NECTAR ABUNDANCE IN SEMI-NATURAL SPECIES-RICH GRASSLAND COMPARED TO AGRI-ENVIRONMENTAL WILDFLOWER STRIPS

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Abstract

Land use change including agricultural intensification have caused pollinators to decline. Given the extensive pollinator loss, it has become important to quantify their food resource to help develop and improve conservation actions. Nectar resources have been measured within urban and rural landscapes, but few studies have undertaken a comparative quantification for agri-environmental schemes such as semi-natural species-rich grasslands and planted agri-environmental wildflower strips, leaving an important knowledge gap in plant-pollinator interactions and food resources within these habitats.

This study quantified the nectar supply of two habitats - semi natural species rich grasslands and planted agri-environmental wildflower strips in three farms in the Cotswolds and West Midlands. Overall, thirty-six flowering plants were identified and assigned a nectar sugar value using secondary data to enable the comparison of nectar between the two habitats. Twenty-eight pollinator species were identified within the 90 quadrats and were assigned a behaviour of feeding, collecting pollen, foraging, and resting to investigate how pollinators within the groups Hymenoptera, Lepidoptera, Diptera and Coleoptera interacted with the flowering plant species identified.

The planted agri-environmental wildflower strips provided twice as much nectar than the semi-natural species-rich grassland. Which was underpinned by a low number of flowering plants, *Centaurea nigra* and *Leucanthemum vulgare* providing most of the nectar resources.

A total of 228 pollinator visits were recorded. Though no significant results the pollinators did use the habitats differently. Butterflies were found mostly in the semi-natural species-rich grassland and bumblebees and hoverflies mostly found within the planted agri-environmental wildflower strips. These results indicate that pollinators are using the habitats differently for their resources needs of food and nesting. *Centaurea nigra* was highest visited flowering plant with 48% of the total visits.

There were two significant relationships with floral traits that attracted pollinator visits, which were the amount of open floral units and the nectar value.

Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Gloucestershire and is original except where indicated by specific reference in the text. No part of the thesis has been submitted as part of any other academic award. The thesis has not been presented to any other education institution in the United Kingdom or overseas.

Any views expressed in the thesis are those of the author in no way represented those of the university.

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1 Introduction

Wild pollinators are inextricably connected to the wellbeing of humans through the maintenance of ecosystem functions and health, crop production and wild plant pollination (Potts *et al*., 2016). Wild pollinators have been declining steadily since 1970 (Thomas *et al*, 2004; Beismeijer *et al.,* 2006; Carvell *et al*., 2006). Long term declines for butterflies have been 16% and moths 25%, whilst habitat specialist butterflies have declined by 68% between 1976 and 2018 (Hayhow *et al*., 2019). Bee abundance has declined by 25% between 1980 and 2013, and hoverflies 24% (Powney *et al*., 2019). There are four pollinating insect groups, these being, Hymenoptera, Lepidoptera, Diptera and Coleoptera. In semi-natural and natural habitats up to 90% of the flowering plants species rely on these pollinating groups for pollination (Corbet *et al.,* 1991; Buchmann & Nabhan, 1996). It is estimated that 75% of agricultural food crops rely upon pollinators particularly bees (Potts *et al*., 2010a). where the pollinator is rewarded in the form of a resource either pollen or nectar (Renner, 2006; King, 2012).

Declines in both insect pollinators and wildflowers in the UK and globally have been reported (Potts *et al*., 2010a; Goulson *et al*., 2015). Key factors contributing to the decline of pollinators is the loss of floral resources, because of changes in land use and inappropriate management (Carvell *et al*., 2006; Klein *et al*., 2007; Roulston *et al*., 2011; Scheper *et al*., 2014). Along with pressures of habitat loss, pesticides, urbanization, and climate change possibly more so for species within upland areas (Potts et al., 2010a; Vanbergan, 2013; Baude et al., 2016). Geographical location presents a considerable difference in the decline of pollinators in Great Britain. For example, there is a 55% decline in upland species and an average of 25% decline in southern pollinator species (Powney *et al*., 2019).

The management factors include increased use of herbicides (Robinson *et al*., 2002), removal of landscape features such as hedgerows (Garrett *et al*., 2017), degradation and loss of semi-natural species-rich grasslands (Ratcliffe, 1984; Fuller, 1987; Blackstock *et al*., 1999) and habitat fragmentation (Winfree *et al*., 2009).

Semi-natural species-rich grasslands are areas of conservation importance in the UK and throughout Europe. These grasslands support a high diversity and richness of pollinators including butterflies (Van Swaay *et al*., 2002) and other invertebrates such as flies and hoverflies (Barnett *et al*., 2004). In England and Wales semi-natural species-rich grassland has an estimated decline of 97% between 1932 and 1984 (Fuller, 1987). This decline has been attributed largely to the intensification of agriculture along with the conversion of the remaining semi-natural species-rich grassland (Fuller, 1987; Bourn and Thomas, 2002; Poschlod and WallisDeVries, 2002) to arable land and more productive grassland (Burnside et al., 2003).

Government policies and initiatives were developed and introduced in England through a statutory protection defined as Sites of special scientific interest (SSSI) to reduce further losses of species-rich semi-natural and unimproved grasslands being converted to agricultural land (Ridding *et al*., 2015). SSSIs were introduced into The National Parks and Access to the Countryside Act 1949 that was later updated under The Wildlife & Countryside Act 1981, then The Countryside and Rights of Way Act 2000 strengthening SSSIs in England and Wales. These acts enabled enhanced protection to allow refusal of consent for damaging activities and penalties for deliberate damage and third-party damage (Defra 2009; *Ridding et al., 2015*). Most SSSIs are either owned privately and are part of working farms, estates, or forests. However, there some that are owned by public bodies or non-government organisations (JNCC, 2015b).

By 2008 68% of semi-natural grasslands in England were designated as an SSSI (Natural England, 2008). Though SSSI designations have land management advisors to provide guidance on management of these areas, only 48% of these semi-natural grasslands were considered as a status of ‘favourable condition’ i.e., that these grasslands met the SSSI criteria of high botanical standards and size and 40% were designated as recovering (JNCC, 2015b).

Further to the SSSI statutory protection for semi-natural species-rich grassland, voluntary agri-environmental schemes were made available to farmers for payment. These agri-environmental schemes were introduced in 1987 and devised by DEFRA under the Common Agricultural Policy (CAP) even though historically farmers have provided a range of eco system services through agricultural and forestry (Reed *et al.*, 2014), CAP enabled farmers to enhance and protect biodiversity and receive payment for doing so (JNCC, 2020). For farmers to receive payment for these services. There are objectives that consist of a set of measures to enable these schemes to increase biodiversity conservation for areas such as semi-natural species-rich grassland and unimproved grassland. (Kleijn & Sutherland, 2003). However, agri-environmental schemes also include options for farmers and land managers to create new habitats within the landscape with wild seed mixes for pollinators, for example wild seed mix buffer strips that are adjacent to cropped fields. These buffer strips can provide a stronger variation of flowering plants and a higher nectar concentration at community level when compared to cropped fields (Pamminger *et al*., 2018).

Society relies upon the diversity of pollinating insects not only for natural wildflower communities, but also for the pollination of some 90 commercial crops within the UK and worldwide (Benjamin and McCallum, 2008). Agricultural practices have progressively become intensively managed leading to monocultures of agricultural crops that are associated with a reduced diversity of wildflower species and pollinating insects.

2 Literature Review

2.1 Plant-pollinator interactions

Plant-pollinator interactions represent a mutualism between plants and their pollinators whilst triggering an exchange of food for pollinators and instigating an exchange of food for pollinators and an effective vectoring of sexual reproduction for flowering plant species (Nicolson, 2007).

Along with nutritional rewards there are secondary rewards such as warmth, detection of protection from potential predators and mating partners (Woodcock *et al*., 2014).

Most flowering plants species have evolved to reward pollinators with nectar and pollen, creating competition between pollinators for access. Influencing the flowering plant in shape, colour, and scent (Nepi *et al*., 2008). If there is an inadequate quantity or quality of pollinators, reproductive success of flowering plant species can be reduced and therefore having a reduction of visiting pollinator numbers (Ashman *et al*., 2004).

Honeybees and bumble bees are purchased and used as a management tool for the pollination of crops, including fruits such as apples and cherries (Gallai *et al*., 2009), indicating a limited pollination services from wild pollinators. However, relying on using only honeybees or a single managed pollinator for crop pollination can be a risky approach due to health threats of diseases and pests such as the ectoparasitic mite (Rader *et al*., 2016). In a study measuring how often domesticated honeybees visited flowering plants within agricultural landscapes. The results showed that domesticated honeybees only enhanced pollination in 14% of crops compared to wild insect pollinators who enhancing 100% of the crops. Suggesting that domesticated honeybees can only be used for supplementing wild insect pollinators and not a substitute (Garibaldi *et al*., 2013). Further literature research on the impact that honeybees have on wild bees reviewed 146 studies. These studies address the effects the domesticated honeybees have on wild bees via competition (n=72), changes in plant communities (n=41) or transmission of pathogens (n=27). Fifty-three percent of the studies for honeybee and wild bee competition reported a negative effect or potentially could negatively affect wild bees, though there was very little evidence that this led to a decline in wild bee populations. However, farmers or landowners should evaluate the use of domesticated/managed honeybees and their impact (Mallinger *et al*., 2017).

Hoverflies are beneficial to agricultural landscape and could be used as an alternative to honeybees as a managed pollinator. They can provide some level of robustness in the face of environmental change (Doyle *et al*., 2020). For example, European hoverflies were discovered operating in green houses during spring and summer in high temperatures in south-eastern Spain. Seemingly being able to adapt to drier and hotter conditions (Pineda,& Marcos-García, 2008). Flowers are vital as a resource as hoverflies drink nectar and consume pollen grains for ovarian development and function (Schneider, 1969; Holloway 1976; Doyle *et al*., 2020). Hoverflies are not limited to a home range and are able to transport pollen over long distances and connect to otherwise isolated plant populations. Therefore, providing connectivity within disturbed and fragmentated habitats (Wotton *et al*., 2019).

Butterflies in agricultural landscape play an equally important part as bees and bumble bees by pollinating wild plant species (Jennersten 1988). They are normally associated with open, grass, flower-rich habitats (Ouin *et al.,* 2004). Agricultural landscape variation does more for diversity and abundance that farming practices as results suggest in a study compiled by Ouin *et al*. (2004). In general, an agricultural landscape that is heterogeneous for example where fields are smaller and there are habitats islands, can provide shelter for butterflies. Shelter for butterflies is an important element for their survival by providing places for their eggs and larval host plants (Dover *et al*., 1997). Further results show that butterfly species composition was no different between organic or conventional farms, but the large-scale heterogeneity displayed the differences in species composition.

2.2.1 Managed pollinators

Honeybees develop in a very narrow temperate range and require a constant temperature of range of 33° to 36° for production of eggs, larvae, and pupa (Kleinhenz *et al.,* 2003; Kavac *et al.,* 2009). The workers actively regulate and maintain the hive temperature using high amounts of energy especially in the brood areas. Changes of temperature in the hive can affect the pupal development (Wang *et al*., 2016). However, solitary bees are temperature generalist and can tolerate a wider range. Solitary bees can take up to a few months to develop unlike the honeybee which can take weeks (Jay, 1985). In an experiment undertaken by Kieret *et al*., (2017) on red mason bees found that the bees were able to develop in a temperate range of 19.9° to 31.5°C demonstrating their tolerance for temperature range.

Honeybees are not the only pollinators, taking part in free pollination are red mason bees. These successfully be used to pollinate crops or plantations such as onions and can replace efficiently species such as honeybees (Wilkaniec *et al*., 2004). The rest, are wild pollinators providing an ecosystem service to pollinate crops. Studies have indicated that these wild pollinators are equally, if not as important, for crop production (Bartomeus *et al*., 2014). However, this can be crop specific for example bean crops predominantly attract bumble bees possibly because of the morphology of the bean flowers, that limit access to nectar for smaller solitary bees and honeybees (Garratt *et al*., 2014). Due to the open accessible flowers, oilseed rape crops are visited by a more diverse pollinator community with no significant differences in visitation rates between pollinator taxa (Garratt *et al*., 2014).

Studies indicate having diverse pollinator groups provides a pollinating service that can benefit floral plant species. However, there are many conditions that can affect pollinators, for example different habitats, grass sward height (Hoehn *et al*., 2014), weather conditions and times of the day (Rader *et al*., 2013). For example, if an early or late spring were to happen this could lead to an interaction mismatch (Ogilvie & Forrest 2017). A sudden rise in temperature at the end of winter / beginning of spring could affect the timing of plants flowering earlier but the flying activity of pollinators are not affected (Fisogni *et al.,* 2020). If pollinators are unable to find plants that they rely, they could face a shortage of resources (Schenk *et al.,* 2018).

2.3.1 Plant-Pollinator networks

Plant-pollinator networks are a distinct type of ecology network that encompasses specific characteristics such as asymmetry of interactions, with specialist species frequently interacting with generalists and nestedness (Bascompte, 2009). This pollination service benefits both the flowering plant and the pollinator in the form of food rewards such as pollen or nectar (Michener, 2007). Darwin observed this interaction and realised the significance when he was writing about natural selection “I can understand how a flower and a bee might slowly become, either simultaneously or one after the other, modified and adapted in the most perfect manner to each other, by the continued preservation of individuals presenting mutual and slightly favourable deviations of structure" (Darwin, 1859).

There has been a rapid decline in wildflower and insect species since 1945 including many species associated with agricultural habitats. Contributing to the decline of botanical and zoological diversity was the rapid conversion of grasslands to arable land (French, 2017) particularly in the years of 1941 and 1942 where many grasslands were ploughed. However, with development of new crop varieties, chemicals and machinery saw a further decline of these grasslands in the post war years seeing a loss of 97% by 1980 (Fuller, 1987). Though still used for agricultural purposes such as intensive grazing or cut for hay, semi-natural species rich grassland are still in decline.

Heithaus (1974) posed the question 'How important are plant-flower-visitor interactions in determining the diversity of visitors and plants that rely on animals for reproduction?'. Heithaus (1974) observes that it is obvious that where there is pollen and nectar there are bees and butterflies. What is not obvious is the structure of entire flower-visitor communities that are largely a function of the number of flowers and resources present. Competition theory for diversity regulation predicts that the diversity of pollinators and flowering resources are positively correlated (MacArthur, 1972). Further studies indicate that diversity of nectar, pollen, and flowers type e.g., long corollas, colour and smell may influence the structure of bee communities and form these positive relationships (Petanidou & Ellis 1996; Steffan-Dewenter & Tscharntke 1997; Potts *et al*., 2003).

2.3.4 Biodiversity decline

Agri-environmental schemes were introduced due to concerns of biodiversity declines. In 1985 a green paper was published addressing the impact of agriculture on the environment (EEC Regulation 797/85) (Kleijn & Sutherland, 2003). A reform of EU agricultural policy later evolved and introduced a novel set of measures for the protection of the environment. However, with the introduction of the regulation, agri-environmental schemes have had variable results. There is limited information on how the agri-environmental schemes have affected biodiversity conservation (Peach *et al*., 2001;

Kleijn & Sutherland, 2003). However, results show they do enhance pollinator species, though mainly generalist pollinator species (Bommarco *et al*., 2010; Ekroos *et al.,* 2010; Scheper *et al*., 2013). To maintain high overall biodiversity of both flowering plants and pollinator in the agricultural landscape, there is a need to combine both flowering fields and semi-natural grassland (Boetzl *et al*., 2020).

Habitat fragmentation is considered to be the largest key threat to pollinators (Brown and Paxton 2009). There is an understanding as to how habitat fragmentation affects biodiversity patterns in species richness. However, less is known about how fragmentation affects the complex stability and structure of ecological network interactions that exist between plants and their pollinators. Loosing either pollinators or a plant population would negatively affect ecosystem stability and biotic interactions (Hagen & Kraemer, 2010). Steffan-Dewenter & Tscharntke (1999) tested the negative effects of habitat isolation and fragmentation that takes place on bee diversity and self-incompatible plants at increasing distances away from species rich semi natural grassland. Steffan-Dewenter & Tscharntke, (1999) confirmed that isolation from existing habitats reduces the abundance and richness of the bee species studied. This affected plant pollinator interactions and therefore affected the seed set of the plants of mustard and radish reducing the number of seeds per plant (Steffan-Dewenter & Tscharntke, (1999).

2.3 Nectar value of the plant community

Wild pollinators use nectar as a primary source of carbohydrates for essential health, energy and development (Roulston & Goodell, 2011). Changes to land use through urbanization and agricultural intensification can have a detrimental effect on the quality, abundance, and availability of flower-derived resources that support wild pollinators for food and larval plants. This can cause nutritional stress for pollinator population with potential adverse effects. For example, pollinated crops are providing an abundance of flowers and food resources during the crop's flowering period, for a relatively short amount of time. After the crop has flowered there could be lack of alternative food resource in a monoculture dominated agricultural setting. Where potentially this could put a strain on foraging bee and other pollinator species outside of the crop flowering period (Potts *et al*., 2010; Roulston & Goodell, 2011; Goulson *et al*, 2015).

2.4 Pollination Syndrome

Pollination syndrome or floral syndrome has been defined as a set of floral traits e.g., morphology, odour, size, colour, rewards, phenology and are associated with particular pollinator groups that underlie pollinator mediated selections (Faegri & Van der Pijl, 1979; Dellinger, 2020). Pollination syndromes have often been used to predict pollinators, implying that this theory of pollination syndrome specialises on functional groups of pollinators that exert similar selective pressure on floral traits (Faegri & Van der Pijl, 1979; Fenster et al., 2004).

It is debated whether pollination syndrome is a reliable tool for predicating the effective pollination of flowering plants. Roasas-Guerrero et al, (2014) and their review of literature relating to pollinator syndromes and whether the most effect pollinator species can be inferred from floral traits. Their result from the review supports the theory of pollination syndrome indicating that convergent floral evolution is driven by the most effective pollinator group. However, there are still studies of pollinator groups such as beetles, butterflies and flies that are needed to better determine their predictability.

Stebbins (1970) observed that flowers of the same species were visited by numerous insects and claimed the effectiveness and frequency implied that there were multiple relationships and therefore the pollinator that visits most often must be the most important selective force. Through such studies observation of species of pollinator is more easily observed than pollination deposition than whether pollen is indeed deposited on the stigma (Fenster *et al*., 2004). However, the most frequent pollinator visits are the most effective could be misleading (Fenster & Dudash, 2001; Tandon *et al*., 2003). This has prompted researchers to look at community-scale properties to find empirical patterns. One finding from this is that most pollinator-plant interactions are asymmetrically specialised. Biologists have assumed frequently that in species interactions, symmetric specialisation take place. Either specialists interact with specialists or generalists interact with generalists (Vazquez & Aiden, 2004). However, this orderly pattern may not be followed within nature. Only a fraction of specialists would interact with specialists and the rest interacting within moderate to extreme generalist i.e., rare plants and pollinators interact with a core group of abundant generalist species (Vázquez & Aizen, 2004; Bronstein *et al*., 2006).

Correlations between traits e.g., flower type, nectar provision, size, colour etc of plants and their pollinators, have been discovered for all aspects of floral nectar, these being volume, concentration, and competition. However, there has not been a satisfactory evolutionary explanation (Nachev *et al*., 2013; Pyke *et al.,* 2020). One theory for nectar sugar composition has been connected to pollinator type such as bees, flies, and bats, and the dietary needs of these pollinators being attracted to different plant species (Pender *et al*., 2014; Pyke *et al*, 2020). However, observed nectar feeding animals and their dietary preferences are often inconsistent with the occurrence of sucrose, glucose, and fructose i.e., floral nectar that occurs in the plant that these pollinators pollinate (Rodriguez-Pena *et al*., 2016).

There are three ways a pollinator can extract nectar for its source, active suction, capillary suction, and viscous dipping (Kim *et al*., 2011). Nectar for pollinators is made from a solution containing proteins and amino acids (Baker & Baker 1986; Nepi *et al*., 2012) and minerals (Afik *et al*, 2014). The benefit for the pollinator is the food resource that has been produced by the plant, in most cases this would be nectar. Making the nectar resource easily collected, ingested, digested, and absorbed by a variety of animals, means nectar is an energy source that can be used instantly (Nicolson, 2007). Consequently, this nectar-based plant-pollinator relationship has been present for 100s years and is an example of symmetric mutualism, where a service has been provided by an animal to plants in exchange for a food resource provided from the plants (Nepi *et al.*, 2012).

Nectar can be classed as either sucrose-rich or hexose-rich. The concentration and composition of individual nectar varies amongst species (Perrett *et al*., 2001). Nectar sugar composition has frequently been related to the flower trait (pollination syndrome) of the plant species and producing different varieties of nectar, where sucrose, fructose and glucose vary in proportion and would be adapted to the dietary requirements of pollinators. For example, studies have suggested that species such as butterflies, long tonged bees, hummingbirds, and moths tend to secrete sucrose-rich nectar and short-tonged bees, flies, and perching birds tend to secrete hexose-rich nectars (Baker & Baker 1983; Dupont *et al*., 2004). The motivation for the pollinator visit appears to be volume of nectar available. Krömer et al. (2008) acknowledges that studies have found varying results and showed no significant differences (e.g., Nicolson & van Wyk, 1998; Elisens & Freeman 1988; Galetto *et al*., 1998) and it could be possible that throughout the day the pattern of nectar distribution is different for different plant groups.

2.5 Semi-natural species-rich grassland decline

Semi-natural species-rich grassland is a valuable habitat and delivers ecosystem services such as carbon sequestration (Hopkins and Holz, 2006), reducing pollution to water i.e., water quality (Bengtsson *et al*., 2019). and livestock contribution for grazing and hay (Bullock, 2011). Semi-natural grassland is mostly the result of human activity usually for domestic livestock which has been achieved through forest clearance or wetland drainage (Natural England, 1999). It has been estimated that unimproved grassland has declined by 97% between 1930 to 1984 (Fuller, 1987) and has continued to decline over the last two decades (Natural England, 2008; Dadds & Averis, 2014). Loses to semi-natural species rich grassland lands have predominately been converted due fertiliser and herbicides being added and becoming improved grassland or arable, also urbanisation such as housing (Ridding *et al*., 2015).

Seminatural species rich grassland is normally maintained by tradition grazing and cutting for hay. To classify as species rich, it should consist of more than 15 vascular plants per square metre and 30%

sedges and wildflower cover (DEFRA, 2021). These types of grasslands could have National Vegetation Classification (NVC) of MG4, MG3, CG2 MG5. NVC is a useful resource and can track the course of vegetation succession especially if a pre-defined target has been put in place (Pywell et al., 2002). Such species found within these classifications are common bent *Agrostis capillaris*, sweet vernal-grass *Anthoxanthum odoratum*, black knapweed *Centaurea nigra*, crested dog's-tail *Cynosurus cristatus* and red fescue *Festuca rubra* (Natural England, 1999). Semi natural species rich grassland is defined by Natural England (1999) to have a high proportion of native grasses and dicotyledonous herbs, it is largely absent from scrub and is less than one metre in height.

Within Great Britain calcareous grassland has declined drastically and the Countryside survey results show there is only a small fraction remaining. Fuller (1987) estimated that by 1984 lowland semi natural grassland within England and Wales was estimated to be 3% of what was present before 1939.

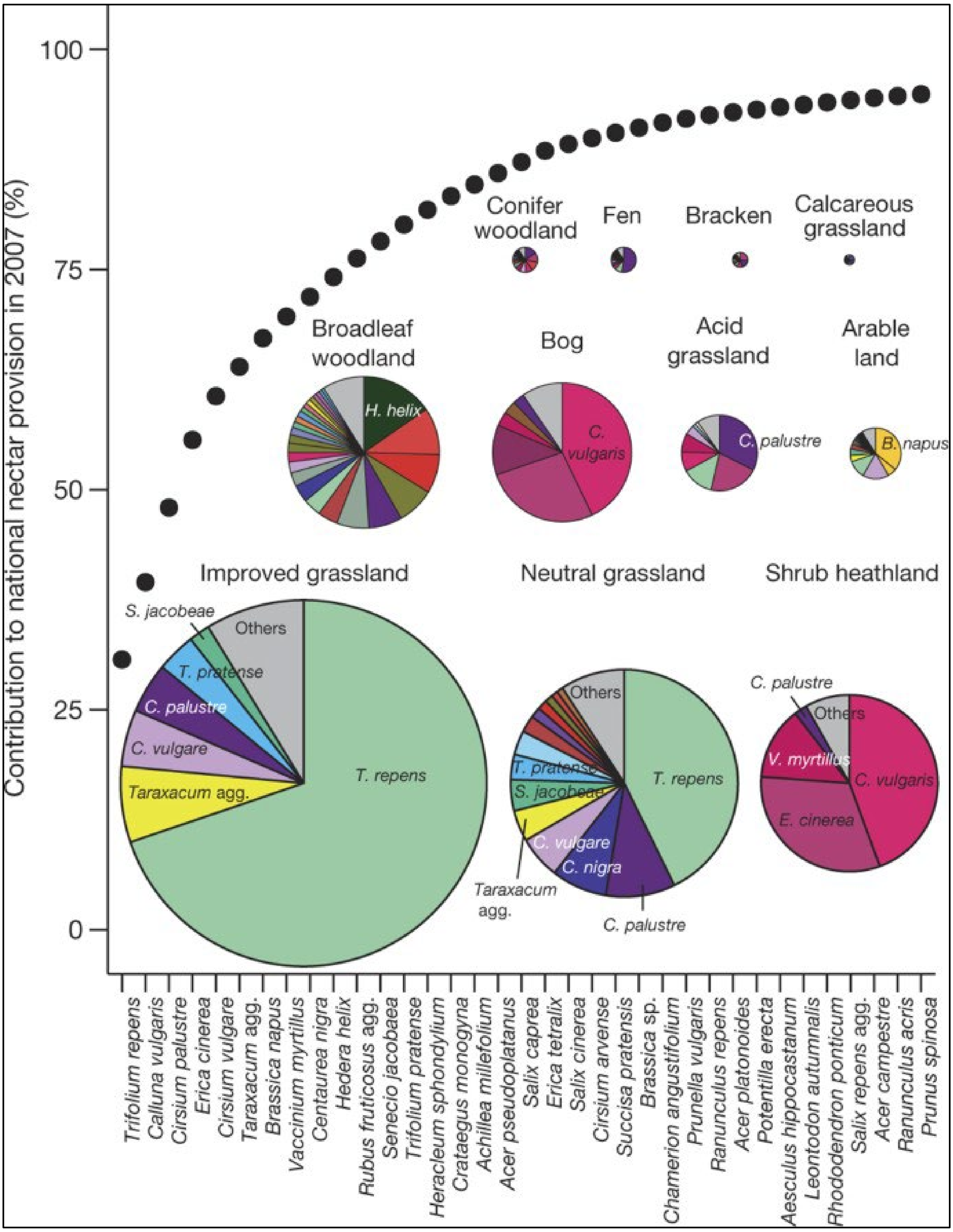


Figure 4.1 Seasonal nectar productivity in Great Britain, based on 2007 Countryside Survey land cover and nonlinear vegetation data (Carey *et al*., 2007).

Between 1978 and 1990 annual nectar productivity decreased significantly particularly within arable areas. From 1998 to 2007 within the habitats of neutral grassland and arable land nectar production did increase significantly. The decline is due to post war changes in land use and habitat management for example the use of herbicides and nitrogen deposition in grasslands (Carey *et al*., 2008; Baude *et al*., 2016).

Restoring agriculturally improved grassland to species-rich semi-natural grassland could positively increase ecosystem services and biodiversity. As a result, grassland plant specialists have been replaced with generalist plants that can cope and thrive within these anthropogenic driven and altered environments (Mckinney and Lockwood, 1999). Unless semi-natural and natural habitats are sympathetically managed, models of habitat loss have indicated that these areas could become extinct (Dobson *et al*., 1997; Walker *et al*., 2004).

Prior to the wide availability of commercial seed mixes, farmers would transport grasses between sites through practices of hay-strewing and shepherding and moved on farmed machinery between hay fields. These traditional practices are no longer being undertaken and it has potentially left many grassland species isolated in intensively farmed landscape. Replacing traditional hay meadows with intensive commercial grassland production (Bullock *et al*., 2002). Depletion of grasslands through intensive management practices have attributed to pollinator decline because of practices such as re-seeding and high fertilization application rates. Along with intensive grazing and cutting for silage two – three times a year to optimize harvest forage quality, these now being characterises of modern livestock farming (Vickery *et al*., 2001). Therefore, these practices are reducing food resource availability for pollinators and plant diversity as many plants will be out competed (Duffey et al, 1974; Tilman 1987; Pott et al., 2009).

In recent years, the research and the availability of comprehensive datasets and modelling into multifunctionality systems, i.e., providing multiple ecosystem functions and services, has become more increasingly common. Therefore, becoming a holistic idea of a 'whole ecosystem' (Manning *et al.*, 2018). However, an integration of these two to create multifunctional ecosystem services has recently become the idea that agricultural land should be delivering to society in the form of public goods (Fanin *et al.*, 2018; Manning *et al.*, 2018). Klaus et al., (2020) suggested that for nature and biodiversity conservation there are two stakeholders that are important for input into environmental strategies and policies, these being farmers and conservationists. The motivation for research is growing into the multifunctional ecosystem services concept, due to the increasing pressure for resources that is being placed on agricultural landscapes. Resulting in a need to design and manage these landscapes that can provide multiple ecosystem services simultaneously for example food and bioenergy production, flood regulation, carbon storage and biodiversity conservation (Isbell *et al.,* 2003; Nelson *et al.,* 2009; Bateman *et al.*, 2013). However, to date, there is no agree or accepted definition or means of measuring the multifunctional ecosystem services concept (Manning *et al*., 2018).

Through management, enhancement, or establishment of semi-natural species-rich grassland can increase beneficial insect diversity (Gill *et al*., 2016). Studies have found that these habitats within agricultural landscapes have increased bee diversity within local areas (Rickets *et al*., 2008; Nicholson, 2010). However, designing these habitats for optimum use by pollinators is not simple (Gill *et al*., 2016).

2.5.1 Pollinators in semi-natural species-rich grassland

Invertebrate community assemblages are derived from the plant species composition within habitats and vice versa (Schaffers *et al*., 2008) i.e. co-occurring species which have matching morphological and physiological traits will interact (Noreika *et al*., 2019). The richness of semi-natural grasslands through plant community, combined with an abundance of structures proving suitable nest sites. Making semi-natural grasslands an important habitat source for pollinators in agricultural landscapes (Steffan-Dewenter *et al*., 2002; Öckinger *et al*., 2009). Results shows and clearly demonstrations that the presence of semi-natural species-rich grasslands do have a positive effect on species rich ness and abundance on bumble bees and butterflies (Öckinger *et al*., 2009). Supporting the theory that these semi-natural species rich grasslands support pollinators by proving food resource, larval host plants and nesting sites. They are comparably richer than distant linear habitats explaining why there is a higher species richness and density because these grasslands provide tussocks, stones, and similar structures.

Knowledge of these pollinator-plant interactions and pollinator services in agricultural crops would give an understanding as to how beneficial their services are. Semi-natural species rich grasslands provide a sufficient floral resource in habitat size and plant species richness which is important for supporting pollinators. However, attracting pollinators is also essential for the persistence and spread of the plants in these habitats. As the main mode of reproduction through pollen dispersal is either by vertebrates or arthropods (Buchmann & Nabhan, 1996; Ashman *et al*., 2004; Ollerton *et al.*, 2011). Decreased visitation from pollinators due to for example, habitat fragmentation can result in insufficient pollen dispersal (Wilcock & Neiland 2002) decreased fruit (Quesada *et al*., 2004) and seed set (Ashman *et al*., 2004).

Abundance of nectar resources (Pywell *et al*., 2004; Saarinen *et al*., 2005), adjacent land use (Saarinen *et al*., 2005), and timing of cutting these semi-natural species-rich grasslands (Feber *et al*., 1996). Have been investigated in how these elements affect species richness and abundance of pollinators (Clausen, *et al*., 2001; Tscharntke *et al*., 2005) and have shown that the abundance of insects and species richness in an intensively managed agricultural environment may be dependent on dispersal from nearby semi-natural species rich grasslands. Alongside this, studies indicate that increasing wildflower patch sizes result in greater seed set of native wildflowers, therefore, corresponding to increase in pollinator densities (Meyer *et al*., 2007; Blaauw & Isaacs,2014).

2.5.2 Habitat Fragmentation

If nearby small uncultivated semi-natural grassland habitat fragments are of low quality i.e., less than 15 vascular plants per meter squared (Magnificent meadows, 2020a), these fragments may contain sink populations. Sink habitats may support large populations of species, but without continued immigration the species would eventually disappear (Pulliam, 1988). Larger habitat fragments of semi natural grassland could support these populations, with the possibility that these small fragments of habitats appear to sustain low density populations, giving the impression they are doing well, but in reality, they are not. They could have an increase in population sizes from the source immigration, therefore so-called pseudo-sinks (Watkinson & Sutherland 1995). However, these small non cropped habitats are of equal quality compared to larger semi natural grasslands, the populations of insects and pollinators may still be reliant on immigration if they are too small to support viable populations of the species in focus. An area of grassland surrounded with these small uncultivated habitat fragments would be able to function as a mainland-island metapopulation system (Harrison, 1991). Enabling these populations within the small uncultivated habitat fragments to be able to persist because of the rescue effect (Brown & Kodric-Brown, 1977).

Pollinators such as butterflies, hoverflies and bumblebees feed on nectar and pollen when they are adults. However, population structure and life histories are likely to be influenced by landscape composition because of different mechanisms. For example, many species of butterfly, are highly specialised with respect to larval host plants and require a sufficient supply of nectar resources along with these larval host plants within the same area (Ouin *et al*., 2004). Butterflies tend to exhibit sedentary behaviour and a high proportion would stay within their natal patch (Wilson & Thomas, 2002).

2.6 Agri-environmental schemes, wildflower strips

Agri-environmental schemes (AES) where first introduced in 1980 as part of the Common Agricultural Policy (CAP) and have been an integral management tool in conserving beneficial insect groups (Campbell *et al.*, 2012). AES can strengthen regulating ecosystems services, such as crop pollination and natural pest control, therefore enhancing crop production (Holland *et al.,* 2012; Korpela *et al*., 2013). Several agri-environmental schemes have been designed to promote and maintain biological diversity in agricultural landscapes (Pe'Er *et al*., 2019). For example, perennial wildflower strips provide a food and nesting resource for invertebrates particularly pollinators (Ouvard *et al*., 2018). Using sown flower strips can effectively enhance natural enemies of crop pests (Landis *et al*., 2002; Isaacs *et al.*, 2009; Haaland *et al.,* 2011; Ramsden *et al.,* 2014). Results from Tschumi *et al,* (2016) study on cereal leaf beetle (CLB) demonstrated that perennial species rich wildflower strips can reduce CLB eggs by 44%. Therefore, these wildflower strips can prevent thresholds being reached in nearby crops and the use of insecticide application is reduced. These results support the correlation evidence that wildflower strips increase natural pest control to account for the observed increase in crop yield.

These findings provide a strong argument that such AES are adopted for biodiversity conservation and natural pest control in crop productions (Tschumi *et al.,* 2016). Further advantages using wildflower strips is that they could act as buffers if insecticides treatments were to be used and avoids non-target effects of insecticides on biodiversity and disservices such as water contamination (Hahn *et al*., 2014; Stehle and Schulz, 2015).

2.7 Knowledge research gap

Through the literature review studies have used an approach of comparing sown wildflower strips with crops or crop edges and/or other margin types. However, there is a knowledge gap of comparing agri-environmental schemes of sown wildflower strips and semi-natural species rich grassland. Upon review most of these studies have found a higher abundance of investigated species in wildflower strips. Which leads to the question of what is attracting the pollinators to these habitats, where this thesis could address this knowledge gap whilst using comparison methods along with secondary nectar abundance data. There have been limited studies that can contribute holistically with regards to plant-pollinator communities, species richness, and nectar abundance. However, innovative studies investigating nectar abundance in different habitat are (Baldock, *et al.*, 2015; Hicks *et al.*, 2016; Timberlake *et al.*, 2019; Tew *et al.*, 2021).

Recent studies undertaken by Baude *et al* (2016); Hicks *et al* (2016) and Timberlake *et al* (2019) analysed nectar found with within habitats of rural, gardens, and agricultural. However, this thesis is the first to compare planted agri-environmental wildflower seed mixes schemes following a similar methodology to quantify the nectar supply by combining floral abundance and published nectar sugar values.

2.8 Research Rationale

Often studies have been undertaken on plant species life traits and nectar sugar values (Prasifka *et al.,* 2018). However, studies have often concentrated on either a single species of pollinator or plant species and concluding that there are few studies that demonstrate the nature and strength of pollinator services Wood *et al.* (2015). Investigations on spatial distribution on nectar sugar rewards studies are starting to be recognised and there are studies now combining flower counts with empirical values of nectar sugar to be able to quantify and compare nectar supply in habitats, therefore being able to provide nectar values on these habitats. However, there is still a knowledge gap on how habitats supply nectar resources and the interactions that take place with pollinators.

2.9 Aims and objectives

The aim of this study, for the first time, is to quantify the nectar sugar supply within two habitats in agricultural landscapes, allowing a direct comparison between two agri-environmental schemes of semi-natural species-rich grassland versus planted agri-environmental wildflower strips. The study will go on to examine the pollinator-plant interactions and determine the plant traits that are attracting the pollinators species that utilise these two habitats and whether natural occurring wildflowers or planted wildflowers through agri-environmental schemes determine pollinator composition and diversity.

The research aims:

(i) To compare and calculate the nectar sugar from the quadrats to investigate which agri-environmental schemes of semi-natural species-rich grassland versus planted agri-environmental wildflower strips are producing the highest nectar resources

(ii) To compare the pollinator visits at each quadrat and quantify richness and community composition to investigate which agri-environmental schemes of semi-natural species-rich grassland versus planted agri-environmental wildflower strips are receiving the highest pollinator visits.

(iii) Asses the pollinator visit behaviour to determine which floral trait is attracting the pollinator within semi-natural species-rich grassland and planted agri-environmental wildflower strips.

3 Experimental Design

3.1 Ethics

No protected species were sampled or harmed within this field study. Ethics review reference number 20202614-King.

3.2 Study Area

The study was undertaken in the Cotswolds, and the West Midlands, England (Fig 3.1). Each point on the map represented each farm within this study, Hollow Fosse Farm, Guiting Manor farm and Southfields Farm. All three farms had both agri-environmental schemes of sown wildflower strips and semi-natural species-rich grassland. In context the landscape for both Hollow Fosse Farm and Guiting Manor Farm are predominately surround by agricultural landscape. In a habitat mosaic of lowland calcareous grassland, broadleaved and deciduous woodlands with small pockets of semi-natural species-rich grasslands (Magic Maps 2022).

Southfields Farms is within the town of Coleshill and approximately 55 miles north of Hollow Fosse Farm and Guiting Manor Farm. The river Blythe (SSSi) runs through the farm along the lowland meadows. Towards the east of the farm is agricultural landscape with a mosaic of lowland meadows, semi-natural species rich grasslands and broadleaved deciduous woodlands (Magic Maps 2022). Southfields Farm have few beef cattle, sheep and mixed arable, mainly rapeseed and cereals.

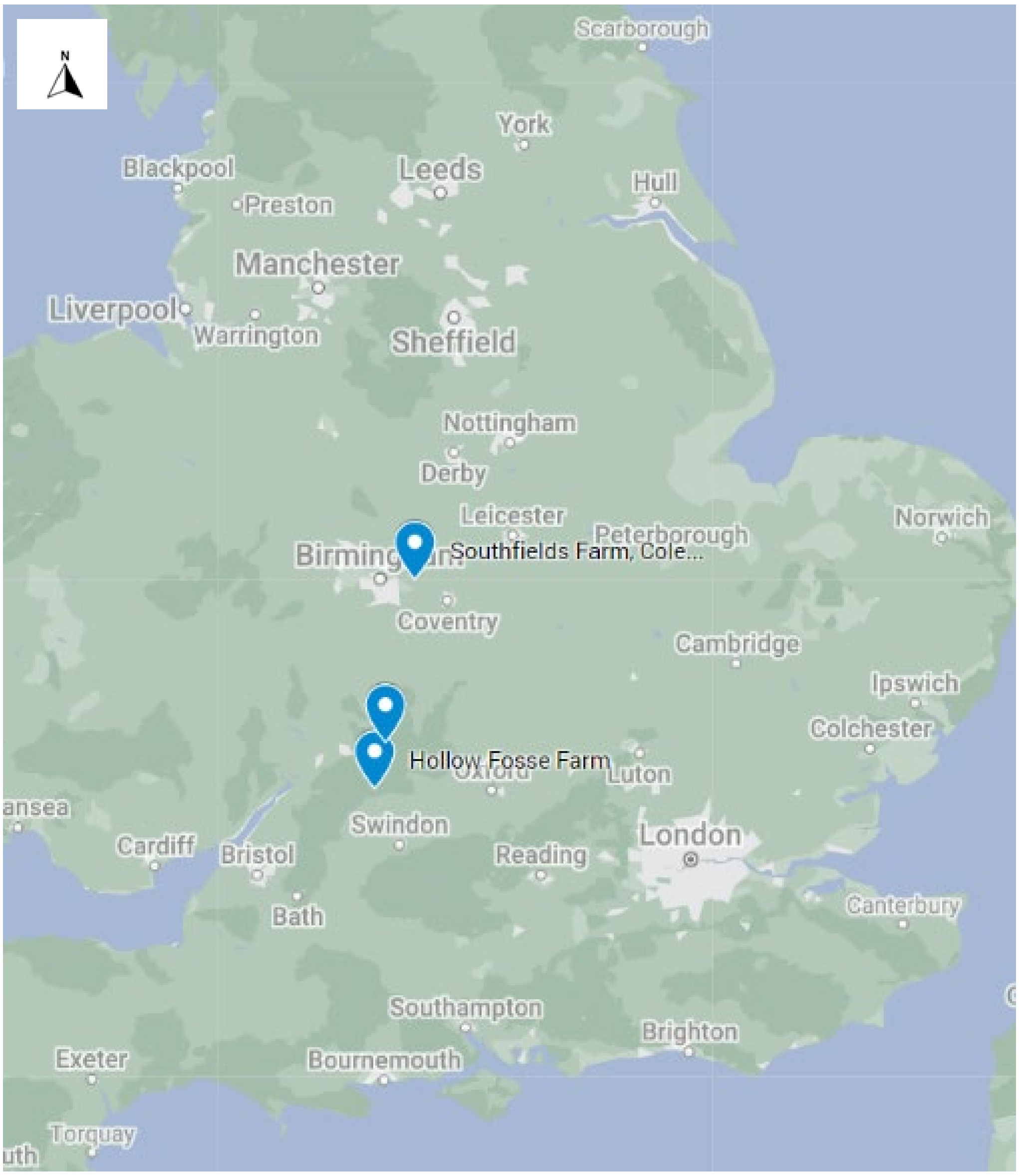


Fig 3.1 Map of the three farms studied

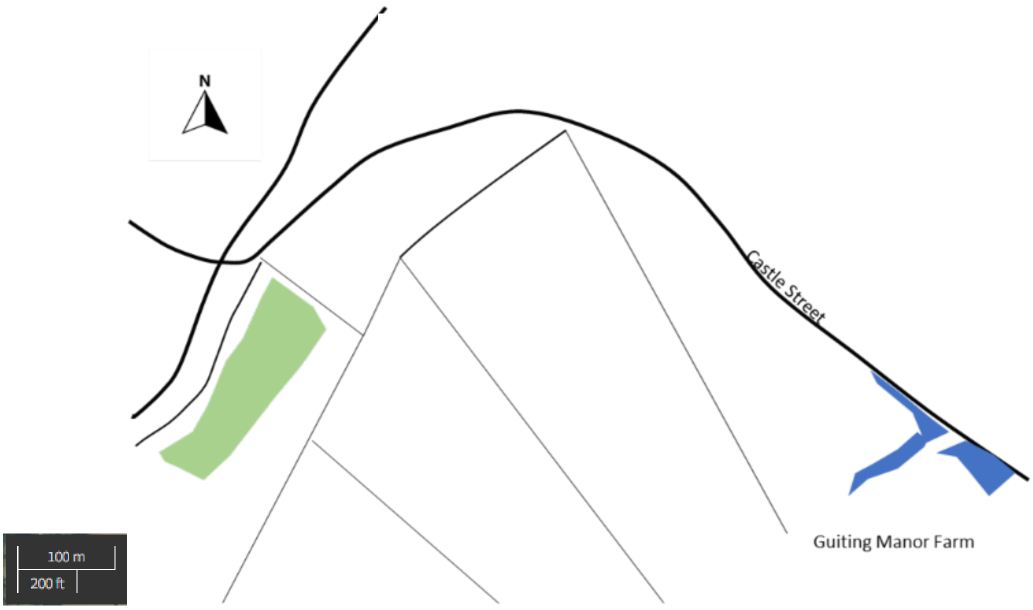
Table 1. List of farms used for the study and details

Site #	Name	Map figure	Location	Size HA	Farming type
1	Hollow Fosse Farm	3.3	Cotswolds	125ha	Conventional arable farm and grazing livestock.
2	Guiting Manor Farm	3.4	Cotswold	800ha	Conventional arable farm

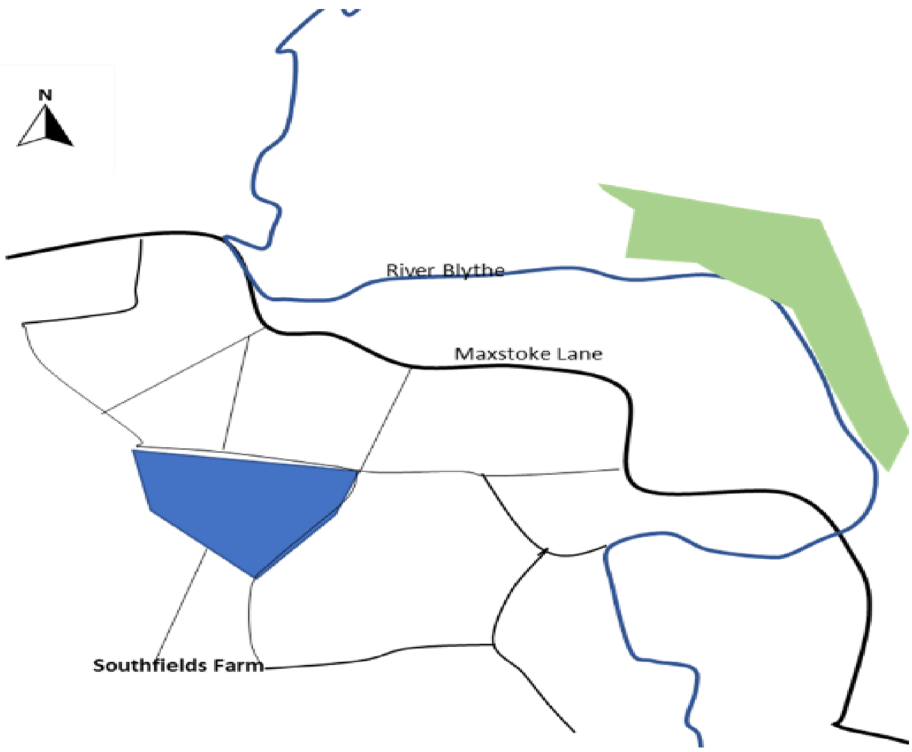
3	Southfields Farm	3.5	West Midlands	120ha	Conventional arable farm

3.2 Hollow Fosse Farm site map and study areas

3.3 Guiting Manor Farm site map and study areas



3.4 Southfields Farm site map and study areas



Key

planted agri-environmental wildflower strips

Semi-natural species-rich grassland

Fig (3.2 – 3.4) Farm sites showing both the agri-environmental schemes of planted agri-environmental wildflower strips and semi-natural species-rich grassland

3.3 Paired design

A paired-site sampling design approach was used to compile three sets of fifteen 3m x 3m plots in the farm habitats (treatment) of semi-natural species-rich grassland and sown wildflower strips 1m x 1m quadrats and 2m x 2m buffer. This paired design method is effective in quantifying the nectar and flowering plant traits that are attracting pollinators to these habitats along with their confounding variables.

The choice of three farms was based on a desktop study involving QGIS and magic maps, as well as knowledge of the local area and data provided from the Warwickshire County Council records department. The data that was used to identify the agri-environmental schemes were downloaded from the Rural Payment Agency and imported into QGIS software. These records contained the OS coordinates for species rich grassland and the nectar and pollen mix agri-environmental schemes. This enabled an overview of species rich grassland stronghold areas along with the agri environmental nectar and pollen schemes along with its proximity to each other. After identification of paired study sites, contact and site walkovers were conducted to assess suitability of the habitats and preliminary test for the research.

3.4 Flower and pollinator count

The core method in obtaining the data for the research was through surveying, identification and recording count data of observed pollinators and flowering plants. Surveying was carried out between 23 June 2021 to the 16 July 2021 providing a snapshot view of the presence of these variables required for statistical analysis. The study sites included all three farms and fifteen plots within each of the habitats at each farm totalling 90 plots altogether. The maximum distance between the two habitats at each site was 1km and minimum was 0.5km (Fig 3.2 – 3.5).

Each study plot recorded the flower head count, number of pollinator visits and the pollinator's behaviour by using 1x1m quadrat with a 2-metre buffer zone from the 1m x 1m quadrat, the buffer zone enabled surveying of the behaviours of the pollinators. The distance between each of the plots was a minimum of 10 metres starting at the edge of the buffer zone to the next buffer zone. Each 3m x 3m plot consisted of a count of all pollinators observed and the total number of floral units in flower. At each plot, every pollinator and flowering plant species were identified, and no samples were removed for further identification. Flowering plants were counted as one flowering unit, unless identified as a composite type of flower where an average of the flowering head was used from Tew et al (2019) study.

3.5 Pollinator behaviour

The activity observation for the pollinators and were recorded for a duration of ten minutes. After each quadrat was set up a few minutes wait was undertaken for the disturbance caused to settle then counting started. For recording pollinators, the survey technique counts of pollinators and ethogram recording their behaviours of either foraging, collecting pollen/nectar, or resting. This technique has been adapted from the UK Pollinator Monitoring Scheme produced by UK Centre for Ecology & Hydrology. The UK Centre for Ecology & Hydrology established a citizen science survey to count pollinating insects within a flower-insect time count (FIT Count) where the survey was to record pollinating insect for a period of ten minutes (UK Centre for Ecology & Hydrology, 2021)

3.6 Survey criteria

Guidelines from organisations such as the Butterfly Conservation (undated) and Bumblebee Conservation Trust (Undated) were used to inform the criteria for pollinator surveys. As a range of pollinator families were surveyed, the study combined the criteria from these organisations to give the optimum surveying criteria (UKBMS, undated; Bumble bee conservation, undated), these being:

Weather is warm and dry

>13 degrees Celsius if sky is clear and less than half cloud

>15 degrees Celsius if more than half cloud

Time of survey 10:45 to 15:45

Wind speed - Beaufort scale <5 i.e., light breeze

15 minutes per quadrat

4 Comparing the nectar value of the plant community

4.2 Introduction

Floral nectar is the primary reward that is offered to pollinators from most angiosperms. The amount, composition and placement of nectar are important elements of plant-pollinator interactions. Nectar composition varies considerably, its non-aqueous solution consists in varying proportions of sucrose, fructose, and glucose (Heil, 2011). Nectar, bridges interactions between plants and mutualists such as pollinators (De la Barrera & Nobel, 2004; Heil, 2011; Parachnowitsch *et al*., 2019). However, the complexity of nectar across angiosperms contains proteins, amino acids, minerals along with other compounds such as colour and scent (Parachnowitsch *et al*., 2019). Literature refers to nectar as an attractant, and few mention how nectar can influence pollinator behaviour throughout the visit and after and rarely is the later labelled manipulation (Bailey *et al*., 2007). Pyke (2010) suggests a flowering plant can therefore manipulate the foraging behaviours by altering the volume, composition, and concentration of the nectar and decreased with increasing body size.

Since the late 19th century, the extinction of pollinating insects has increased significantly (Ollerton *et al*., 2004), with species and abundance decreasing across the United Kingdom for example, wild bees have decline by 52% (Biesmeijer *et al*., 2014). 77 European wild bee species have a status of 'threatened' including six of these species being native to the UK (Neito *et al*., 2014). The UK Biodiversity Indicator for habitat specialist butterflies showed a decline of 68% between 1976 and 2018 (State of Nature report, 2019). The State of Nature report (2019) states there has been a loss of nearly one species of wildflower per year per county since the 1950s.

Through intensification and expansion of agriculture in western Europe (Robinson & Sutherland 2002; Foley *et al*., 2005). This has been the major driver for the loss of semi-natural species rich grassland (Bullock *et al.*, 2011). Large scale changes in land use and management intensity have greatly depleted the floral resources that provide food and nesting resources for pollinators (Baude *et al*, 2016; Carvell *et al*., 2006), causing pollinators to decline due a reduction in quantity of nectar and pollen resources (Goulson *et al*., 2015; Roulston & Goodell, 2011). However, testing for historical changes within nectar and pollen resources has been hampered due to lack of quantitative historical data (Tew *et al*., 2021).

4.2.1 Pollination syndrome

This study focuses on the nectar from floral plants rather than hedgerows or arable crops, which is essential for health and development and an important energy resource in the diet of pollinators. Along with the energy resource it provides a common currency as in total sugars in which can be expressed as nutritional contribution of all plant species (Willmer, 2011). Baude *et al.* (2016) modelled nectar productivity data per unit for 260 plant species along with estimates of historical vegetative cover that were retrieved from British Countryside Survey, detailing the national scale of plant community composition (Carey *et al*., 2007). The study allowed contributions of species, habitats and agri environmental schemes to national nectar provision to be assessed. Baude et al. (2016) used the data from historical countryside surveys dated 1978, 1990, 1998, 2007 and observed and identified that there had been shifts within nectar provision.

Using the results of the most recent Countryside survey dated 2007, Baude et al. (2016) had identified that there are significant differences in annual nectar productivity, species nectar diversity and functional nectar diversity among habitats. Total area habitats such as calcareous grassland, broadleaved woodland and neutral grassland resulted in being the most productive with regards to nectar resources. Where arable farmland crops were consistently the poorest of the habitats (Fig 4.1.).

There are pioneering studies into the nectar value of plant communities, nectar supply has been quantified in urban and some rural landscapes (Baude *et al*., 2016; Flo *et al*. 2018; Timberlake *et al*., 2019; Tew *et al*., 2019). Timberlake et al. (2019) identified within their studies the different types of habitats that are available within a rural setting, particularly at farm settings and have identified that there are times within the nectar season there are gaps when nectar is low especially at the beginning of spring for when the queen bees are looking for an energy resource. Hicks et al. (2016) identified significantly more diverse plant taxa in urban areas due to non-native seed mixes in urban parks compared to other habitats, with a majority of species not native to the UK. However, the nectar supply showed no significant difference between urban and rural landscapes.

4.2.2 Agri-environmental schemes

Agricultural landscapes are a monoculture of mass flowering crops, when these crops have finished flowering, there is a potential lack of alternative resources that is putting a strain on pollinators outside of this mass flowering period (Liczner & Colla, 2020) due to providing only one resources type for a limited range of species (Vaudo *et al.*, 2015). To provide a more consistent resource within the landscape throughout the year habitats have been created agri-environmental schemes within the UK and EU. These options are intended to provide a diversity of resources to assist wildlife include pollinators throughout the year.

Baude et al. (2016) highlighted the low availability and diversity of floral nectar sources within arable habitats and that additional supplementary support for pollinators on farmland is needed. This would be in the form of nectar flower mixes and native wildflower seed mix. However, Baude et al. (2016) implies that their contribution to nectar provision remains low. There are several options that contribute to pollinators in terms of floral resources that could also be eligible for grants under European Union funded agri-environmental schemes such as pollen and nectar mix, sown grassy field margin.

4.2.3 Semi-natural species-rich grassland

Species-rich semi-natural grassland is not intensely cultivated or fertilised, and one of the most species rich ecosystems in the world (Squires *et al*., 2018). In pre-intensification agriculture these grasslands were feed for livestock, enabling the livestock to produce manure for crop production as an additional advantage (Lennartsson *et al*., 2016). With the introduction of new techniques, mineral fertilisers, and pesticides, species rich grassland with their diversity of flower resources became abandoned in favour of cultivated fodder, causing a rapid decline during the 19th and 20th century, which therefore has caused a severe decline in abundance and diversity of flowering plant taxa along with wild pollinators (WallisDeVris & Van Swaay, 2009).

Semi-natural species-rich grassland habitat is considered a key core resource for the conservation of biodiversity wildflower diversity and pollinators. To maintain these areas of grassland several European countries have agri-environmental schemes to restore, increase and maintain the quality of such habitats (Kleijn & Sutherland 2003; Wehn *et al*., 2018). These grasslands are considered important due to their high floral richness and for sources of pollen and nectar (Pywell *et al*., 2005).

4.3 Aims

The overarching aim of this chapter within the study is to quantify, compare nectar sugar resource differences between the two agri-environmental schemes of semi-natural species-rich grassland and planted agri-environmental wildflower strips by calculating the nectar sugar value from the open flowering plants recorded at each quadrat.

4.4 Methods

Study location

The study was undertaken in the Cotswolds, and the West Midlands, England. Each point on the map represented each farm within this study, Hollow Fosse Farm, Guiting Manor farm and Southfields Farm. All three farms had both agri-environmental schemes of sown wildflower strips and semi-natural species-rich grassland

Cross reference to section 2.2

Paired Design

A paired-site sampling design approach was used to compile three sets of fifteen 3m x 3m plots in the farm habitats (treatment) of semi-natural species-rich grassland and sown wildflower strips 1m x

1m quadrats and 2m x 2m buffer. This paired design method is effective in quantifying the nectar and flowering plant traits that are attracting pollinators to these habitats along with their confounding variables.

Cross reference to section 2.3

Flower Count

The core method in obtaining the data for the research was through surveying, identification and recording count data of observed pollinators and flowering plants. The information that was gathered at the time of the study in July 2021 was able to provide a snapshot view of the presence of these variables required for statistical analysis.

Cross reference to section 2.4

Survey Criteria

Guidelines from organisations such as the Butterfly Conservation (undated) and Bumblebee Conservation Trust (Undated) were used to inform the criteria for pollinator surveys. As a range of pollinator families were surveyed, the study combined the criteria from these organisations to give the optimum surveying criteria (UKBMS, undated; Bumble bee conservation, undated), these being:

Weather is warm and dry

>13 degrees Celsius if sky is clear and less than half cloud

>15 degrees Celsius if more than half cloud

Time of survey 10:45 to 15:45

Wind speed - Beaufort scale <5 i.e., light breeze

15 minutes per quadrat

4.4.1 Nectar data sources

Nectar bagging was not undertaken within this study. Secondary data for the nectar sugar content values for the floral plant species identified were obtained from Baude et al. (2016), Hicks et al. (2016) and Timberland et al. (2019). Some of the flowering species did require an alternative measure from a similar flower species or received a value of zero due to no nectar sugar content value for example the bee orchard.

Nectar values per 1m x 1m quadrat were quantified for all open flowers (appendix 1 for full list of species) of each nectar-producing species that were identified within each of the 90 quadrats. Each open flower was assigned a daily nectar sugar production value (mean mass of nectar sugar per single flower or capitulum (µg/day) using published values from Baude et al. (2016) and Timberlake et al. (2019). For flowers classed as a composite, for example *Leucanthemum vulgare* or *Centaurea nigra*, the numerical value from the 'mean open flowers per floral unit' was used from the study compiled by Hicks et al. (2016). These types of flower species are classed as a cluster of flowers within a single flower head not one singular flower head. To achieve a nectar value for these flower species the flowers heads were multiplied by the nectar values from these studies.

Example of calculations	
Lotus corniculatus – single flower	*Centaurea nigra* – composite floral unit
Sum of flower count x nectar amount = nectar amount for species	Average number of florets in one flower unit x sum of flower count x nectar amount = nectar amount for species

4.4.2 Statistical Analysis

Statistical analysis was conducted within IBM SPSS Statistics for Windows, version 27, to compare the nectar value of the plant community within semi-natural species-rich grassland and planted agri-environmental wildflower seed mixes. As nectar values did not form a normal distribution, a non-parametric test Mann-Whitney U was conducted to test whether there was a significant difference in nectar values per m2 between the two habitats, then for each habitat at farm level. Bonferroni correction was applied to multiple tests.

4.5 Results

The overall result at habitat level with all the quadrats total nectar values combined were found to be significantly lower within the semi-natural species-rich grassland than the planted agri-environmental wildflower strips ($p<0.00$) (Fig 4.1).

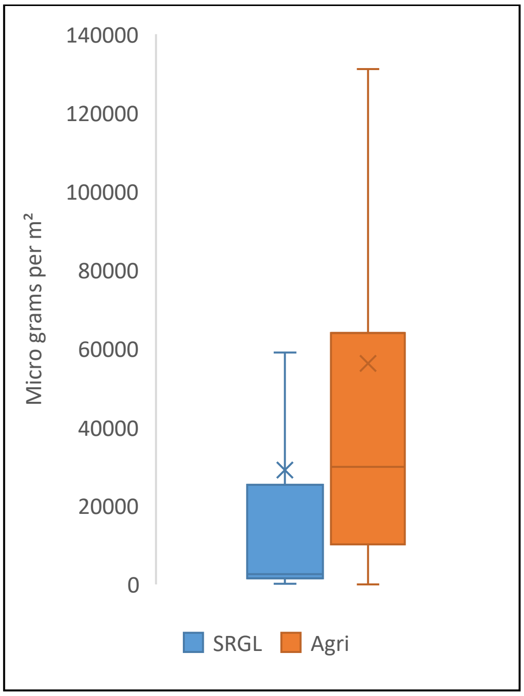


Fig 4.1 Mean of nectar total recorded within this study in semi natural species rich grassland (SRGL) (n-45) and planted agri-environmental wildflower strip (AGRI) (n=45). Box plots showing the metrics of Range, IQR and median

However, at farm level between the two habitats that there was a significant difference between the two farms of Hollow Fosse Farms (fig 4.2) and Guiting Manor Farm (Fig 4.3). The two habitats of semi-natural species rich grassland and the planted agri-environmental wildflower strips nectar values were not distributed in the same. Semi-natural species-rich grassland nectar value is less within the planted agri-environmental wildflower strips indicating planted agri-environmental wildflower strips are producing considerably more nectar Semi-natural species-rich grassland. Though at Southfields farm (4.4) nectar Semi-natural species-rich grassland is producing more nectar than planted agri-environmental wildflower strips

Table 4.1 Mean nectar recorded within this study Hollow Fosse (n=15, n=15), Guiting Manor Farm (n=15, n=15) Southfield Farms (n=15 and n15). Nectar value richness between both habitats semi natural species rich grassland (SRGL) and planted agri-environmental wildflower strip (AGRI). at all three farms were tested using Mann-Whitney U with post-hoc pairwise Mann-Whitney U and Bonferroni correction.

Farm	Mean SRGL µg	Mean Agri µg	Sig.
Hollow Fosse Farm	2,688	33,583	0.01
Guiting Manor Farm	3,577	105,376	0.00
Southfields Farm	80,957	29,750	0.13

Key	
	Semi-natural species-rich grassland
	Planted Agri-environmental wildflower strip

Hollow Fosse Farm

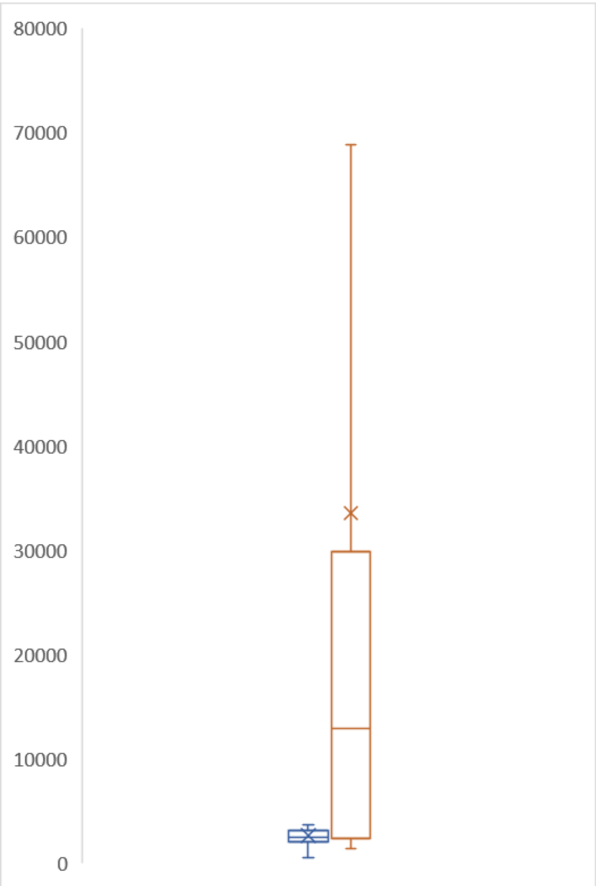


Fig 4.2 Mean nectar recorded at Hollow Fosse Farm both habitats, semi-natural species rich grassland (n=15) and plant agri-environmental wildflower strip (n=15). Box plots showing the metrics of Range, IQR and median

Guiting Manor Farm

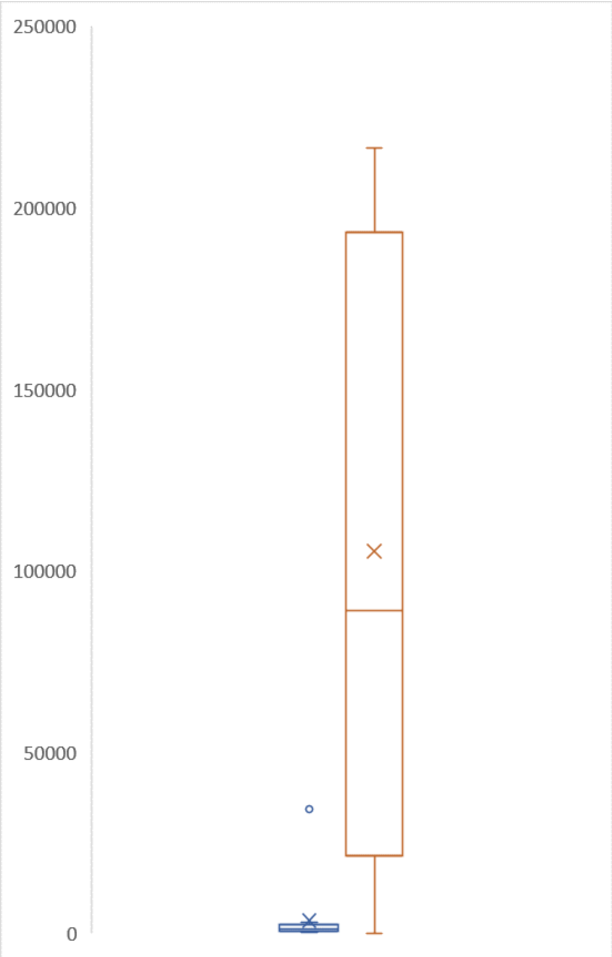


Fig 4.3 Mean nectar recorded at Guiting Manor Farm both habitats, semi-natural species rich grassland (n=15) and plant agri-environmental wildflower strip (n=15). Box plots showing the metrics of Range, IQR and median

Southfields Farm

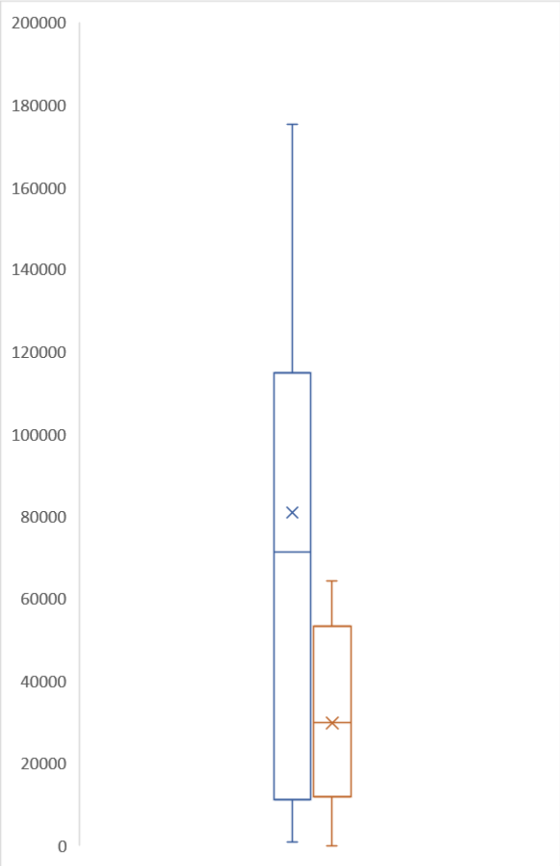


Fig 4.3 Mean nectar recorded at Southfield farm both habitats, semi-natural species rich grassland (n=15) and plant agri-environmental wildflower strip (n=15). Box plots showing the metrics of Range, IQR and median

The open flower per floral unit count was 10,766 over the 90 plots between the two habitats of semi natural species rich grassland and sown agri-environmental wildflower seed mixes. Fifty-seven different plant species were identified including 10 grasses, of which, forty-seven plant were assigned a daily nectar sugar production value. Eleven flowering plant species were assigned a zero-amount nectar sugar production value, due to the flower having no recorded nectar value.

Planted agri-environmental wildflower strips produced more nectar than semi-natural species-rich grassland (figs 4.3 & 4.4), with 66% of total nectar. In the planted agri-environmental wildflower strips (Fig 4.5) the higher nectar flower species were Oxeye daisy *Leucanthemum vulgare* (49%), Common knapweed *Centaurea nigra* (35%), and field scabious *Knautia arvensis* (9%). Birds-foot trefoil *Lotus corniculatus* (81%), red clover *Trifolium pratense* (4%), and selfheal *Prunella vulgaris*

(4%) contributed to the highest nectar flowering plants within the semi-natural species-rich grassland (4.6). Semi-natural species-rich grassland had the lower mean sugar production with 5383µg and the agri environmental schemes the average was 16,755µg.

a) Planted agri-environmental wildflower strips

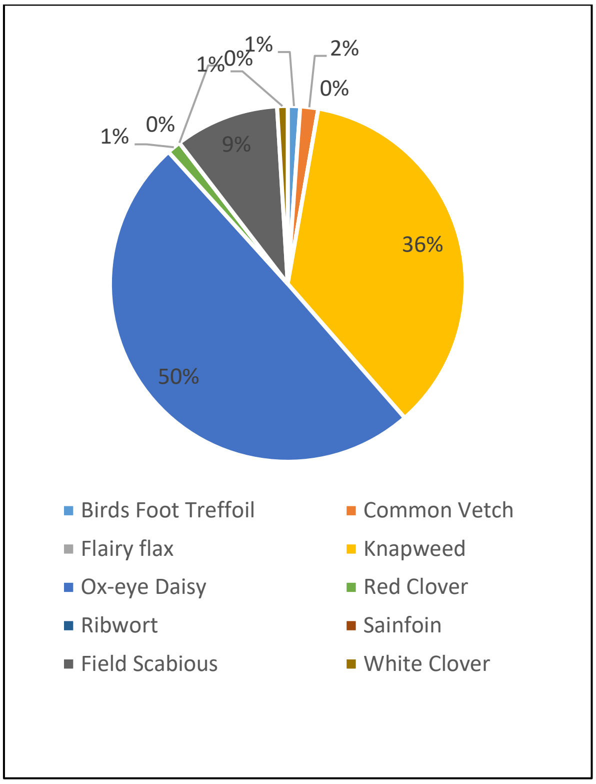


Fig 4.5. The percentage of nectar values of the flowering plants identified for Planted agri-environmental wildflower strips

b) Semi-natural species- rich grassland

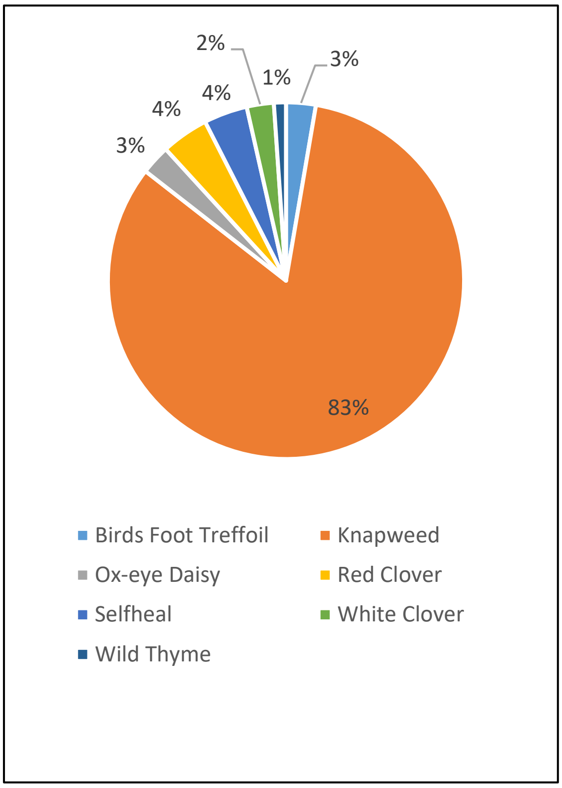


Fig 4.6. The percentage of nectar values of the flowering plants identified for semi-natural species-rich grassland.

4.6 Discussion

The study aims were to quantify the nectar value within the flowering plant community and analyse how both habitats of semi-natural species-rich grassland and agri-environmental schemes provided nectar resources between 23 June 2021 to the 16 July 2021. The planted agri-environmental wildflower strips produced a greater amount of nectar than semi natural species rich grassland. However, the semi-natural species-rich grassland of the farm at Southfields had and significant higher amount of nectar compared to the planted agri-environmental wildflower strips due to the presence of *Lotus corniculatus* and *Leucanthemum vulgare*. There were varying degrees of nectar abundance, and only a small number of flowering plants species provided most of the nectar resource. The less productive flowering species are still an important resource by providing a continuity of nectar and diversity of nectar resources.

The results of the data within this study showed that the planted agri-environmental wildflower strips contributed the greatest amount of nectar sugar produced per unit area. These results reveal the importance of the contribution that these schemes make for nectar resources within the summer months of June and July. The highest contributing flowering plants for example *Leucanthemum vulgare*, , and *Lotus corniculatus* vary flowering times from May, June to September (Blamey *et al*., 2013). With the absence of other flowering plant species that flower earlier or later in the year are potentially leaving a nectar resource gap for early spring for pollinators especially within the planted agri-environmental wildflower strips. Several studies have identified a food resource deficit for pollinators, when oil seed rape has finished flowering and the summer floral resources have yet to produce flowers (Westphal *et al*., 2003). Timberlake *et al*. (2019) identified a limitation of floral resource timings and the limits this has on pollinators and that designing of any restoration or agricultural schemes phenology of both plants and pollinators are critical. In line with current study, Timberlake *et al*. (2019) recorded a small number of floral plants that provided the most nectar resources e.g., white clover, red clover, and creeping thistle.

Semi-natural species-rich grassland however could fill this gap with species such as cuckooflower, *Cardamine pratensis* and louse wort, *Pedicularis sylvatica* that flower within early spring are typical species within these types of habitats (Magnificent meadows, undated) though these species were not identified at the time when surveying to indicate there were early nectar producers. These diverse arrays of nectar sources would likely to produce a nutritional diversity of rewards within flowering plants to support the pollinator community (Woodard & Jha, 2017).

The flowering plant species that were identified providing a nectar resource were provided by native plants and are a favoured floral resource for pollinators through their peak pollination period. As a managed mix for the agri-environmental schemes the highest producing nectar sources such as *Leucanthemum vulgare*, *Lotus corniculatus, Centaurea nigra* was found across all three farms making them essential resources for nectar production. Flowers species of common vetch, lady's bedstraw, *Galium verum* and creeping buttercup, *Ranunculus repens* where only found at one farm and are not listed as a species within the planted agri-environmental pollen and nectar wild flower mix. These flowering plants have high nectar values and add to the diversity and quality of the floral resources available and provide a positive floral functioning group that been found to positively correlate with pollinator health, diversity, and abundance (Balzan *et al*., 2014; Hicks *et al*., 2016). A diverse and species rich floral mixture would attract a great variety of pollinators and the less abundant / rare plants would benefit by virtue due to the increasing number of pollinators that show a preference for the rarer plants because of the rise of pollinator abundance in one area (Ghazoul, 2006).

Baude *et al*. (2016) had implied that the contribution from agricultural landscapes had low availability and diversity of floral nectar sources and that additional supplementary support for pollinators on farmland is needed through sown wildflower strips with wildflower mixes seed. Carvell et al. (2006) supports Baude et al. (2016) suggesting that nectar provision and diversity remains low within sown agricultural wildflower strips schemes. This study supports this due to the small variety of floral resources proving most of the nectar resources.

With development in the future, estimates can be undertaken on total flower production through flower abundance over a given time, treatment, and site, and therefore provide total floral resource for variety of given scenarios. These pioneering studies that are researching nectar values will start to give a tangible value on habitats and their potential to be used a tool to support pollinators.

In conclusion this chapter provided an analysis of nectar resources for the main species producing nectar that were identified at the time of the study. At the time the data was recorded for this study the planted agri-environmental wildflower strips had the higher nectar value compared to the semi-natural species-rich grassland. The planted agri-environmental wildflower strips were a created anthropogenic habitat of wildflowers that provided a higher food/energy resource than the naturally regenerated habitat of semi natural species rich grassland.

5 Pollinator abundance and community composition

5.1 Introduction

Pollinators provide an important ecosystem service for angiosperms and agricultural crops (Klein *et al*., 2007). However, a pollinator is not just dependant on nectar, there are other factors that need to be accounted for such as pollen, nesting sites, nesting material and host plants for larvae. Habitats and pollinators that support the fundamental needs of pollinators are declining due to primary causes such as habitat loss and fragmentation, and agriculture intensification. The loss of pollinators and the interactions that are established with flowering plant species may threaten the wild plant communities further (Aguiler *et al*., 2006; Potts *et al*., 2010a; Thomann *et al*., 2013). This decline could threaten agricultural yields of pollinator crops that are dependent on pollinators for pollination (Deguines *et al*., 2014). For populations of pollinators to survive and thrive, appropriate management of pollinator habitats should be maintained for the abundance and richness of pollinator (Falk *et al*., 2006).

5.2.1 Ecological needs of pollinators

Garibaldi et al. (2014) suggests that for wild pollinators there should be key habitats available for them, including semi natural areas, habitat heterogeneity, hedgerows, and nesting resources. To increase the abundance and diversity of wild pollinators should be within close proximity to these semi-natural areas and natural vegetation (Kremen *et al*., 2002; Klein *et al*., 2003; Ricketts *et al*., 2008; Garibaldi *et al*., 2011; Blaauw & Isaacs 2014). The intensification of agriculture affects the availability and quality of foraging resources and has been shown to reduce nesting sites appropriate for some pollinators (Kremen *et al*., 2002; Requier *et al.,* 2015). With the results of intense agriculture can cause habitat loss and degradation of habitat quality, which reduced the population sizes, composition, and species richness of pollinator communities (Steffan-Dewenter & Westphal, 2008).

Populations of pollinator species to grow and be maintained for conservation, appropriate management of existing habitats and the restoration of poor-quality habitat is required (Falk *et al*., 2006). Studies conclude insects including pollinators that are in an agricultural landscape conclude that establishing wild flowering plants can enhance biodiversity and contribute to supplying of the ecosystem service of pollination (Fitz Gerald and Solomon, 2004; Fielder and Landis 2007a; Hogg *et al*., 2011). Bennet & Gratton (2013) concluded that there is a positive relationship between the anthropoid richness including pollinators and plant diversity supporting the evidence. From the collection of 37 taxonomic invertebrate groups over a field session conducted in 2006 resulted in the plots that have seven or more flower species increased the pollinator species richness compared to single or double flower species plots, reason being that the resource that is offer at the time of flowering an intense short burst of resource offering a large temporary reward (Bennet & Gratton, 2013).

The study demonstrated that each flower taxa attracted a distinct arthropod species, including pollinators. Floral characteristics for example flower size, structure, rewards of pollen and nectar all influence pollinator visitations (Conner & Rush, 1996; Silva & Dean 2000; Kudo & Harder, 2005; Fielder & Landis 2007b). Due to the flowering plant diversity being able to support more than one species of pollinator these studies have found that flowering plant diversity increases arthropod and pollinator diversity (Bangert *et al.*, 2005; Crutinger *et al*., 2006; Scherber *et al.*, 2010).

These studies suggest the reason for a large abundance of a pollinator at a single or two species plot is that there is a dominant flowering species, therefore increasing nectar production or visual quality of neighbouring plants causing one species to be more successful in attracting visitors. Ghazoul (2006) observed a plot of two flowering species, one of the flower species pollination increased when it was planted within a mixed flower planting. However, when co-occurring species within the plot increased its inflorescences and become the dominate plant and the target species pollination declines. Therefore, reducing a floral species for example by dominate plants such as grasses may then prevent disproportionate visitation resulting in a less variable pollinator community (Bennet & Gratton, 2013).

5.3.2 Diversity of pollinator and flowering plant species

Diverse planting is expected to increase the diversity of pollinators due to being able to offer greater resources (Bangert *et al*., 2005; Johnson *et al.*, 2010; Scherber *et al.*, 2010). Agri-Environmental schemes have proven how important they are in supporting the low remaining semi-natural species rich grasslands in providing flowering strips for a variety of insect groups (Carvell *et al*., 2011; Korpela *et al.,* 2013; Scheper *et al.,* 2013, 2015; Grass *et al.,* 2016) with the increased wildflower plant species available, increases higher pollinator abundance and diversity (Carvell *et al*., 2007; Korpela *et al.,* 2013).

5.3.3 Land use change

Rapid declines of global animal species diversity can be directed at land use change (Sala *et al*., 2000) contributing to these changes are the anthropogenic habitats that are losing vegetation or being transformed into small patches through habitat fragmentation (Garibaldi *et al*, 2011; Winfree *et al*., 2011). However, taxa found within these landscapes do not all respond in the same way. Studies have shown how species richness has changed through habitat fragmentation and the extent of these changes, for example identifying compositional shifts will enable whether more common generalist species have forced out specialist species that were once found present in the habitat (Radar *et al*., 2014).

Landscape fragmentation can alter pollinator communities along with pollination and therefore causing a decrease in pollinator abundance and diversity within small fragments. Radar et al. (2014) and Jauker et al. (2013) concluded that sociality and diet alone do not mitigate a negative response for being to exist within land use changes, there are other traits such as body size, non-floral larval food resources and solitary behaviour. Small bodied social bees within halictid family encountered a negative impact and were more likely to be susceptible to decline than larger bumble bees to land use changes.

5.4 Aims

The aim of this chapter within the study is to quantify richness and community composition of pollinating taxa in semi-natural species-rich grassland and planted agri-environmental wildflower strips. With further analyses on the influence that the flowering plant species has on pollinator abundance, and the flower species that cause the most attraction as regards to resources.

Questions to achieve the aim are (i) quantify the pollinator species visits to flowering plants between semi-natural species-rich grassland and planted agri-environmental wildflower strips. (ii) analyse the association between the number of flowering plant species and the pollinator visits. (iii) analyse the association of flowering plant species richness and the different pollinator families.

5.5 Materials and methods

5.5.1 Study location

The study was undertaken in the Cotswolds, and the West Midlands, England. Each point on the map represented each farm within this study, Hollow Fosse Farm, Guiting Manor farm and Southfields Farm. All three farms had both agri-environmental schemes of sown wildflower strips and semi-natural species-rich grassland

Cross reference to section 2.2

5.5.2 Paired Design

A paired-site sampling design approach was used to compile three sets of fifteen 3m x 3m plots in the farm habitats (treatment) of semi-natural species-rich grassland and sown wildflower strips 1m x 1m quadrats and 2m x 2m buffer. This paired design method is effective in quantifying the nectar and flowering plant traits that are attracting pollinators to these habitats along with their confounding variables.

Cross reference to section 2.3

5.5.3 Survey Criteria

Guidelines from organisations such as the Butterfly Conservation (undated) and Bumblebee Conservation Trust (Undated) were used to inform the criteria for pollinator surveys. As a range of pollinator families were surveyed, the study combined the criteria from these organisations to give the optimum surveying criteria (UKBMS, undated; Bumble bee conservation, undated), these being:

Weather is warm and dry

>13 degrees Celsius if sky is clear and less than half cloud

>15 degrees Celsius if more than half cloud

Time of survey 10:45 to 15:45

Wind speed - Beaufort scale <5 i.e., light breeze

15 minutes per quadrat

5.5.1 Pollinator Surveys

Surveying was carried out between 23 June 2021 to the 16 July 2021. On each farm and in each habitat all open flower heads and pollinator visits were recorded within 1 x 1-metre quadrats with a 2-metre zone around each quadrat with a distance of 10 metres between each plot and zone. The Cotswold farms were split i.e., half of the plots in each habitat were completed one day then a second visit to collect the rest of the plots. Southfields farm had two visits and equal amounts of plot observations where collected.

The ninety plots were surveyed for 15 minutes. Each pollinator survey was carried out at the optimal time of the day and favourable weather conditions (Pollard & Yates 1993), these requirements are between 10.45am to 3.45pm. Temperatures over 13°C and no rain and light wind i.e., Beaufort scale <5. All pollinators observed within the quadrats or within the 2-metre buffer zone were identified to species. Pollinator behaviour and any flowers visitations were recorded at each plot and within 2 meters of the quadrat.

5.5.2 Statistical Analysis

To quantify richness, diversity and community composition of pollinator taxa in semi-natural species-rich grassland versus planted agri-environmental wildflower strips and to test for any significant differences between the habitat types, Chi-squared test was calculated in Excel between the pollinator species and the habitat to determine if there was a significant difference in composition. Shannon diversity index was calculated within Excel for pollinator species and wildflower plant species diversity between the habitats of semi-natural species-rich grassland and planted agri-environmental wildflower strips. The calculations were also carried out to individual farm level.

5.6 Results

There was no significant difference in the pollinator family count between the semi-natural species-rich grassland and the agri-environmental wildflower strips (p=0.677). There were 228 pollinator visits were recorded and 20 pollinator species and 7 pollinator families identified. 10,766 wildflower units were recorded and identified across all 90 plots across the three farms between 23 June 2021 to the 16 July 2021. Table 5.1 below gives the total pollinator observation count. A high percentage of the visits were made by butterflies being 52% of the total of pollinators and the meadow brown making most of the visits at 18%. Common Knapweed was the most visited flowering plant within the planted agri-environmental wildflower strips and rock rose within the semi-natural species-rich grassland (fig 5.1).

The results from the Shannon Diversity index represent that between the habitats of semi-natural species-rich grassland and planted agri-environmental wildflower strips there is a diversity of pollinator community and the wildflowers identified. The results for Shannon Diversity index also represented that there was pollinator diversity between the two habitats at each of the farms.

Table 5.1 Pollinator visitation at family level across all 90 plots on the two habitats of semi natural species rich grassland and planted agri-environmental wildflower strips.

Pollinator Family	Agri total	SRGL total
Beetle	1	1
Bumblebee	41	16
Butterfly	41	78
Hoverfly	20	6
Moth	1	0
Social Bee	9	13
Solitary bee	1	0
Grand Total	114	114

Table 5.2 Pollinator species visitations across all 90 plots and 22.5 hours observation on the two habitats of semi-natural species-rich grassland and planted agri-environmental wildflower strips.

Family	Common Name	AES	SRGL
Beetle	Solider beetle	0	1
	Thick Thighed beetle	1	0
Bumblebee	Red-tailed bumblebee	27	8
	White-tailed bumblebee	9	2
	Buff-tailed bumblebee	1	5
	Carder bee	4	1
Butterfly	Meadow Brown	16	27
	Marbled White	12	21
	Gatekeeper	2	18
	Large Skipper	1	9
	Large White	6	1
	Small Tortoiseshell	3	0
	Red Admiral	1	0
	Ringlet Butterfly	0	1
	Silver-washed Fritillary	0	1
Hoverfly	Marmalade hoverfly	19	6
	Drone fly	1	0
Moth	Silver Y Moth	1	0
Social bee	Honey bee	9	13
	Orange legged furrow bee	1	0

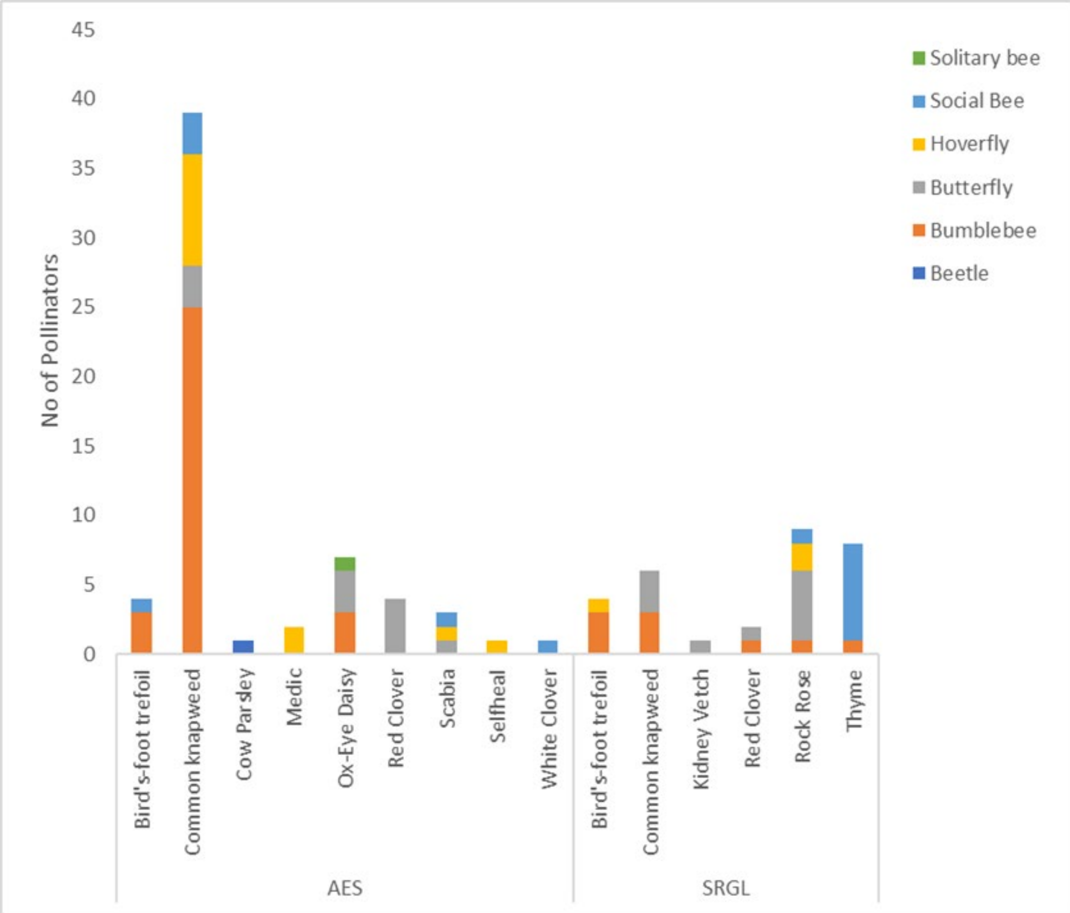


Fig 5.1 The interaction between the wildflower and pollinator species within the two habitats of semi-natural species-rich grassland and the planted agri-environmental wildflower strips

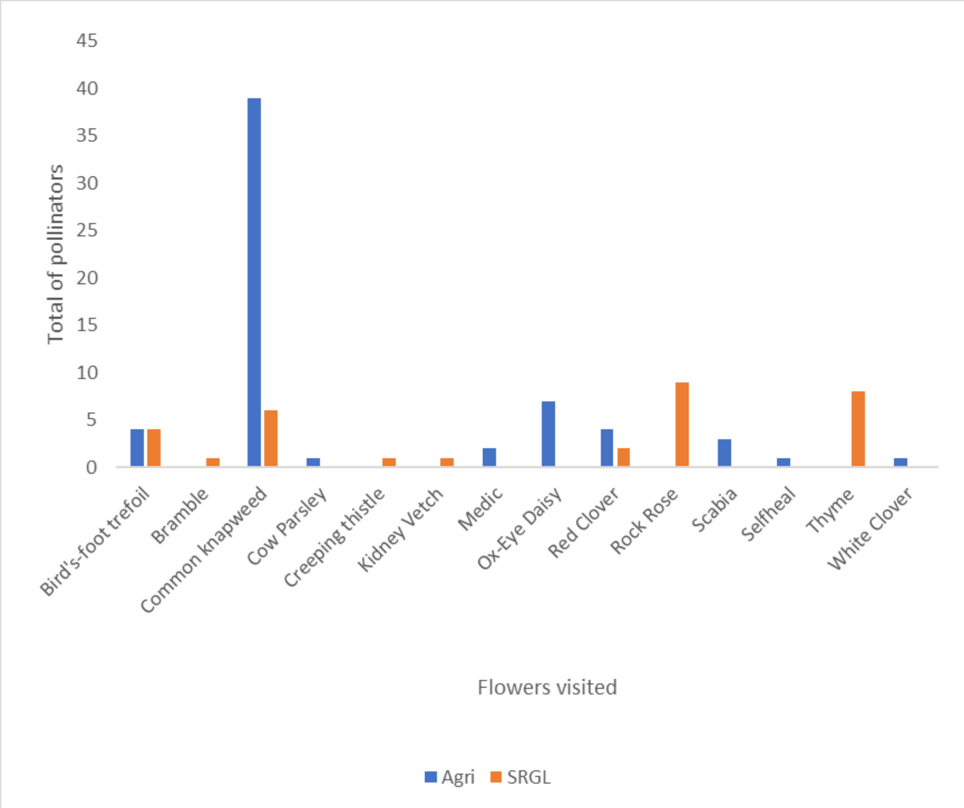


Fig 5.2 Comparison of pollinator visits within the two habitats of semi-natural species-rich grassland and the planted agri-environmental wildflower strips

5.8 Discussion

The results reported there was no significant difference of pollinator visits between the two agri-environmental schemes of semi-natural species-rich grassland and the planted agri-environmental wildflower strips. However, there was a diversity of pollinators and wildflower plant species overall between the two agri-environmental schemes and at farm level. Butterflies accounted for the highest number of visits from the pollinators recorded with the majority being within semi-natural species-rich grassland habitats, this result supports studies that habitat heterogeneity in the shape of semi-natural species rich grassland is key for maintaining farmland biodiversity and supporting pollinators such as butterflies (Steffan-Dewenter *et al*., 2002; Öckinger & Smith 2007). With 18 identified pollinator species in the species pool of pollinators. Most of these species were generalists that had good dispersal capabilities (Kleijn *et al*., 2006). The agri-environmental wildflower strips within this study were suitable for the pollinator species identified.

However, the richness and abundance of the flowering plant species within the semi-natural species-rich grassland compared to the planted agri-environmental wildflower strips would have expect to see a result of higher number of bumbles bee visits due to the variety of wildflowers within semi-natural species-rich grassland. However, there was a higher number of bumble bee visits to the planted agri-environmental wildflower strips, because of the high nectar sugar value flowering plant species and the number of pollinator visits these flowers received, for example, *Leucanthemum vulgare* and *Centaurea nigra* had the highest amounts of visits. As calculated within chapter 4 these species do have a high nectar sugar value, therefore with the abundance of these species within the planted agri-environmental wildflower strips the expectation was to see a higher number of pollinators simply because of the high resource available to them. The decisions undertaken by the pollinator are repeated throughout the foraging sessions.

The quantity of nectar is likely to influence the biological fitness of nectar feeding pollinators (Pyke et al., 2020). This combination could be described as a 'currency' by which outcome of alternative decision are determined. Therefore, when a pollinator is foraging optimally, it would be expected to make decisions that maximise this currency (Pyke *et al*., 2016). However, there is still limited information on how these attributes of nectar volume, sugar concentration and sugar composition and the effects of these qualities and how combined the influence they have on pollinating animals (Dreisig *et al*., 2012).

However, overall, the results showed there was diverse flowering plants attracting a diverse set of pollinator species. Though some individual quadrant results within the study had only one or two species of flowering plants at the time of the study. These individual quadrats still did well with pollinator visits because there was a high concentration of nectar sugar resource at the optimum nectar/pollen time over a short intense period of time due to a selection of efficient key plant species. Even though there were plots with seven to eight species of flowers, potentially offering a wider diversity of flowering plants. The concentration of the nectar sugar resources were considerably less than plots with one to two flowering species, therefore limiting the support of the pollinator and the resources required.

Overall, the results showed the farms and the two agri-environmental schemes to be diverse in terms of flowering plants species and pollinators. However, although the abundance of *Centaurea nigra* and *Leucanthemum vulgare* was the dominant flowering plant proving the highest nectar resource within planted agri-environmental wildflower strips means these schemes may not need to be diverse to support large numbers of pollinators within agricultural landscape. However, this could further limit further the pollinator species using the planted agri-environmental wildflower strips as resources are not suitable and limited.

With a few key species of flowering plants within the two habitats would finish flowering before the end of July and therefore depleting the resources towards the end of the summer for species such as carder bees that are still feeding and foraging well into September. It is recommended that flowering plants should cater for early and later flowering species should be considered within the wild seed mixes composition for the agri-environmental schemes going forward to support the earlier and later pollinators. Without a continuous cycle of flower resources for pollinator conservation over the whole of season, which is necessary, as potential pollinator groups might be prevented from completing their life cycles and therefore, limiting future pollinator generations (Memmott *et al*., 2010; Burkle *et al*., 2013).

6 Plant species floral traits attracting number of pollinator visits

6.1 Pollinator decisions

Pollinator decision and selection on floral traits is an important evolution for the underlying diversification of flowering plants (Darwin, 1862; Rosas-Guerrero *et al*., 2014). To promote floral divergence requires different pollinators (Fenster *et al*., 2004). Floral traits include colour, odour, size, rewards such as nectar and phenology. These traits are associated with pollinator groups and are known as floral or pollinator syndromes (Faegri & Van der Pijl, 1979). The concept of pollination syndrome suggests that plants focus on a particular family of pollinators that exert similar selective pressures on floral traits (Fenster *et al*., 2004). Variations within individual flower or inflorescence traits frequently translates into individual fitness differences of the plant. Because of these differences it affects the behaviour of pollinators, including frequency of visits and pollinator spectrum composition. Visitation rates from pollinators to flowering plants firmly depend on the pollinator abundance and the overlap between the flowering plants and the flying period. However, pollinators do not all visit at the same rates.

Pollinator interaction with their chosen flower either through colour, scent, nectar, or pollen may be of greater importance than the number of pollinator species that visit a certain plant (Ashworth *et al.,* 2015). The floral resource that is provided from pollinators is dependent on the frequency of visits, time spent and pollinator body size. (Murua, 2020). Valverde et al. (2019) found that large bees made short visits enabling the bee to handle the flowers quickly, therefore increasing their pollen load. With the bee's ability to extract pollen quickly and efficiently on each visit whilst increasing the pollen load with longer visits to enable extraction of more grains per visit (Ohashi, 2002).

Bumblebees differ in proboscis length, long-tongued pollinators feed on flowers with deep corollas and short-tongued species feed on flowers with short corollas (Ranta & Lundberg 1980). Short tongued species are likely to forage more efficiently on short-tubed mass flowering crops and therefore could benefit from crops such as oilseed rape as well as having large foraging ranges (Walther-Hellwig & Frankl 2000; Westphal, *et al*., 2006a; Greenleaf *et al*., 2007). Short tongued species are usually more dominate in agricultural landscapes (Glouson *et al*., 2005; Westphal *et al*. 2006a, b). Long tongued bumbles are more associated with extensive managed, semi-natural habitats and habitat heterogeneity which are becoming increasingly rare in modern agricultural landscapes (Pywell et al., 2005). With short tongued species using these habitats they could cause competition exclusion on generalist plants or resource depletion on specialised long tubed plants because of nectar robbing by short-tongued bumblebees (Ishii *et al.,* 2008). Therefore, it could cause pollen limitations in long tubed plants and populations of long-tubed plants over time (Dohzano *et al*., 2008).

6.2 Pollinator visits

Within landscapes of different species of flowers, during their foraging trips pollinators will make visitations to flowering plant species and would be carrying a mixture of pollen grains (Vaudo *et al.*, 2015). With the continued decline of pollinating invertebrates in the United Kingdom (Powney *et al.,* 2019), a further loss of pollinating invertebrates would lose the complex systems of interactions that are established with the flowering plant species therefore endangering the maintenance of wildflower communities (Aguilar *et al.*, 2006; Potts *et al.*, 2010; Thomann *et al.*, 2013). These loses would drive down several ecosystem services for example agricultural yields (Deguines *et al.*, 2014). The complexity of these plant-pollinator networks is linked to the dependence of interactions on the space-time turnover of plant, composition and pollinator communities which contribute to the temporal and spatial differences between local networks (Poisot *et al.*, 2012). However, when a pollinator species is present these interactions may not happen due to temporal mismatches or competition (Olson *et al.*, 2001).

Such flower rich habitats within agricultural landscapes, provide pollinators with the resources of pollen, nectar, and nesting sites within crop fields (Carreck and Williams, 2002, Pywell *et al.,* 2005, Russo *et al.,* 2013). However, these flower rich habitats could be ineffective in supporting key species of pollinators if not chosen correctly (Campbell *et al*., 2012, Olson and Wäckers, 2007) and could cause incompatibility between flowers and pollinator causing a decline of pollinators and limit fitness benefits of pollinators. Temporal overlap between flowering periods and insect foraging periods and the pollinator feeding structures (Campbell *et al.,* 2012, Junker *et al*., 2013, Russo *et al.,* 2013). Therefore, establishment of flower rich habitats that are directly within crops fields are crucial to pollinators to exist and be provided with resources within agricultural landscapes (Carvalheiro et al., 2012),

6.3 Aims

This aim is to investigate the behaviour of pollinators and to see what floral trait is attracting the pollinators to the two habitats. To understand the pollinator and floral trait relationship the following questions were considered: (i) which floral plant species was visited the most frequently? (ii) do pollinator species prefer a particular floral trait?

6.4 Methods

Study location

The study was undertaken in the Cotswolds, and the West Midlands, England. Each point on the map represented each farm within this study, Hollow Fosse Farm, Guiting Manor farm and Southfields Farm. All three farms had both agri-environmental schemes of sown wildflower strips and semi-natural species-rich grassland

Cross reference to section 2.2

Pollinator behaviour

Individual pollinator species were recorded once and verified insitu. On the few occasions further identification was required detailed photographs were taken with a DLSR camera and macro lens and identified either by reference books or an Entomologists. The behaviour of the pollinator visits was recorded within the quadrat and within the two-meter buffer. The behaviours were recorded as foraging (flying whilst looking for resources), feeding i.e. if the pollinator landed and you could see the proboscis feeding on the flower head or collecting pollen and resting i.e., no movement or feeding.

Paired Design

A paired-site sampling design approach was used to compile three sets of fifteen 3m x 3m plots in the farm habitats (treatment) of semi-natural species-rich grassland and sown wildflower strips 1m x 1m quadrats and 2m x 2m buffer. This paired design method is effective in quantifying the nectar and flowering plant traits that are attracting pollinators to these habitats along with their confounding variables.

Cross reference to section 2.3

Flower Count

The core method in obtaining the data for the research was through surveying, identification and recording count data of observed pollinators and flowering plants. The information that was gathered at the time of the study in July 2021 was able to provide a snapshot view of the presence of these variables required for statistical analysis.

Cross reference to section 2.4

Survey Criteria

Guidelines from organisations such as the Butterfly Conservation (undated) and Bumblebee Conservation Trust (Undated) were used to inform the criteria for pollinator surveys. As a range of pollinator families were surveyed, the study combined the criteria from these organisations to give the optimum surveying criteria (UKBMS, undated; Bumble bee conservation, undated), these being:

Weather is warm and dry

>13 degrees Celsius if sky is clear and less than half cloud

>15 degrees Celsius if more than half cloud

Time of survey 10:45 to 15:45

Wind speed - Beaufort scale <5 i.e., light breeze

15 minutes per quadrat

6.4.1 Statistical Analysis

To assess the floral life traits and the pollinator visits several variables will be analysed: floral unit colour (as perceived by humans); mean open flowers unit per square metre; nectar value and flower size (small, medium, or large). A series of binary logistic Generalised Linear Models were run using IBM SPSS Statistics for Windows, version 27 to assess variables (floral unit colour; mean open flowers unit per square meter nectar value and flower size). The dependant variable was the behaviour of the pollinator to enable the GLM to process the behaviour was assigned a 0 if the pollinator behaviour was feeding or 1 if another form of pollinator behaviour.

The variables underwent separate univariate Generalised Linear Models using (floral unit colour; mean open flowers unit per square meter nectar value and flower size). Then a single multivariate test (size, colour, and mean floral unit). The results from the Generalised Linear Model and the Akaike information criterion (AIC) values were compared, the highest Akaike information criterion (AIC) result was considered for best model strength and fit. The pollinator behaviour ‘foraging’ has not been included in the Generalised Linear Models due to not having an interaction with an open floral species.

6.5 Results

There were two significant relationships in the results following based on the behaviours of feeding and not feeding (excluding collecting pollen and resting). Were nectar value and number open flowers per floral unit. Generalized Linear Model, and the Akaike's information criterion (AIC) (table 6.1) presented a set of models defining the pollinator-plant interaction relationship between floral unit colour, mean open flowers per floral unit, nectar value and flower size. The best fit model was the nectar value along with the number open flowers per floral unit.

Table 6.1 Generalised Linear Model for explanatory variables of pollinator visitations (n=95). Akaike's Information Criterion scores are used to compare all models. Univariate single predictors of floral unit colour, number of open flowers, nectar value and flower size. Multivariate multi models to predict pollinator of size, colour, and mean floral unit.

Model #	Model Name	Sig	Degrees of freedom	AIC
1	Nectar value & Behaviour	**0.02**	1	34.336
2	Number of open flowers & behaviour	**0.04**	1	35.346
3	Floral unit size & behaviour	0.12	1	36.880
4	Floral unit colour, floral unit size & behaviour	0.30	2	38.875
5	Floral unit colour & behaviour	0.64	1	39.065
6	Floral unit colour, floral size, mean & behaviour	0.26	3	39.292

There was an overall count of 228 pollinator observations. All observations were allocated a behaviour: feeding on nectar; collecting pollen; resting; foraging (table 6.1). With foraging behaviour being the top result of n=131 pollinator individuals. Butterflies constituted the highest number of foragers within semi natural species rich grassland n=67 (Table 6.2). *Centaurea nigra* was the most visited wildflower, and this species was present in both habitats apart from Barnsley Warren within the semi-natural species- rich grassland. The dominant pollinator visitor was the red-tailed bumble bee with a total of 35 visits. 34 visits were made to *Centaurea nigra* and one visit to *Leucanthemum* *vulgare*. The total pollinator visits that were made to open flowers, purple was the preferred colour of flower with 52% of visits, then pink, yellow and white wildflowers (Fig 6.3).

Table 6.2 Total pollinator count for both habitats and the behaviour of the pollinator (excluding foraging)

Pollinator Behaviour	**AES Wildflower strip**	**Semi-natural species rich grassland**
Feeding	40	24
Not feeding	23	8

Table 6.3 Pollinator species behaviour count across all 90 plots on the two habitats of semi natural species rich grassland and planted agri-environmental wildflower seed mixes.

AES	Collecting pollen	Feeding	Foraging	Resting
Beetle	0	0	0	1
Bumblebee	16	16	9	0
Butterfly	0	11	30	0
Hoverfly	0	12	8	0
Moth	0	0	0	1
Social Bee	5	1	3	0
Solitary bee	1	0	0	0
SRGL				
Beetle	0	0	0	1
Bumblebee	7	2	7	0
Butterfly	0	11	67	0
Hoverfly	0	3	3	0
Social Bee	1	8	4	0

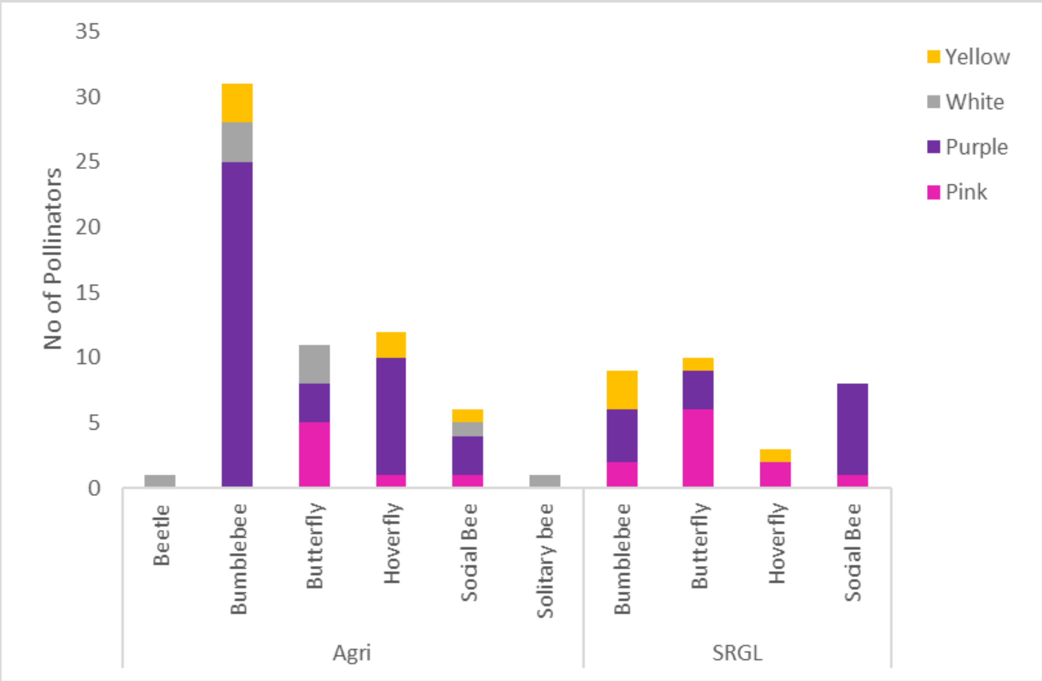


Fig 6.2 Composition of pollinators visiting wildflower plants and the colour preference within the two habitats of semi natural species rich grassland and the agri-environmental wildflower strip

6.6 Discussion

This chapter examined the floral traits of individual plants with open flowers to assess the behaviour of pollinator species that were identified and what their attraction was to the two habitats within the study. The two habitats appeared to be used in different ways by the pollinators. The agri-environmental wildflower strips showed a larger proportion of pollinators especially bumble bees were feeding, whilst in semi natural species rich grassland, pollinators particularly the butterflies appeared to use the area more for foraging and not landing very often, possibly indicting that the floral planted species available within the semi-natural species-rich grassland may not have been suitable for resources for their needs (Woodcock *et al.,* 2015).

The strongest interaction that is affecting the feeding decisions of pollinator behaviour and the visits the pollinators are undertaking to these floral plants is the nectar resource that is available between the study period of 23 June 2021 to the 16 July 2021. The results indicated that the floral traits or size and colour that were recorded at each pollinator visit was not a strong attraction to the pollinator as a choice. Pollinators need nectar for food and energy resources, if floral plant species was not offering this as a reward for pollination, then the floral plant is reducing its reproductive success (Parachowitsch *et al.,* 2019).

Where the species of the *Centaurea nigra* and *Leucanthemum vulgare* were abundant within the two habitats there were a higher number of pollinators feeding on nectar and collecting pollen. The pollinators were making behavioural decisions of probing the flower and receiving the nectar. This encounter influenced the decision whether to move onto the next flower or not, thereafter the next plant expecting the same yield as previously encountered. Therefore, manipulating the behaviour of the pollinator and therefore this manipulation from plant influences the pollen transfer (Hodges & Wolf, 1981; Pyke, 2010).

Nectar and mean open flowers per floral unit are an introduction into understanding plant-pollinator interactions and the influence that floral plant species have on pollinators and their behaviours. The results from the study have assisted in explaining the relationship that involves nectar and the attraction that this has to the pollinators along with the influence of the plant floral species trait of a composite floral type. However, in this the study there were key flowering plant species that dominated and received visits more frequently, particularly within the planted agri-environmental wildflower strips and supports the optimal foraging strategy. This floral type does determine a higher yield of nectar and therefore a larger nectar food resource for the pollinator for example *Centaurea nigra* contains an average of 54 units within one flower head compared to *Lotus corniculatus* that is one single unit. However, there are studies discussing whether nectar is a food resource reward and whether the floral plant species is manipulating the pollinator for pollination. Essentially some floral plants species do not have nectar to offer, but they are able to pretend there is a reward by giving off signals that a nectar reward is available, therefore manipulating the pollinator for pollination (Pyke *et al.,* 2016).

To determine the reward gains on a larger level and the benefits planted agri-environmental wildflower strips have for pollinators as a food resources that specific floral plants could make. Maintaining floral plant species within the planted agri-environmental wildflower strips that are already existing and providing a high resource, for example *Centaurea nigra* and *Leucanthemum vulgare*. However, consideration of introducing other floral plant species of high producing nectar composite floral plants to the planted agri-environmental wildflower strips would provide a continued nectar resource throughout the spring and summer for the pollinators that are encountered within agricultural farmland.

7 Overall Discussion

Pollinators are one of the most important elements in the maintenance and promotion of biodiversity providing a functioning role in pollinating ecosystem to crops and wild plants (Lippert *et al*., 2021). There is strong evidence that with recent declines in pollinators that there is a parallel decline of wild flowering plant species that these pollinators depend upon (Potts *et al*., 2010). These parallel declines and the dynamics between the pollinators and wild flowering plant species suggest these are linked, and that bee diversity declines, in particular, have had an influence on bee pollinated plants. Beismeijer et al. (2006) studied this relationship and even though there was a strong population of hoverflies pollinating flowers, bee pollinated plant species declined without the bee species providing their pollination service. Although these results were correlative the mechanism is still unknown for the parallel decline; further research is needed.

This aim of this study was to quantify the nectar supply of two habitats – semi-natural species-rich grasslands and planted agri-environmental wildflower strips across threes farms in the Cotswolds and the West Midlands, UK. Floral abundance was combined with published secondary data of nectar sugar values of 57 flowering plants, enabling comparison of nectar resource. To compare pollinator abundance between the two habitats, pollinator visit counts were conducted across Hymenoptera, Lepidoptera, Diptera and Coleoptera. Plant-pollinator interactions were investigated by modelling pollinator behaviour: feeding, collecting pollen, foraging and resting according to plant species and floral traits of size, colour, and nectar.

7.1 Synthesis of key findings

Values of nectar (Chapter 4) were significantly higher within planted agri-environmental wildflower strips than within the semi-natural species-riches grassland. However, these results showed there was diversity of floral plant species in the seed mixes - these being *Leucanthemum vulgare*, *Centaurea nigra*, and *Lotus corniculatus*. With a relatively high amount of nectar recorded in a short period of time within this study and the methodology undertaken, there could be a potentially ‘hunger gap’ especially at the beginning of spring and late summer when bumble bee queens are emerging from hibernation ready to start new colonies and find new nesting spots. Baude et al. (2016) and Timberlake et al. (2019) studies found that nectar supply was seasonally limited and over 50% of the nectar resource was produced with three key species of flowering plants dominating farmland these being wild garlic, creeping thistle, and white clover. The results within this study comparing the nectar values between the two habitats of semi-natural species-rich grassland and planted agri-environmental wildflower strips found there were three key species of flowering plants, however this study was only undertaken between 23 June 2021 to the 16 July 2021. These two habitats of semi-natural species-rich grassland and planted agri-environmental wildflower strips may not be able to provide adequate nectar resources when demand is at its peak and therefore there could be a deficient of nectar for pollinators which could potential results in decline of pollinator species within these habitats.

The results found from the pollinator visits that there was no significant relationship in pollinator species composition between the two habitats of semi-natural species-rich grassland and planted agri-environmental wildflower strips. However, results found there was diversity of pollinators overall at the two habitats and at both habitats at farm level. A numerically greater number of butterflies visited the semi natural species rich grassland, and their behaviour was foraging/flying rather than landing and feeding on nectar. Whilst bumblebee and hoverfly visits were found more within the planted agri-environmental wildflower seed mixes and feeding on nectar or collecting pollen. Indicating that the two habitats appeared to be used in different ways by the pollinator species that were identified within the study’s data collection.

In the UK there are 57 species of lepidoptera and the data collection between the two habitats identified nine species altogether. Öckinger & Smith (2015) results found there was a significant relationship of both habitat quality and landscape composition affecting species richness and visits of butterflies. However, the habitat area was not significant within the relationship. There are limited studies on how landscape composition does influence butterfly richness and abundance. Bergman et al. (2004) indicated that where there was a higher proportion of butterflies at suitable habitats that these habitats have connectivity, not fragmented pockets of habitats.

The results in this thesis have supported that the agri-environmental schemes that were on the farms are supporting nectar rich flowers and diverse pollinator populations. Studies that have been complied, for example by Wood et al. (2015) suggest that the agri-environmental schemes and the management techniques seem to be supporting only a limited suite of generalist pollinators and are not providing a suitable resource to support a diverse pollinator community. There are key reasons why there is such a limited diversity of pollinators within the two habitats and these include that they do not exist in the area, only visit sites once a year.

Agricultural intensification being a cause of decline due to the loss of flowering plant species that provide nectar and pollen resources, therefore negatively impacting on pollinator fitness (Carvalheiro *et al*., 2013; Alaux *et al*., 2017). Having semi-natural species rich grassland that are close to agricultural crops provide benefits to agriculture through effects on productivity. For example having a diverse community of plant and pollinator species would hold a probability of containing species that would be able to cope with environmental pressures of drought, frosts or other extreme environmental change whilst maintaining ecosystem functions such as pollination, retention of soil nutrients and natural pest control (Minns *et al*., 2001; Villalba & Provenza, 2009; French *et al.,* 2017). The results from chapter 5 of this thesis showed there was diversity of pollinators and specific pollinators were using different habitats, for example meadows brown and honeybees were found both habitats, whereas red tailed bumblebees were predominantly in the planted agri-environmental wildflower seed mixes.

The ability to assess the nectar value within this study has been an important role in determining the importance of the two habitats and nectar provision they provide within the agricultural landscape.

Other floral traits of size, type of flower i.e., single, or composite and colour did not influence the pollinator visitation. The results have been able to give a tangible value on how available nectar resources within the flowering period have contributed within the two habitats. To support and sustain pollinator population of the four pollinator groups Hymenoptera, Lepidoptera, Diptera and Coleoptera Carvell et al. (2011) research concludes that 1ha of good quality nectar flower mix would provide a sufficient pollen resource. Though bumblebee's pollen demands have been calculated that they would require 7-8ha or legume and herb rich sward needed per 100ha. (Dicks *et al.,*2015).

The study did demonstrate that semi-natural species-rich grassland does have a positive effect on at least the abundance of butterflies within the agricultural landscapes. These habitats are potentially hosting larval host-plants due to grasses identified in the quadrats of common bent, cocks' foot and Yorkshire fog which support butterflies such as marble white, meadow brown and ringlets. The planted agri-environmental wildflower strips did not contain as much of these hence the reasons for higher flying rather than feeding on the available nectar. However, for the bumblebees this has been the opposite where they were feeding or collecting pollen in higher bee numbers than flying and where there was sufficient continuous food resource. Hoverflies were also present and more dominate within the planted agri-environmental wildflower strips providing an essential ecosystem service such as pollination (Radar *et al.,* 2020). Pest control (Ben-Issa *et al*., 2017). Can tolerate environmental changes especially in increasing temperatures and changing climate (Pineda *et al*., 2008). Transport pollen over vast distances, potentially proving connective to isolated patches of wildflowers (Doyle *et al.,* 2020). Therefore, the pollinators were using each of the habitats for different needs and the habitats that were closest together could be supporting both suitable nest sites due to the structure of tussocks, stones, and a plentiful food resource.

Habitat management recommendations

To boost the value of semi-natural species-rich grasslands and the diversity of wildflowers that are within these habitats, management treatments need to be reviewed and addressed to protect the ecosystems that exist within these habitats. Whilst conserving species that are dependent on semi-natural species-rich grasslands and to stay in good condition (Babai & Molnar, 2014). Management of semi-natural species-rich grasslands are managed for hay and mowing is undertaken each year in June. Semi-natural species-rich grasslands do require management either from mowing or grazing, if left i.e., mowed every third year, then there is a loss of biodiversity attributes in the vegetation compared to semi-natural species-rich grasslands that are mowed annually (Milberg *et al.,* 2017).

For semi-natural species-rich grasslands conservation, an appropriate mowing regime is to be established. It is recommended that late mowing is undertaken which is favourable for biodiversity (Cizek *et al.*, 2012; Dahlström *et al*., 2013; Humbert *et al*., 2012; Wehn *et al.,* 2018). It is recommended that where there are only small fragments of semi-natural species-rich grasslands is mown at varying times and different parts each year to enable continuous resource availability (Johansen *et al*., 2019). Later mowing in the year can ensure undisturbed wildflower resources can still contribute resources to pollinators. Whilst early mowing could aid decline of wildflowers species and therefore resources for pollinators could decline (Valtonen *et al*., 2006; Kühne *et al.,* 2015; Bruppacher *et al.,* 2016).

Semi-natural species-rich grasslands within the UK are small and fragmented through agricultural landscapes and perhaps now semi-natural species-rich grasslands should be looked at for biodiversity only and not managed for hay to conserve these habitats and the eco systems they provide.

7.2 Limitations

A limitation of the study that it was 'snapshot' at a point in time within summer. There was no comparison undertaken with another season for example comparing spring and summer or comparing peak abundance of pollinators at different points. Which would be a good opportunity to undertake and study further.

Another limitation of this study has been the time and practical constraints of recording the flowering phenology. Within the year of 2021 a cold spring had caused a delay to wild flowering plants in both of the habitats, combined with the mowing of the semi-natural species-rich grassland within this study gave a small window of opportunity to survey and monitor these three farms which may have impacted on flowering species and the species recorded.

8 Conclusion

The results from this study have revealed that the planted agri-environmental wildflower strips were producing more nectar sugar than the semi-natural species-rich grassland within the period of 23 June 2021 to the 16 July 2021. The planted agri-environmental wildflower strips contained *centaurea nigra* and *leucanthemum vulgare* that were producing most of the nectar sugar and bumble bees were the dominate species. In the semi-natural species-rich grassland the wildflowers of *helianthemum oelandicum and thymus polytrichus* produced most of the nectar sugar, with butterflies being the dominate species.

The driving force for the pollinator visitations to the wildflowers appears to be the nectar sugar content rather than colour, size or if the flower is composite or not. Making nectar sugar the most important floral evolution that influences the pollinator's decision for the visit.

Evaluating the nectar value on both semi-natural species-rich grassland and the planted agri-environmental wildflower strips can start to provide a more ecological description of the resource value that these habits have available. Ongoing studies can provide recommendations for conservation that can be made within evidence-based habitat valuations. The nectar studies that have been undertaken by Baude *et al*., 2016; Hicks *et al*., 2016 & Timberlake *et al*, 2019 have provided a new way of comparing different habitats providing a tangible value that these habitats within this study have given, and therefore can be extended into further understanding of the available floral plants and their importance to pollinators.

9 Appendices

Appendices 1. List of species and mean nectar sugar content and mean open flowers per floral unit

Species	Common Name	Mean nectar sugar content in /flower/ day	Mean open flowers per floral unit	Source of pollen quality data
Ophrys apifera	Bee Orchid	0	1	
Betonica officinalis	Betony	0	1	
Lotus corniculatus	Birds Foot Treffoil	61.82	1	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Silene vulgaris	Bladder Campion	251.47	1	Baude *et al.*, 2016
Rubus fruticosus	Bramble	1892.83	1	Baude *et al.*, 2016
Ranunculus bulbosus	Bulbous buttercup	49.33	1	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Veronica persica	Common field Speedwell	31.59	1	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Cerastium fontanum	Common mouse-ear	26.93	1	Baude *et al.*, 2016
Dactylorhiza fuchsii	Common spotted orchid	0	1	
Vicia sativa	Common Vetch	300.34	1	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Anthriscus sylvestris	Cowparsley	11.33	119	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Ranunculus repens	Creeping Buttercup	104.51	1	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Cirsium arvense	Creeping Thistle	76.22	114	Baude *et al.*, 2016
Bellis perennis	Daisy	0.84	95	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Filipendula vulgaris	Drop Wort	0.00		Unavailable data
Cirsium acaule	Dwarf Thistle	66.45	1	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Linum catharticum Knautia arvensis	Fairy Flax Field Scabious	N/A 146.31	1	Unavailable data Baude *et al.*, 2016
Lathyrus nissolia	Grass Vetchling			Baude *et al.*, 2016
Anthyllis vulneraria	Kidney Vetch	2.22	1	Jablonski & Keltowski, 2005
Centaurea nigra	Knapweed	198.99	53	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Galium verum	Lady's bedstraw	0.66	1	Baude *et al.* 2016
Geranium columbinum	Long stalked crane's-bill	2.69	1	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Ranunculus Acris	Meadow Buttercup	78.83	1	Baude *et al.*, 2016 & Timberlake *et al.*, 2019
Filipendula ulmaria	Meadowsweet	0.00	57	Baude *et al.* 2016

Medicago lupulina	Medick	1.63	1	Baude *et al*. 2016
Polygala vulgaris	Milk Wort	N/A	1	Baude *et al*. 2016
Pilosella officinarum	Mouse-ear hawkweed	7.08	1	Baude *et al*. 2016
Leucanthemum vulgare	Ox-eye Daisy	15.92	135	Baude *et al*., 2016 & Timberlake *et al*., 2019
Papaver rhoeas	Poppy	5.35	1	Baude *et al*. 2016
Senecio jacobaea	Rag Wort	22.60	24	Baude *et al*. 2016
Trifolium pratense	Red Clover	116.86	24	Baude *et al*., 2016 & Timberlake *et al*., 2019
Plantago lanceolata	Ribwort	N/A	54	Baude *et al*. 2016
Helianthemum Nummularium	Rock Rose	0.00	1	Baude *et al*. 2016
Sonchus asper	Rough Sow-thistle	0.13	116	Baude *et al*. 2016
Onobrychis viciifolia	Sainfoin	NA	1	Baude *et al*. 2007
Sanguisorba minor	Salad Burnet	0.11	1	Baude *et al*. 2016
Prunella vulgaris	Selfheal	138.62	1	Baude *et al*. 2016
Crepis capillaris	Smooth Hawksbeard	9.02	22	Baude *et al*. 2016
Vicia tetrasperma	Smooth Tare			Not included
Vicia cracca	tuffed vetch	484.40	1	Baude *et al*. 2016
Trifolium repens	White Clover	48.97	23	Baude *et al*., 2016 & Timberlake *et al*., 2019
Daucus carota	Wild Carrot	7.35	1	Baude *et al*. 2016
Thymus polytrichus	Wild Thyme	24.65		Baude *et al*. 2016
Achillea millefolium	Yarrow	7.56	1	Baude *et al*., 2016 & Timberlake *et al*., 2019
Rhinanthus minor	Yellow Rattle	108.90	1	Baude *et al*. 2016

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