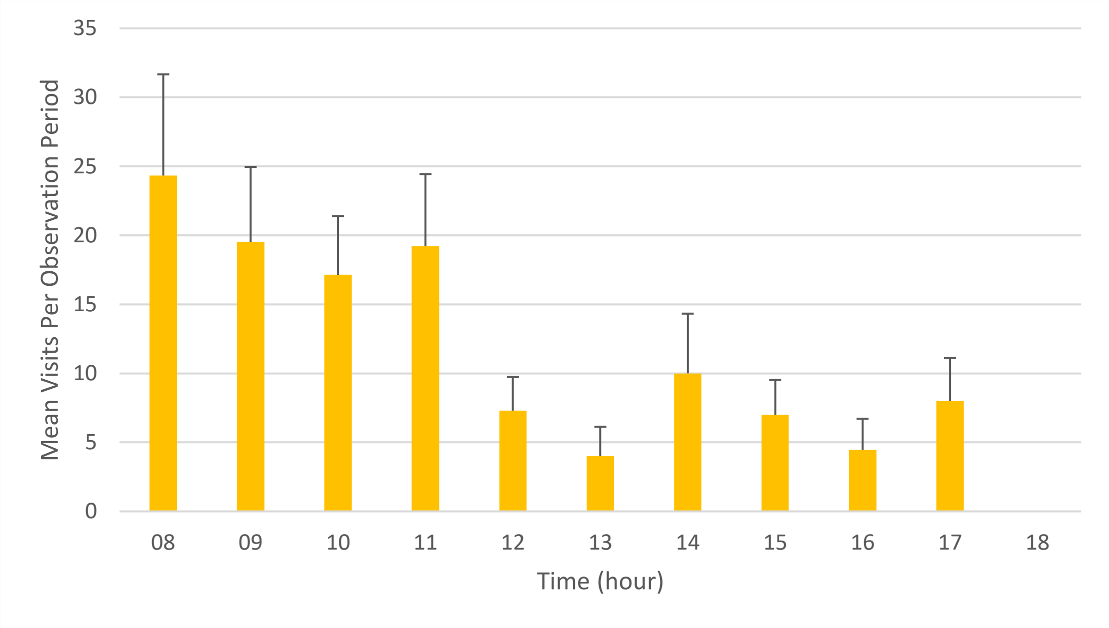


Figure 4.10: The mean number of visits each observation period for all bird species combined per time category for Olderwood feeders. Each hour indicates when the observation period started, as above. No observations were undertaken at 18 in Olderwood. The error bars show standard error of the mean.

The total number of visits observed on all feeders per woodland per bird for the entire study period is shown in Table 4.5, along with the total number of observation periods during which they were seen (denoted regularity). The ratio between visits and regularity is shown in column 3, which is the average number of visits per observation period in which each bird was observed. There are large differences between Olderwood and Denham Woods in the number of visits, and this was confirmed as significant to a 95% confidence level using chi-squared testing; with 1 degree of freedom, an X^2 value greater than 3.84 shows significance. Along with the total number of visits being significantly greater in Olderwood than Denham Woods, the birds Great Tit (GT), Robin (R.), and Chaffinch (CH) individually show significantly greater visits to Olderwood than Denham Woods, as well as Nuthatch (NH) not being seen in Denham Woods. However, Siskin (SK), Greenfinch (GR), and Field Mouse/Bank Vole (FMs) were not seen visiting the feeders in Olderwood unlike in Denham Woods. There are two birds for which Denham Woods received more visits, the Coal Tit (CT) and Blue Tit (BT), but the difference was not found to be significant. However, the difference in visits of the Grey Squirrel (GSq) is significantly greater in Denham than Olderwood. The trend of Olderwood receiving more feeder visits is not reflected in the regularity of sightings, as the only bird with significantly greater regularity of visits in Olderwood being the Robin. The Grey Squirrel was found to be visiting the feeders significantly more regularly in Denham Woods. The ratio of visits/regularity is similar for most birds between the two woodlands for the species that were observed in both, which is reflected by the chi-square tests, but this test could only be accurately conducted for three species as the rest had expected values less than 5.

Table 4.5: The total number of visits, the regularity (the number of observation periods in which the species was seen) and the ratio between the two (or the mean visits per observation) for each species separated by woodland; Denham Woods in green and Olderwood in yellow. Chi-square testing results are shown, comparing Denham and Olderwood, with X^2 results greater than 3.84 indicating significant difference to 95% confidence level highlighted in red. Blank cells in the chi-squared columns indicate that testing could not be done, either as one value was zero or the sum of both values was less than or equal to 5. BTO bird codes and mammal coding was used to refer to each species (see p. 51).

	Visits			Regularity			Ratio		
Species	**D**	**O**	**X²**	**D**	**O**	**X²**	**D**	**O**	**X²**
GT	98	250	66.39	21	30	1.59	4.7	8.3	0.5
NH	0	459	-	0	51	-	0	9	-
CT	380	329	3.67	42	40	0.05	9	8.2	0.04
BT	110	106	0.07	23	23	-	4.8	4.6	-
M/WT	246	264	0.64	21	15	1	11.7	17.6	0.59
R.	8	65	44.51	5	31	18.78	1.6	2.1	-
GS	3	10	3.77	2	7	-	1.5	1.4	-
CH	22	46	8.47	13	23	2.78	1.7	2	-
D.	3	4	-	2	3	-	1.5	1.3	-
SK	34	0	-	9	0	-	3.8	0	-
GR	4	0	-	1	0	-	4	0	-
GSq	27	5	15.13	17	5	6.55	1.6	1	-
FMs	56	0	-	12	0	-	4.7	0	-
Total	991	1538	118.31	168	228	9.09	-	-	-

Figure 4.11 shows feeder preference between the hanging feeder, the ground feeder, and the woodland floor for each species seen excluding Greenfinch due to there being only minimal data. The results are variable between each species, but most show a preference for a singular feeder type. The extent of preference was tested for significance using chi square between each species' two most visited feeders overall, shown in Table 4.6. Great Tit (GT), Coal Tit (CT), Blue Tit (BT), and Siskin (SK) all show a significant preference for the hanging feeder, whereas the Marsh/Willow Tit (M/WT), Nuthatch (NH), and Robin (R.) significantly prefer the ground feeder. Only the Field Mouse/Bank Vole (FMs) show a preference for the woodland floor, although the Chaffinch (CH) and Grey Squirrel (GSq) do not show a significant preference between the floor and the ground feeder. The Dunnock (D.), Grey Squirrel, Robin, and Chaffinch show large variation between woodlands. Greater Spotted Woodpecker and Dunnock are displayed in Figure 4.11 but have too few data points for chi square analysis and so are excluded from Table 4.6.

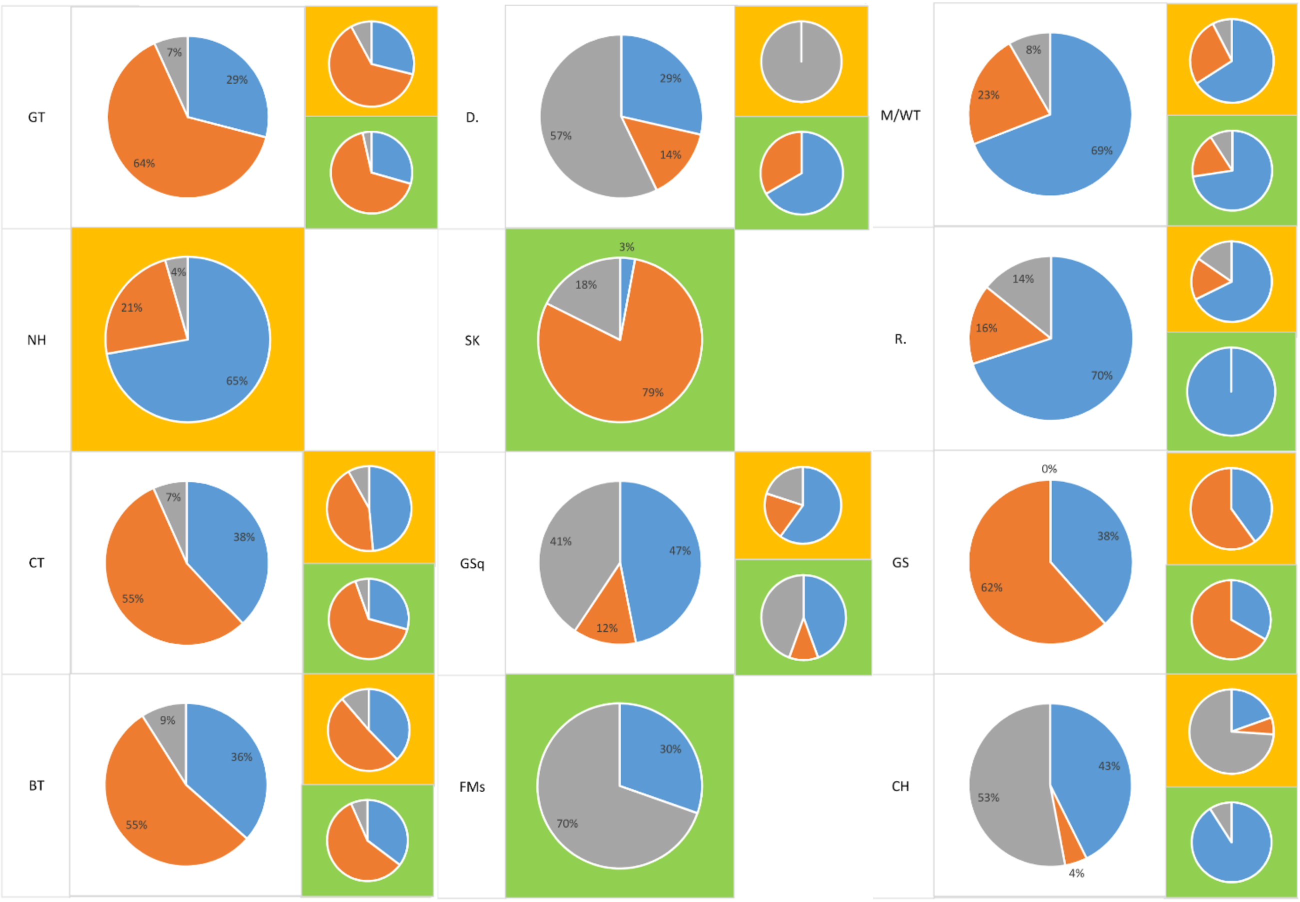


Figure 4.11: Feeder preference of different species (using coding from p.51), with hanging feeder (hf) in orange, ground feeder (gf) in blue and the woodland floor in grey (fl). Olderwood results are shown with a yellow background, and Denham Woods with green with the totals of both woodlands white; not all species were present in both woodlands, and so only have the one set of results.

Table 4.6: Chi-square results, testing the significance for feeder preference - the difference between the two most visited feeders for each species, combined across both woodlands except for Nuthatch (NH), Siskin (SK), and Fieldmouse//Bankvole (FMs). Not every species is included due to some having too few data points to conduct the analysis.

Species	X^2	Feeder preference
GT	43.03	hf
NH	105.09	gf
CT	21.43	hf
BT	7.52	hf
M/WT	119.28	gf
R.	24.07	gf
CH	0.75	gf & fl
SK	13.36	hf
GSq	0.14	gf & fl
FMs	8.64	fl

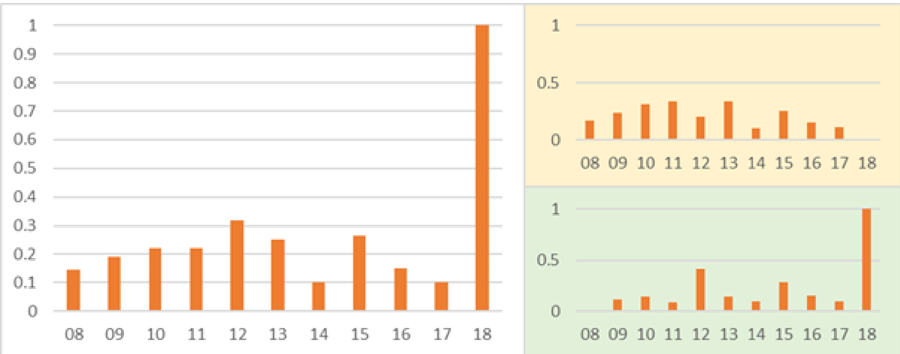
Figure 4.12a shows the frequency of Great Tits seen during each time category classified as between 0, never seen at this time, to 1, always seen at this time; the proportion of observation periods the species was seen. This was calculated by dividing the number of observation periods conducted in the given hour during which the bird was seen visiting the feeders (not taking into account the number of visits made) by the total number of observation periods conducted at that time. This is shown for both of the woodlands combined as well as Olderwood in yellow and Denham Woods in green. For the overall data, it appears that the Great Tit was most likely to be seen between hour 18 and 19, during which Great Tit was always seen; a total of 3 observations were taken during this time period. The second most likely time was at hours 12 or at 15, but the least likely time period was at hour 14. There were some differences between Olderwood observations and Denham Woods, most notably during hour 12, which was the second least likely in Olderwood, but the second most likely in Denham Woods. However, hour 14 was consistent in having a low frequency between the two woodlands.

Figure 4.12b shows the frequency of observing Nuthatch per time category. As Nuthatch was not seen in Denham Woods, only the results from Olderwood are given. Visitation was comparatively constant, although with higher frequencies before 12, and the lowest in hour 16. The hours before 12pm appear to have the highest frequency of Coal Tits presence in both woodlands, shown in Figure 4.12c, although in the morning Olderwood peaks in hour 11, whereas in Denham Woods this peaks in hour 08 with a frequency of 1. The latter is not necessarily a reliable result however, as only one observation at this time occurred at the feeders for which Coal Tit was present. Denham Woods has a small peak at the 18 time category, and this is more noticeable when the results for both woodlands are combined. From the Olderwood observations in Figure 4.12d, Blue Tit is most likely to be seen in the morning hours prior to 11am. However, this is not reflected in Denham woods, for which the frequency of observations in the hours 08 to 09 were zero. There is little discernible pattern to Denham's results beyond this, other than a drop in frequency in hour 14. When both woodlands are combined, no significant patterns seem visible. The frequency results for Marsh/Willow Tit are shown in Figure 4.12e. From the results of both woodlands combined, there are peaks in frequency in the morning, in hour 08, and in the early evening in hour 17. However, this pattern is less apparent in either Olderwood or Denham, in which both woodlands seem to have sporadic frequency results.

Robin was seen during only 5 observation periods in Denham, and on only one feeder. This means a clear pattern is not discernible in this woods in Figure 4.12f, but in Olderwood, there appears to be a peak at hours 10-11 as well as 17. Similarly to many of the bird species, hour 14 has the lowest frequency of visitation. The only time Greater Spotted Woodpecker had a frequency greater than 0.5 was at hour 10, whereas hour 11 had the second lowest frequency at 0.14 shown in Figure 4.12g. The lowest frequency was at hour 17 with 0.11. Greater Spotted Woodpecker was not seen during 6 of the total 11 hours. The

Chaffinch frequency results are shown in Figure 4.12h. From both of the woodlands combined, little pattern is visible. However, from Olderwood the frequency rises to a peak at the 11 category and the 17 category; the probabilities in Denham Woods are small for every time category. Chaffinch was not seen at hour 14 in Olderwood, as well as hours 8-9 in Denham. Siskin, which was only seen in Denham, was seen during 5 of the total 11 hours, with hour 12 being the highest at 1, and hour 17 being the lowest at 0.17 shown in Figure 4.12i. Figure 4.12j shows the frequency per time category for the Grey Squirrel in Denham Woods. There is an apparent trend of increasing frequency later in the day, with the frequency of 1 at hour 18. Field Mouse/Bank Vole was seen in hours of 09, 10, 11, 14, 15, & 18 during more than 50% of the observations undertaken at each in Denham Woods, as shown in Figure 4.12k. For hours 14 and 18, Field Mouse/Bank Vole was seen during every observation undertaken during these hours, and the species was seen during 7 hours out of a total possible 11. From the hours in which Field Mouse/Bank Vole was seen, 17 was the least frequent, seen in 33% of observations.

a



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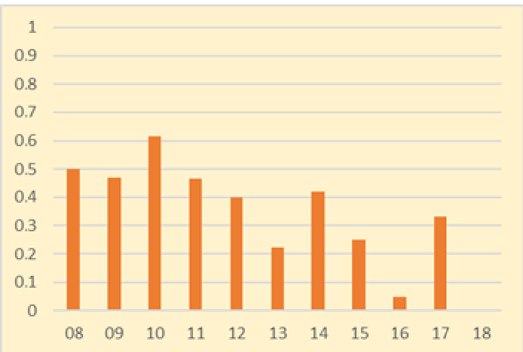

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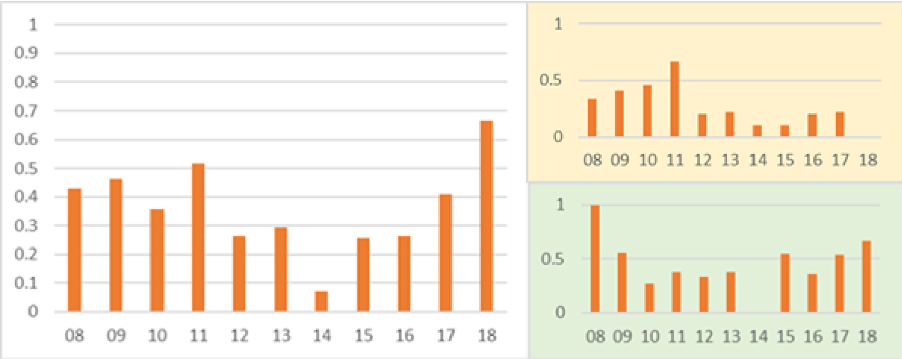
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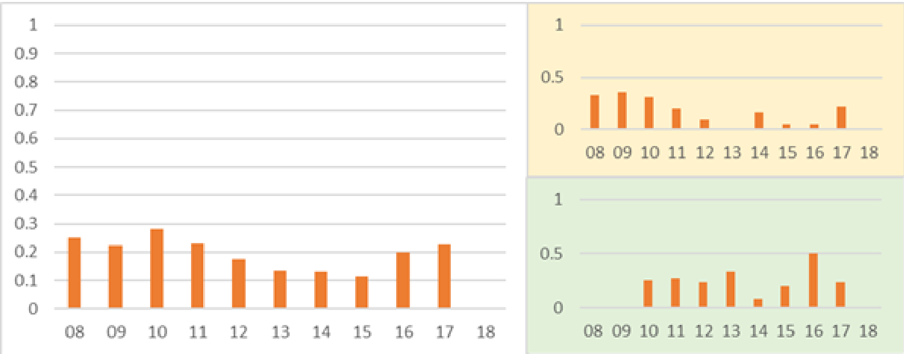
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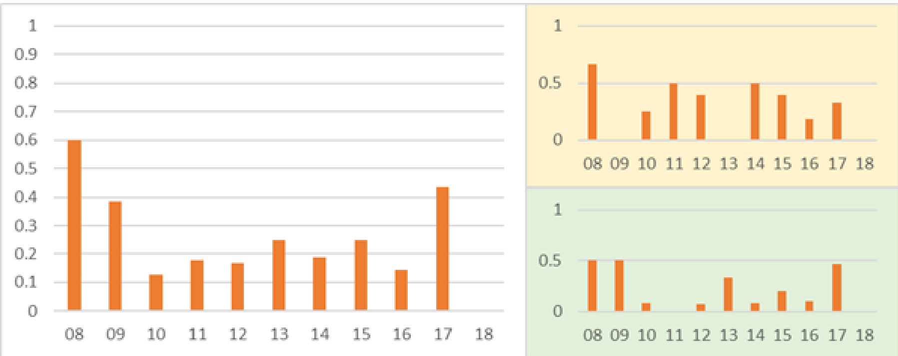
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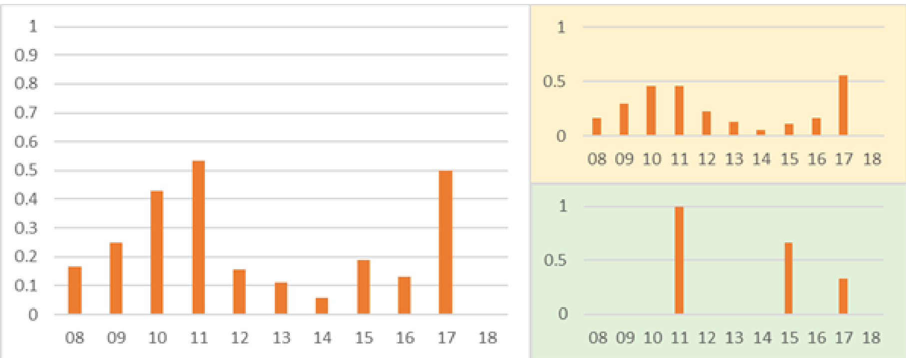
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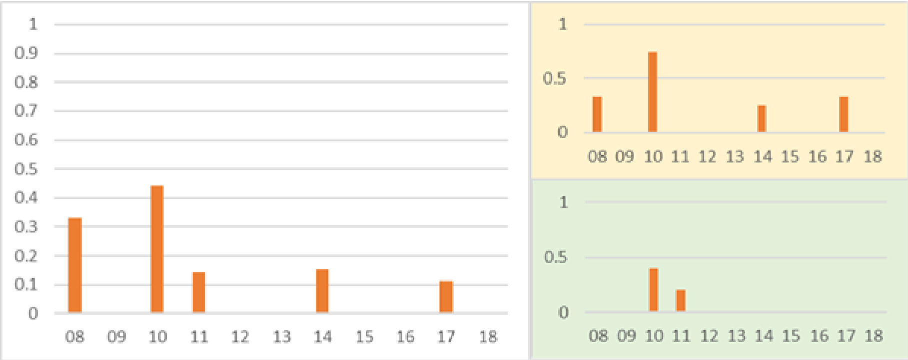
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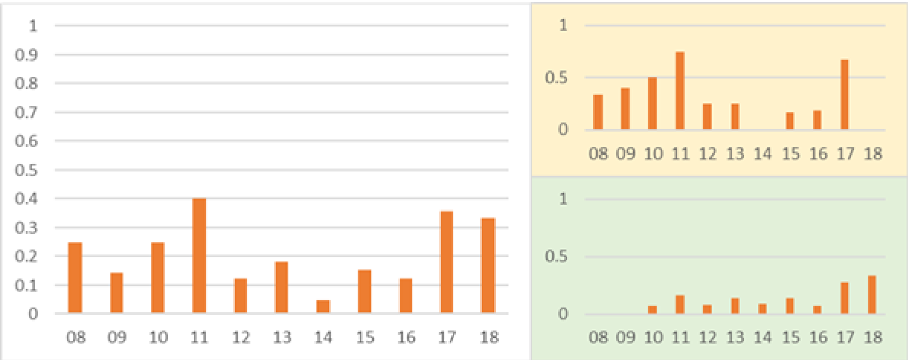
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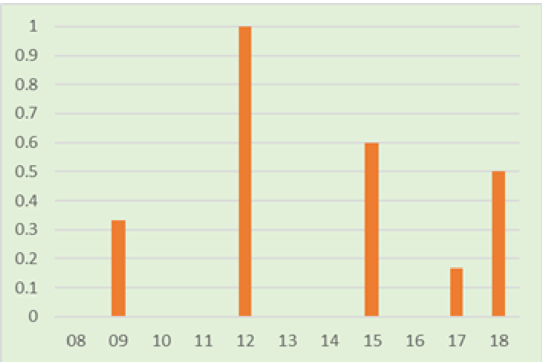
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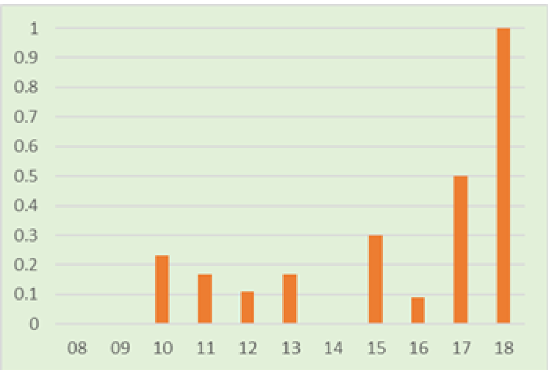
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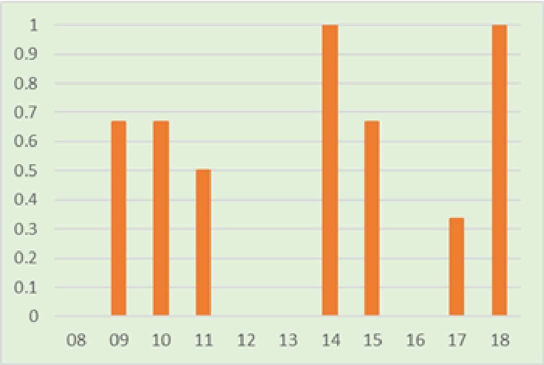

Figure 4.12: The number of times each feeder was visited in proportion to the total number observation periods undertaken at each hour for;

a: Great Tit

b: Nuthatch

c: Coal Tit

d: Blue Tit

e: Marsh/Willow Tit

f: Robin

g: Greater Spotted Woodpecker

h: Chaffinch

i: Siskin

j: Grey Squirrel

k: Field Mouse/Bank Vole

Olderwood results are displayed in yellow, Denham in green, and both woodlands combined in white when the species was present in both woodlands. As in Figures 4.9 and 4.10, each hour indicates when the observation period started, so that 08 is every observation period beginning from 07:46 until observation periods beginning at 08:45.

4.2.3 Camera Traps

Table 4.7 summarises the camera trap data for Olderwood. The number of pictures taken of each of the 11 species captured is shown, with Field Mouse/Bank Vole (FMs) being the most common. Great Tit (GT), Sheep (SHp), and Fox (FOx) were only caught in one photo respectively. Most birds seen during the observation periods were not caught by the camera trap. The number of pictures in which the animal is feeding from either the ground or hanging feeder is also given, with Field Mouse/Bank Vole captured the most, but Great Tit and Greater Spotted Woodpecker (GS) captured the most by proportion of total pictures. The maximum number of the species caught in one photo is given in column 4. For the majority of species, only one individual was caught, the exceptions being Nuthatch (NH), with 2, Field Mouse/Bank Vole, with 4, and Pheasant (PH) with 5.

Table 4.7: The number of pictures taken by the camera trap for each species in Olderwood, as well as the number pictures in which the species was using one of the feeders, and the maximum number of individuals caught in one photo. Coding from p.51 was used to refer to each species.

Species	Pictures	Feeding	Max no. present
FMs	201	60	4
J.	13	3	1
GT	1	1	1
RoD	11	1	1
PH	45	18	5
GS	9	8	1
GSq	18	8	1
NH	21	8	2
MP	24	11	1
SHp	1	0	1
FOx	1	0	1

For species with more than 5 pictures taken, and from more than one occasion (seen in more than one hour category), the times to the nearest hour for each photograph are given in Figure 4.13. Greater Spotted Woodpecker (GS), Jay (J.), Nuthatch (NH), and Pheasant (PH) all have peaks at 07:00, whereas Grey Squirrel (GSq) and Roe Deer (RoD) have peaks at between 19:00-20:00. Field Mouse/Bank Vole (FMs) does not share a peak with any other species, being far more active between 23:00-04:00.

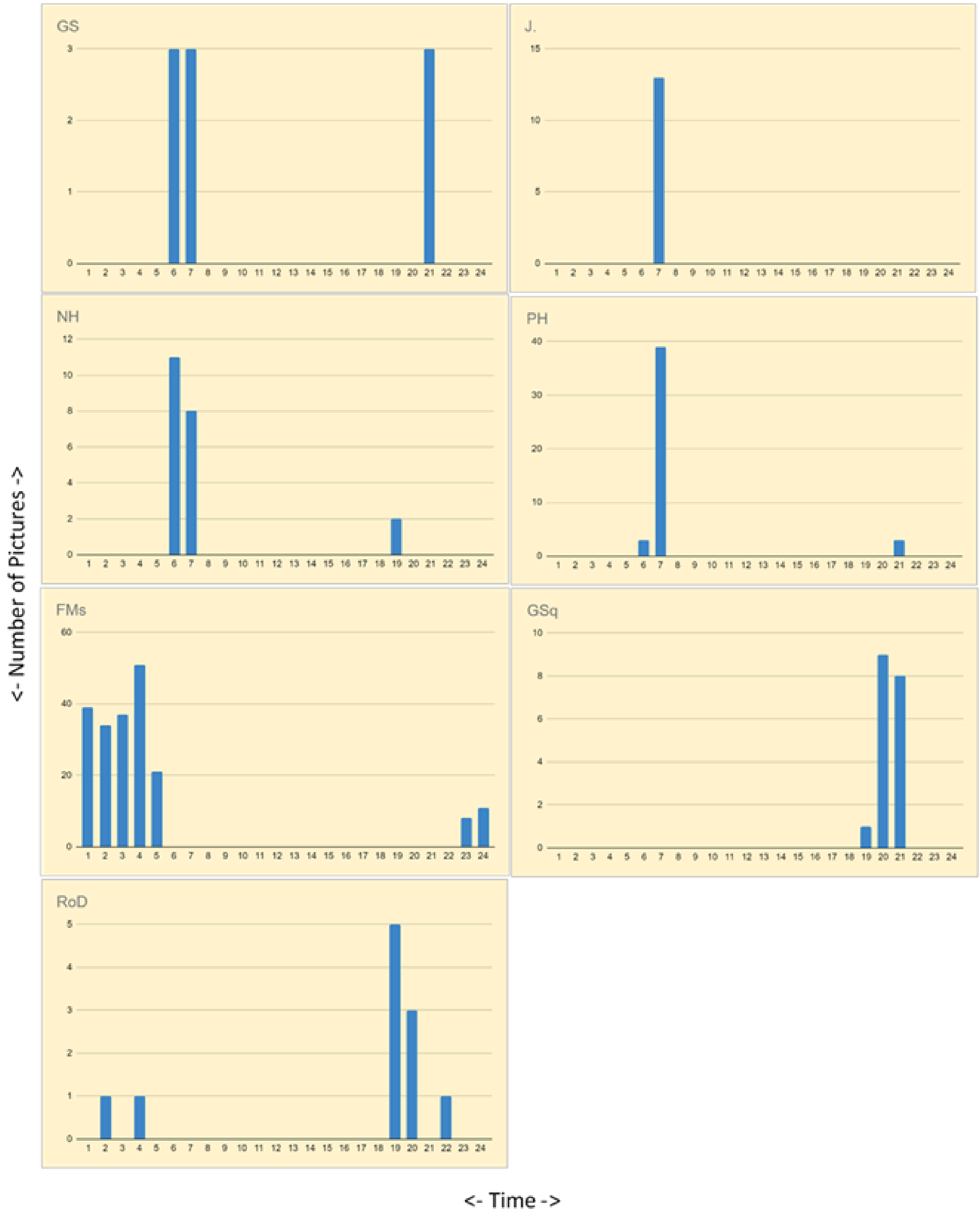

Figure 4.13: The number of photographs taken by the camera trap at each time for Greater Spotted Woodpecker (GS), Jay (J.), Nuthatch (NH), Pheasant (PH), Field Mouse/Bank Vole (FMs), Grey Squirrel (GSq), and Roe Deer (RoD). Species with fewer than five pictures, or from fewer than three occasions, were excluded. The x-axis differs in scale for each species graph.

Grey Squirrel (GSq) was pictured by the camera trap far more than any other species in Denham Woods, as shown in Table 4.8. This is over three times the next most frequent, the

Field Mouse/Bank Vole (FMs). Blue Tit (BT) and Roe Deer (RoD) were pictured the least, with 1 photo each. Table 4.8 also shows the number of photos of each species in which they were feeding, which was 100% of occasions for Greater Spotted Woodpecker (GS), Blue Tit (BT), Marsh/Willow Tit (M/WT), and Coal Tit (CT). A total of 11 species were captured in Denham, which although the same number as captured in Olderwood, the majority are different species. Roe Deer was not captured feeding from any of the stations in Denham Woods. The maximum number present in any photograph of each species is given in column 4. Similarly to Olderwood, only one individual was captured at a time for most species. However, two individuals were seen for Chaffinch (CH), three for Grey Squirrel, and five for Field Mouse/Bank Vole.

Table 4.8: The number of pictures taken by the camera trap for each species in Denham Woods, the number pictures in which the species was using one of the feeders, and the maximum number of individuals caught in one photo.

Species	Pictures	Feeding	Max no. present
GSq	534	130	3
GT	6	4	1
GS	4	4	1
SK	2	1	1
BT	1	1	1
RoD	1	0	1
CH	6	2	2
J.	6	1	1
FMs	174	11	5
M/WT	2	2	1
CT	3	3	1

Similarly to most species in Olderwood, Chaffinch were photographed most often by the camera trap in the morning, as shown in Figure 4.14, although in Denham Woods this is at hours 08-09 as opposed to 07. Great Tit was also caught at hour 09 and peaked in the evening hour 20. Field Mouse/Bank Vole and Grey Squirrel show opposite patterns to each other; the Field Mouse/Bank Vole is caught between 23:00 to 05:00, whereas the Grey Squirrel is caught from 07:00 to 22:00. The Grey Squirrel shows a dip in the afternoon, before peeking again in the evening.

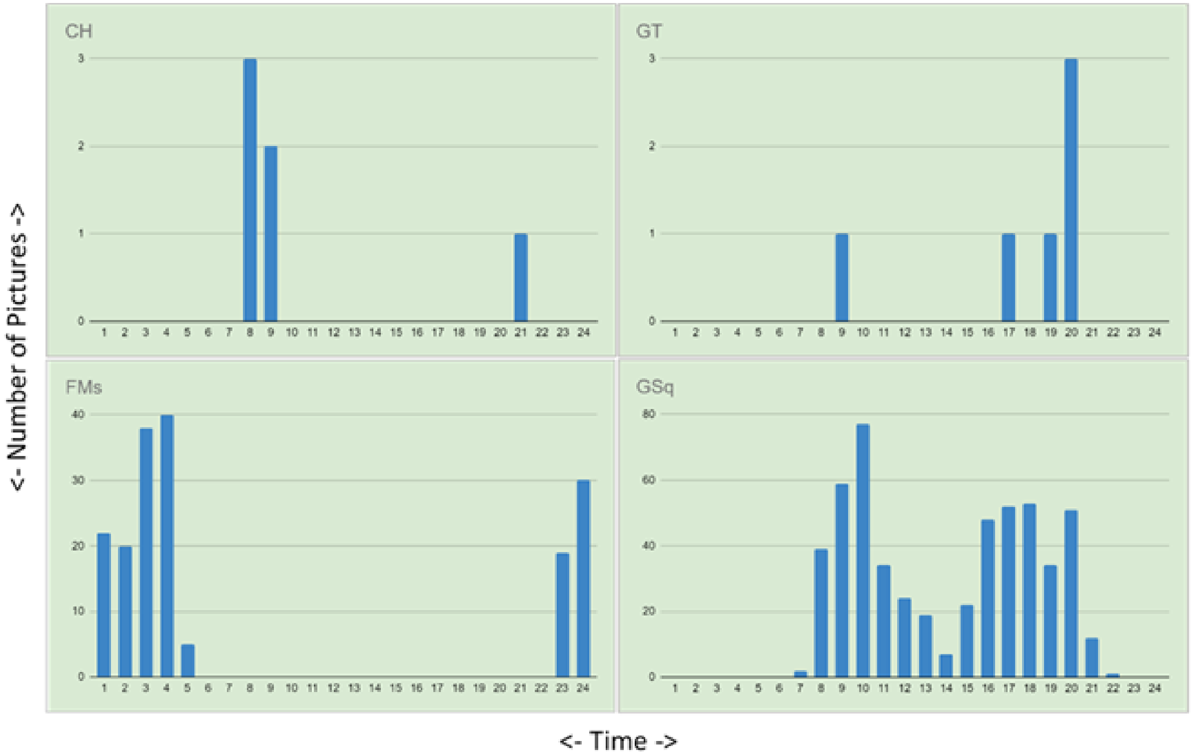

Figure 4.14: The number of photographs taken at each hour in Denham Woods for Chaffinch (CH), Great Tit (GT), Field Mouse/Bank Vole (FMs), and Grey Squirrel (GSq). Species with fewer than five pictures, or from fewer than three occasions, were excluded. The x-axis differs in scale for each species graph.

4.2.4 Seed Consumption from Feeders

The results in Figure 4.15 show the seed consumption from the hanging feeders in weight from Olderwood. The feeders were refilled and weighed every 1 to 3 days, and so the weight of seed consumed shown in Figure 4.15 was averaged over the intervening days. All but station 5 seem to show an increasing trend of consumption throughout the study period. All feeders show at least one gap, which indicates disruption to supply for which no average consumption could be determined. For O1, 2, 3 & 4 they have coinciding dates of disruption around the 16th/17th of April. Table 4.9 shows the total number of disrupted days, which varies from just 1 for station O1 to 9 days for O6. As a proportion of total days, this represents 52% of the period O6 was in operation. All feeders also had days in which all the seed was consumed before resupply, which is indicated in red, and the number varies between the stations. Between the 4th and 14th of May, feeders 1, 2 and 6b experienced a complete consumption of seed, as well as feeders 3 and 4 experiencing some days in this period where all seed was consumed. Table 4.9 shows the number of days with consumption figures affected by the seed being fully consumed before resupply. Feeder 2 had the most days whereby the estimated consumption is inaccurate due to complete consumption, however as a proportion, feeder 6 was affected most by inaccuracy from complete consumption with 29% of data. On the other hand, feeder station O3 and O4 only had 4 days influenced by complete consumption, and both these feeders had the highest number of accurate consumption estimates per day when also taking into account disruptions.

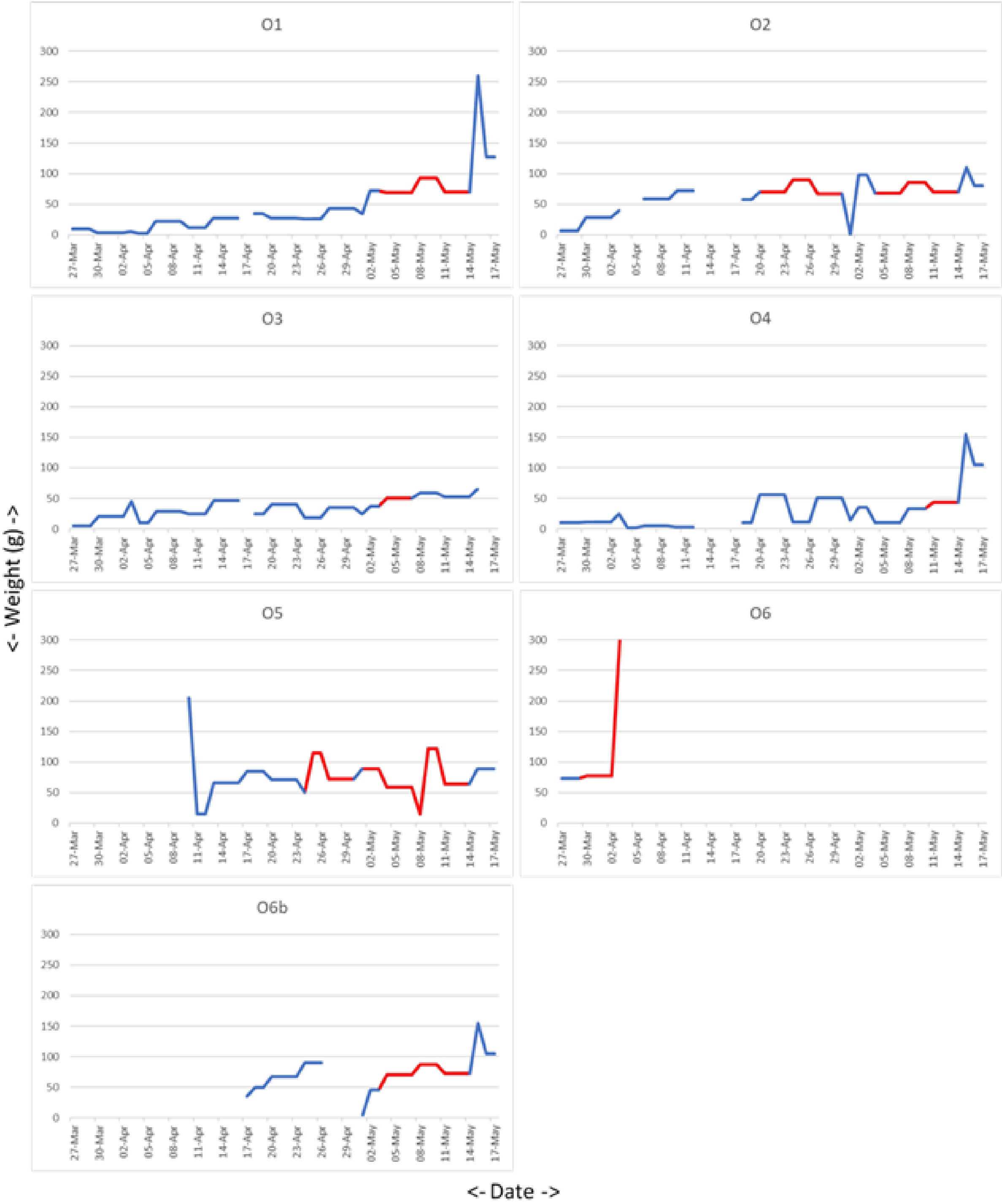

Figure 4.15: The approximate weight of seed consumed from the hanging feeders at each station in Olderwood per date. Gaps in the data represent where the feeder was disrupted and red data points indicate where all the seed was consumed before refilling (and so is an inaccurate estimate as consumption was limited by supply).

Table 4.9: The number of data points of seed consumption in weight per day that are an accurate figure, the number for which is an incorrect estimate due to the feeder being empty by the time of refill, and the number for which no estimate was possible due to the feeder being disrupted.

	O1	O2	O3	O4	O5	O6	O6b
Accurate	40	24	45	43	39	3	15
Empty Feeder	11	21	4	4	6	5	11
Disrupted	1	7	3	5	7	9	4
Total	52	52	52	52	52	17	30

Figure 4.16 shows the seed weight data for Denham Woods. In the same way as for Olderwood, these are averages for the days between the weighing of seed consumption, which ranged between 1 to 3 days. It can be seen that there are a greater number of days for which the estimate is inaccurate due to seed being fully consumed than in Olderwood. There are also a larger number of gaps from disruption to the supply, to the extent that trends are hard to qualify and could be inaccurate. Table 4.10 shows the number of days whereby weight of consumption accuracy is affected by complete consumption before resupply, the number of days affected by disruption, and the number of days for which there are accurate estimates. In no feeder does the number of accurate estimates exceed the number of inaccurate. Feeder station D5 has the largest number of accurate data points with 34%, and is the only feeder to have accurate estimates exceeding those influenced from complete consumption. Station D6 had the largest number of days affected by disruption, but had the joint lowest number influenced by complete consumption. Station D1 had the lowest number of days affected by disruption, but conversely had the largest number of data points affected by complete consumption.

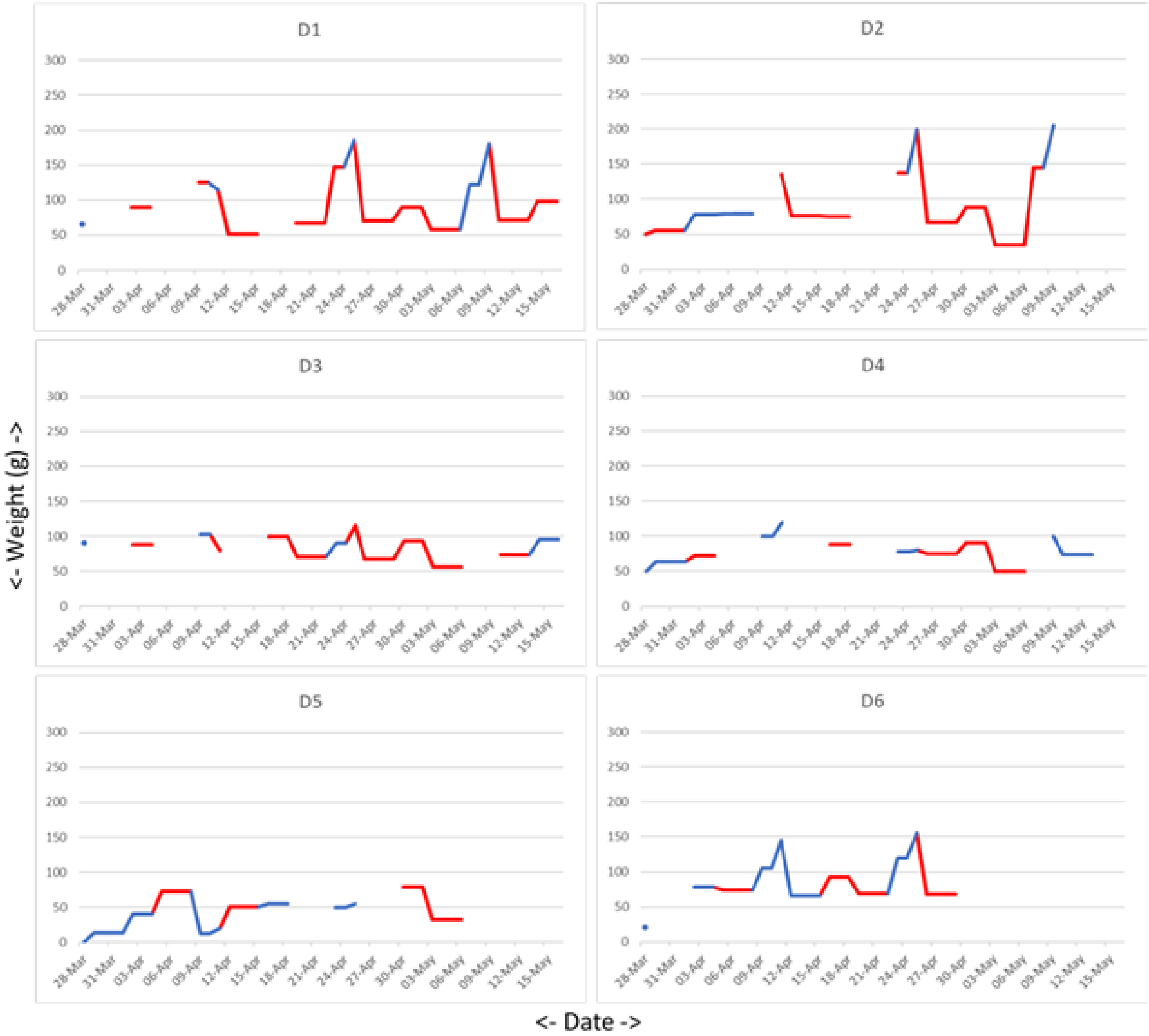

Figure 4.16: The approximate weight consumed from the hanging feeders at each station in Denham Woods per date. Gaps in the data represent where the feeder was disrupted, the red indicates where the weight consumed was limited by the supply (i.e when the seed was fully consumed).

Table 4.10: The number of data points of seed consumption in weight per day that are an accurate figure, the number for which is an incorrect estimate due to the feeder being empty by the time of refill, and the number for which no estimate was possible due to the feeder being disrupted for Denham Woods.

	D1	D2	D3	D4	D5	D6
Accurate	6	11	8	12	17	14
Empty Feeder	33	26	27	21	15	15
Disrupted	11	13	15	17	18	21
Total	50	50	50	50	50	50

4.2.5 Woodland Diversity

Table 4.11 shows the canopy cover results for Olderwood, in yellow, and Denham Woods, in green. The mean canopy cover range in Olderwood is relatively narrow, between 62.9-81.6% whereas Denham has a wider range, with one site being completely open, and the maximum average cover estimated at 92%. In addition, the Denham study site shows a greater variety in the canopy cover results than Olderwood, with the smallest difference between station averages in Olderwood being just 0.5 compared to the smallest difference in Denham being 5.5. The largest recorded value was the Western measure for Denham feeders station 2 at 97.8% compared to the largest value recorded in Olderwood at 87.0% at the Northern measure of feeder station 6.

Table 4.11: The canopy cover calculated using photos analysed in 'ImageJ' at five points around each feeder station as well as the average for each in Denham Woods and Olderwood. Each result is a value between 0 (fully open canopy) to 100 (fully closed canopy).

Olderwood	N	E	S	W	C	Av.
F1	45.1	72.5	74.2	59.7	-	62.9
F2	86.2	76.0	77.0	82.4	78.3	80.0
F3	77.5	59.7	53.0	73.1	65.6	65.8
F4	81.5	76.8	72.9	84.2	77.4	78.6
F5	80.5	77.3	78.7	80.2	79.5	79.3
F6	87.0	74.9	66.6	80.3	80.3	77.8
F6b	86.9	78.1	78.5	84.1	80.2	81.6
Denham	N	E	S	W	C	Av.
F1	0.0	0.0	0.0	0.0	0.0	0.0
F2	86.2	72.5	69.2	97.8*	96.1*	84.4
F3	84.0**	81.6	66.5	45.5	74.5	70.4
F4	61.6	50.4	57.6	62.3	57.5	57.9
F5	95.1*	80.1	96.0*	93.0*	96.1*	92.0
F6	80.5	80.6	68.1	73.6	81.6	76.9

Figures 4.17 and 4.18 show the number of stems above 5cm diameter at breast height (DBH) recorded within a radius of 10m from each hanging feeder in Olderwood and Denham Woods respectively. Any stems with a diameter of less than 5cm were recorded as saplings, the number of which is also shown in Figure 4.17 and 4.18. The only saplings recorded within the 10m radius in Olderwood was at feeder station 6, which had just 2. The number of stems in Olderwood is largely consistent between the feeder stations, with all but one within the range of 11-16 stems. The consistency of stem number between stations was tested using chi square to 95% accuracy, shown in Table 4.12. In Olderwood, a significant difference is found when all the stations are included; excluding station 2, the result indicates no significant difference. Feeder station 2 has the largest number with 29 stems along with

station 6 in Denham Woods, and O2 is the only one in Olderwood to have more than 20 stems. Over half of the stations in Denham Woods have more than 20 stems within a 10m radius, and chi square testing shows that only station 1 is significantly different to the others; testing inclusive of D1 produced a significant result, and indicated no significant difference when excluding D1, shown in Table 4.12. Denham also has a large number of saplings, the largest being estimated at station 1 with 143, which is more than double the next largest number estimated at station 3. The other stations have far fewer, with less than 20 at each. Chi square testing produces significant results when including stations D1 and D2, but does not produce a significant result when both of these stations are excluded.

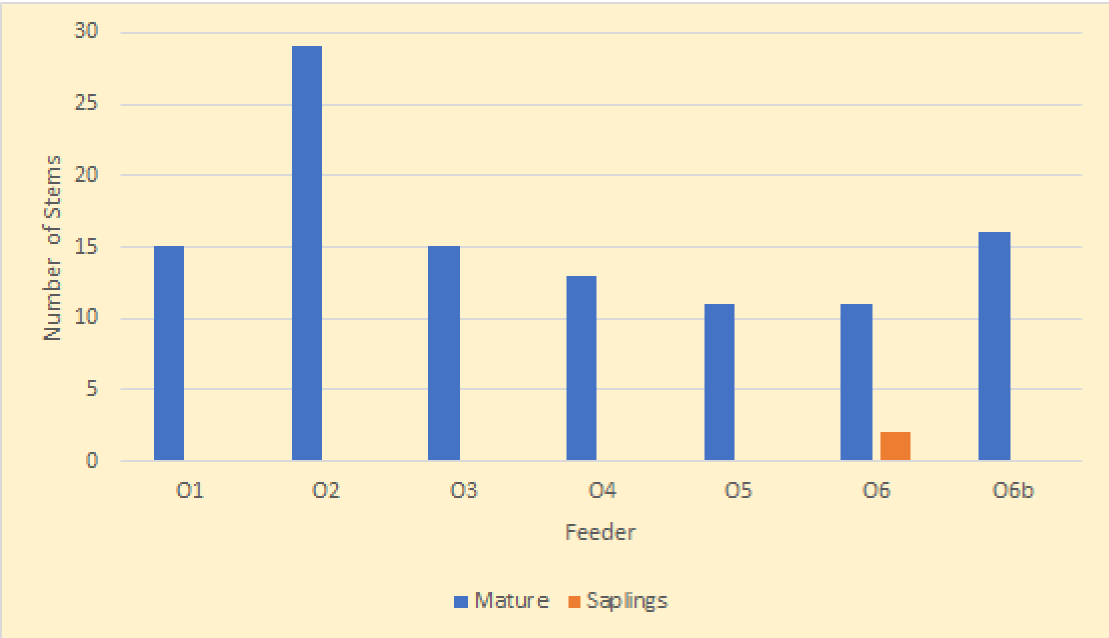

Figure 4.17: The number of stems and saplings (stems <5cm DBH) within a 10m radius of each feeder station in Olderwood.

Figure 4.18: The number of stems and saplings (stems <5cm DBH) within a 10m radius of each feeder station in Denham Woods, the locations of which can be found in.

Table 4.12: Chi-square test results on the number of mature stems and saplings between feeder stations; the stations included in each test are indicated in column three. All significant results to a 95% confidence level are highlighted in red.

		Stations	X2
Olderwood	Mature stems	O1 to O6b	14.6
	Mature stems	O1, O3 to O6b	1.74
Denham	Mature stems	D1 to D6	19.31
	Mature stems	D2 to D6	7.27
	Saplings	D1 to D6	326
	Saplings	D2 to D6	100.62
	Saplings	D2, D4 to D6	4.77

Figures 4.19 and 4.20 show the species richness and Simpson's Index results for Olderwood and Denham Woods respectively within a 10m radius around each feeder station, including the SI for saplings <5cm DBH. Within Olderwood, the species richness is similar between each station, with each having between 2-5 different species. This is confirmed by chi-

square testing, which produced a X^2 value of 2.25, less than the significance factor for 95% confidence level. However, the total Simpsons Index (SI) value is more variable, with a range between 0.33 for O1 and 0.83 for O5. As there is only one feeder station with saplings present, the mature SI and total SI are largely the same, and for O6, the difference is only 0.02 between them. The sapling SI in Olderwood is 0.5, but this is only comprised of two trees. In Denham Woods, the species richness is greater for every station than any of those in Olderwood. Station 1 in Denham is notable for its lack of mature stems, with only 6, however it has the second-highest total SI value for both woodlands. Similarly to Olderwood, chi square testing between species richness of all stations produced a X^2 of 3.75, less than the significance level. The range of total SI in Denham Woods is smaller, with the lowest being 0.28 and the highest 0.52. In feeder stations 1 and 4, the difference between the mature and sapling diversity values is greatest, with Mature having a D value of 1 in both cases compared to 0.53 and 0.3 for sapling diversity respectively. In 4 instances, the D value is greater for the Mature stems than the saplings, with only stations D3 and D5 having a larger SI value for saplings. Chi square testing between the sum of species richness in each woodland for each station produces an X^2 of 9, which indicates that Denham Woods has a greater overall species richness than Olderwood.

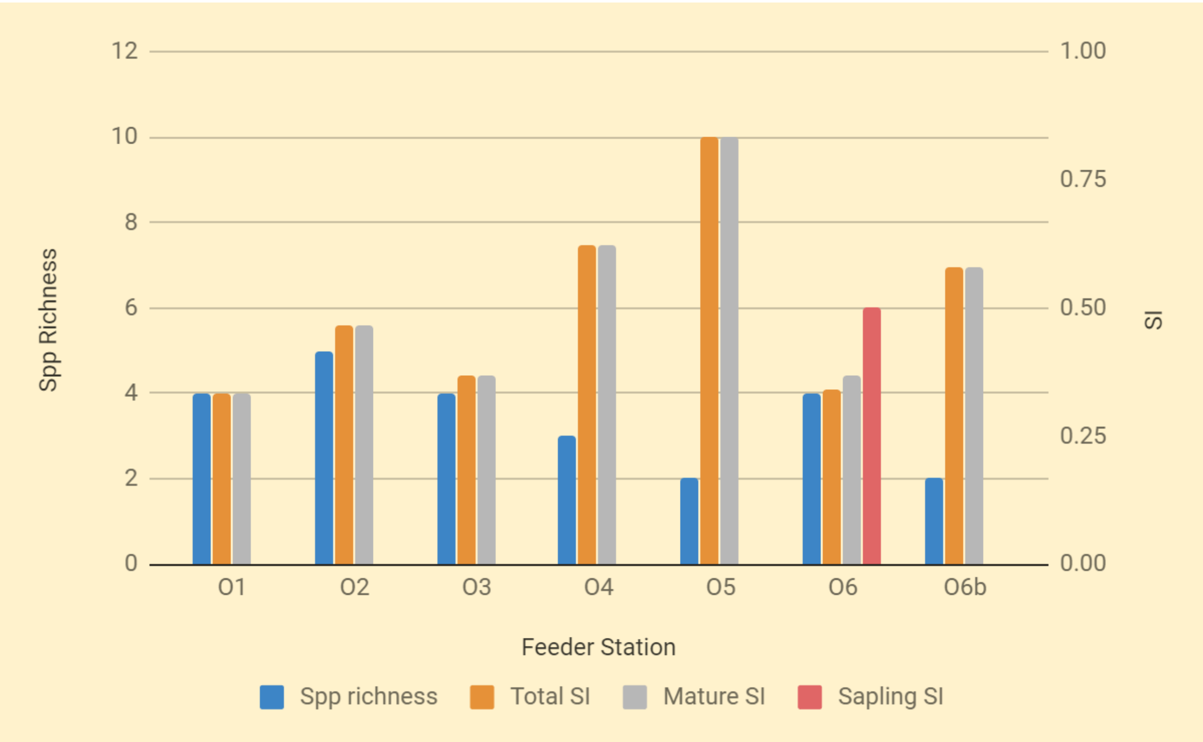

Figure 4.19: The species richness (left axis), and the total, mature and sapling Simpson's Index of diversity on the right axis, where 1 is no diversity and 0 is maximum diversity, within a 10m radius area around each feeder station in Olderwood.

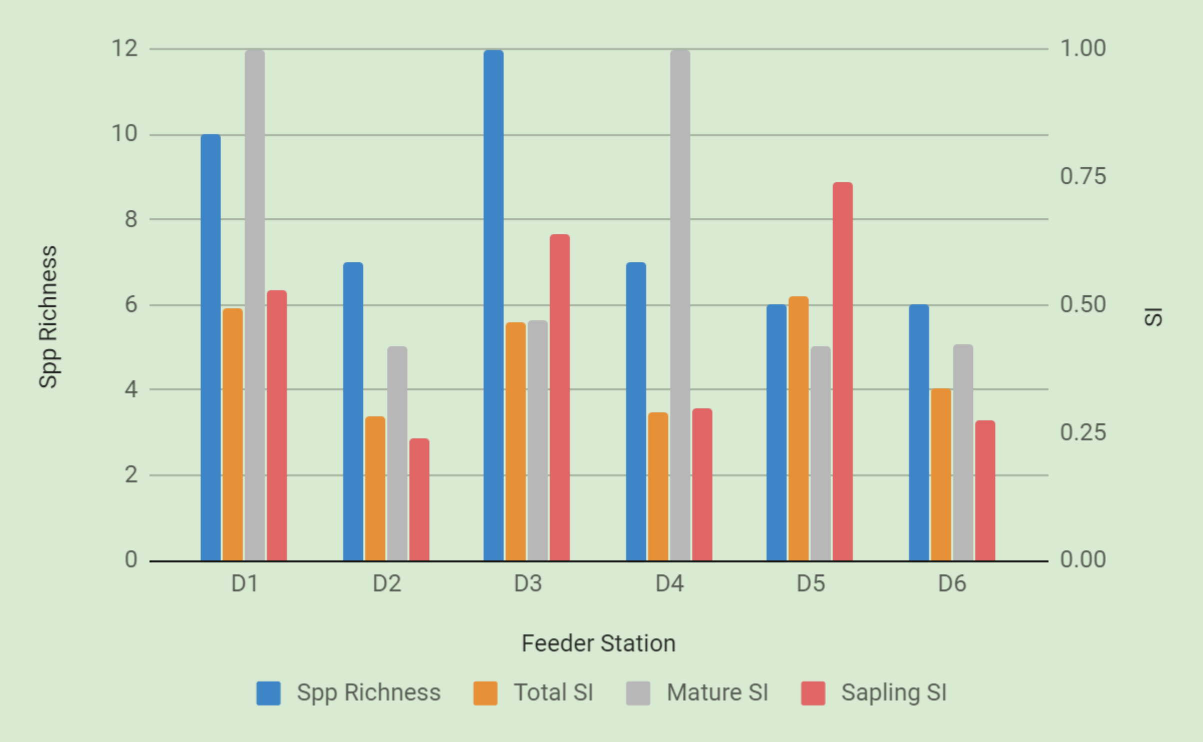

Figure 4.20: The species richness (left axis), and the total, mature and sapling Simpson's Index of diversity on the right axis, where 1 is no diversity and 0 is maximum diversity, within a 10m radius area around each feeder station in Denham Woods.

Table 4.13 shows the correlation results between the number of occurrences of each bird species (in how many observation periods were each seen) and stem data consisting of the number of stems, the species richness, and the Simpson's Index for stems above 5cmDBH for Olderwood. Number of saplings and sapling SI (as well as total SI) were not included for Olderwood, unlike Denham in table 4.14, as there were too few data points for saplings. The significant results with a P-value of less than 0.05 are highlighted in red, and include a positive correlation between Marsh/Willow Tit (M/WT) and the number of stems >5cmDBH. Greater Spotted Woodpecker (GS) was also found to have a strong negative correlation with species richness as well as a positive correlation with the Simpson's Index for stems >5cmDBH, and Chaffinch (CH) likewise had a positive correlation with SI above 5cmDBH.

Table 4.13: The correlation results between the number of observation periods in which each species was seen and the total number of stems, species richness, and mature SI in Olderwood for different bird species. Results that have a P-value less than 0.05 indicate a significant correlation, and are highlighted in red. The r value, indicating type of correlation is also given. Coding from p.51 was used for the common species names.

Species	Correlation	stem no.	Spp richness	Mature SI
GT	r	-0.232	-0.384	0.419
	P-value	0.617	0.395	0.349
NH	r	-0.396	-0.592	0.463
	P-value	0.379	0.161	0.295
CT	r	0.391	0.450	-0.605
	P-value	0.385	0.310	0.150
BT	r	-0.105	-0.709	0.646
	P-value	0.824	0.075	0.117
M/WT	r	0.769	0.626	-0.383
	P-value	0.043	0.133	0.396
R.	r	0.702	0.265	-0.046
	P-value	0.078	0.566	0.922
GS	r	-0.244	-0.946	0.779
	P-value	0.599	0.001	0.039
CH	r	0.015	-0.415	0.794
	P-value	0.975	0.354	0.033
D.	r	-0.613	-0.136	0.138
	P-value	0.143	0.772	0.769

Table 4.14 displays the correlation results between species occurrence and stem data for Denham Woods, with the additional variables of the number of stems less than 5cm DBH (saplings), the Simpson's Index for saplings and matures combined, and the SI for Saplings. The correlations found in Olderwood are not supported by the results from Denham. Marsh/Willow Tit (M/WT) shows some significant correlations, but for different variables to Olderwood; strong negative correlation between the Total SI and the Saplings SI. Significant strong negative correlations were also found between Robin (R.) and Stem no. and Dunnock (D.) and Stem no., as well as a positive correlation with Dunnock and Mature SI. All other results did not meet the significance criteria of P-value<0.05.

Table 4.14: The correlation results between the number of observation periods in which each species was seen and the total number of stems, total number of saplings, species richness, and total, mature, and sapling SI in Denham Woods for different species. Results that have a P-value less than 0.05 indicate a significant correlation, and are highlighted in red. The r value, indicating type of correlation is also given. For each species, coding from p.51 was used.

Species	Correlation	stem no.	sap. no.	Spp richness	Total SI	Mature SI	Sapp SI
GT	r	0.400	0.119	0.235	0.308	-0.688	0.209
	P-value	0.432	0.823	0.654	0.553	0.131	0.690
CT	r	-0.422	-0.155	-0.395	-0.563	0.461	-0.736
	P-value	0.405	0.769	0.439	0.245	0.357	0.095
BT	r	-0.371	0.284	0.207	-0.316	0.241	-0.551
	P-value	0.470	0.585	0.694	0.541	0.645	0.257
M/WT	r	0.230	-0.279	0.056	-0.848	-0.052	-0.836
	P-value	0.661	0.593	0.916	0.033	0.921	0.038
R.	r	-0.871	0.751	0.442	0.437	0.603	0.217
	P-value	0.024	0.085	0.380	0.386	0.180	0.608
GS	r	0.547	-0.499	-0.475	-0.631	-0.537	-0.739
	P-value	0.262	0.314	0.341	0.179	0.272	0.094
CH	r	0.600	-0.239	-0.056	0.033	-0.808	-0.020
	P-value	0.208	0.648	0.917	0.950	0.052	0.970
D.	r	-0.923	0.390	0.215	-0.052	0.997	-0.106
	P-value	0.009	0.444	0.683	0.922	0.00	0.842
GR	r	0.581	-0.023	0.415	-0.181	-0.597	-0.124
	P-value	0.227	0.965	0.413	0.732	0.211	0.816
J.	r	-0.297	-0.257	-0.17	-0.503	0.63	-0.35
	P-value	0.568	0.623	0.747	0.309	0.18	0.496
GSq	r	0.641	-0.293	-0.221	-0.404	-0.408	-0.316
	P-value	0.17	0.573	0.674	0.427	0.422	0.542
FMs	r	0.409	-0.401	0.097	-0.431	-0.43	-0.367
	P-value	0.421	0.43	0.855	0.393	0.395	0.474

4.3 Weather Data

Figure 4.21 shows the temperature from the Plymouth weather station for the period between March 1st and May 31st. This includes the highest temperature recorded for each day in orange and the lowest temperature in blue. From this figure, it can be seen that there is an overall increasing trend in the temperatures as Spring progresses, although it is highly variable between days. However, there is an anomalous peak between the 16th and 22nd of April, where the temperatures were highest. On May the 6th, there is also an exception to the trend of temperature increase, as the Low temperature dropped below the trend. The lowest temperatures were recorded in April on the 3rd, both for the High temperature and the Low.

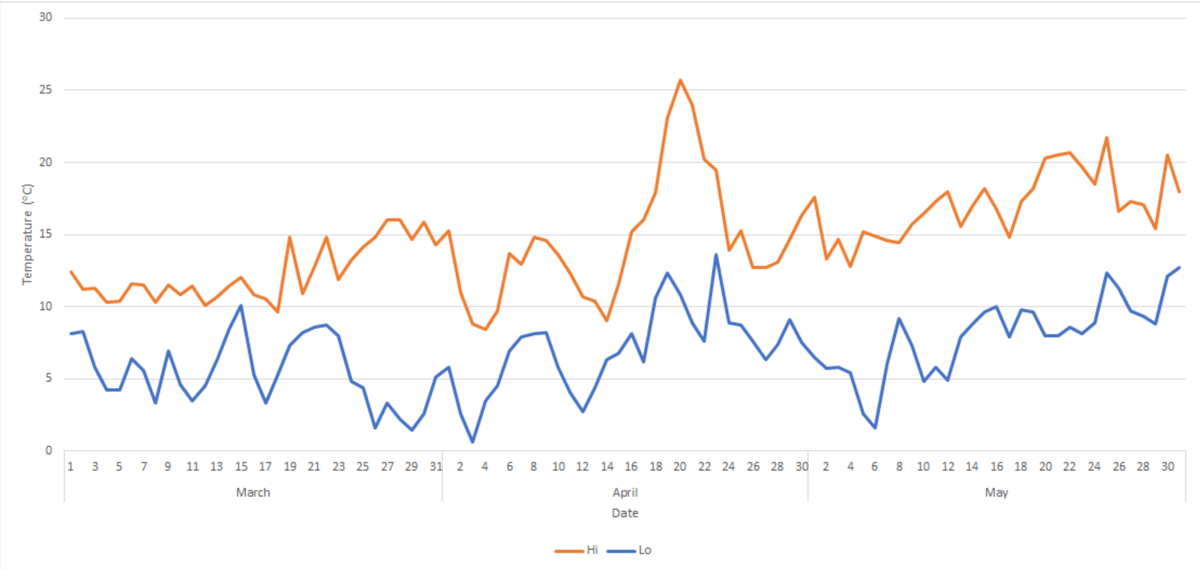

Figure 4.21: The highest daily temperature recorded and the lowest for each date recorded in the Plymouth weather station.

Precipitation recorded in Plymouth is displayed on Figure 4.22. Rain days were more frequent in March, with 20 days receiving at least 0.1mm, and were least frequent in May receiving 7 days >0.1mm. April received 15 days with above 0.1mm of rain. March also received the day with the highest level of rainfall at 15.2mm on the 5th, but April received 14.6mm on April the 5th. May received one day of rainfall at 13.4mm on the 8th, and all other days during the March to May period were below 10mm.

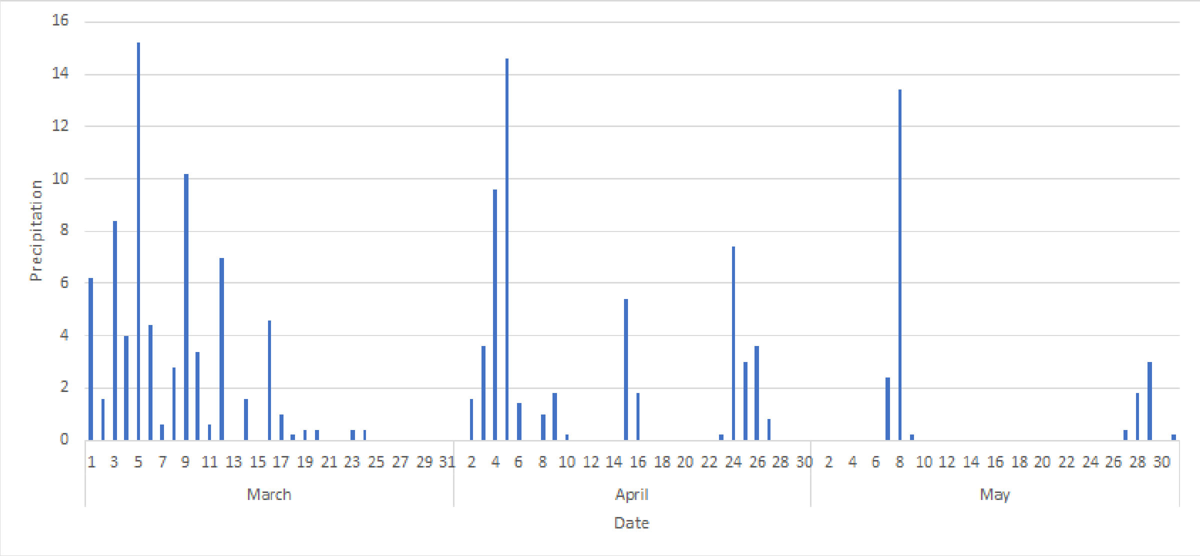

Figure 4.22: The daily total precipitation figures from March to May 2019 recorded by the Plymouth weather station.

Wind data from Plymouth weather station is shown in Figure 4.23 as a daily average, shown in grey, and as a daily highest recorded shown in orange. Early March receives the highest wind speeds, and the number of days with an average speed above 10mph were most frequent in this month. After the 17th of March, average wind speeds only reach above 10mph on the 27th of April, and fluctuate but remain consistent for the rest of the period. Highest recorded speeds rarely reach above 30mph in April and May, with the 15th, 26th and 27th of April being exceptions along with the 7th of May. However, 14 days in March received high wind speeds of over 30mph, with the highest on the 10th at 49.5mph.

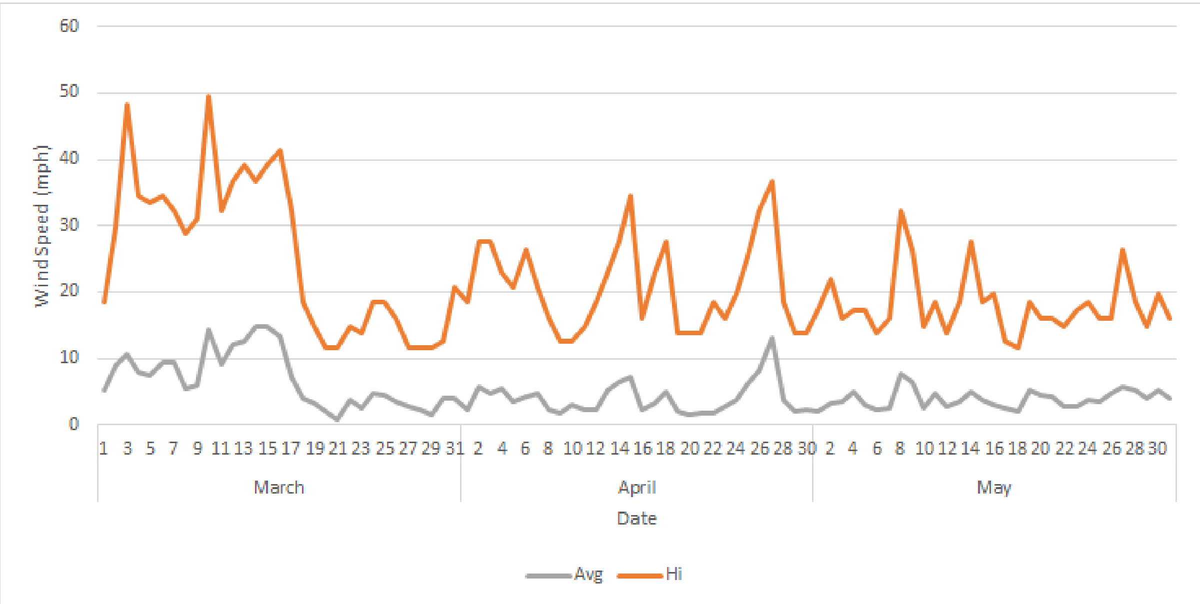

Figure 4.23: The average daily wind speed as well as the daily highest maximum for March to May 2019 recorded by the Plymouth weather station.

Figure 4.24 displays the number of sunshine hours per day during March-May. May has the largest number of days with sun hours above 4, with March having the least. The maximum appears to rise from March at 10.7 hours to 11.5 hours in April and to 13 in May. May is the only month in this period with no days containing 0 sunshine hours.

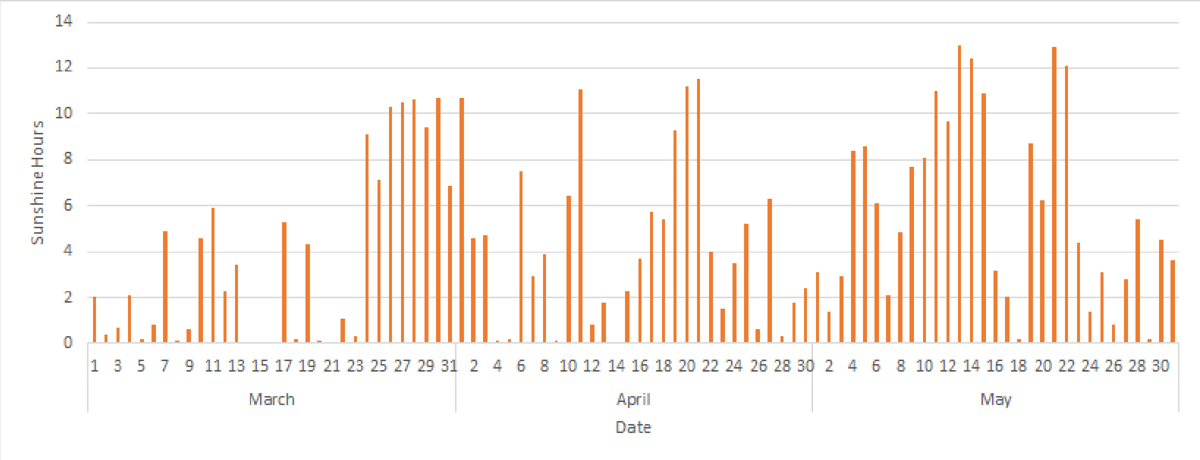

Figure 4.24: The total sunshine hours per day from March through to May 2019 recorded from Plymouth weather station.

The dew point temperature for Crownhill, Plymouth is shown in Figure 4.25. There is a general increase seen in the latter half of May, but otherwise the maximum dew point temperature remained just below 10ºC. April contained two of the three coldest dew point temperatures, with the third being found in May. The smallest range in results was found in March.

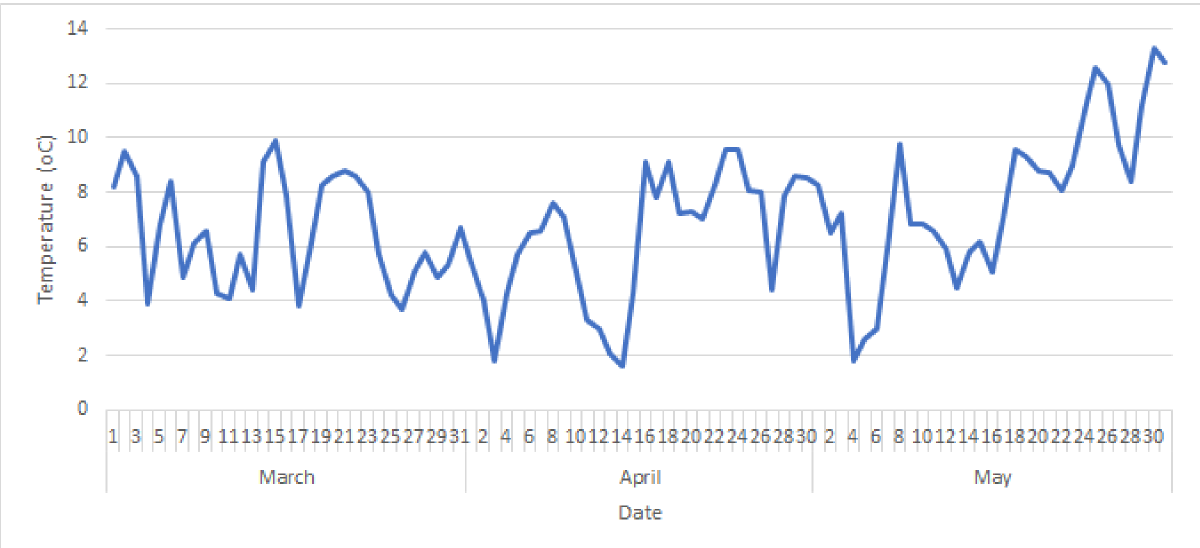

Figure 4.25: The daily dew point temperature recorded in Plymouth for the months of March through to May 2019.

Table 4.15 shows the results from the Pearson-correlation tests comparing individual bird species observations (the number of visits) in Olderwood with the data from key weather variables. Results with a P-value below 0.05 are considered significant to a 95% confidence level, and all significant results are highlighted in red. The only correlations considered significant in this way are between Great Tit (GT) and average wind speed, with an r value of 0.477 indicating positive correlation, and between Greater-Spotted Woodpecker (GS) and daily maximum temperature, with a strong positive correlation of 0.84. All other results had a P-value above the specified confidence level, and so are not indicative of a significant correlation with any of the weather variables.

Table 4.15: Correlation results between the number of visits by species in Olderwood and daily highest temperature, lowest temperature, precipitation, average wind speed, highest wind speed, dew point temperature, and sunshine hours. Results for which the P-value is lower than 0.05, indicating significant correlation, are highlighted in red.

		T.Hi	T.Lo	Precip.	W.Av	W.Hi	D.Point	Sun
GT	r	-0.162	-0.173	0.015	0.477	0.332	-0.017	0.163
	P-value	0.482	0.453	0.947	0.029	0.142	0.942	0.481
NH	r	-0.104	-0.093	0.155	0.337	0.05	-0.132	0.114
	P-value	0.653	0.689	0.502	0.136	0.828	0.57	0.622
CT	r	0.228	0.322	-0.079	-0.045	-0.065	0.251	0.099
	P-value	0.321	0.155	0.735	0.845	0.78	0.272	0.668
BT	r	-0.069	0.244	0.159	0.187	0.182	0.282	-0.259
	P-value	0.766	0.286	0.492	0.416	0.429	0.216	0.258
M/WT	r	-0.034	-0.285	0.133	-0.353	-0.160	-0.224	0.121
	P-value	0.902	0.284	0.622	0.180	0.553	0.405	0.655
R.	r	-0.173	-0.207	0.093	0.229	0.174	-0.137	0.115
	P-value	0.522	0.442	0.731	0.393	0.519	0.612	0.672
GS	r	0.840	0.612	-0.582	0.278	0.195	-0.150	0.692
	P-value	0.036	0.197	0.225	0.594	0.711	0.777	0.128
CH	r	0.032	0.191	-0.081	-0.033	0.458	0.370	-0.209
	P-value	0.896	0.434	0.741	0.893	-0.181	0.119	0.390

The Pearson correlation test results are shown in Table 4.16 for species visits in Denham Woods. The results with a P-value indicating significance are likewise highlighted in red. The visitation observations do not share a correlation with average wind speeds in Denham as they did in Olderwood. Denham Woods shows significant positive correlations that were not found in Olderwood with Marsh/Willow Tit (M/WT) and high wind speeds, as well as between Chaffinch (CH) with rain and average wind speed. No other significant result was discovered.

Table 4.16: Correlation results between the number of visits by species (coded as per p.51) in Denham Woods and daily highest temperature, lowest temperature, precipitation, average wind speed, highest wind speed, dew point temperature, and sunshine hours. Results for which the P-value is lower than 0.05, indicating significant correlation, are highlighted in red.

		T.Hi	T.Lo	Rain	W.Av	W.Hi	D.Point	Sun
GT	r	-0.171	-0.415	-0.067	-0.016	-0.122	-0.122	0.098
	P-value	0.485	0.077	0.786	0.949	0.620	0.620	0.688
CT	r	-0.394	-0.134	0.202	0.331	0.324	-0.293	-0.147
	P-value	0.095	0.585	0.407	0.166	0.175	0.224	0.547
M/WT	r	0.188	-0.021	-0.172	-0.309	-0.464	0.150	0.197
	P-value	0.441	0.932	0.482	0.198	0.045	0.541	0.420
BT	r	0.028	-0.145	0.128	0.026	-0.057	0.348	-0.155
	P-value	0.906	0.543	0.59	0.915	0.811	0.133	0.515
CH	r	-0.294	-0.004	0.605	0.610	0.442	0.091	-0.302
	P-value	0.209	0.985	0.005	0.004	0.051	0.704	0.196
SK	r	-0.073	-0.101	0.169	0.075	-0.165	0.018	0.276
	P-value	0.759	0.672	0.475	0.753	0.488	0.941	0.239
GSq	r	0.003	0.288	0.118	0.242	0.198	0.191	0.038
	P-value	0.991	0.218	0.622	0.303	0.402	0.419	0.873
FMs	r	-0.047	-0.176	0.025	-0.167	-0.145	0.006	0.185
	P-value	0.862	0.515	0.926	0.537	0.592	0.984	0.492

5 - Discussion

5.1 Residents Survey

The results of the resident's survey pertain to completing objectives 1f, and 2a set out in section 1.4 as follows:

1. To examine whether supplementary bird feeders used in natural or semi-natural environments can be used to study woodland bird populations
    f. Compare bird species seen in woodland environments to those in garden environments
        i. Residents survey
        ii. Regular observation of woodland feeders
2. Assess whether birds could be transferring spores of the tree pathogen *Phytophthora ramorum*
    a. Test residents bird feeders proximate to woodland with potentially infected material
        i. Door knocking residents of Keele University campus and taking swab samples
        ii. Residents survey to gain information on how much time spores would have to build up (before the feeder is cleaned)

Gauging the ownership rate of feeders in Keele can be useful to compare whether the location represents a ‘typical’ residential area of the country. Davies *et al* (2009) estimated that 48% of households across Britain fed birds in some way, although the study further estimated that 28% of households did this with a feeder. This means that the 40% feeder ownership found in Keele is higher than the national average. This could be for a multitude of reasons, but Davies *et al* (2012) found a positive relationship between feeder ownership and the age of the head of the household as well as the type of house (i.e detached, terraced, etc.). From personal observation, the residents of Keele often fit an older demographic; or there was a bias in data collection as surveys were taken in person during hours which may have been unsuitable for collecting responses from working households. However, it could be that houses closer to wooded or more natural environments have a greater connection with nature and are therefore more likely to seek interactions with it through the use of bird feeders. This could be seen at Keele as group A (which had the lowest proportion of feeder ownership) was the furthest away from wooded areas on campus whereas group D with the largest ownership rate was proximate to the largest area of woodland near to the occupied campus. This could be an important consideration for further study relating to aim 2, as if there was a correlation with bird feeder ownership and proximity to wooded environments, it could increase the likelihood that birds visiting these feeders from the woodlands are transferring spores at these locations.

Question 1 of the survey on how long feeders have been present at each respondents address contribute to objective 2aii, to gauge over how long spores could build up at each feeder, as well as to gain an understanding of how long these feeders have had to establish. For example, the minimum length of two weeks would indicate that this feeder may not yet be established in the bird community as a food source. Therefore, the birds visiting the feeder might be expected to be less diverse and/or frequent. This is backed up by the observation and seed consumption rate found with the woodland feeders discussed in section 4.2.1, as it was found that in woodland environments it took between 1.5 and 3 weeks for the feeders to establish. From the fact that the median response was 5 years and the mean being 13 years, the feeders at Keele could generally be seen as well established. The number of responses for this question was 24, as although 25 residents were asked the survey questions it was decided at the researcher’s discretion how appropriate each question was on a case by case basis (for example if the resident expressed they did not have much time); therefore not all questions were asked to all residents.

If *Phytophthora* was detected on feeders at Keele, estimating the number of feeders present at Keele would give an approximation of the scale. And, even if *Phytophthora* being present is unlikely, the large density of feeders in the area, estimated at 150, could still lead to many instances of spore transfer. No spores were detected in this study, but this data still contributes to a growing number of studies estimating bird feeder use by households, and demonstrates that further work would be justified as the scale of supplemental feeding could provide a large data set for studying bird vectoring of tree disease such as *Phytophthora*.

Although residents were asked for an overview of what birds they typically saw on their feeders in question 5, the type of feeder and the food used was also asked (questions 3 and 4) to gain a more detailed approximation of what birds could be expected to visit. Different feeder types attract different birds, with birds adapted to living and feeding in trees more likely to visit hanging feeders, such as Blue Tits. Feeders with perches enable less agile species to feed, such as Chaffinch, and ground feeders attract ground-feeding birds such as Thrushes (Burton, 2003). Furthermore, different foods are preferred by different species, with nyjer seed being favoured by Goldfinch and Siskin for example. The developing picture of which birds are visiting feeders most often can be used to give an understanding of which species could be moving the spores and approximate distances could be estimated as birds have varying ranges, as well as migrational movements. As well as providing data that could be used in the same way, these results can give an indication of the diversity in the local bird community. For example, Hawfinch was reported once, which is a red-listed, winter migrant bird to the study area (RSPB, 2019). As a shy bird, it could perhaps be overlooked without the aid of a feeder giving observational access without disturbance, and as a winter migrant, it can be moving great distances. As the Hawfinch often feeds on the ground before perching in trees, this would give it the proximity to transfer spores.

Grey Squirrels were a commonly reported pest visiting residents bird feeders, and all households reported seeing them feeding. Hanmer *et al* (2018) found a positive correlation between proximity to woodland and Grey Squirrel presence at feeders, and so this 100% presence rate in Keele could reflect the fact that all households on the Keele campus are within varying distances to woodland, but all relatively proximate within 300 meters. This indicates that Grey Squirrel are another potential vector, and if spore transfer did occur at bird feeders it would be unclear without further study whether this was due to Grey Squirrel, birds, or both. However, Grey Squirrel presence prevents bird visitation (Hanmer *et al*, 2018), and damage from Grey Squirrels were frequently reported by residents not only to feeders but food waste bins for example. Because of this, many residents would actively discourage the squirrels, and so their presence at each household may vary depending on the effort made to deter them.

To investigate objective 2fii on how much time spores would potentially have to accumulate on the feeders, residents were asked how frequently they were cleaned. 24% reported that they never cleaned their feeder, with a further 24% reporting every year and the same number every few months. This would provide long periods for possible spore transfer from bird to bird via feeders. However, it is not clear whether different materials used to make the feeders could have an effect on how adherent spores would be to the feeders, and would require further investigation as to the effects of rain on different materials to determine if spores could be displaced naturally. This data also provides an insight into how successfully information on the importance of feeder hygiene is being disseminated. A blog in the Scottish Wildlife Trust recommends every month, the RSPB reports on their website that feeders should be cleaned at least every few weeks, and the BTO recommends every two weeks (RSPB, 2019b; Sheperd, 2014; BTO, undated). These recommendations come as there is growing concern over disease transmission in garden bird feeders, and as it is thought that hygiene becomes a risk after 2-4 weeks of use, there is the risk of transfer that could also apply to phytopathogens (Lawson *et al*, 2018). Even if spores of phytopathogens are present in lower quantities than avian diseases, the results from Keele residents show that 72% of residents clean their feeders less frequently than recommended, and so there could be enough time for spores to be present and to transfer at these sites.

The swab samples tested for *Phytophthora* with rapid diagnostics test kits failed to show any positive results on residents bird feeders. This could be indicative that spore transfer of *Phytophthora* diseases including *P. ramorum* does not occur in the manner theorised. However, it is also possible that the entirely negative results are due to the sensitivity of the lateral flow device, and that PCR (Polymerase Chain Reaction) testing or isolation and cultural testing could have greater sensitivity (Lane *et al*, 2006). There are other considerations that could be investigated further to determine whether swab sampling in combination with lateral flow devices can be used in this way:

- A trial of the methodology by which known infected material was swabbed could be used to determine the effectiveness of this methodology. This was not determined during this study as known infected sites are heavily controlled, and preliminary studies of recently infected sites were inconclusive (see section 3.1).
- Sampling of the resident's feeders occurred during a period of intermittent rain. Although rain can stimulate spore production (Davidson *et al*, 2005), it is possible that different materials could hold spores to varying degrees and rain could potentially displace spores. Further study would be needed to see if spores would be present during a period without precipitation.

The void results for which the control line did not become visible is most likely due to a combination of user error and cold. The sampling took place during cold temperatures (unmeasured) in which care needs to be taken with the lateral flow devices to warm them so as to still operate effectively. Although there are some factors that could be studied further to decisively determine the use of swab sampling in combination with lateral flow devices for *Phytophthora*, their use proved effective for household feeder sampling; their cost and ease of use means that a number of tests can be undertaken, which would prove effective for sampling residents over great areas and collecting a greater number of samples for more accurate conclusions on the role of feeders in spore dispersal. It is also important when using citizen sampling to be able to demonstrate to the citizen how the data they assisted with is used, and lateral flow devices give an immediate result with which the resident can engage.

5.2 Woodland Feeders

The woodland study was designed to achieve the following:

1. To examine whether supplementary bird feeders used in natural or semi-natural environments can be used to study woodland bird populations
    a. Identify which species will use feeders in woodland environments
        i. Regular observations
        ii. Identification of which type feeder is visited, ground or hanging
    b. Assess how long it takes for feeders placed in woodland environments to establish
        i. Regular observations
        ii. Regular measurements on how much food is being eaten
    c. Assess how frequently feeders are used by birds in woodland environments
        i. Regular observations
        ii. Regular measurements of how much food is being eaten
    d. Identify any other animals that might be using the feeders (i.e. mammals)
        i. Regular observations
        ii. Camera traps
    e. Identify reasons for variations in objectives 1a to 1d
        i. Stem maps to compare environment differences
        ii. Digital photographs to compare canopy cover differences
        iii. Identify weather events that could cause differences
    f. Compare bird species seen in woodland environments to those in garden environments
        i. Residents survey
        ii. Regular observation of woodland feeders
2. Assess whether birds could be transferring spores of the tree pathogen *Phytophthora ramorum*
    b. Test feeders in a *P. ramorum* site for proof of concept
        i. Establish feeders in *P. ramorum* affected woodland
        ii. Test feeders after allowing time for spore build up using swab samples

5.2.1 *Phytophthora*

No positive results for *Phytophthora* presence were found on the bird feeders using swabs combined with lateral flow devices in either woodlands, even in the woodlands classified as having current *Phytophthora* infections. As discussed previously (section 5.1), this could indicate that birds are not significant vectors of *Phytophthora*, or that spores are not being transferred significantly on feeders, or that swabs combined with lateral flow devices are not sensitive enough to register a potentially small number of spores transferred in this way. A further possibility is that the disease is no longer present at either site, or is present asymptomatically and not producing spores. Further study into the effectiveness of the methodology is required to address aim 2 conclusively. The next step would be to investigate whether spores are moved by birds in active sites, under the difficult circumstances of intensive management which will limit the number of birds using the site at that time.

5.2.2 Observations

The total number of visits by birds for each date, as well as providing data to begin to understand what causes fluctuations in visits, can be used to determine how long it takes for feeders to become established. One method for estimating at which point the feeder could be considered 'established' would be to calculate the standard deviation from the mean, and consider the point at which visits first exceed the lower deviation to be an estimated date from which the feeder can be considered established. Using this method, it can be seen from Figure 4.7 in section 4.2.1 that the feeders in Denham Woods become established from the 28th of March, and in Olderwood (Figure 4.8 section 4.2.1) from the 5th of April. This data would support a conclusion that it would take between 1.5 - 3 weeks for feeders to establish in typical deciduous woodlands at this time of year.

Determining the time of day at which average visitation rate peaks is useful to ascertain the optimal time at which to make observations in future studies. The data for Denham feeders shows a three-peak pattern (Figure 4.9 section 4.2.1), at 09:00, 13:00, and 18:00 which is consistent with behaviour reported by the BTO on garden bird feeder behaviour (BTO, 2019). However, other foraging patterns of birds have been observed. Bonter *et al* (2013) found no peak in foraging with birds feeding consistently throughout the day, perhaps as increasing fat reserves at peaks in the day can decrease flight manoeuvrability, and so spreading out feeding throughout the day improves the ability to avoid predation. Other studies for wild birds support either morning or afternoon peaks, depending on food availability whereby food uncertainty leads to increased foraging effort earlier in the day, and 'bimodal' feeding behaviour has also been theorised (Bonter *et al*, 2013; Koivula *et al*, 2002; Olsson *et al*, 2000). The average visitation rates in Olderwood seem to support a peak in foraging in the morning, suggesting that the birds in this area are used to more limited supplies of food than those in Olderwood, which is perhaps a reflection of the relative uniformity in the Olderwood stands. However, considering the standard error in the averages, displayed in Figure 4.10, the data appears inconclusive, with the error ranging between 25-53% of the means. Looking at the standard error bars may likewise contradict the conclusions for Denham Woods, as although the mean data suggests peaks in foraging, the standard error for most of these is large. For Denham woods, the lowest standard error as a proportion of the mean is at 17:00 hours, but this is still 30% of the mean (2 s.f). At 08:00 hours in Denham, the standard error is as large as the mean itself. There are likely twofold reasons for this; either the sample size to abstract the mean was not large enough, and indeed for 08:00 hours the sample was only from 3 observation periods, or that the feeding visits were largely inconsistent with time of day, perhaps being determined by other environmental factors.

Olderwood received significantly more visits (total number of times a species was seen arriving at the bird feeder) and observations (total number of observation periods during which the species was seen) than Denham Woods overall and for the birds Great Tit, Nuthatch, Robin, and Chaffinch. This could be an indication of greater avian abundance in Olderwood, or it could indicate that there is less availability of natural food sources. The morning peak in Olderwood for foraging times supports a limited food supply, and so the bird feeders may be a greater attraction here than to the birds in Denham Woods; the risk of predation by returning consistently to one food source is outweighed by the need for supply. However, this could also be a reflection of less shrub-cover in Olderwood making the feeders easier to find, or perhaps the most likely explanation comes from the difference in Grey Squirrel presence. Significantly more squirrels were seen in Denham Woods, and these were observed to frequently consume much of the food as well as damaging the feeders. This caused significant disruption, leaving many of the feeders completely broken and without seed for large periods, discussed further in section 5.2.4.

Coal Tit, Marsh/Willow Tit and Blue Tit occurred in similar abundance in both woodlands, with Coal Tit being the most observed species in Denham and the second most in Olderwood. As the two sites are approximately five and a half kilometres apart, these will not be the same individuals. The fact that these three species were seen often and are not significantly different between woodlands could be related to the fact that outside of breeding season, Tits can form interspecies flocks to forage through woodlands (Burton, 2003). This greater abundance at feeders would make them most likely to be vectors within any place, but as they generally do not travel far as do migratory birds it is less likely they will take them between areas. As they are similar in both woodlands, it is possible that both locations have reached the limit to each of these species' populations; that the territory ranges are the same for both woodlands. Coal Tit and Nuthatch are highly specialised woodland birds, with Davey *et al* (2012) giving them Species Specialisation Indices (SSI), a measure based on species density across different habitats, of 1.709 and 1.263 respectively. This compares to an SSI of 0.614 for the more generalist Great Tit species. As the Coal Tit is highly specialised, it would be expected that in the appropriate environment the species would be found in abundance. This is also true of the Nuthatch, and the fact that none were found in Denham Woods would mean that the species' niche does not exist at this location. Comparing visits by observations shown in column 3 of Table 4.5 (p.57) indicates the behaviour of each bird at the feeders. Visits cannot be seen as an indication of how much food each species consumes as different birds will spend differing amounts of time at the feeder. For example, in Denham Woods the ratio of visits per observation for Marsh/Willow Tit is 11.7 compared to 4.7 for Great Tit and they were both observed 21 times. However, based on observations, it is likely this difference is due to behaviour, whereby the Marsh/Willow Tit will make fleeting visits, but often, compared to the Great Tit which will make fewer visits but spend more time on the perch feeding. They can, however, be used to compare birds on an interspecies level. The ratios mostly seem consistent between each woodland, although most have values too small to be reliably tested for significant difference. The exception is the difference in the ratio between Denham and Olderwood for the Great Tit, which shows more visits per observation in Olderwood. This could be because of the larger number of overall bird visits in Olderwood indicating there is more competition for time spent at the feeder, whereas in Denham Woods the Great Tit could have remained at the feeding perch undisturbed by other birds. Understanding the behaviour of birds at feeders as well as how often each species visits could be used to determine the likelihood of each transferring spores of *Phytophthora* on supplementary food sources.

Data on the proportion of visits to either the hanging feeder, the ground feeder, or the forest floor provides insight into the best use of different feeder types. The hanging feeder was more often preferred by 5 bird species although not proven with chi square for the Greater Spotted Woodpecker. This is perhaps not surprising for the Tit species as they specialise in perching on twigs, feeding among trees and shrubs. In contrast, the ground feeder which was preferred by 3 species. This may seem surprising for Nuthatch and Marsh/Willow Tit, as they are perching birds that one may expect to prefer the hanging feeders, whereas Robin are much more likely to use groundfeeders though they have learnt to use the hanging variety (Burton, 2003). These differences in expectation to what was observed may be explained by there forming a natural pecking order amongst the species that visited, whereby the dominant birds were able to feed from their preference and intimidate the others to use the next available; although it could also be that some species preferred using the ground feeder as the food was more readily available and easier, before moving onto the hanging feeder once this supply was gone. Although the Robin in Denham is the only species that used only one of the feeders, and the Dunnock in Olderwood which only fed from the surrounding forest floor, this provides justification for use of both feeders in future studies to capture a wider range of birds. However, the supply of ground food is less reliable than for hanging feeders as they proved more prone to food being taken by non-avian species, with Grey Squirrel showing a preference for them as well as Field Mouse/Bank Vole in Denham Woods, and the supply was depleted by the end of each day. This would mean for more effective use of ground feeders, food would have to be distributed daily. Although not seen during an observation period, Pheasants in Olderwood would also feed from the ground feeders, causing their rapid depletion even with the absence of squirrels. Another aspect to consider for their use in future study is that for the Coal Tit, Robin, Chaffinch and Dunnock, there were significant differences in their feeder preference between the two woodlands. This could be because of differences in natural food availability favouring perching or ground feeding in each woodland.

It was also observed that the mix of birds using the feeders led to a hierarchy in feeder use; the larger passerines would sometimes get preferential use of the hanging feeders, with the smaller species moving to the ground feeder as the larger birds came to feed. The variation in species use of the feeders between the woodlands could account for the variation in feeder preference, for example the Great Tit was seen significantly more in Olderwood, and the smaller Coal Tit was seen to use the hanging feeder significantly less in Olderwood than Denham Woods. This priority access has been observed in garden feeders in other studies; in Wojczulanis-Jakubas *et al* (2015) this was found to be linked with body size. The larger species tended to win confrontations over feeder access, and these interactions were observed as winter flocks of mixed species, usually in different niches, came together at a singular food source. In this study, aggressive behaviour was not directly observed, but the larger species appeared to have priority over the favoured feeding perches.

Different species can have different foraging timing patterns, and so individual species are considered in Figures 4.12 to 4.22 (Lilliendahl, 2002). Great Tit and Grey Squirrel appear to show evening peaks in feeding, whereas Coal Tit, Marsh/Willow Tit, and Robin perhaps show a bimodal pattern. There are no apparent peaks in the other species. The lack of clear peaks could suggest consistent effort in feeding in these species, but it could also be that observations did not begin early enough to detect morning peaks, and likewise for the evening. The experimental design aimed to keep observation timings regular due to compromising with other woodland users, but the sunrise and sunset times changed over the course of the study period, which could prevent timing preferences being detected.

5.2.3 Camera Trap Data

The camera trap data provides insights into how the feeders were being used that observations did not capture. As mentioned previously, Pheasants were not seen to be feeding from the ground feeders in the observation periods, but the Olderwood camera trap captured 18 instances of direct feeding, with a total 45 captures of their presence near the feeding stations. The camera traps also captured Field Mice using the feeders at night, shown in Figure 5.1; however, Bank Voles were observed in person in some instances, with a picture taken from nearby feeder 6b shown in Figure 5.2. When observing the feeders, a definitive identification could not be made. Half of the species captured in Olderwood were mammals, which is in contrast to the observations which only found two different mammal species. However, this does not represent a complete picture of feeder use, and the camera trap displays a greater sensitivity to mammals than birds, with the birds seen during observations not captured by the camera; conversely, the pictures of the Fox and the Sheep were at a greater distance than the bird pictures taken.

Figure 5.1: A picture taken of a Field Mouse, identified by the large ears, by the camera trap at feeder station 3 in Olderwood.

Figure 5.2: A photograph captured of a Bank Vole near to Olderwood feeder station 6b.

Morning peaks in activity are shown in Figure 4.23 for Jay, Nuthatch, and Pheasant indicated by the number of pictures taken during the morning hours of these species. However, for the Nuthatch, a morning peak is not reflected in the observation results (Figure 4.13), and the bimodal peaks for the Greater Spotted Woodpecker are likewise. This could be explained as the observations did not start until 08:00, whereas these peaks are 06:00-07:00. As no pictures of nuthatch were taken between 08:00-19:00, this could mean that there was such an increase in visits in this time that the camera trap was able to capture some of these. It could, however, indicate that the camera operates most efficiently during the half-light early morning and late evening hours and does not reflect a true pattern in visits. Observation during these hours would be required to determine this, and it appears that these early morning and late evening hours would be most beneficial to future studies. Grey Squirrels were captured entirely in the evening hours between 19:00-21:00, and this is supported by observations in Denham Woods which found an evening peak in activity. Field Mice/Bank Voles were captured only at night, which reflects their mostly nocturnal nature, and demonstrates the benefit of camera traps in gaining a complete understanding of how feeders are used by different animals as these would not have been observed.

Other activity was captured with the camera traps that would have otherwise been unobserved. For example, Pheasants were seen feeding from damaged feeders outside the observation periods, but were captured on camera providing evidence for what was causing damage to the ground feeders as shown in Figure 5.3a. Sheep were also suspected of entering the woodlands and causing some damage, and although were not captured using the feeder, they were photographed entering the woodlands in Figure 5.3b. Deer were also suspected of feeding from the ground feeders and were captured in pictures. These can be used to create measures to protect the feeders from mammal grazing and damage by birds or otherwise, or to provide evidence feeders could be used to target these species in any future study.

a

b

c

Figure 5.3: Pictures taken by the camera trap in Olderwood, showing

a: Pheasants causing damage to the ground feeder by bending the mesh out of the frame

b: Sheep utilising the woodland from the adjacent field

c: Roe Deer feeding from the ground feeder.

The camera trap in Denham Woods captured 11 species in total, but unlike Olderwood this captured more bird species than mammals. However, 72% of all the pictures taken were of Grey Squirrel, and Field Mice were also photographed shown in Figure 5.4. Similarly to Olderwood, the camera trap at feeder station 3 didn't photograph any Bank Vole, but one was photographed on a ground feeder at station 1 shown in Figure 5.5 and the species could not positively be split for Denham woods. This is due in part to the positioning of the camera nearby the feeder that was being damaged more frequently than the others to discover the cause, which is shown in Figure 5.6 (p.97) to be the Squirrels, and so it could be expected that they were far more frequent at this station than the others. It does also show a contrast with Olderwood where the Squirrel population is controlled, even if not through a deliberate programme. Roe Deer was captured once on the Denham camera but unlike Olderwood was not seen to be feeding. Based on personal observations, there were fewer deer spotted in Denham Woods in the study area. The Denham camera captured more bird species than Olderwood, but as the model is different, this could be due to difference in sensitivity whereby the Denham model is better able to capture birds.

Figure 5.4: Field Mice photographed by the camera trap at feeder station 3.

Figure 5.5: A Bank Vole photographed using the ground feeder in Denham feeder station 1.

Chaffinch, Great Tit, Field Mice/Bank Vole, and Grey Squirrel for which the camera trap pictures are organised by time in Figure 4.24 show a different pattern to those from observations discussed in section 5.2.2. This could be for many reasons, including those discussed for the Olderwood camera trap whereby the observation periods do not capture the optimum feeding time of species, or that the camera trap has a greater sensitivity to movement in the light levels in the early morning and late evening. It could also be that the camera was used for a limited time period and so could not distinguish the same time patterns with a small number of days in use. Another consideration is that the camera trap was only used at one feeder station in Denham Woods, unlike the observations which combined several stations to abstract the patterns in feeding behaviour. However, it could be that it is the presence of the observer that deters the animals, even at a distance, or that after resupplying the feeders a greater amount of time prior to observations than the 1-2 hours allotted is needed before the birds and mammals return. This seems to be the case with the Grey Squirrel, as the camera traps show them to be using the feeders throughout the entire day, although peaking at 10:00 and 18:00, and this pattern was not determined through observations which found only one peak at 18:00.

As aforementioned, the camera trap in Denham Woods was placed to gain information as to what was causing the damage in section 4.2.3, as well as to ascertain whether the observations were missing use of the feeders. The cause of damage was captured on video and is shown in Figure 5.6 to be the Grey Squirrel. Being acrobatic, curious and intelligent they were able to learn how best to access the food, and can be seen to carry off parts of the feeders rendering them unrepairable. From observations and camera trap evidence, the large population of Squirrel in Denham Woods means that without further protective measures to prevent damage or use from them, any study using bird feeders in similar UK woodlands would have a limited timespan before the squirrels become too adept at disassembling the apparatus causing too much disruption to continue a similar study. However, if birds are able to vector *Phytophthora* in the means discussed in previous sections, then Grey Squirrel might also be a potential vector, and this demonstrates the benefit that bird feeders would have to study them using a non-invasive method.

Figure 5.6: Still image taken from camera trap video in Denham Woods at feeder station 3 showing Grey Squirrel removing parts from the hanging feeder.

5.2.4 Food Mass

From the mass of food taken from hanging feeders, a general trend of increasing seed consumption seems apparent in Olderwood. This could be because the feeders were being found by more animals, and were being incorporated into a foraging routine as a reliable source of food. The benefit of taking feeder mass is that it can capture feeder use outside of observations, and this gives an estimate of use across periods in between observations as well as being a more accurate depiction of how each feeder is being used. However, no pattern is discernible from the Denham feeders. Multiple problems ensued from this form of data collection affecting both woodlands, but in particular Denham Woods. Gaps in the data are the result of disruption to supply, which in Denham Woods is frequent. Grey Squirrels were frequently seen utilising the feeders in Denham Woods, and were captured on camera traps to be damaging them. In Olderwood, disruption to supply was less frequent, perhaps reflecting the fact that Grey Squirrels were observed far less. However, in Olderwood Pheasants seemed to be able to displace the hanging feeders to access the seed, shown in figure 5.7. This led to an improved method of securing the hanging feeders being incorporated. It is likely to also be the case in Denham Woods as Pheasants were seen outside of the observation periods. Indicated in red on Figures 4.25 and 4.26 is when the feeders were found fully depleted before refill. This means that more seed could have been consumed had the supply allowed, and therefore makes the estimate for food consumed an underestimate, meaning activity at the feeders was limited. If feeder mass would be incorporated more accurately, it would have to be maintained and data recorded daily. Because of these issues, the data has not been analysed further. However, if these recommendations were followed, more accurate estimates for feeder activity could be calculated, which could be important information for ascertaining the role in feeders with *Phytophthora* spore transportation. Furthermore, correlations between feeder activity and key variables (for example temperature) could be tested for.

Figure 5.7: A female Pheasant pictured feeding from a hanging feeder it displaced.

5.2.5 Woodland Character

The canopy cover results provide information on the woodland, which can be used to infer information about the site and to assist in replicating the methodology as well as to ascertain areas for future study. The results for Olderwood show that the differences in canopy cover between feeder station sample points are not significant. This is most likely a reflection of the stands being even aged, with little undergrowth forming a lower canopy. On the other hand, Denham Wood canopy cover between the feeder stations results proved significantly different to each other, which could be because of the number of different species and tree ages in varying proportions forming variations in crop densities. Software to analyse digital photographs of the canopy was used to create more accurate estimations with limited resources, and is easily replicable with little influence by the data collector. It could feasibly be used in citizen science projects with ease. However, with canopy cover above 90%, an alternative method had to be used, which if using volunteers for citizen science involves additional steps which could create difficulties.

Denham Woods generally had a greater density of stems above 5cm DBH than Olderwood, reflecting the fact that it is a denser woodland. Likewise, Denham Woods had far more saplings than Olderwood. This could perhaps be due to thinning management, as Denham Woods is largely managed in this way, and Olderwood may have been thinned more recently than Denham (Robinson, 2016). Less information is available on Olderwood, but many Sweet Chestnut had been recently felled, perhaps accounting in part for the lower stem number (B. Robinson *per comm*, 2019). Table 4.12 (p.73) shows that the study area in Olderwood was uniform in density other than the location of feeder station O2, and in Denham Woods this is also the case with the exception of station D1. In Denham Woods, there was some diversity in the density of saplings around each station, with stations D1 and D3 being significantly different to the rest. This reflects the nature of the pen in station 1, which was recently felled other than some coppiced sweet chestnut, creating an open canopy in which saplings are restocking. At feeder station D3, the saplings were concentrated around an opening on a bank to the access road, where light was more accessible to support the saplings.

The richness in species of stems and the total diversity is also generally higher in Denham Woods compared to Olderwood, and this is probably due to the same reasons. Correlations between stem number and bird species occurrence were found for Marsh/Willow Tit (positive) in Olderwood, and Robin and Dunnock in Denham (negative). This could be due to a balance between having cover close to the feeders to avoid predators and the ease in which the feeders were found. Correlation with species richness was only found for Greater Spotted Woodpecker in Olderwood in a negative association, which could be due to a high preference for a particular tree species, but this is contradicted by a positive correlation with stem diversity. Chaffinch in Olderwood was also associated positively with stem diversity, as was Dunnock in Denham, which could show that these species have a preference for areas with a diversity in food sources, or a preference for a more diverse canopy topography created by different tree shapes to provide cover. Marsh/Willow Tit shows a negative correlation with total diversity in Denham Woods and with sapling diversity, which could be for the opposite reason; a preference for one food type based on particular species. However, due to the number of correlation tests, it cannot be ruled out without further study that these relationships are coincidental, even with a 95% confidence level.

These correlations of avian visits and woodland characteristics begin to demonstrate how feeders can be used in woodlands to study *Phytophthora*; by describing how the choice of woodland, or even location within a particular wood, can influence the bird species that are seen and the ease in which the feeders will be found by the local population. If the correlations are replicated, then the woodland characteristics can be used to focus a study on particular avian species. From the characteristic results and correlations, there seems to be a balance between choosing locations where woodland diversity increases the number of avian species the area can support versus being easily able to observe the feeders in a woodland with a more uniform canopy. As well as this, the high natural food availability could possibly lead to less visitation to the bird feeders in natural environments.

5.3 Weather

Met Office data from a nearby location, Mount Batten, can provide an average for 1981-2010 to ascertain whether the patterns seen at Crownhill during the study period deviate from this longer-term mean (MET Office, undated). Compared to these Met Office averages, March, April and May saw a warmer average maximum temperature of 1.8 ^{O}C, 2 ^{O}C, and 1.5 ^{O}C respectively. March and April also saw warmer average minimum temperatures of 0.7 ^{O}C and 1.2 ^{O}C respectively, but May had a negative 0.8 ^{O}C deviation. Rainfall compared to the Met Office averages, March, April and May all had less rainfall with a negative deviation of 4.6mm, 10.9mm, and 42.4mm respectively (MET Office, undated). Average windspeeds cannot be compared to the Met Office averages due to differing methods, however the maximum gust speeds are greater for March and April by 3.7mph and 0.5mph, and less in May by 1.4mph (MET Office, undated). However, all months show a decrease in total sunshine hours from the Mount Batten 1981-2010 average of -9.4, -58, and -43.1 hours chronologically. Average dew point temperatures are also not available from the Mount Batten 1981-2010 average, but is positive 1.6^{O}C for March and 1.1 ^{O}C for May from the average of 2011-2018 Crownhill data, with no deviation in April.

Warmer temperatures in early spring could be better for bird populations as there is less energy expended on maintaining body temperature, as well as more abundant food supply beginning earlier. Although it could also be continuing a trend of unequal phenological changes whereby animals higher up the food change are responding to changes in food availability more slowly, and furthermore increased temperatures in early spring can lead to a decrease in plant productivity (Hatfield & Prueger, 2015; Both et al, 2009). Changing rainfall patterns could also be influencing bird interactions with the feeders; less energy could be expended by the birds on maintaining temperature with less rainfall, and in addition it could possibly extend foraging time if the birds do not have to take shelter. If this was the case, it might be expected that the birds do not need to rely on bird feeders as much as otherwise, as an extended foraging time and less energy expenditure would require less dependence on supplementary food sources. In contrast, it could be the case that the change in rainfall conditions creates pressure on certain plants by reducing available soil water and therefore affects the food chain such that less natural food sources are available (Zeppel, 2013). Windspeed can have an impact from windchill as well as the effect of gusts potentially causing windthrow to trees in the woodlands, though there was not a large difference between observed and the average. Sunshine hours could affect plant productivity and therefore natural food availability, from changes to photosynthesis rate and evapotranspiration. Dew point temperature reflects humidity, with a higher dew point meaning more moisture in the air. This can influence energy expenditure for birds for temperature regulation.

Spore genesis in Phytophthora species are temperature dependent, with chlamydospores being produced between 10-26 ^{O}C and sporangia forming between 10-30 ^{O}C, the optimum being about 22OC for both (Kliejunas, 2010). This is thought to be the case for other species including forest Phytophthora pathogens. From the Crownhill temperature results shown in Figure 4.31, it could therefore be suggested that throughout the study period, the temperature requirements for spore genesis were met, with the exception of the 18th of March, and the 3rd, 4th, 5th, and 14th of April. Rainfall can lead to the release of Phytophthora spores, as well as dispersing them with the potential for rain splash transferring spores onto birds, and so is an important consideration combined with the Phytophthora test results (Coleman & Clark, 2010; Davidson et al, 2005). The number of rainfall days from March to May decreased per month, and so it could be expected that the potential for spore vectoring decreased throughout the study period. As May had much less rainfall than the average, there could be expected that there could be less zoospore release than previous years, and less movement of the spores. As Sunshine hours and dew point temperature influence evaporation, the change in these variables may affect the amount of water available in which the short-lived zoospores of Phytophthora need to survive (Walker & van West, 2007).

Despite potential influences weather may have to bird energy expenditure and feeding, the correlation tests largely found no link to species visits and the weather. The few correlations found are likely to be due to the large number of correlation tests conducted, and therefore beneficial weather for future investigations seeking to attract particular birds in woodland environments cannot be ascertained from this data. However, rainfall as an abiotic dispersal method for Phytophthora spores is well documented and although no active spores were discovered in this study, it could be expected that there would be a greater chance for birds to transfer spores during or shortly after rainfall as the spores disperse (Davidson et al, 2005). Moreover, as rainfall is likely to lead to a greater energy expenditure from birds and mammals, if an increase in foraging activity is observed after rainfall of natural food sources or otherwise, there is a higher chance to collect spores and transfer them at feeding stations. Further investigation into the ability of different materials to hold the spores of Phytophthora would be needed, but if the feeders were able to hold spores throughout rainfall then this would make the period following rainfall events the optimum for future study into birds as vectors of the disease.

5.4 Bird Feeders as a Monitoring Tool

Using bird feeders for ecological monitoring appears to have many benefits from the data collected during this study. They proved successful in attracting a range of bird species, receiving large numbers of visits from Great Tit, Coal Tit, Blue Tit, Nuthatch, and March/Willow Tit, all birds that may typically be found in the garden (Burton, 2003). There are however ways this methodology may be better adapted for future use as an ecological monitoring tool. As already discussed, observing them at earlier and later times in relation to the sunrise and sunset could gain more complete results. The bird feeders also lend themselves to being monitored via camera traps both in natural environments and gardens, as sensitive models are able to capture visits by even small birds as shown in section 5.2.3. Furthermore to adapting how the feeders are used, additional equipment and measures could improve their use for ecological studies. As aforementioned, there were frequent visits by some species typically considered pests to garden feeders that may not be the focus of studies. Using cages around the ground feeders may deter the larger of these, such as Deer, Sheep, and Pheasants seen in Olderwood; though this could prevent larger birds of interest from utilising the feeder, and would be unlikely to stop voles and mice. Of greater concern to future studies utilising bird feeders is deterring Squirrels, if the study location has a high population. As found in Denham woods, the Grey Squirrels were highly capable of destroying the feeders. A simple solution was used in this study to prevent the hanging feeders being knocked of the shepherds crook poles by Squirrels and Pheasant, by securing the feeders on with zip-ties. This was effective at preventing Pheasant interfering with the hanging feeder as a monitoring tool and prevented the perching method of Grey Squirrels from dislodging the hanging feeder, but the damage caused by Squirrels was not halted for long. Further measures to prevent squirrel damage would be beneficial; from anecdotal evidence from residence at Keele whilst undertaking the survey, this could be as simple as creating a cone midway up the hanging feeder pole as some residents had done with repurposed plastic bottles. There are also specialised feeders designed to prevent squirrels, though these would risk deterring some bird species as well as increase the cost and therefore scope of any study.

6 - Conclusion

6.1 The Literature

From the literature, the role of animal vectors of *Phytophthora* is unclear. Humans have been documented to transport the disease across the globe through the nursery trade, as well as more locally by transporting spores in the soil via vehicles or even footfall (Brasier, 2008; Cushman *et al*, 2008; Weste & Taylor, 1971). Vertebrates could be transporting spores in a similar way, moving spores present in the soil (Krull *et al*, 2013). As rain releases zoospores that can splash onto neighbouring plants, it is also possible birds and other animals could be transporting them after picking spores up from wet surfaces or splash from rainfall (Coleman & Clark, 2010). Birds could be a significant vector, as many species travel great distances in migration, and so could introduce the disease to new locations. Furthermore, woodland species spend a significant amount of time in areas where they are potentially in contact with large quantities of spores, and so may also be a significant local vector.

Bird feeders may present a non-invasive method of studying whether birds are vectors of *Phytophthora,* by providing a means of testing what they come into contact with, to determine whether they are carrying spores of the disease. Bird feeders are known to be places where avian diseases can be transmitted, but nothing was found in the literature about whether phytopathogens could be transferred in a similar manner (Adelman *et al*, 2015). The number of households providing supplementary food for birds has been increasing in recent years, and so there is a large base from which to study bird populations (Boothby, 2017). Bird feeders attract a wide range of bird species including woodland species, which are particularly relevant in the context of tree disease (Burton, 2003).

A method of testing whether birds could be transferring spores to bird feeders would be to establish them in woodlands with known *Phytophthora* presence. Few studies were found that used supplementary feeders in natural environments to study bird populations, and so using this method also has the benefit of seeing if they could be used in this way for future studies. By observing which species use the feeder and how long they took to establish in natural environments, the usefulness of this method can be ascertained.

6.2 Residence Survey

Semi-rural residences such as Keele present an ideal area for study on bird feeders and *Phytophthora*. The feeder ownership here is higher than the national average, and most had been there a considerable time so are well established in the bird communities. The large densities of well-established feeders at Keele could suggest that other semi-rural residences would provide an ample data set to investigate in future studies. Hanging feeders were by far the most common type, and the most frequent foods supplied were fatball, mixed seed, and nuts. However, a large range of birds were reportedly seen, many of which are typical woodland species. Species that are commonly viewed as pests on household feeders were also commonly reported such as Grey Squirrels. Furthermore, as 24% reportedly never cleaned their feeder, and a further 24% reported annual cleaning, there is time for spores to transfer at these sites as well as potentially avian diseases . No *Phytophthora* spores were detected in this study on residential feeders using the rapid diagnostic test kits in combination with swabs.

6.3 Woodland Feeders

Much was learnt on the use of feeders in woodland environments from this study. This includes timings of when species visit the feeders as well as how long it took for the feeders to establish in the woodland environments, indicating how the choice of woodland influences the results gained; natural food abundance will influence how the supplementary feeders are used in the semi-natural environment. The different feeders were preferred by different species, but both have their problems when used to study woodland species. However, they can be used to maximise the species seen if the method of placement and the routine of supplying food is adapted from this study.

The observations of bird feeders in woodland environments showed that they take between 1.5-3 weeks to establish in these environments. The optimum time of day for observation based on total visits differs between the woods from a morning peak to a three-peak pattern, which may be dependent on natural food availability. On an individual species level, few showed a regular time pattern in visits to the feeders. However, if observations were taken both earlier and later, time preferences that were not otherwise seen may be captured as inferred from the camera trap data; keeping the observations in relation to sunrise and sunset rather than fixed times may be better still for finding foraging patterns in individual species.

The camera traps were able to capture visits to the feeders that were unseen during observations. They were able to capture mammal, and some bird visits, that were either outside the times of observation or were deterred by the presence of the observer. Using the camera traps, the effectiveness of observations can be commented on, and by discovering the cause of damage to the feeders better recommendations can be made as to how to utilise them in future study.

All the birds seen feeding during the study were non-migratory residents, and the most common birds observed were Great Tit, Coal Tit, and Marsh/Willow Tit. These species are frequently present at garden bird feeders, and could be implicated with local spore transfer if spores are found on feeders in future study. They also share a woodland environment with further travelled species, such as Redwing (seen outside of an observation period, close to feeder stations), with implications for spore transfer at greater distances.

Taking the food mass from the hanging feeders before resupplying the stations was able to show that there was a general trend of increasing consumption throughout the study period, indicating higher activity. However, there were numerous issues with this method of data gathering, primarily in that the food supply was often disrupted leading to gaps in the data. This does provide relevant information in itself, in that for Denham Woods it is another indicator of the activity of Squirrel, and the lack thereof in Olderwood. It provides supporting information into the effectiveness of using supplementary feeders in different woodlands, by showing how the regularity of supply is impacted by species that were present in different numbers in both locations.

6.4 Woodland and Weather Correlations

The woodland correlation results further demonstrate how supplementary feeders can be used in semi-natural environments, by showing how the woodland characteristics of the study sites can affect the birds that are seen as well as the ease in which they are found by the local avian community. The weather correlation results could also show how there is a need to balance unfavourable conditions causing a higher energy demand, therefore need to feed, and sheltering from adverse weather; favourable weather is better for foraging, but there is less demand from energy expenditure to keep warm. However, with these weather and woodland characteristics, they are often contradictory between species and between the woodlands. Because of the large number of variables tested for correlation, it is therefore likely that these are coincidental, even with a 95% confidence level. This would need corroborating with future studies to see if they are true correlations or not.

6.5 Recommendations

No positive results for the presence of *Phytophthora* spores were found in either Denham or Olderwood. This is likely because recent management measures have limited the number of spores present, perhaps limiting *P. ramorum* on the sites to very few infections or as resting/saprotrophic presence. For future study, these tests would have to be used in an active *Phytophthora* site before or during management. This presents many difficulties, not least that the bird populations would be disturbed by any management, however, a positive *Phytophthora* result would be required to be able to test the swabbing methodology in combination with the rapid diagnostic test kits. Once this is determined, the residents survey suggests that household feeders in semi-rural locations by infected woodlands would present a large study area that attracts a variety of woodland bird species that could be transporting *Phytophthora*.

Observations and use of camera traps were able to capture different uses of the woodland bird feeders, while neither were able to give a full account by themselves. Therefore, the use of camera traps along with these observations in a systematic way is recommended in any future study looking into what species are utilising bird feeders. Camera traps have become a financially viable tool in ecological studies, but care must be taken in the model of camera as they have different sensitivities to birds, and can miss many visits especially by the smaller passerines. Their ease of use and non-invasive nature means they could be a way to monitor the use of household feeders which could present difficulties in observing in person. A combination of camera traps used in a systematic way alongside observations would be highly beneficial to use with feeders for ecological monitoring; the camera traps can provide data for a greater range of time without disturbing the birds, which in this study gave useful insights into when the feeders were being used. Observations are still necessary to compensate for the sensitivity by the camera traps, and gave anecdotal evidence into the behaviour of species using the feeders that was not captured in this study by the cameras such as the hierarchy of species at the feeders.

Using both hanging feeders and ground feeders has the advantage that they are preferred differentially by different bird species, and so encourages diversity of animals seen. However, for the ground feeders to be effective, and not to deplete so quickly as to be unlikely to be incorporated in the foraging routines of the local population, they would have to be filled daily. Or, the use of a bird table may encourage many of the same avian species while discouraging some of the mammals and Pheasants that depleted them so rapidly. Furthermore, ground feeders and tables are often made from different materials to hanging feeders; wood as opposed to plastic. There is a possible difference in the ability of spores to persist on different materials, which could be the focus of future study, or both feeder types could be incorporated to allow for one material holding spores to a greater extent than the other.

Squirrels may be another potential vector for *Phytophthora*, and so supplementary feeders could be a method of analysing their role in local *P. ramorum* dispersal. However, for the purposes of this study, their persistence meant that if *Phytophthora* spores were found, it would not be possible to determine if this was from birds or mammals. In addition, the Squirrels ability to disassemble the hanging feeders limited their useful lifetime. For future study, in woodland environments where squirrels have a high population, methods to exclude the squirrels would be recommended or more robust feeders that could withstand the squirrels attempts to use them.

Although the correlations between bird visits and woodland characteristics are inconclusive, there appears to be a balance between using a woodland with high plant diversity and one in which the feeders are both more easily found by the birds as well as more easily accessed and observed by the researcher. Olderwood was a more uniform woodland, both in stand species as well as the canopy height and cover, but still had a diverse range of bird species and had a higher number of overall visits. Therefore, similar woodlands would be recommended for future studies, so long as they fulfil the previous recommendations of having confirmed, current presence of *Phytophthora*. The weather correlations are similarly inconclusive due to the number of tests undertaken, but March-April fulfils the temperature requirements of *Phytophthora* as well as having high rainfall which could cause the release of *Phytophthora* spores.

Studying bird feeders to investigate whether birds can vector plant pathogens has proven to be an effective non-invasive method. Household feeders provide a large base of study, and a positive way to engage the public on ecological sciences, while establishing feeders in natural environments enables investigation into woodland bird populations. The method of testing for the presence of *Phytophthora* would benefit from further trial in a site with confirmed current cases, but this study has been able to establish the groundwork for further investigation onto such a site.

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Appendix

i) The survey used as a transcript to ask the Keele residents questions on their bird feeders.

Script for Residents Questions

My name is Matthew, I'm a research masters student at Keele looking at tree disease and associations with bird feeders. As bird feeding attracts a number of birds, I am researching whether birds are carrying the tree disease *Phytophthora* and if bird feeders could transfer disease spores.

Do you own a bird feeder?

Would you have a couple of minutes to answer some anonymous questions about it?

1) How long have you had a bird feeder at this address?

2) How many bird feeders do you have?

3) What type of feeder/s do you have?
    a) Hanging
    b) Table
    c) Ground

4) What type of food do you use?

5) What birds do you see at your feeder?

6) Do you see squirrels feeding from them?
    a) No
    b) Regularly
    c) Irregularly/only occasionally

7) How often do you clean your feeders? This is not intended to suggest there is a correct frequency, nor will there be any repercussions for you if bird feeders are associated with *Phytophthora*.
    a) Every week or two
    b) Every month (3-5 weeks)
    c) Every few months
    d) Every year
    e) Never

Thank you for answering my questions.

I would also like to ask if you could further help my study by allowing me to collect a swab sample from your bird feeder/s? This would take up to ten minutes of your time. This would be able to give me valuable data on established bird feeders that would really help to make my project more meaningful.

ii) BTO bird species codes that are used as shorthand for studies such as the Breeding Bird Survey

BTO SPECIES CODES

AC	Arctic Skua	GA	Gadwall	LE	Long-eared Owl	SM	Sand Martin
AE	Arctic Tern	GX	Gannet	LT	Long-tailed Tit	SS	Sanderling
AV	Avocet	GW	Garden Warbler	MG	Magpie	TE	Sandwich Tern
BO	Barn Owl	GY	Garganey	MA	Mallard	VI	Savi's Warbler
BY	Barnacle Goose	GC	Goldcrest	MN	Mandarin Duck	SQ	Scarlet Rosefinch
BA	Bar-tailed Godwit	EA	Golden Eagle	MX	Manx Shearwater	SP	Scaup
BR	Bearded Tit	OL	Golden Oriole	MR	Marsh Harrier	CY	Scottish Crossbill
BS	Berwick's Swan	GF	Golden Pheasant	MT	Marsh Tit	SW	Sedge Warbler
BI	Bittern	GP	Golden Plover	MW	Marsh Warbler	NS	Serin
BK	Black Grouse	GN	Goldeneye	MP	Meadow Pipit	SA	Shag
TY	Black Guillemot	GO	Goldfinch	MU	Mediterranean Gull	SU	Shelduck
BX	Black Redstart	GD	Goosander	ML	Merlin	SX	Shorelark
BJ	Black Tern	GI	Goshawk	M.	Mistle Thrush	SE	Short-eared Owl
B.	Blackbird	GH	Grasshopper Warbler	MO	Montagu's Harrier	SV	Shoveler
BC	Blackcap	GB	Great Black-backed Gull	MH	Moorhen	SK	Siskin
BH	Black-headed Gull	GG	Great Crested Grebe	MS	Mute Swan	S.	Skylark
BN	Black-necked Grebe	ND	Great Northern Diver	N.	Nightingale	SZ	Slavonian Grebe
BW	Black-tailed Godwit	NX	Great Skua	NJ	Nightjar	SN	Snipe
BV	Black-throated Diver	GS	Great Spotted Woodpecker	NH	Nuthatch	SB	Snow Bunting
BT	Blue Tit	GT	Great Tit	OP	Osprey	ST	Song Thrush
BU	Bluethroat	GE	Green Sandpiper	OC	Oystercatcher	SH	Sparrowhawk
BL	Brambling	G.	Green Woodpecker	PX	Peafowl/Peacock	AK	Spotted Crake
BG	Brent Goose	GR	Greenfinch	PE	Peregrine	SF	Spotted Flycatcher
BF	Bullfinch	GK	Greenshank	PH	Pheasant	DR	Spotted Redshank
BZ	Buzzard	H.	Grey Heron	PF	Pied Flycatcher	SG	Starling
CG	Canada Goose	P.	Grey Partridge	PW	Pied Wagtail	SD	Stock Dove
CP	Capercaillie	GV	Grey Plover	PG	Pink-footed Goose	SC	Stonechat
C.	Carrion Crow	GL	Grey Wagtail	PT	Pintail	TN	Stone-curlew
CW	Cetti's Warbler	GJ	Greylag Goose	PO	Pochard	TM	Storm Petrel
CH	Chaffinch	GU	Guillemot	PM	Ptarmigan	SL	Swallow
CC	Chiffchaff	FW	Guineafowl (Helmeted)	PU	Puffin	SI	Swift
CF	Chough	HF	Hawfinch	PS	Purple Sandpiper	TO	Tawny Owl
CL	Cirl Bunting	HH	Hen Harrier	Q.	Quail	T.	Teal
CT	Coal Tit	HG	Herring Gull	RN	Raven	TK	Temminck's Stint
CD	Collared Dove	HY	Hobby	RA	Razorbill	TP	Tree Pipit
CM	Common Gull	HZ	Honey Buzzard	RG	Red Grouse	TS	Tree Sparrow
CS	Common Sandpiper	HC	Hooded Crow	KT	Red Kite	TC	Treecreeper
CX	Common Scoter	HP	Hoopoe	ED	Red-backed Shrike	TU	Tufted Duck
CN	Common Tern	HM	House Martin	RM	Red-breasted Merganser	TT	Turnstone
CO	Coot	HS	House Sparrow	RQ	Red-crested Pochard	TD	Turtle Dove
CA	Cormorant	JD	Jackdaw	FV	Red-footed Falcon	TW	Twite
CB	Corn Bunting	J.	Jay	RL	Red-legged Partridge	WA	Water Rail
CE	Corncrake	K.	Kestrel	NK	Red-necked Phalarope	W.	Wheatear
CI	Crested Tit	KF	Kingfisher	LR	Redpoll (Lesser)	WM	Whimbrel
CR	Crossbill (Common)	KI	Kittiwake	RK	Redshank	WC	Whinchat
CK	Cuckoo	KN	Knot	RT	Redstart	WG	White-fronted Goose
CU	Curlew	LM	Lady Amherst's Pheasant	RH	Red-throated Diver	WH	Whitethroat
DW	Dartford Warbler	LA	Lapland Bunting	RE	Redwing	WS	Whooper Swan
DI	Dipper	L.	Lapwing	RB	Reed Bunting	WN	Wigeon
DO	Dotterel	TL	Leach's Petrel	RW	Reed Warbler	WT	Willow Tit
DN	Dunlin	LB	Lesser Black-backed Gull	RZ	Ring Ouzel	WW	Willow Warbler
D.	Dunnock	LS	Lesser Spotted Woodpecker	RP	Ringed Plover	OD	Wood Sandpiper
EG	Egyptian Goose	LW	Lesser Whitethroat	RI	Ring-necked Parakeet	WO	Wood Warbler
E.	Eider	LI	Linnet	R.	Robin	WK	Woodcock
FP	Feral Pigeon	ET	Little Egret	DV	Rock Dove (not feral)	WL	Woodlark
ZL	Feral/hybrid goose	LG	Little Grebe	RC	Rock Pipit	WP	Woodpigeon
ZF	Feral/hybrid mallard type	LU	Little Gull	RO	Rook	WR	Wren
FF	Fieldfare	LO	Little Owl	RS	Roseate Tern	WY	Wryneck
FC	Firecrest	LP	Little Ringed Plover	RY	Ruddy Duck	YW	Yellow Wagtail
F.	Fulmar	AF	Little Tern	RU	Ruff	Y.	Yellowhammer

iii) The common and scientific name species list of birds and mammals recorded in Devon woodlands

Common Name	Scientific Name
Birds	
Blue Tit	*Cyanistes caeruleus*
Chaffinch	*Fringilla coelebs*
Coal Tit	*Periparus ater*
Dunnock	*Prunella modularis*
Greater Spotted Woodpecker	*Dendrocopos major*
Great Tit	*Parus major*
Greenfinch	*Chloris chloris*
Jay	*Garrulus glandarius*
Long Tailed Tit	*Aegithalos caudatus*
Magpie	*Pica pica*
Marsh Tit	*Poecile palustris*
Nuthatch	*Sitta europaea*
Pheasant	*Phasianus colchicus*
Robin	*Erithacus rubecula*
Siskin	*Carduelis spinus*
Willow Tit	*Poecile montanus*
Mammals	
Bank Vole	*Myodes glareolus*
Field Mouse	*Apodemus sylvaticus*
Fox	*Vulpes vulpes*
Grey Squirrel	*Sciurus carolinensis*
Roe Deer	*Capreolus capreolus*
Sheep	*Ovis aries*