Oilseed rape and pollinators: the impact of variety on resource availability and pollination resilience

Stacey Michael Fairhurst

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Abstract

Mass-flowering crops help support the nutritional demands of insect pollinators in agricultural environments. With an estimated 70% of crops experiencing increased yields through animal pollination, recent declines in pollinator abundance and diversity have severe consequences to global food production. Oilseed rape (*Brassica napus*) is the most abundant oilseed crop in Europe and experiences enhanced yield from insect pollination. Subject to intensive commercial breeding programmes, growers face continuous annual variety selection, with new varieties offering increased yields and more favourable agronomic characteristics. At a critical time for pollinators, little is known about the effects that variety selection may have on resource provisioning. This thesis examines the impact of pollination on oilseed rape and the inter-dependence between pollinators and growers, with an emphasis on variety type and the breeding systems used to produce them.

The value of oilseed rape to the insect community was studied. Insect visitor surveys were undertaken in fields of conventional and hybrid varieties of oilseed rape, comparing the abundance and species composition between the field centre and crop edge, adjacent to semi-natural habitat. Overall, insects were more abundant and diverse at the edge of the crop than the field centre. While conventionally recognised pollinators (e.g. bees) were scarce during flowering, bumblebees were most abundant, particularly in the crop centre, whereas solitary bees favoured the crop edge. However, Diptera abundance was high, suggesting that their contribution to oilseed rape pollination in Scotland is more significant than that of bees. Conversely, the contribution of insect pollination to oilseed rape yield was estimated through pollinator exclusion experiments. Insect pollination increased seed set by 23% and seed weight per pod by 29%. Evidence of resource allocation was found, where plants with flowers subject to pollen limitation redirected resources to other parts of the plant. Increased pollinator abundance did not have a positive effect on the proportional contribution of pollinators for any of the yield metrics measured.

To measure the effect of pollination on plant development and reproduction, glasshouse experiments, comparing wind- and insect-simulated pollination against a control were undertaken. The addition of supplementary pollination had significant effects on vegetative and reproductive metrics. Both wind- and insect-simulated pollination produced shorter plants, a reduced flowering period and the number of flowers produced per plant. Although plants receiving supplementary pollination produced lighter individual seeds, they produced a greater number of seeds per pod. In combination with increased fruit set, this resulted in a greater overall seed weight per plant.

The prediction of floral resource availability (i.e. nectar and pollen) using oilseed rape agronomic characteristics was also investigated. Multiple regression analysis and predictive modelling were used to conclude that agronomic traits influence nectar sugar content and pollen quantity in oilseed rape. Contrary to the expectation that developing varieties with desirable traits for growers may come at a cost to floral resources, the opposite was found. Varieties with a higher tolerance to stressful environmental factors, particularly those found during winter, offered more nectar sugar per flower. The opposite was found for pollen, where early maturity, a desired trait for growers, had a negative effect on pollen quantity. Statistical analysis also highlighted the influence of short-term climatic changes on the sugar content of nectar.

Conclusions indicate that the inter-relationship between oilseed rape and pollinators is complex but has the potential to be mutually beneficial. The floral rewards offered by oilseed rape attract a plethora of insect pollinators during a period of resource scarcity. In return, pollinators have a significant effect on plant development and seed production. Furthermore, by making considered varietal choices, oilseed rape growers can increase the potential to financially

benefit from this mutualistic relationship by exploiting this valuable ecosystem service.

Lay summary

Agricultural crops that provide abundant floral resources such as nectar and pollen are an essential food supply for insect pollinators (including bees, hoverflies and butterflies). In return, pollinators increase seed production by transferring pollen between the male and female parts of flowers. For over a century, farming practices have had to change to feed the growing human population. These changes have contributed to a decline of pollinator numbers. Since animal pollination has the potential to improve the harvest in 70% of crops, the decline of insect pollinators could have severe consequences to global food production.

Oilseed rape is an important crop that benefits from insect pollination. It has multiple uses, such as vegetable oil for human consumption and biodiesel. Oilseed rape is commercially valuable, prompting intensive breeding programmes to develop new varieties. These varieties have different characteristics (e.g. plant height, stem stiffness and hardiness) designed to maximise harvest and improve disease resistance. Presently, we know very little about whether prioritising these characteristics comes at a cost to the food resources for pollinators.

In my research, I used different methods to investigate the relationship between pollinators and oilseed rape. Firstly, I surveyed sixteen commercial farms in Mid and East Lothian, Scotland, to discover which insects inhabit the crop. I then performed experiments to see how insect pollination affected seed

production, and whether the number of pollinators present was relevant. In the glasshouse, I experimented further with different methods of pollen transfer to determine the effect on growth, flowering and seed production of eight varieties of oilseed rape. Finally, I compared nectar and pollen for nineteen varieties of oilseed rape with the features that plant breeders focus on when producing new varieties. I built statistical models to predict the amount of sugar and pollen that new varieties would produce if only the breeding features of the variety were known.

Results show that oilseed rape attracts many insect visitors during flowering. Higher numbers were found in the crop edge than in the centre of the field. These insects increased the average number of seeds per pod by 23% and the average pod weight by 29%. When pollen is in short supply, plants reinvest energy from seed production into growth and flowering. Results also show that nectar and pollen quantity differ across varieties and is influenced by the characteristics selected by the breeders.

This thesis shows that oilseed rape and pollinators depend on each other in several ways. Oilseed rape provides resources for a wide range of insects when food is in short supply elsewhere. In return, pollinators improve the seed production of oilseed rape and can influence their growth and development via pollen transfer. By encouraging more pollinators into their fields, through the

improvement of wildlife-friendly habitat, farmers can reap the rewards associated with more productive harvests.

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General introduction

1 Introduction

1.1 The insect pollination of crops

1.1.1 Plant reproduction

Pollination is the transfer of gametes (pollen) from the male part of the flower, the anther, to the receptive female part, the stigma. This transfer is mediated using abiotic (i.e. wind and water), and biotic (i.e. animals) vectors. Pollination occurs within plants (self-pollination) or between plants (cross-pollination) (Pannell and Voillemot, 2017). Self-pollination is executed in two ways, either through the transference of pollen within a flower, autogamy or between flowers on the same plant, geitonogamy (Free, 1993). The success of self-pollination is greatly influenced by morphological and phenological mechanisms (Richards, 1997, Sargent, 2004). Self-pollination has several advantages; when insufficient pollination is available (e.g. low pollinator populations), self-compatible plants can maintain reproductive success by reducing expenditure on pollinator attractiveness and redirecting resources into seed-production (Ornduff, 1969, Kalisz *et al.*, 2004). However, allocating resources to self-pollination rather than pollinator attractiveness reduces insect visitation, subsequent cross-pollination, and increases inbreeding depression (Lloyd, 1979, Barrett, 1998, Fishman, 2000).

Sexual reproduction through cross-pollination offers the benefit of genetic diversity of progeny and allele purging through selection (Smith and Maynard-Smith, 1978). Although a large proportion of global food production comes from crops that are self- or wind-pollinated (Aizen *et al.*, 2009, Gallai *et al.*, 2009), 39 of the 57 leading global food crops benefit from insect pollination through increased fruit, vegetable or seed production (Klein *et al.*, 2007). With global food requirements expected to double by 2050 (Tilman *et al.*, 2011), insect pollination is crucial, not only in terms of fulfilling demand but also for providing nutrients essential for human health (Eilers *et al.*, 2011, Chaplin-Kramer *et al.*, 2014, Potts *et al.*, 2016b).

1.1.2 Biotic pollination

Almost 90% of all flowering plant species rely on the transfer of pollen by animals for reproduction (Ollerton *et al.*, 2011). Although pollinators include birds, bats and some mammals, the most influential group of animals are the insects (Proctor *et al.*, 1996). Plants that benefit from insect pollination require high productive pollinator activity. Failure to attract pollinator visitors may result in an insufficient supply of viable pollen (Engel and Irwin, 2003), causing plants to suffer from a reduction in reproductive output (Burd, 1994, Wagenius *et al.*, 2007). Reproductive success is determined by the quantity of compatible pollen received by a plant (Bernhardt *et al.*, 2008). It is measured by the number of viable fruits (fruit set) and seeds (seed set) produced (Stephenson, 1981). Pollen deposition is fundamental to reproductive success in terms of both quantity and quality and has positive relationships with seed set in many plants (Shore and Barrett, 1984, Spigler and Chang, 2008, Briggs *et al.*, 2016). Conversely, limitations in pollen supply have adverse effects on seed set (Groom, 1998).

The mutualistic interactions between plants and pollinators are central to many plants' reproductive success (Thomson, 2003, Bascompte and Jordano, 2007, Mitchell *et al.*, 2009). As a reward for the gamete transfer delivered by visitors through pollination, plants provide floral resources in the form of nectar and pollen (Proctor *et al.*, 1996). Providing these rewards is expensive, and the amount of energy allocated to their provision is representative of their dependency upon attracting pollinators. Not all flower visitors are effective pollinators of all plant species. The floral characteristics of a plant (e.g. corolla length, anther and stigma position) play an essential part on which insects visit particular plant species. These characteristics must align with the functional traits of the pollinators (e.g. tongue length, body size and foraging behaviour) that visit them (Campbell *et al.*, 1991, Harder and Barrett, 1996, Kobayashi *et al.*, 1997, Adler and Irwin, 2006, Willcox *et al.*, 2017). The maximum productivity of a patch is related to the time pollinators spend within it (Dreisig, 1995), with multiple pollinator visits often required to fertilise all ovules (Bernhardt *et al.*, 2008). Therefore, the foraging behaviour of pollinators plays a crucial role in reproductive success.

1.1.3 Foraging behaviour of insect pollinators

Foraging for floral resources involves a series of complex spatial and temporal decisions. These include minimising the flight distances between patches of floral richness (Carvell *et al.*, 2012), reducing the flying times between searching and handling resources (Goulson, 1999, Spaethe *et al.*, 2001), and avoiding recently visited flowers (Dreisig, 1995). The spatial arrangement of floral resources is integral to the foraging distances of wild pollinators (Lander *et al.*, 2011, Jha and Kremen, 2013, Lander *et al.*, 2013). For example, in landscapes where semi-natural habitat coverage was high, bumblebees (*Bombus* spp.) are found to make shorter foraging trips (Carvell *et al.*, 2012, Redhead *et al.*, 2016). Another example of pollinator efficiency is the multiple visits by pollinators to individuals of the same plant species (pollinator consistency) (Raine and Chittka, 2007). This behaviour is particularly favoured by bumblebees (Goulson, 2003, Cresswell and Osborne, 2004, Gegear, 2005), making them efficient pollinators of mass-flowering crops. Therefore, pollinator movement between plants, and subsequent pollen transfer, is a question of energetics, with optimal foraging theory hypothesising that individuals will leave a flower once the rate of return from floral resources falls below that of moving to another (Charnov, 1976).

1.2 Insect diversity in agroecosystems

1.2.1 Value of insect pollinators

With pollinating insects having a significant effect on the reproductive output of flowers and crops, they are of particular value, both environmentally and economically (Senapathi *et al.*, 2015b). From an environmental perspective, pollinators enhance the genetic diversity of wild and cultivated flowers, essential for a functioning ecosystem (Potts *et al.*, 2016a). With reports of yield increases in 70% of major crops grown worldwide (Klein *et al.*, 2007), and a contribution to global crop production estimated between €213 and €523 billion (Lautenbach *et al.*, 2012, Potts *et al.*, 2016a, Potts *et al.*, 2016b), maintaining this valuable ecosystem service, and its contributors, is economically important.

1.2.2 Insect pollinator community

The diversity of insect pollinators is broad and includes species of beetles (*Coleoptera*), butterflies and moths (*Lepidoptera*) and flies (*Diptera*). However, in most communities, the most dominant pollinators are bees (*Hymenoptera*; Figure 1.1), which depend on the rewards of flowers throughout their larval and adult life (Proctor *et al.*, 1996). Managed bees, for example, the European honey bee (*Apis mellifera*), are of great importance to the pollination of crops (Carreck *et al.*, 1997, Delaplane *et al.*, 2000). Considered generalists, they forage on a wide variety of plant species (Winston, 1991). In addition to the most sophisticated non-primate communication system that enables resource availability information to be transferred between individuals (Riley *et al.*, 2005), physical adaptations such as branched hairs, pollen baskets and combs allow for efficient pollen transfer (Abrol, 2007). Their pollinating efficiency benefits from the recruitment of a large workforce and can be managed and transported with ease (vanEngelsdorp and Meixner, 2010). Nevertheless, managed honey bees are only capable of supplying 64% of pollination services in Europe (Breeze *et al.*, 2014), with that number almost halved (34%) for the needs of the United Kingdom (Breeze *et al.*, 2011). Additionally, the increased demand for insect-pollinated crops is growing faster than honey bee stocks (Aizen and Harder, 2009). This shortfall highlights the concerns raised about the failure to maintain wild bee populations.

Several non-*Apis* bee species are managed for the pollination of crops. Examples include the pollination of tomato crops by bumblebees (Velthuis and van Doorn, 2006), and apples and alfalfa by solitary bees (Gruber *et al.*, 2011, Pitts-Singer and Cane, 2011). However, a large proportion of crop pollination is performed by wild pollinators (Ollerton *et al.*, 2012, Garibaldi *et al.*, 2013, Mallinger and Gratton, 2015). These include bumblebees and solitary bees as well as non-bee taxa, including hoverflies (*Syrphidae*) (Biesmeijer *et al.*, 2006, Jauker and Wolters, 2008, Meyer *et al.*, 2017).

Figure 1.1 Examples of insect pollinators found in oilseed rape: **A** honey bees and **B** bumblebees. (Photo: SMF)

1.3 Threats to pollination services

1.3.1 Pollinator decline

Biodiversity loss in response to anthropogenic activity has already exceeded its boundaries, affecting Earth system functions (Rockström *et al.*, 2009). While comprehensive data on pollinator populations is lacking, the available evidence suggests that since the mid-twentieth century, pollinator diversity has declined in industrialised areas including Europe and North America (Biesmeijer *et al.*, 2006, vanEngelsdorp and Meixner, 2010, Carvalheiro *et al.*, 2013, Vanbergen *et al.*, 2014, Senapathi *et al.*, 2015b, Potts *et al.*, 2016b). These declines raise serious concern over human food security, health and ecosystem services (Potts *et al.*, 2010a, Garibaldi *et al.*, 2011, González-Varo *et al.*, 2013, Vanbergen and The Insect Pollinators Initiative, 2013). Honey bees in Europe and North America have also experienced a decline, despite the number of managed hives increasing (Aizen and Harder, 2009, Kulhanek *et al.*, 2017, Soroye *et al.*, 2020). However, it is the populations of wild pollinators that have suffered most (Goulson *et al.*, 2008, Ollerton *et al.*, 2014, Gill *et al.*, 2016). For example, of almost 2,000 European bee species, only the populations of 12.6% show stability, while less than 1% exhibit population increases (Nieto *et al.*, 2014). Examples of bumblebee decline are reported in Scandinavia (Bommarco *et al.*, 2012a, Dupont *et al.*, 2011), as well as the United Kingdom and Ireland (Fitzpatrick *et al.*, 2007, Williams and Osborne, 2009). Distribution losses of other essential pollinators, such as butterflies and hoverflies, have also been recorded (Warren *et al.*, 2001, Keil *et al.*, 2011).

The reduced diversity of pollinators is closely associated with the decline of plant species diversity (Biesmeijer *et al.*, 2006, Carvell *et al.*, 2006, Carvalheiro *et al.*, 2013, Albrecht *et al.*, 2016). With almost 60% of plant species in arable habitats declining (Preston *et al.*, 2002), this may lead to some plant species becoming over-reliant on a few abundant pollinator species (Pradervand *et al.*, 2014). Plants with specific floral traits, reliant on specialist pollinator species, have been severely affected (Fontaine *et al.*, 2006, Vanbergen *et al.*, 2014). Pollen limitation, as a result of pollinator decline, hinders the yield growth of insect-pollinated crops, in comparison to other crops, resulting in yield instability (Garibaldi *et al.*, 2011). Indeed, 50% of farmers perceive there to be pollination deficits in at least one of their crops (Breeze *et al.*, 2019).

1.3.2 Drivers of pollinator decline

The production of food through agriculture, to meet the requirements of an ever-growing human population, is considered the primary driver of environmental change, with adverse effects on climate, water resources and biodiversity (Foley *et al.*, 2005, Rockström *et al.*, 2009). Studies conclude that pollinator declines are not attributable to a single cause, but linked to complex interacting pressures and processes (Potts *et al.*, 2010a, Roulston and Goodell, 2011, González-Varo *et al.*, 2013, Vanbergen and The Insect Pollinators Initiative, 2013, Potts *et al.*, 2016b), with pollinators in agricultural environments particularly under threat (Kovács-Hostyánszki *et al.*, 2017).

Since the early twentieth century, enhancements in methods and technology, in the pursuit of higher yields, has led to agricultural intensification and anthropogenic land-use change (Robinson and Sutherland, 2002, Hodgson *et al.*, 2005, Kim *et al.*, 2006, Storkey *et al.*, 2012). Increases in field sizes and subsequent losses of semi-natural habitat, such as hedgerows, has been identified as the primary driver in pollinator declines (Aguirre-Gutiérrez *et al.*, 2015, Senapathi *et al.*, 2015b, Corlett, 2016, Potts *et al.*, 2016a). Loss of species-rich semi-natural grasslands and flower-rich field borders has reduced nesting site opportunities (Osborne *et al.*, 2008) and floral resource diversity (Rundlöf *et al.*, 2008, Öckinger *et al.*, 2009, Scheper *et al.*, 2014). With pollinators requiring adequate food supplies, nesting sites and shelter to maintain stable populations (Torné-Noguera *et al.*, 2014), these losses have led to malnutrition and colony stress (Vanbergen and The Insect Pollinators Initiative, 2013, Baude *et al.*, 2016). Land-use changes are not the only impact of agricultural intensification that has affected pollinator numbers. In an attempt to reduce weeds and pest abundance, the increased use of insecticides and fungicides has led to sub-lethal effects on pollinators and a loss of in-field floral diversity (Gill *et al.*, 2012, Whitehorn *et al.*, 2012, Godfray *et al.*, 2014, Goulson, 2015).

Agricultural intensification is not the only driver of pollinator decline. Non-native pollinator species out-compete local pollinators and upset complex plant and pollinator networks (Morales and Traveset, 2009, Dohzono and Yokoyama, 2010). Also, the spread of pathogens between managed and wild bees present severe fitness consequences to their hosts (Martin *et al.*, 2012, Fürst *et al.*, 2014, Traveset and Richardson, 2014). Finally, changes in geographic range and phonologic changes associated with climate change are of particular concern (Memmott *et al.*, 2007, Hegland *et al.*, 2009, Kerr *et al.*, 2015, Franklin *et al.*, 2016).

1.3.3 Reducing the impacts of pollinator decline

These pressures impact wild pollinator populations in numerous ways. In addition to abundance and diversity losses, shifts in foraging range, dietary selection, life history and genetic variation are also affected (Roulston and Goodell, 2011, Lebuhn *et al.*, 2013, Ollerton *et al.*, 2014, Senapathi *et al.*, 2017). Reversing the impacts of wild pollinator decline must first address the current scarcity of nutritional resources available to pollinators in agri-environments (Carvell *et al.*, 2007, Pywell *et al.*, 2011). Indeed, European policy has looked to reverse and mitigate these impacts for decades, identifying the improvement of habitat quality as a priority (Bignal, 1998, Cole *et al.*, 2020).

Agri-environment schemes (AES), where farmers are financially rewarded in exchange for farming practice adjustments to benefit the ecological status of their land have been adopted throughout Europe. However, the objectives of the schemes (e.g. biodiversity enrichment, reduction in pesticide use, restoration of landscapes) vary by country (Kleijn and Sutherland, 2003). Examples include the targeting of specific taxa, such as long-tongued bumblebees, by planting flower-rich nectar and pollen mixes with a focus on flowers with longer corollas (Carvell *et al.*, 2011, Wood *et al.*, 2015) and planting of buffer strips to increase ecological connectivity (Cole *et al.*, 2015). Experimentally, these schemes have been successful in increasing diversity of target species (Carvell *et al.*, 2015, Pywell *et al.*, 2015). However, the complexity of the surrounding landscape is considered to be more important than patch size (Heard *et al.*, 2007, Batary *et al.*, 2011, Scheper *et al.*, 2013).

1.3.4 Pollinator-friendly habitat

Habitat quality, composition and diversity all play a vital role in maintaining wild pollinator communities, with increases in density and diversity of pollinators attributed to wildlife-friendly habitats (Jönsson *et al.*, 2015, Campbell *et al.*, 2017). Uncropped and uncultivated field margins are particularly effective at increasing plant species richness, resulting in pollinator-friendly habitat (Albrecht *et al.*, 2016). Hedgerows, commonplace in UK agricultural environments (Norton *et al.*, 2012), not only offer shelter and floral resources for many invertebrates (Staley *et al.*, 2012, Amy *et al.*, 2015), but their linear features show an increase in pollinator visitation (Cranmer *et al.*, 2012). These benefits highlight the importance of protecting and maintaining current hedgerows, particularly in intensely managed arable landscapes (Garratt *et al.*, 2017). However, individual habitats do not typically provide the resources required for all pollinators. Thus, more diverse habitats may be required to support pollinators throughout their life cycle, with mass-flowering crops contributing (Mandelik *et al.*, 2012, Cole *et al.*, 2017).

1.4 Resource availability and allocation in crops

Plant fitness is characterised by several traits, such as size, biomass and reproductive output, present during its life history (Younginger *et al.*, 2017), and attributed to the interactions between genetic and environmental conditions (Walisch *et al.*, 2012). Environmental conditions that influence resource availability can have detrimental effects on development and seed production (Herrera, 2000, Ågren *et al.*, 2012, Skálová *et al.*, 2015). When faced with resource scarcity, crops, in particular, are capable of compensating and allocating resources within the plant from one yield parameter to another (Grosse *et al.*, 1992, Bos *et al.*, 2007). For example, when resources are insufficient to produce large amounts of seed, plants have been shown to release growth inhibitors which promote fruit abortion, resulting in large flower to fruit ratios (Tamas *et al.*, 1979, Stephenson, 1981). With the cost of seed production high, this highlights the importance of resource availability (e.g. water and soil nutrients) to a plant's reproductive success (Stephenson, 1981, Haig and Westoby, 1988).

1.5 Floral resource availability in agroecosystems

1.5.1 Nectar

The rewards offered by plants in exchange for providing pollination services include pollen and nectar. These provide essential proteins and nutrients, vital to the survival of pollinators (Proctor *et al.*, 1996), specifically bees that depend on these resources throughout all stages of their life history (Michener, 2000). Nectar, a sugar-rich solution, provides the primary source of energy and fulfils the main food requirements of winged adults, and is used by flowers to attract pollinators (Brandenburg *et al.*, 2009). As well as sugars (i.e. sucrose, glucose and fructose), the other major component of nectar is water (Corbet, 2003, González-Teuber and Heil, 2009). Trace elements also include lipids, phenols, alkaloids and volatile organic compounds (Nicolson and Thornburg, 2007). The essential carbohydrates, found within the sugars, provides energy for flight, wax production and brood incubation and represents the majority of the energetic value of nectar (Free, 1993, Willmer, 2011). Minor components of nectar include lipids and volatile organic compounds (Nicolson and Thornburg, 2007), while amino acids influence the taste of nectar (Gardener and Gillman, 2002).

The production and secretion of floral nectar are costly to plants. It is produced in the nectary glands, usually situated at the base of the flowers (Willmer, 2011). Sugars are produced by photosynthesis in nectar parenchyma cells and may be stored as starch molecules before secretion (Pacini *et al.*, 2003). Commonly used nectar measurements include standing crop (the quantity of nectar available at a particular point in time) and secretion rate (the quantity secreted within a given time) (Corbet, 2003). Nectar volume is highly variable and sensitive to fluctuations in response to micro-climatic effects such as precipitation, condensation and evaporation (Nicolson and Thornburg, 2007). Although less variable than nectar volume, sugar concentration can also differ (Real and Rathcke, 1988, Willmer, 2011). If concentration, and subsequent viscosity, is too high, efficient consumption becomes difficult, while too low reduces the energy content for pollinators. For most bees, the optimal concentration is estimated at 55% (Kim *et al.*, 2011). In addition to environmental conditions, nectar production is influenced by flower age (Burquez and Corbet, 1991), physiological changes in the flower post-pollination (Gillespie and Henwood, 1994), exposure to light (Nicolson and Nepi, 2005) and soil nutrients (Baude *et al.*, 2011).

1.5.2 Pollen

For plants, pollen grains exist to transfer the genetic information from the anthers to the stigma for fertilisation. However, to flower visitors, they provide a valuable source of protein and nutrients essential for larval development and growth (Roulston and Cane, 2000, Behmer and Nes, 2003, Somme *et al.*, 2015). For example, the number and size of buff-tailed bumblebee (*Bombus terrestris*) offspring has been found to have a positive relationship with the protein quantity of the pollen they feed on (Génissel *et al.*, 2002, Vanderplanck *et al.*, 2014). The size of pollen grains ranges from 4 µm to 350 µm in diameter, with an average size of 15-60 µm for most plant species (Willmer, 2011). The outer layer comprises of sticky, lipid-rich material (Piffanelli *et al.*, 1997), which as well as providing protection, attracts pollinators and adheres grains to foragers and receptive stigmas (Edlund *et al.*, 2004, Pacini and Hesse, 2005). Post dehiscence, pollen viability is relatively short-lived (Pacini *et al.*, 1997, Wilcock and Neiland, 2002).

Many factors adversely affect seed production. For example, an early or late harvest can result in reduced seed quality and quantity (Król and Paszko, 2017), as can extreme weather conditions (Zipper *et al.*, 2016, Li *et al.*, 2019). Pollen limitation has also been as a primary contributor to reduced seed production (Ashman *et al.*, 2004, Knight *et al.*, 2005b, Jakobsson *et al.*, 2009). Insufficient pollen deposition, either in terms of quantity or quality, has a detrimental effect on a plant's capability to achieve its potential seed set (Waites and Ågren, 2004, Campbell and Husband, 2007).

1.5.3 Pollinator needs

Pollinator species vary in their metabolic needs (Sedivy *et al.*, 2011) and nutritional demands (Archer *et al.*, 2014, Vaudo *et al.*, 2016). Specifically, some pollinators need only nectar, while others require nectar and pollen (Proctor *et al.*, 1996). These nutritional demands are plastic and can vary with life stage (Stabler *et al.*, 2015). For instance, in the early stages of adulthood, honeybee preferences switch from an amino acid to a sugar-rich diet (Paoli *et al.*, 2014). Pollinators are also capable of altering their foraging behaviour based on nectar sugar concentration and pollen quality (Elisens and Freeman, 1988, Vaudo *et al.*, 2016). Bumblebee species are observed to preferentially visit only those plants that fulfil their amino acid requirements (Somme *et al.*, 2015). Further consideration of floral preference is dictated by the physical adaptations of the pollinator species. Flowers with long corollas are mostly suitable for long-tongued pollinator species (Stang *et al.*, 2006). However, these morphological mismatches are often overcome by nectar-robbers. These are flower-visitors who 'steal' nectar from the flower by perforating the corolla tissue without contributing to pollination (Inouye, 1983). Nectar-robbing also contributes to reduced pollinator visitation through flower mutilation (Varma *et al.*, 2020). Regardless of foraging strategy or nutritional needs, direct relationships exist between food supply and wild pollinator populations, with increased populations observed in solitary bees and bumblebees alongside the availability of mass-flowering crops (Westphal *et al.*, 2003, Jauker *et al.*, 2012b, Holzschuh *et al.*, 2013, Riedinger *et al.*, 2015).

1.6 Oilseed rape

1.6.1 Cultivation

Oilseed rape (*Brassica napus*; OSR) is the largest oilseed crop in Europe, cultivated on 34.9 million hectares in 2019 and producing over 18 million metric tons of seed annually (USDA, 2019). Although oilseed brassica cultivation has existed for thousands of years, OSR cultivation is relatively young and has only been a significant crop since the mid-twentieth century (Snowdon *et al.*, 2007, Friedt and Snowdon, 2009). While grown briefly in Europe during the 19th century for lamp oil (Kramer, 2012), cultivation increased rapidly for the production of margarine following World War II (Snowdon *et al.*, 2007). With improved methods for crop establishment and crop protection, the mean yield has increased globally by 230% since 1970 (Rondanini *et al.*, 2012). Grown as a break crop to reduce weeds and pathogens in fields usually sown with cereals, the value of OSR is in its seeds. These oil-rich seeds have many uses, including as vegetable oil and margarine for human consumption as well as various industrial uses (e.g. as a bio-lubricant) (Friedt and Snowdon, 2009, Salimon *et al.*, 2010). However, the dominant use of total oilseed rape production across the European Union in 2019 (46%; USDA Gain Reports, 2020) was processed into biodiesel, with increased cultivation to meet biofuel targets (Van Der Velde *et al.*, 2009, Solis *et al.*, 2017). OSR yield measurements include fruit set, seed set and seed weight (Habekotté, 1997, Diepenbrock, 2000), with seed quality parameters being chlorophyll and oil content, the latter having direct economic value to growers in the form of an oil bonus (Limagrain UK Ltd, 2018). Modern varieties are the result of intensive, ongoing commercial breeding programmes. Traditionally bred using classic line-breeding methods (conventional), the discovery of male sterility systems has resulted in restored-hybrid varieties (Friedt and Snowdon, 2009, Ollerton *et al.*, 2011).

1.6.2 Oilseed rape growth and development

In northern and central Europe, the subspecies *B. napus oleifera* is the dominant crop, available as spring-sown or the more common winter-sown varieties (Friedt and Snowdon, 2009). The Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale is a framework used to identify the phenological development of plants (Lancashire *et al.*, 1991). For oilseed rape, the entire OSR plant cycle is divided into nine primary phases, then subdivides it further into secondary phases (Figure 1.2). Before the onset of winter, winter-sown varieties form rosettes of leaves (BBCH: 10-19) before vernalisation (a cold period required to enable flowering) (Waalen *et al.*, 2014). These rosettes elongate in spring (BBCH: 30-39) and develop flower buds that begin to open in April (BBCH: 50-59). Flowering (BBCH:60-69) starts on the main stem, followed by the secondary, lateral racemes (Daniels *et al.*, 1986). Flowering lasts approximately four weeks before the fertilised ovules develop into seeds (BBCH:70-79) (Nedić *et al.*, 2013). Plants are dead and dry by BBCH stage 97 and harvested at stage 99).

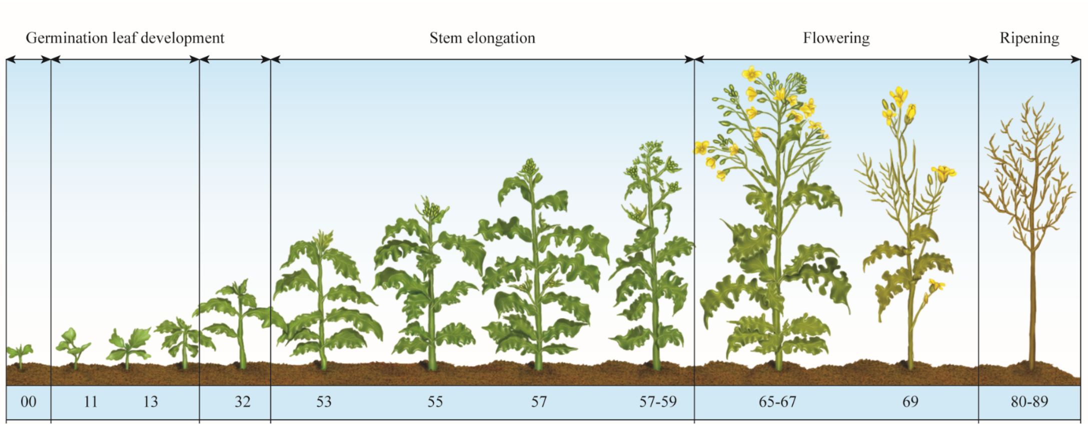

Figure 1.2 Growth stages of oilseed rape. Numbers represent the coding phenology of the BBCH-scale. (Illustration courtesy of Bengtsson (2013)).

An OSR flower has a lifespan of approximately three days (Eisikowitch, 1981). Pollinators are attracted to the bright yellow flowers, for the nectar provided by two pairs of nectary glands situated at the base of the flower (Abrol, 2007). The inner pair of nectaries account for 95% of the nectar sugar produced by the flower (Figure 1.3) (Davis *et al.*, 1994). Six male stamens surround the female style. Two pairs of long stamens encircle the style, with a shorter pair located outside. Anthers on the long stamen dehisce outwards, while synchronously, the inner stamen anthers dehisce inwards (Bell and Cresswell, 1998). This arrangement allows for some degree of self-pollination (Eisikowitch, 1981). Pollen grains are sticky, a characteristic associated with insect-pollinated plants (Cresswell *et al.*, 2004).

Figure 1.3 The nectary position of an oilseed rape flower with the petals removed. Two pairs of nectary glands are present: **1** the inner and **2** the outer. (Photo: SMF).

1.6.3 Insect pollination of oilseed rape

OSR provides an early season forage for many insect pollinators including managed honey bees, wild bees and hoverflies (Hayter and Cresswell, 2006, Jauker and Wolters, 2008, Stanley *et al.*, 2013). With sugar-rich nectar and pollen with high protein content, OSR fulfils the nutritional requirements of many insect visitors (Roulston and Cane, 2000, Farkas, 2006). Honey bees increase their median lifespan when fed on OSR pollen (Schmidt *et al.*, 1995), while Tasei and Aupinel (2008) found that bumblebee larval weight was higher when fed 96% OSR pollen when compared to other pollen mixes. Although OSR is self-fertile, outcrossing by insects has been shown to affect yield, both in terms of quantity (Bartomeus *et al.*, 2014, Hudewenz *et al.*, 2014) and quality, by increasing seed germination and oil content (Kevan and Eisikowitch, 1990, Adegas and Couto, 1991, Bommarco *et al.*, 2012b).

1.7 Varietal differences in oilseed rape

Oilseed rape is an important cultivated crop with an estimated annual global value of $7.5 billion (USDA, 2019) and is subject to intensive commercial breeding programmes. New varieties are the culmination of lengthy research, to increase seed yield and quality, selecting favourable functional growth traits and improving disease resistance. New varieties are made commercially available once they outperform current varieties. However, little is known about the extent that these breeding programmes have on aspects associated with insect pollination. Has the pursuit of varietal improvement from a grower's perspective resulted in a trade-off with factors favourable to pollinators? Some evidence exists that may suggest varietal differences. For example, the extent to which insect pollination enhances seed production differs between varieties and breeding systems, but results are inconsistent (Hudewenz *et al.*, 2014, Marini *et al.*, 2015). Varietal differences are also present in floral resource availability, but again results are unpredictable. For example, Bertazzini and Forlani (2016) report that nectar secretion was up to 50% higher in varieties bred conventionally than as restored hybrids. In contrast, Carruthers *et al.* (2017) suggest that although mean nectar volume is higher in hybrid varieties, nectar secretion remains consistent between breeding systems. With new varieties regularly being brought to market, studies involving specific OSR varieties become outdated quickly; therefore, comparisons using alternative parameters would offer longer-term answers to questions involving varietal dissimilarity.

1.8 Study objective

The overarching objective of this thesis was to explore the impacts of pollination on oilseed rape and the interdependence between pollinators and growers, with an emphasis on cultivar breeding system differences. I approached this through four main questions:

1. *How important is the relationship between oilseed rape and insect pollinators, with respect to resource provisioning?*

   To approach this, I evaluated the abundance, diversity and structure of the insect community found within oilseed rape. With semi-natural habitat providing valuable resources such as nesting and overwintering sites, I investigated whether proximity to field margins affected insect abundance in oilseed rape fields (Chapter two). To assess the floral resources available to visiting insects, I collected nectar and pollen from varieties of oilseed rape with different functional agronomic traits. Using these data, I built predictive models to determine if floral resource availability is influenced by agronomic traits (Chapter five). This offers insight into the importance of oilseed rape to pollinating insects and the wider insect community.

2. *What role do insect pollinators play in the pollination of oilseed rape?*

   To estimate the contribution of insect pollinators on oilseed rape yield in commercial farms, I compared a range of yield metrics, encompassing measures of both quantity and quality, from open-pollinated and pollinator-excluded flower heads (Chapter three). To explore the impact of pollinator abundance on yield, the relationship between pollinator abundance on pollinator contribution was also explored.

3. *How does oilseed rape distribute resources between growth and reproductive metrics under different pollination treatments, and what are the implications to quantity and quality of yield?*

   To delve deeper into the role of pollination on resource utilisation, I investigated the effect of wind and insect simulated pollination on phenotypic plasticity, in a controlled environment, with an emphasis on how plants redistribute resources when encountering pollen limitation (Chapter four). This evaluates the importance of insect pollinators to the grower to determine if improving pollination services can be economically rewarding.

4. *How can oilseed rape growers increase the pollination services of oilseed rape through varietal selection and management practices?*

   I approach this by first answering the questions above to see where potential solutions may be present. I offer some practical solutions to key issues arisen from previous chapters (chapter six).

1.9 Thesis structure

1.9.1 Chapter two

This chapter assesses the insect community found during the flowering of oilseed rape in the Central Lowlands of Scotland. It evaluates the spatial variability of insect abundance over two consecutive years. This was addressed by evaluating the effect of field margin proximity on insect abundance and structure, and whether insect communities differ across varieties bred conventionally or as hybrids. This offers a greater understanding of the spatial distribution of insect visitors to oilseed rape and explores the importance of field margins and oilseed variety to insect populations nearby.

1.9.2 Chapter three

Pollinator abundance data from chapter two was used to assesses the value of insect pollination to oilseed rape in an agricultural landscape. Through pollinator exclusion experiments in sixteen field over two consecutive years, the contribution of insect pollination was estimated for conventional and hybrid varieties of oilseed rape. I investigated whether, when faced with localised pollen limitation, plants redistribute resources to other parts of the plant. Additionally, using pollinator abundance as a proxy for visitation, I explored the differences between the proportional change of yield metrics resulting from pollination, with pollinator visitation. This study highlights the importance of insect pollination to oilseed rape yield and how plants can reallocate resources when faced with challenges.

1.9.3 Chapter four

To explore the resource allocation found in Chapter two further, Chapter four explores the effect of pollen deposition on the growth and reproduction of oilseed rape plants within a controlled environment. With a focus on breeding system differences, supplementary pollination techniques were used to simulate wind- and insect-pollination and these were compared with self-pollination by autogamy only. I investigated the degree to which plants altered their functional growth and reproductive traits in response to variable pollen deposition. This offers a greater understanding of the effect of pollination on the phenotypic plasticity of resource allocation across oilseed rape breeding systems.

1.9.4 Chapter five

Building upon the variability associated with variety and breeding system from the previous three chapters, chapter five investigates if floral resource availability can be predicted using the agronomical trait data of conventional and hybrid varieties of oilseed rape. Floral resources (i.e. nectar and pollen) were quantified for nineteen varieties undergoing varietal recommendation testing. Combined with agronomic trait data for each variety, multiple regression analysis was used to create predictive models to allow for resource prediction in future varieties. This provides an opportunity to explore potential synergies and trade-offs between agronomic traits desirable to growers and the provisioning of pollen and nectar resources. Predictive modelling of pollinator resources based on traits offers a practical application to oilseed rape breeders and growers to quickly evaluate the resource availability of oilseed rape varieties without additional data collection.

1.9.5 Chapter six

Finally, the conclusions and key findings from the previous four chapters are drawn together to address the overarching questions of the research. In addition to fundamental practical applications derived from my research findings, I identify experimental design and method limitations and make suggestions for future research.

Chapter 2

Insect community composition in a mass-flowering crop: assessing abundance and diversity in oilseed rape fields in Scotland.

2 Insect community composition in a mass-flowering crop: assessing abundance and diversity in oilseed rape fields in Scotland.

2.1 Abstract

Agricultural intensification has been identified as a primary driver of biodiversity decline. Semi-natural habitat provides valuable nest, shelter and forage resources for a diverse group of invertebrates in agricultural landscapes. To increase farming efficiency, much of this habitat has been lost, with consequential effects for invertebrate communities. Biodiversity is an essential factor in maintaining healthy ecosystems and underpins many ecosystem services critical to agricultural production (pollination services, natural pest control, nutrient cycling). To determine the effect of semi-natural habitat on insect community composition in oilseed rape (*Brassica napus*), we assessed how proximity to semi-natural field margins influenced the abundance and diversity of four taxonomic guilds: (i) common pollinators, (ii) pest species, (iii) beneficial insects and (iv) non-syrphid flies over two consecutive years. We also investigated if insect abundance and diversity were affected by the varietal breeding system. We found that proximity to semi-natural habitat affected most taxonomic groups. Abundance and diversity were higher in the field margins than the centre of the crop for the invertebrate community as a whole. However, this was influenced by the most abundant taxa: pollen beetles and natural enemies of many common pests, parasitic wasps. Although the pollinators were more abundant in the centre of the field, small sample sizes were unable to confirm these differences statistically. Insect populations were mostly consistent across oilseed rape breeding systems for all taxonomic guilds. We also found that bee and pest abundance differed between years, with lower numbers found in 2017 when flowering started earlier. Our results show that field margins support diverse invertebrate communities. The higher abundance of invertebrates found in the crop edge indicates that field margins provide sufficient resources for both pests and beneficial insects, highlighting the importance of field margin management. Providing sufficient forage before and after flowering of mass-flowering crops enables pollinators and beneficial invertebrates to reproduce; while also providing alternative host plants to control populations in the crop.

2.2 Introduction

2.2.1 Biodiversity in agricultural ecosystems

Traditionally, the biodiversity of European agricultural landscapes has been high (Potter, 1997). However, over the last fifty years, the intensification of farming has led to marked declines in flora and fauna, including birds, small mammals and insects (Chamberlain *et al.*, 2000, Tilman *et al.*, 2001, Kleijn *et al.*, 2009, Van Swaay *et al.*, 2013). Specifically, insect decline has adverse effects on ecosystem functioning as they contribute to essential ecosystem processes, such as herbivory (Mattson and Addy, 1975) and nutrient cycling (Yang and Gratton, 2014). Insects are also an important food source for higher trophic levels (Morse, 1971). However, with an estimated contribution to global crop production of between €213 and €523 billion, insect pollination is arguably the most economically valuable ecosystem service under threat (Öckinger and Smith, 2007, Ollerton *et al.*, 2011).

2.2.2 Insect pollination of crops

Crop pollination can enhance productivity for approximately 70% of crops (Klein *et al.*, 2007). Although managed honeybees (*Apis mellifera*) are effective pollinators of crops (Free, 1993), recent studies place greater emphasis on the value of wild pollinators (e.g. bumblebees, solitary bees (*Hymenoptera: Apoidea*) and hoverflies (*Diptera: Syrphidae*) as primary providers of pollination services (Winfree *et al.*, 2008, Breeze *et al.*, 2011, Lowenstein *et al.*, 2015, Rader *et al.*, 2016). Pollinator decline has been widely reported (Biesmeijer *et al.*, 2006, Potts *et al.*, 2010a, Weiner *et al.*, 2014). As many crops benefit from increased yields through greater pollinator abundance (Garibaldi *et al.*, 2011, Garibaldi *et al.*, 2013) and diversity (Kremen *et al.*, 2002, Hoehn *et al.*, 2008), this decline has implications for pollination services and subsequent global food production (Klein *et al.*, 2007, Eilers *et al.*, 2011).

2.2.3 Drivers of pollinator decline

Agricultural intensification has long been identified as a primary driver of pollinator decline. It presents a threat to pollination services for crops (Kearns *et al.*, 1998, Kremen *et al.*, 2002, Klein *et al.*, 2007, Potts *et al.*, 2010a, Kennedy *et al.*, 2013). The need to increase production to feed a growing population has led to agricultural landscape simplification by replacing polycultures with homogeneous monocultures (Benton *et al.*, 2003). Larger field sizes to facilitate the increasing mechanisation of farming practices have also contributed to a gradual loss of semi-natural habitats (Steffan-Dewenter *et al.*, 2002, Tscharntke *et al.*, 2005, Rundlöf *et al.*, 2008, Bommarco *et al.*, 2010) and subsequent loss of foraging resources (Carvell *et al.*, 2006, Goulson *et al.*, 2008, Persson *et al.*, 2015). These losses in resources are still present despite those provided by mass-flowering crops (Westphal *et al.*, 2003, Rundlöf *et al.*, 2014). The resources from mass-flowering crops are limited to short flowering periods and followed by a period of scarcity (Westphal *et al.*, 2009, Diekötter *et al.*, 2010, Kallioniemi *et al.*, 2017). This 'hunger gap' is considered a severe threat to many pollinator groups.

2.2.4 Insect diversity in agricultural landscapes

Agricultural habitats are unsuitable for pollinators for several reasons: (i) regular ground disturbance impedes the nesting habits of ground-nesting species (Shuler *et al.*, 2005); (ii) the increased use of agrochemicals, particularly pesticides can have lethal effects on flora and fauna (Robinson and Sutherland, 2002, Desneux *et al.*, 2007) and (iii) floral resources are considerably sparser in comparison to other habitats (Baude *et al.*, 2016). As a result, biodiversity is mostly concentrated in field margins that contain hedgerows, non-cultivated field margins and wildflower meadows (Bianchi *et* *al.*, 2006, Billeter *et al.*, 2008, Landis, 2017). These habitats provide valuable nesting and overwintering sites which increase pollinator abundance and diversity (Steffan-Dewenter *et al.*, 2002, Öckinger and Smith, 2007, Le Féon *et al.*, 2013, Martins *et al.*, 2015) and pollination services (Ricketts *et al.*, 2008b). Positive associations between habitat and insect diversity have also been found in non-pollinating insects (Kruess and Tscharntke, 2000, Letourneau *et al.*, 2012).

2.2.5 Oilseed rape

Oilseed rape (*Brassica napus;* OSR) is a mass-flowering crop of global economic importance, grown primarily for biodiesel (Van Der Velde *et al.*, 2009). Varieties are bred either conventionally using traditional crossing methods or as hybrids using selected inbred lines (Friedt and Snowdon, 2009). OSR provides a readily available source of nectar and pollen during the flowering season. These resources attract a wide range of insects, such as bees and adult hoverflies (Sabbahi *et al.*, 2005, Jauker and Wolters, 2008, Stanley *et al.*, 2013) as well as parasitic wasps (Russell, 2015), with many also exploiting the resources of nearby co-flowering wildflowers (Stanley and Stout, 2014). With increasing restrictions on pesticide usage and withdrawal of neonicotinoids (European Commission, 2019), farmers have fewer means of controlling pests with negative implications to yield (Hansen, 2004). Pest control services from natural enemies, such as parasitic wasps, are integral to pest management strategies and has particular implications for biocontrol

(Begg *et al.*, 2017). Although OSR is capable of self-pollination (Williams *et al.*, 1986), increased abundance of insect visitors benefits has been shown to increase seed production (Morandin and Winston, 2005, Hayter and Cresswell, 2006). As a result of interspecific differences to environmental conditions, the diversity of pollinators will also help ensure pollination services are resilient to environmental change (Willmer *et al.*, 2017, Winfree, 2013).

2.2.6 Other insect visitors of oilseed rape

Not all interactions between insect visitors and crops are positive. Herbivory by pest species negatively affects the yield of many crops and spreads disease (Oerke and Dehne, 2004, Bos *et al.*, 2007). OSR is host to a diverse group of pest species (Alford *et al.*, 2003). During spring, the most harmful pests are pollen beetles (*Coleoptera: Nitidulidae*) and weevils (*Coleoptera: Curculionidae*), with reported losses of yield ranging from 4% by weevils (Williams, 2010) to 80% by pollen beetles (Nilsson, 1987). Aphids (*Hemiptera: Aphididae*) are also commonly found in OSR crops in Europe (Desneux *et al.*, 2006). With abundant and diverse pest species, OSR is also visited by a diverse group of parasitoids and predators (Büchi, 2002, Nielsen and Philipsen, 2005, Mari and Lohar, 2010, Hatt *et al.*, 2018), with field margins being the preferred overwintering site for many species (Dennis, 1997, Büchi, 2002).

2.2.7 Aims of this study

In this study, we aim to assess the insect communities found during the flowering of OSR over two consecutive years. We will investigate spatial variability by evaluating the effect that proximity to the field margin has on insect abundance and diversity and whether insect communities differ across the two breeding systems used to produce OSR varieties. Using pan traps to sample the invertebrate community of 16 commercial fields in the Central Lowlands of Scotland, UK, we aim to answer the following questions:

1) Which invertebrates inhabit the canopy of OSR in Scotland?
2) How does the OSR invertebrate community differ with proximity to the field margin?
3) Does the breeding system used to produce OSR varieties affect the invertebrate community and if so, are these effects consistent in the field edge and field centre?

We predict that a wide range of invertebrates visit OSR. When compared to communities in the centre, crop edge communities will be more abundant and diverse because of stable resources and nesting availability. We also suggest that nectar-foraging insects, primarily pollinators, will be more abundant in hybrid varieties of OSR because of the increased nectar production (Carruthers *et al.*, 2017).

2.3 Methods

2.3.1 Sites

This study was conducted in eight locations across Mid and East Lothian, Scotland, UK, in 2016 and 2017 (Figure 2.1). These central lowlands of Scotland are temperate in climate. Predominantly used for arable farming, it consists of noncalcareous gleys and brown earth soil (Scotland's Soils, 2019). For each year, four paired-sites were selected and, in each paired-site, two winter-sown oilseed rape fields (mean field size: 9.1 ha; range 3.3 – 20 ha) were selected: one hybrid variety and one conventional variety. All fields had at least one crop edge directly adjacent to semi-natural habitat. For all fields, this was mixed hedgerow (e.g. hawthorn and gorse) and naturally regenerated field margin. Shaded woodland edges were avoided. Sites were paired to minimise landscape variability with a maximum distance between the paired fields of 2 km. To minimise spatial pseudo-replication, the minimum distance between pairs was 4.5 km, further than the maximum foraging distance of most bees (Steffan-Dewenter *et al.*, 2002, Greenleaf *et al.*, 2007, Chifflet *et al.*, 2011). With oilseed used as a break crop and rotated annually, fields selected for sampling were different for each year.

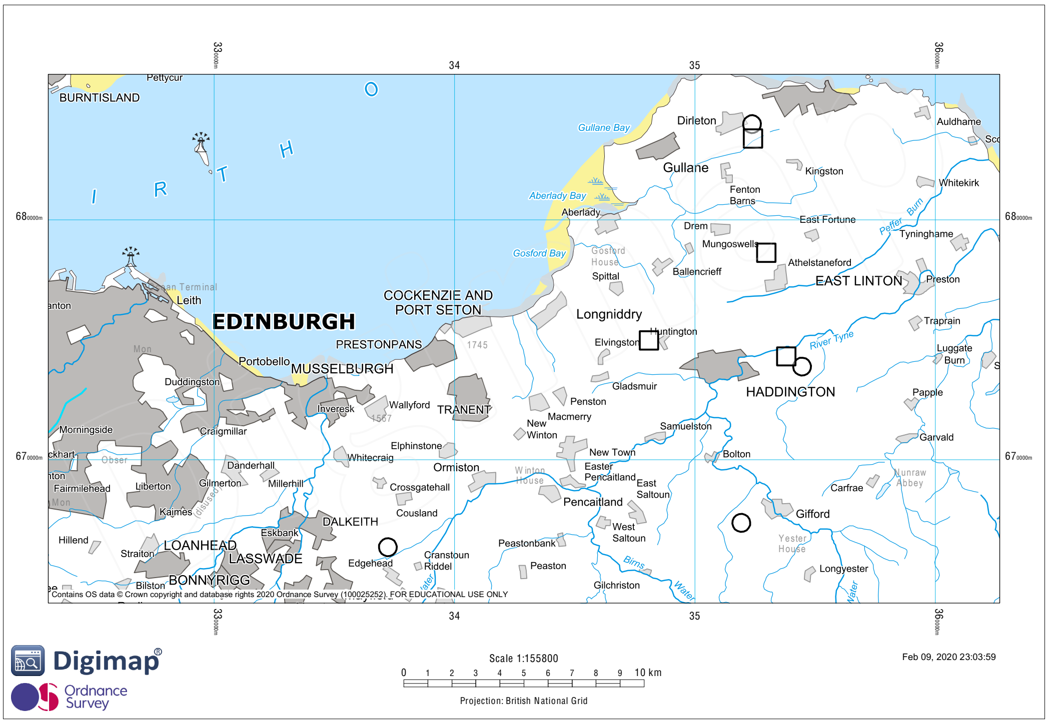

Figure 2.1 Locations used for pan trap sampling in Mid and East Lothian, Scotland for 2016 (circles) and 2017 (squares). Each symbol represents a pair of fields, one sown with a hybrid variety and one with a conventional variety. © Crown copyright and database rights 2020 Ordnance Survey (100025252).

2.3.2 Insect sampling

Insect sampling was undertaken using pan traps. Pan traps are a standard passive insect trapping method for simultaneously sampling multiple locations and are unaffected by collector bias (Kearns and Inouye, 1993, Potts *et al.*, 2005, Westphal *et al.*, 2008). To improve attractiveness to insects and to replicate the colour of OSR flowers, plastic bowls (15 cm diameter and 4 cm in depth; 500 cm^3 volume) with yellow UV-reflecting paint (Sparvar Leuchtfarbe, Spray-Color Gmbh, Merzenich, Germany) sprayed on the inside were used. Three 3 mm holes were drilled below the trap brims to allow rainwater to drain. Traps were fixed to bamboo canes to allow for positioning at crop height (Figure 2.2) (Westphal *et al.*, 2008). Traps in the crop edge were positioned 1 m into the crop.

Figure 2.2 Experimental setup of pan traps in oilseed rape fields. Yellow traps were positioned within the crop canopy before sampling. (Photo: SMF).

Five traps were arranged linearly in the centre of the field. As study sites were of different sizes, these traps were positioned at least 75 m from the nearest crop edge to ensure uniformity within all fields. A further five traps were positioned 1 m in from the crop edge, adjacent to the semi-natural field margin (Figure 2.3). Traps were positioned 25 m apart along the study area and contained water and a few drops of unscented detergent to reduce surface tension. Sampling took place for 48-hours during peak-flowering for four consecutive weeks on days with a dry forecast and low winds (18/5/16 – 8/6/16 and 3/5/17 – 24/5/17). After 48-hours, the trap contents were collected and stored in alcohol before identification. With over 33,000 individuals collected throughout the study, insects were identified to family level, where possible, in the laboratory. Non-syrphid flies were grouped by antenna morphology. To investigate the effect of trap position, year and OSR breeding system on different insect communities, insects were grouped into broad taxonomic guilds (Table 2.1): conventionally recognised pollinators, most common OSR pests, non-pollinating beneficial insects and non-syrphid flies. Microclimate variability was reduced by aggregating samples for the five traps in a transect to analyse the abundance for each field edge and centre. Samples were collected from all 16 sites over the two years. However, inclement weather in the final week of sampling in 2016 resulted in almost a total loss of samples. Hence, only samples collected during the first three weeks of flowering (18/5/16 – 1/6/16) were included in this study, compared to four weeks in 2017.

Table 2.1 Major insect groups divided into broad taxonomic groups.

Pollinators	**Pests**	**Beneficials**	**Non-syrphid flies**
Bumblebees	Pollen beetles	Parasitic wasps	Thread horn
Honey bees	Weevils	Predator beetles	Lower Brachycera
Solitary bees	Aphids	Spiders	Circular-seamed
Hoverflies			

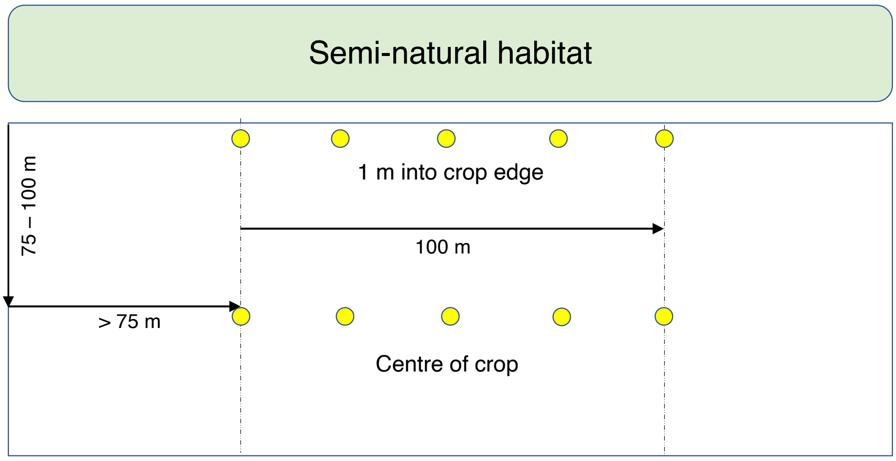

Figure 2.3 Configuration of pan traps in oilseed rape fields. Traps were positioned 25 m apart along the crop edge and the centre of the field. The distance between the semi-natural habitat and the crop edge was no greater than 2 m. Yellow circles represent pan trap position.

2.3.3 Statistical analysis

Before analyses, the five pan trap samples in a specific location and sampling date were summed for each taxa/guild. Due to sparsity of data (i.e. low densities of certain taxa on specific sampling dates) and to control for the differences in sampling dates, data were then averaged across the four sampling dates in a year. Differences in community composition, associated with trap position, breeding system and sampling year, were visually compared using non-metric, multidimensional scaling ordinations (NMDS), based on the Bray-Curtis dissimilarity matrix. To compare insect community composition between trap locations and year, a permutational multivariate analysis of variance (PERMANOVA), using the Bray-Curtis dissimilarity matrix and 999 permutations of residuals was performed. SIMPER (Similarity Percentage) analyses were used to determine the taxa driving the differences within each taxonomic guild (Clarke, 1993). Invertebrate diversity was measured using the Shannon-Wiener Diversity Index (Shannon, 1948). The effects of trap location, breeding system and sampling year on taxonomic guilds and key taxa were examined by aggregating the data and with log transformations used to meet normality assumptions. Linear mixed models (LMM) were used, with trap location (centre vs edge), breeding system (conventional vs hybrid) and year (2016 vs 2017) as fixed effects. To control for spatial variability and to account for different farming practices, pair, representing geographical location, and farm were used as random effects. Models were fitted with and without interactions between the fixed effects. Models were compared using Bayesian information criterion (BIC), with the most parsimonious models selected. Residual plots validated models for normality and standardised residuals for heteroscedasticity. All analyses were conducted using R version 3.6.1 (R Core Team, 2019). Ordination and diversity analyses were performed using the vegan package (Oksanen *et al.*, 2019). LMMs were fitted using the lme4 package (Bates *et al.*, 2015).

2.4 Results

2.4.1. Overview of invertebrate sampling

A total of 33,517 individuals from ten broad taxonomic orders were collected during sampling. Diptera and Coleoptera were most dominant, accounting for 93% of all specimens collected.

2.4.2 Community composition

The communities of invertebrates were significantly different across trap location and/or year for all taxonomic guilds. No differences were found across breeding systems. Pollinator and non-syrphid fly communities differed across trap location and year, whereas pests only differed across sampling years and beneficials only differed across trap location (Table 2.2a; Figure 2.4). SIMPER analysis indicated that wild bees (bumblebees and solitary bees) drove differences in pollinator communities. Pollen beetles and weevils contributed to dissimilarities in pests, and parasitic wasps and circular-seamed flies influenced beneficials and non-syrphid fly community compositions, respectively.

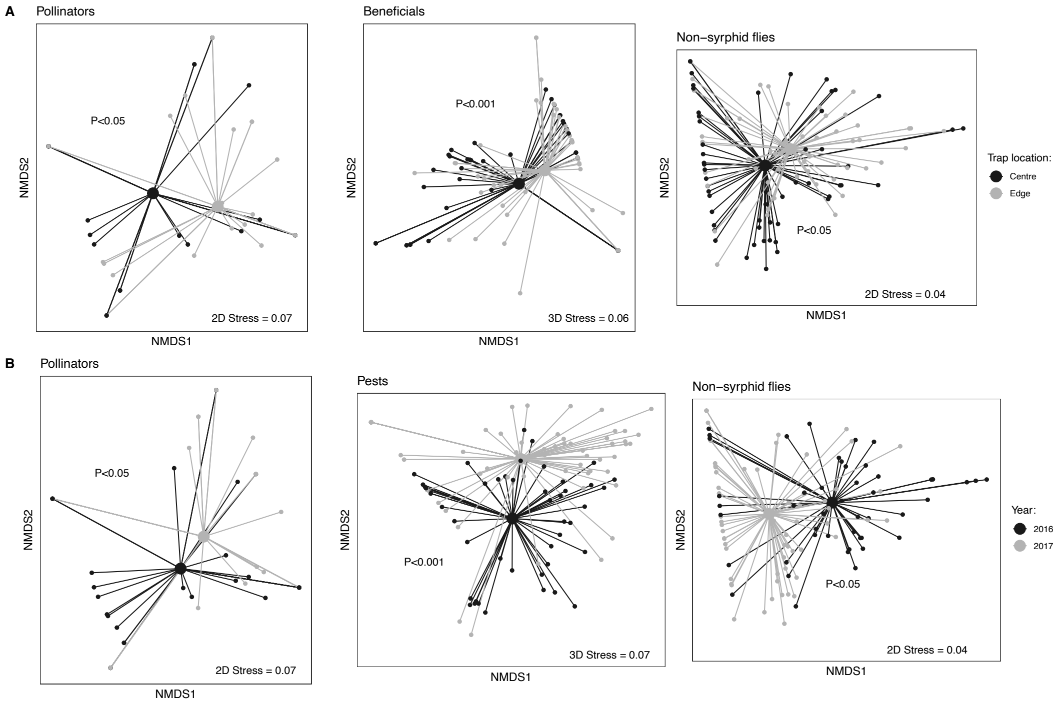

Figure 2.4 Non-metric multidimensional scaling (NMDS) analysis examining differences in community composition for pollinators, beneficials and non-syrphid flies as sampled by pan traps in oilseed rape fields in 2016 and 2017. Plots are grouped by **A** trap location and **B** year. The centre of each spider plot indicates the centroid of the group. The greater the distance between the centroids indicates the differences in community composition. Ordination dimensions, stress values and P values from PERMANOVA analysis are labelled.

2.4.3 Invertebrate abundance and diversity

Invertebrate abundance (F = 9.99, P <0.01) and diversity (F = 39.08, P <0.001; Figure 2.5) were significantly greater in the crop edge than the centre. Invertebrate diversity was also significantly affected by year (F = 13.54, P <0.01; Figure 2.5). Differences resulting from the cultivar breeding system were non-significant for abundance and diversity.

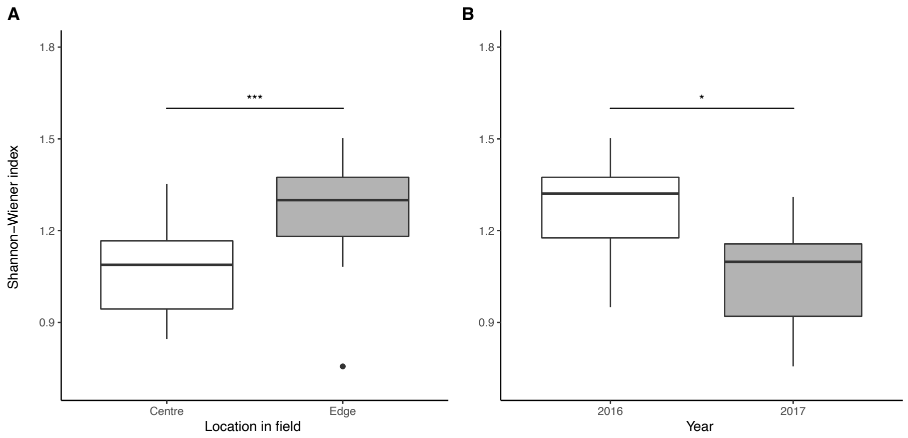

Figure 2.5 Effect of: **A** trap location and **B** year on invertebrate diversity (mean Shannon-Wiener ± SE). Boxes around the median shows interquartile range. Whiskers extend to minimum and maximum values.
Significance: ***P < 0.001; *P<0.05.

Invertebrates typically identified as pollinators (i.e. bumblebees, solitary bees, honeybees and hoverflies) formed approximately 1% of all individuals trapped. Within this guild, wild bees were more abundant than honeybees (Figure 2.6A). Bees comprised of 92% of pollinators sampled and thus were the primary drivers of the LMM results for pollinators. The most abundant taxa of the pest guild were pollen beetles and weevils (64% and 33% of all pests, respectively; Figure 2.6B). Of the non-predatory beneficial invertebrates, parasitic wasps were most abundant, with 84% of all individuals within this taxonomic guild (Figure 2.6C). Rove beetles were the most abundant taxa of the predators (12% of all beneficials). Non-syrphid flies were grouped by antenna morphology. The most abundant group were circular-seamed flies, with 75% of all flies trapped (Figure 2.6D).

Table 2.2 Results for statistical analysis of the insect community in oilseed rape. (a) PERMANOVA tests for differences in community composition across trap location and year. F-values and direction of effect for the linear mixed-effect models fitted to the pan trap data for (b) each of the four guilds and (c) key taxa. No significant differences were found across breeding system and are therefore omitted.

Significance: ***P < 0.001; **P<0.01; *P<0.05.

	Trap location	**Year**
(a) PERMANOVA	*F-value*	*F-value*
Pollinators	3.74*	2.60*
Pests	1.74	12.09***
Beneficials	7.07***	2.23
Non-syrphid flies	2.7*	3.68*

	F-value	*Effect ↑*	*F-value*	*Effect ↑*
(b) Guilds				
Pollinators	0.09		7.77*	2016
Pests	7.59*	Crop edge	3.27	
Beneficial predators	2.66		0.14	
Non-syrphid flies	0.99		1.45	
(c) Key taxa				
Bees	0.00		11.26*	2016
Pollen beetles	9.85**	Crop edge	10.03**	2016
Weevils	0.82		9.46*	2016
Aphids	7.86*	Crop edge	1.33	
Parasitic wasps	23.60***	Crop edge	0.34	

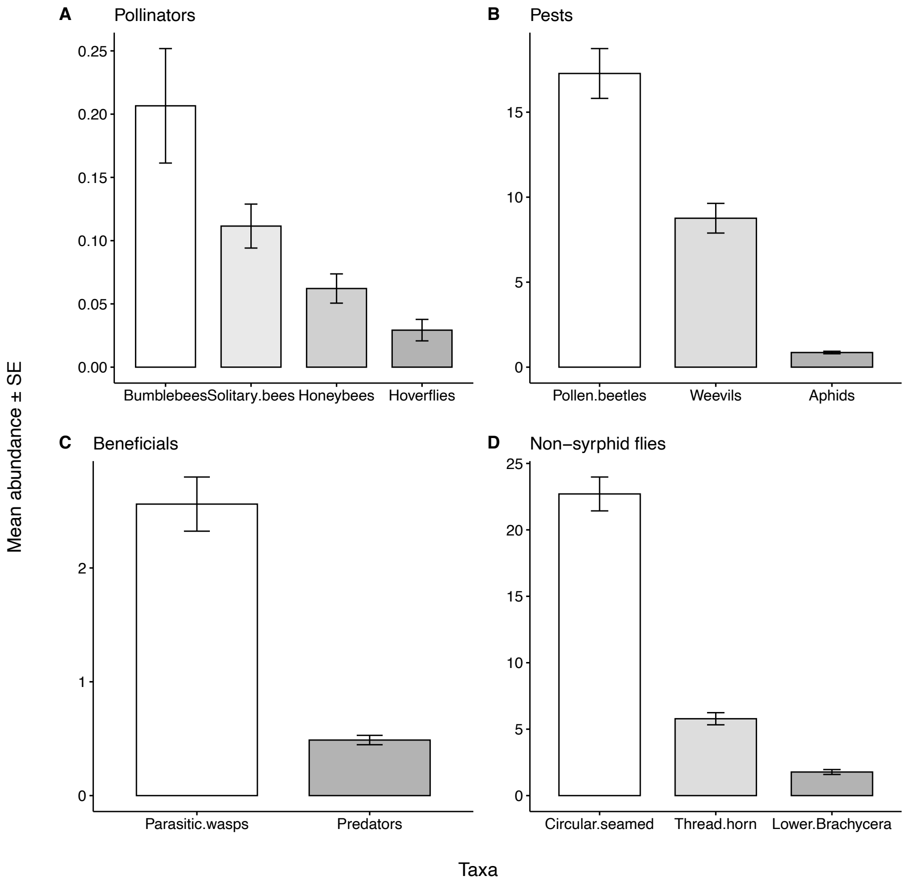

Figure 2.6 Mean abundance per trap (± standard error) for key taxa found in oilseed rape across sample sites; grouped by taxonomic guild.

2.4.4 Effect of spatial variability

Bumblebee and honeybee abundance were slightly higher towards the centre of the crop, but this was not the case for solitary bees and hoverflies. However, these differences were not significant. Similarly, when all pollinators were grouped, there was no significant difference in abundance between field edges and centres (Table 2.2b). Mean abundance was significantly higher in the crop edge than the centre for individuals within the pest guild (Table 2.2b; Figure 2.7A), driven by similar trends for pollen beetles and aphids (Table 2.2c, Figure

2.7B, C). Beneficial predators (i.e. spiders and predatory beetles) were not significantly affected by trap location. The mean abundance of parasitic wasps was significantly higher in the crop edge (Table 2.2c, Figure 2.7D). No differences were found in non-syrphid fly abundance as a result of trap location alone. However, differences across trap location were not consistent between breeding systems, with a larger population in the crop edge in conventional varieties, but more abundant in the centre in hybrid varieties ($F = 4.54$, $P < 0.05$).

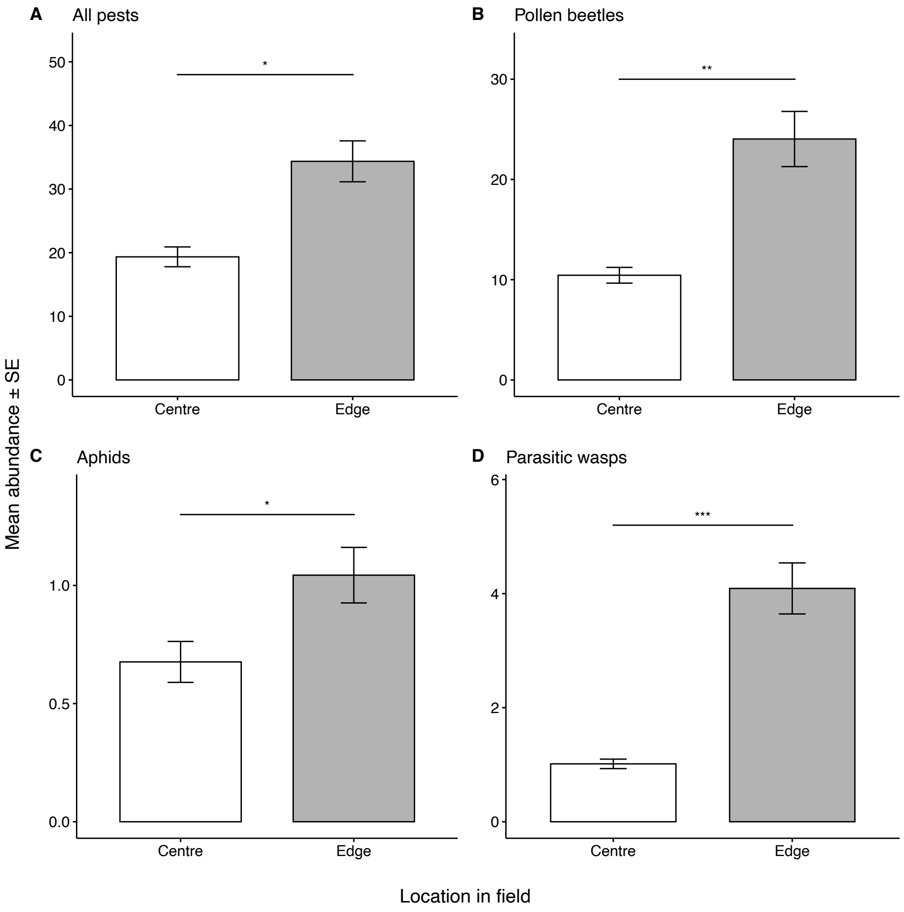

Figure 2.7 Mean abundance per trap (± standard error) for: **A** all pest species, **B** pollen beetles, **C** aphids and **D** parasitic wasps collected in the crop centre and edge of oilseed rape fields. Significance: ***P < 0.001; **P<0.01; *P<0.05 determined via LMM.

2.4.5 Effect of temporal variability

Pollinator abundance, driven by bee abundance, was significantly affected by year (Table 2.2b, c), with higher numbers present during 2016 (Figure 2.8A, B, Table 2.2). Aggregated pests and beneficial predators were not affected by year. Of the key taxa within these guilds, there were significantly more abundant populations of pollen beetles and weevils in 2016 (Table 2.2b, Figures 2.8C, 2,8D). Non-syrphid fly abundance was consistent across sampling years (Table 2.2a).

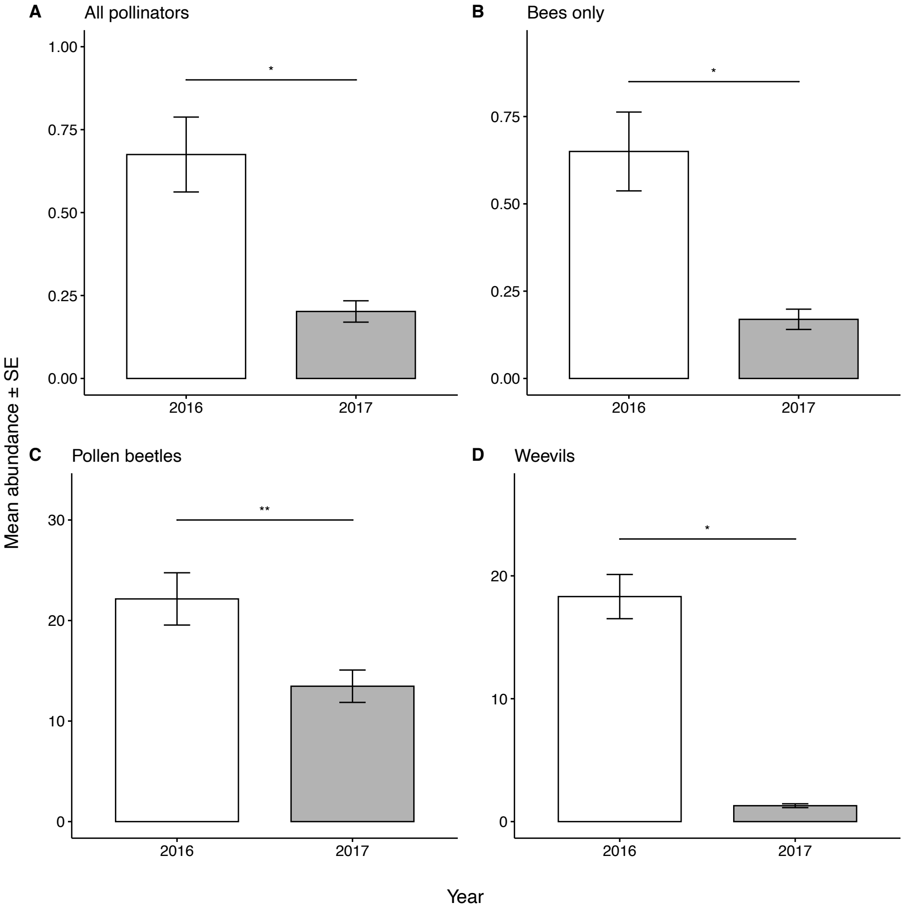

Figure 2.8 Mean abundance per trap (± standard error) for: **A** all pollinator species, **B** bees, **C** pollen beetles and **D** weevils collected in oilseed rape fields during 2016 and 2017. Significance: **P<0.01; *P<0.05.

2.5 Discussion

2.5.1 Pollinators

Oilseed rape provides essential forage for pollinators (Westphal *et al.*, 2003, Morandin and Winston, 2005, Holzschuh *et al.*, 2011), particularly so for winter oilseed rape which flowers early in the season when alternative forage is scarce (Westphal *et al.*, 2009). Despite this, we found that the abundance of key pollinator taxa (i.e. bumblebees, solitary bees, honey bees and hoverflies) were relatively low. A greater abundance of wild bees (i.e. bumblebees and solitary bees) compared to honeybees suggests that wild bees may offer a more significant contribution to pollination in oilseed rape. This agrees with findings from sweet cherry (Holzschuh *et al.*, 2012) and apples (Földesi *et al.*, 2016). Wild bee abundance and diversity is also crucial for providing most of the pollination services in agricultural and urbanised landscapes (Garibaldi *et al.*, 2013, Kleijn *et al.*, 2015, Lowenstein *et al.*, 2015). Hoverflies have also been identified as important pollinators of OSR (Jauker and Wolters, 2008). However, with so few found during sampling (i.e. 16 across the two study years), and since hoverfly densities are required to be five-fold those of wild bees to achieve similar yields (Jauker *et al.*, 2012a), hoverfly contribution to the pollination of winter oilseed rape in this area of Scotland is likely negligible. Although we focused on common pollinators (i.e. bees and hoverflies), the value of non-bee pollinators should not be overlooked. Insects such as non-syrphid flies, beetles and wasps are also regular flower visitors. These flower visitors lack the adapted morphology to enable the movement of large amounts of pollen in a single visit, such as branched hairs and pollen baskets. However, this could be outweighed by higher visitation rates, resulting in a pollination service provision which is not significantly different from that provided by bees (Rader *et al.*, 2016). Inconsistencies in non-syrphid fly populations between crop edge and centre are not surprising. Although limited in pollen-carrying capabilities, flies carry pollen over greater distances (Rader *et al.*, 2011), allowing them to penetrate the agricultural matrix to pollinate OSR.

2.5.2 Pests

Of the pest taxa collected OSR pollen beetles dominated. Although several species of pollen beetle occur on OSR, the most common is *Brassicogethes aeneus,* a species that became a brassica specialist within the first 16 years of oilseed rape cultivation (Hokkanen, 2000). This species is one of the most economically important pests found in OSR (Alford et al., 2003). When found in large numbers, yield reductions as high as 53% are reported (Schneider *et al.*, 2015). Weevils are also common pests found in OSR crops in Europe (Williams, 2010). The most damaging weevil species to winter-sown OSR being the cabbage seed weevil (*Ceutorhynchus obstrictus*) (Free and Williams, 1979). Direct damage caused by adults is minimal, although yield-damaging losses of up to 18% are caused by larvae feeding within the pods (Williams and Free, 1978).

2.5.3 Beneficial invertebrates

Ground beetles (*Coleoptera: Carabidae*), rove beetles (*Coleoptera: Staphylinidae*) and spiders (*Araneae: Linyphiinae*) are among the most abundant invertebrate predators found in arable ecosystems (Sunderland, 2002, Nyffeler and Sunderland, 2003). However, despite trapping several predatory individuals during pan trap sampling, pitfall traps are a more successful method for capturing surface-living invertebrates (Sutherland, 2006). However, pitfall traps are less likely to accurately monitor predatory activity higher in the crop canopy, where pest populations are more prevalent. Parasitic wasps (*Hymenoptera: Apocrita*), which are effectively trapped by pan traps, were unsurprisingly the most abundant within our beneficial invertebrate guild. We found similar trends in abundance for parasitic wasps and the most common pests (e.g. pollen beetles, weevils and aphids). With pests species being host to at least 80 species of parasitic wasps from 15 different families (Ferguson *et al.*, 2010), finding similarities in their assemblages was expected. An example of parasitism in OSR involves the parasitic wasp *Diaeretiella rapae* and the cabbage aphid (*Brevicoryne brassicae*), one of the most common aphid species found in OSR (Desneux *et al.*, 2006). The effectiveness of *D. rapae* at controlling aphid populations is reflected in their successful use as a biological control in cabbages (Zhang and Hassan, 2003).

2.5.4 Non-syrphid flies

Species composition of non-syrphid flies is driven by the vast abundance of circular-seamed flies (*Diptera: Cyclorrhapha*) that were collected. Although Diptera were not identified to species level, the most abundant species of this suborder commonly found in OSR is the root fly (*Delia* spp.) (Alford *et al.*, 2003). As with weevils, plant damage is caused by larvae rather than adults; the primary damage being root destruction, with secondary damage caused by exposure to root rot pathogens as a consequence of larval feeding (Soroka *et al.*, 2004). While the pollination services provided by hoverflies is well documented (Jauker *et al.*, 2012a, Földesi *et al.*, 2016), other taxa within the *Diptera* order are often overlooked. Non-syrphid flies are the most abundant flower-visiting *Diptera* in agricultural environments, capable of delivering pollination services equal to those of hoverflies (Orford *et al.*, 2015, Rader *et al.*, 2016). With non-syrphid flies found in such large numbers during this study, it suggests that they, and not those conventionally recognised as pollinators (i.e. bees and hoverflies) are the key pollinators of OSR in Scotland.

2.5.5 The spatial distribution of insect communities

The value of semi-natural habitat to the abundance and diversity of insects, particularly wild pollinators, is well-documented (Steffan-Dewenter *et al.*, 2002, Öckinger and Smith, 2007, Diekötter *et al.*, 2014, Martins *et al.*, 2018). Semi-natural habitat provides food sources, shelter and nesting sites for a wide range of insects (Cane *et al.*, 2007, Steffan-Dewenter and Schiele, 2008, Földesi *et al.*, 2016, Kremen *et al.*, 2018). Overall, invertebrate abundance was typically higher in the crop edge adjacent to semi-natural habitat than the centre of a homogeneous mass-flowering crop. Although unsupported statistically, more bumblebees were present in the centre of the crop, while solitary bees were more abundant at the crop edge.

Dietary preference may explain differences in bee assemblage. The majority of solitary bees found on farmland are generalist foragers with a polylactic diet, preferring to forage from a broader diet of plants (Wood *et al.*, 2016). Typically, field margins provide a more heterogeneous forage than that found within mass-flowering crops. Solitary bees, particularly early-spring flying *Andrena* species, have been found to favour OSR margins over those of other arable crops (Le Féon *et al.*, 2013). Small-bodied generalist bees are more impacted by habitat loss, although these effects decrease for those with a broader dietary niche, particularly larger-bodied bees (Bommarco *et al.*, 2010). Spatial variability across bee taxa may also be influenced by foraging behaviour, where a relationship has been found between bee body size and foraging range (Greenleaf *et al.*, 2007). Solitary bees have a relatively small foraging range compared to those of bumblebee species (Gathmann and Tscharntke, 2002, Knight *et al.*, 2005a). This suggests that local, more diverse resources may be more important to solitary bees than those distant from nesting sites.

Both aphids and pollen beetles were more abundant in the crop edge than the field centre. This is contrary to the evidence that pollen beetles prefer to overwinter within the oilseed crop than in semi-natural habitat (Sutter *et al.*, 2018). The natural enemies of pest species (e.g. parasitic wasps) were also more abundant in the crop edge than the centre of the field. Many beneficial insects complete their lifecycle in semi-natural habitats, such as field margins and hedgerows (Landis *et al.*, 2000). Emergence from these overwintering sites may explain a greater abundance of parasitic wasps in the crop edge. Additionally, higher prey densities may contribute to this trend, with high predator-prey ratios found within OSR fields (Sutter *et al.*, 2018). Using natural enemies to suppress pest populations offers economic and environmental benefits, including reductions in yield loss and pesticide use (Naylor and Ehrlich, 1997). Semi-natural habitat supports biological control by increasing the species-richness of natural enemies, resulting in higher pest suppression (Letourneau *et al.*, 2009, Holland *et al.*, 2017). Management of field margins to optimise beneficial insects, while minimising pests is an effective strategy. Kaasik *et al.* (2014) report pollen beetles being lured away from OSR, during the stage most affected by damage, by cruciferous weeds. A longer-term study in Germany demonstrated that OSR field margins, undisturbed for six years, improved parasitism in the centre of the crop pursuant to that in the crop edge (Thies and Tscharntke, 1999).

2.5.6 The temporal distribution of insect communities

Although an investigation of temporal variability was not an aim of this study, we found it to influence pollinator abundance, as well as the abundance of pollen beetles and weevils. With only two time points (2016 vs 2017), it is challenging to make distinct assumptions from the statistical analysis without comprehensive information on environmental conditions. In addition to climatic variability between the two years, another explanation may arise from insect emergence phenology. In 2017, OSR crops in the study area started flowering two weeks earlier than in 2016.

Should environmental conditions have such an effect on flowering phenology over consecutive growing seasons, it may highlight the effect of climate change in the future. Indeed, on visual evidence, very few pollinators were spotted in the crop during the early part of the 2017 flowering season. The timing of flowering is essential for pollinator populations. Environmental conditions (e.g. rainfall, temperature and light) contribute to phenological variation, although insect phenology is more sensitive to temperature than plants (Gordo and Sanz, 2005, Parmesan, 2007). These phenological shifts are particularly evident during the spring, creating mismatches between resource availability and pollinator emergence (Hegland *et al.*, 2009, Thomson, 2010, Bartomeus *et al.*, 2011). To suggest a phenological mismatch between insect emergence and OSR flowering in this study, would need to be supported by long-term studies.

2.5.7 The impact of breeding system on insect community

Despite our predictions that pollinator abundance would be higher in nectar-rich hybrid varieties (Carruthers *et al.*, 2017), this was not the case. The breeding systems used to produce OSR cultivars had no effect on abundance or diversity for any of the insect guilds that we sampled. The lack of breeding system effect here may be influenced by the sampling method. Though pan traps have been used to successfully sample pollinator populations (Westphal *et al.*, 2008), they do not accurately reflect visitation (Roulston *et al.*, 2007).

2.5.8 Conclusion

Mass-flowering crops, such as oilseed rape, provide essential habitat for a diverse group of invertebrates, not only in terms of floral resources for pollinators but for pest species and their natural enemies. This study highlights the spatial variability of invertebrate abundance and diversity in an arable landscape. It supports other research that emphasises the importance of semi-natural habitat for shelter and forage resources for a wide range of insects. These include species that provide important ecosystem services with positive (i.e. pollination and pest control) and adverse consequences (pests) (Van Buskirk and Willi, 2004, Power and Stout, 2011, Castle *et al.*, 2019). We show how field edges attract not only more individuals but also a more diverse invertebrate community than field centres. When focussing on key taxa, pollinator numbers were found to be exceptionally low compared to other invertebrate guilds. Potentially due to this data scarcity, pollinator abundance did not differ between field margins or field centres, contradicting studies reporting the opposite (Power and Stout, 2011, Stanley and Stout, 2013). We found populations of pest species (i.e. pollen beetles and aphids) to be higher in field edges than in field centres, as were their natural enemies. These relationships could be due to margins providing refuge for both pests and beneficial insects, or that natural enemies were responding to pest occurrence, congregating in area of high density.

Field margins support diverse invertebrate communities. The higher abundance of invertebrates found in the crop edge indicates that field margins provide adequate resources for both pests and beneficial insects. Consequently, there is a need to determine margin prescriptions that increase beneficial insects while limiting pest numbers. With pest species often particular on the plants they feed on, providing alternative host plants in field margins may control populations in the crop. For pollinators and parasitic wasps, field margins must provide sufficient forage before, and particularly after flowering, to enable assemblages to reproduce.

Chapter 3

Pollinators enhance oilseed rape seed production: the effect of insect pollination in a mass-flowering crop

3 Pollinators enhance oilseed rape seed production: the effects of insect pollination in a mass-flowering crop

3.1 Abstract

Insect pollination is a valuable ecosystem service, with agricultural productivity increasing in 70% of the 124 leading global crops grown for human consumption. Current pollinator declines, driven by habitat loss and the increased use of pesticides, threatens global production through reduced crop yield. Insect visitation has positive effects on seed production for many crops, including the economically important mass-flowering crop, oilseed rape (*Brassica napus*).

The contribution of insect pollination to oilseed rape yield was estimated using pollinator exclusion on flowers in sixteen fields, over two years, in an arable dominated landscape. Three yield metrics were explored, seeds set, seed weight per pod and individual seed weight. Resource allocation, when plants face pollen limitation, was also tested by pollinator exclusion experiments within plants. The relationship between pollinator populations and the proportional change in yield from pollinator visitation was also investigated using pollinator abundance data obtained from pan traps.

Insect pollination had a positive effect on yield, increasing seed set by 23% and seed weight per pod by 29%. These increases were consistent across oilseed rape breeding systems. The reallocation of resources within the plant occurred in one year only. When faced with pollen limitation, plants

redistributed resources from pollinator-excluded racemes to those available to pollinators, resulting in a higher seed weight in comparison to untreated plants. Resource allocation was also present at the pod level, where resources were invested in producing either a larger quantity or heavier seeds. Since seed weight relates to seed quality, this indicates a potential trade-off between yield and quality. However, yield gains in insect-pollinated crops were of a much higher magnitude than seed weight losses.

Evidence suggesting that increasing pollinator abundance, as measured by pan trapping, affects the pollinator contribution of oilseed rape yield was not observed. This indicates that the abundance of pollinators captured by pan traps may not be an appropriate proxy to monitor pollination services in OSR crops.

This study emphasises the benefit of insect pollination and the role it plays in achieving maximum returns from oilseed rape. It highlights the temporal variability found within agricultural landscapes and the consequential unpredictability of crop cultivation. Our study indicates that when faced with pollination deficits, plants can redistribute resources, helping stabilise economic returns under ever-changing conditions. To exploit the effects of pollination to their potential, farmers should invest in pollinator management, such as agri-environment schemes. Providing sufficient resources, particularly before and after the flowering of mass-flowering crops, will support colony management by providing food stability at the farm scale, ensuring pollinators are supported throughout their lifespan.

3.2 Introduction

3.2.1 Insect pollination and potential consequences of pollinator decline

Insect pollination contributes to global food production in an estimated 70% of crop species (Klein *et al.*, 2007) and 88% of wild plants (Ollerton *et al.*, 2011). It is considered an important ecosystem service (Potts *et al.*, 2010a, Gallai *et al.*, 2009, Winfree *et al.*, 2011). Understanding the benefits of insect pollination is fundamental to providing food to a growing global population. However, pollination services provided by wild pollinators are at risk with reported declines, threatening the stability of this ecosystem service and subsequent food production (Biesmeijer *et al.*, 2006, Potts *et al.*, 2010a, Cameron *et al.*, 2011, Goulson *et al.*, 2015). Almost 50% of European farmers perceive pollination deficits in their crops, indicating that yield loss as a result of insufficient pollination services may already be widespread (Breeze *et al.*, 2019). Agricultural intensification is a primary driver of pollinator decline (Kremen *et al.*, 2002, Potts *et al.*, 2010a) with larger field sizes and loss of semi-natural habitat (Steffan-Dewenter *et al.*, 2002, Rundlöf *et al.*, 2008, Kennedy *et al.*, 2013) and increased agri-chemical use (Robinson and Sutherland, 2002) to blame.

3.2.2. The effect of insect pollination on crops

Studies attempting to quantify the contribution of insect pollination to seed production of crops are numerous; including pumpkins (Hoehn *et al.*, 2008), apples (Garratt *et al.*, 2014), strawberries (Horth and Campbell, 2018) and oilseed rape (Kołtowski, 2005, Bommarco *et al.*, 2012b, Adamidis *et al.*, 2019). Insect pollination consistently enhances crop production (i.e. yield and quality) in most crops in response to increased pollinator density (Sabbahi *et al.*, 2005, Gaines-Day and Gratton, 2016), diversity (Hoehn *et al.*, 2008), visitation rate (Jauker and Wolters, 2008, Jauker *et al.*, 2012a) and abundance (Kremen *et al.*, 2002, Brittain *et al.*, 2013). Although managed honeybees are significant pollinators of crops worldwide (Free, 1993), the pollination services provided by wild bees (i.e. bumblebees and solitary bees) are of greater value to crop production and can provide greater stability under environmental change (Winfree *et al.*, 2008, Garibaldi *et al.*, 2013, Lowenstein *et al.*, 2015). Non-bee pollinators, such as butterflies (*Lepidoptera*) and flies (*Diptera)*, are also being recognised as efficient pollinators of crops and are regularly included in pollination studies (Rader *et al.*, 2016, Woodcock *et al.*, 2019).

3.2.3. Resource allocation

Plants have developed strategies to allow them to optimise reproductive fitness when faced with environmental change. Crop plants can redistribute resources within the plant from one yield metric (e.g. seed set) to another (e.g. seed weight) when necessary (Steffan-Dewenter, 2003, Bos *et al.*, 2007). For example, oilseed rape has such capacity when confronted with pollen limitation, where some cultivars produced fewer seeds but with a higher seed weight when pollen was restricted (Williams *et al.*, 1986). Most pollination studies measure the effect of insect pollination between treatments across plants, and the effect of insect pollination on resource allocation within the plant is relatively unknown, particularly in oilseed rape.

3.2.4 Oilseed rape

Oilseed rape (*Brassica napus;* OSR) is an economically important mass-flowering crop, cultivated on 34.9 million hectares with a global commercial value more than $75 billion per annum (USDA, 2019). Primarily grown for vegetable oil and animal fodder, targets to increase the use of renewable fuels in the transport industry have seen OSR cultivated as a source of biodiesel (Van Der Velde *et al.*, 2009). Although OSR is capable of self-pollination by wind (Williams *et al.*, 1986), the production of readily available floral resources encourages the visitation of a wide range of insect pollinators (Jauker *et al.*, 2012a, Stanley *et al.*, 2013, Zou *et al.*, 2017). Indeed, insect pollination has positive effects on the yield of OSR worldwide (Woodcock *et al.*, 2019). Although increases in yield of 46% and 50% have been reported by Sabbahi *et al.* (2005) and Araneda Durán *et al.* (2010) respectively, more modest estimates of the effect of insect pollination are approximately 20% from studies that manipulate insect visitation (Manning and Wallis, 2005, Bommarco *et al.*, 2012b, Bartomeus *et al.*, 2014).

3.2.5 Aims of this study

In this study, we assessed the value of insect pollination to oilseed rape in an agricultural landscape in the Central Lowlands of Scotland. OSR is subject to intense commercial breeding programmes, and varieties are either bred conventionally or as hybrids (Friedt and Snowdon, 2009). Although the effect of insect pollination has been compared between breeding systems in other parts of Europe (Lindström *et al.*, 2016, Steffan-Dewenter, 2003), we believe this to be the first using OSR varieties recommended for cultivation in Scotland. Through pollinator exclusion experiments in 16 fields over two consecutive years, we will attempt to estimate the value of insect pollination to OSR varieties across both breeding systems. We will also investigate whether, when faced with pollen limitation, OSR plants redistribute resources to other parts of the plant by varying the pollinator treatment within the same plant. Using pollinator abundance as a proxy for insect visitation frequency (Woodcock *et al.*, 2019), we will also explore the differences between the pollinator contribution of OSR yield metrics with pollinator populations. During this study, we aim to answer the following questions:

1) What is the contribution of insect pollination on oilseed rape yield metrics (i.e. seed set and weight), and is this effect consistent across both OSR breeding systems?
2) When pollen is limited, are OSR plants capable of redistributing resources to other parts of the same plant?
3) Does the contribution of insect pollination on oilseed rape yield increase with pollinator abundance?

We predict that insect pollination will increase the number of seeds and seed weight per pod but decrease the individual seed weight as the plant allocates resources to produce either more lighter seeds when pollination is adequate, or fewer heavier seeds when experiencing pollination deficits. We also predict resource reallocation to be evident through increased yield metrics in open-pollinated racemes when other racemes are pollen limited. Since conventional varieties having a higher genetic diversity than hybrid varieties (Tommasini *et al.*, 2003), we expect the benefits of insect pollination will be more significant in conventional varieties. Finally, we predict that increased pollinator abundance will have a positive effect on the pollinator contribution of seed set and seed weight per pod.

3.3 Methods

3.3.1 Sites

This study was conducted in eight locations across Mid and East Lothian, Scotland, UK, in 2016 and 2017 (Figure 3.1). These central lowlands of Scotland are temperate in climate. Predominantly used for arable farming, it consists of noncalcareous gleys and brown earth soil (Scotland's Soils, 2019). Each year, eight fields sown with winter varieties of oilseed rape were selected (mean field size: 9.1 ha; range 3.3 – 20 ha). All sites had at least one crop edge directly adjacent to semi-natural habitat (mixed hedgerows containing hawthorn and gorse). Landscape variability was minimised by pairing all sites geographically, with each pair consisting of a field sown with a conventional and a hybrid cultivar. The maximum distance between fields within a pair was 2 km. To minimise spatial pseudo-replication, the distance between different pairs was at least 4.5 km. This is beyond the maximum foraging distance of most bees (Steffan-Dewenter *et al.*, 2002, Greenleaf *et al.*, 2007, Chifflet *et al.*, 2011).

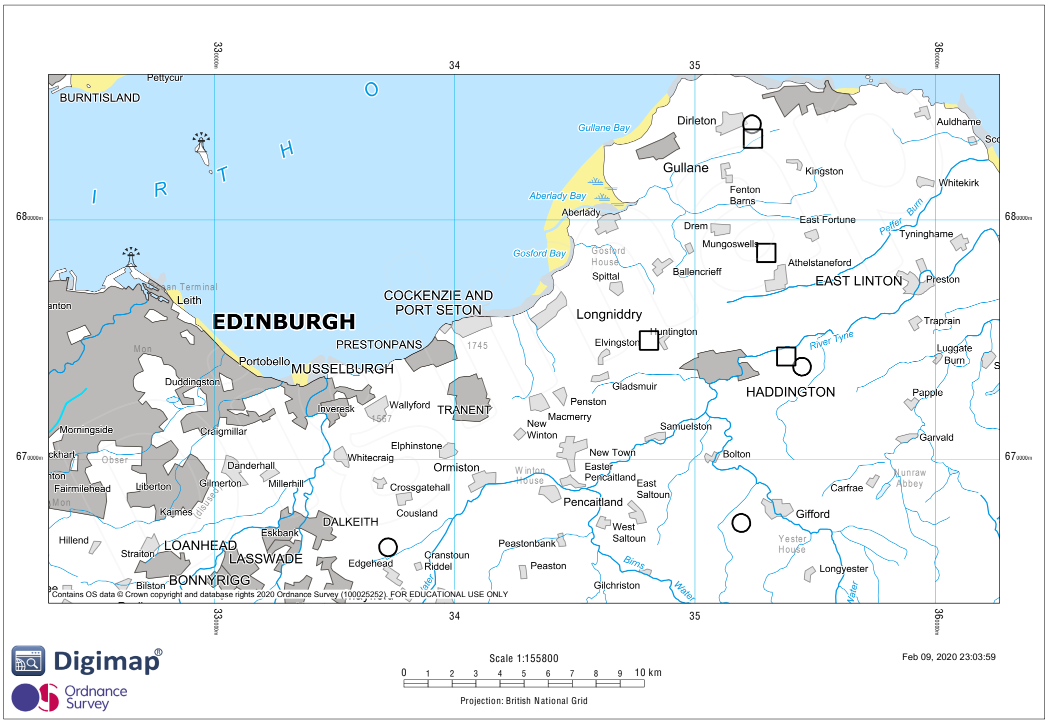

Figure 3.1 Locations of field pairings used for yield analysis in Mid and East Lothian, Scotland for 2016 (circles) and 2017 (squares). © Crown copyright and database rights 2020 Ordnance Survey (100025252).

3.3.2 Insect exclusion

To measure the effect of insect pollinators on yield metrics, a blocking experiment was established with ten blocks in each field. A transect of five blocks, 10 m apart, were positioned within the centre of the crop, a minimum of 75 m from the crop edge. A further five blocks were placed along the crop edge, adjacent to the semi-natural margin, between 75 and 100 m from the corresponding field corner (Figure 3.2).

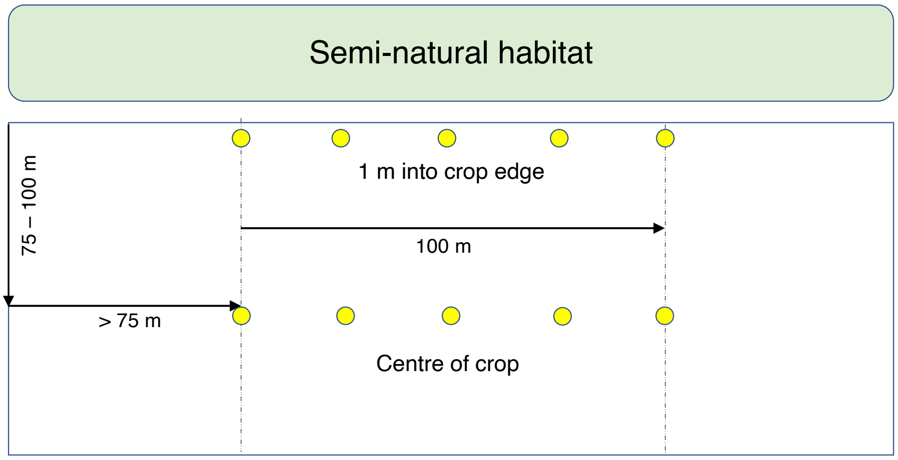

Figure 3.2 Experimental design of yield analysis experiment in oilseed rape fields. Blocks (yellow circles) were positioned 25 m apart along the crop edge and the centre of the field. The distance between the semi-natural habitat and the crop edge was less than 2 m. Semi-natural habitat consisted of mixed hedgerow in all fields.

Each block had two primary treatments: (1) Control, unmanipulated with the entire plant exposed to wind-, self- and insect-pollination and (2) mesh bags over one flower head per plant to exclude insect pollinators. To determine if pollinator exclusion of one flower head resulted in a reallocation of resources to other flowers of the same plant, yield metrics were not only collected from the control flower head and insect exclusion flower head, but also from an insect-exposed flower head of plants subject to treatment 2. Thus, three treatments were compared, specifically: Control, Bagged and Open-pollinated. Mesh bags were made from tulle (15 x 35 cm; 1 x 1 mm mesh size) following Bommarco *et al.* (2012b). Pollen deposition by the wind was still possible, as tulle bags have been shown not to impede pollen flow (Sacchi and Price, 1988, Wragg and Johnson, 2011). Since experimental plants were subject to two treatments, lateral, rather than terminal, racemes were selected for the study. Bags were fitted to the plants at the bud stage of oilseed rape development, before the onset of flowering and the position of the bags was adjusted regularly throughout the flowering period, to prevent obstructing plant growth. Bags were removed immediately following flowering to allow for unimpeded maturing of the pods.

3.3.3 Yield metrics

Crops in all study sites were harvested using desiccation by glyphosate, the most common technique for preparing oilseed rape for harvest in the UK (Cook *et al.*, 2019). Just before commercial harvesting, all experimental racemes were cut and dried to preserve them. Pods from all experimental racemes were removed, and the seeds in each pod were counted and weighed. Three yield metrics were calculated from these data.

1) Seed set: the number of seeds per pod.
2) Seed weight: the weight of all seeds within a pod.
3) Individual seed weight: calculated by dividing pod weight by seed set.

3.3.4 Pollinator abundance data

Insect abundance data was collected using pan traps (see Chapter 2.3.2) were obtained. Abundance for commonly recognised pollinators (bees, butterflies and all flies) were first summed over the five pan traps in a transect (i.e. edge and field). The average was then calculated across sampling dates to give an overall abundance for each location (crop centre and edge) in all fields.

The effect of pollinator abundance on pollinator contribution (i.e. the proportion of yield attributed to insect pollination) was tested. The proportional change of each yield metric as a result of insect pollination was calculated for each plant using the formula:

$$pollinator\ contribution = 1 - \left(\frac{yield\ metric\ value\ for\ bagged\ flowers}{yield\ metric\ value\ \ for\ open - pollinated\ flowers}\right)$$

3.3.5 Statistical analysis

The effects of insect pollination on oilseed rape yield metrics (i.e. seed set, seed weight per pod and individual seed weight) were analysed using general linear mixed-effects models (LMM). Fixed effects were treatment (i.e. Control, Bagged and Open-pollinated) and its interactions with location in field, breeding system and year. Random effects were geographic location (field nested within pair) to control for spatial variability, and farm, to control for farm practice differences, as several farms managed more than one field.

To test the effect of pollinator abundance on pollinator contribution (see 3.3.4), abundance data were centred and scaled to a mean of zero and a standard deviation of one. LMMs were fitted using the same random effects as for the pollination effect models. However, with sample size limitations (i.e. pollinator data was summarised across the five blocks in a transect), only the effect of pollinator abundance on pollinator contribution was tested. For all models, those with the lowest Bayesian information criterion (BIC) were selected. All models were fitted with Residual Maximum Likelihood (REML) using the lem4 package (Bates *et al.*, 2015), with pairwise comparisons of least square means performed using the lsmeans package (Lenth, 2018) in R version 3.6.1 (R Core Team, 2019).

3.4 Results

3.4.1 Effect of insect pollination on yield

Almost eight thousand seed pods were collected over the two years of this study. The mean number of seeds produced was 22 ± 9.24 SD, with a mean seed weight of 0.10 g ± 0.05 SD. Mean individual seed weight was 4.8 mg ± 1.51 SD. Overall, when compared to bagged racemes (i.e. those where insect pollinators were excluded), insect-pollinated racemes had a significantly higher seed set and seed weight per pod. This result was consistent in both the Open-pollinated treatment (i.e. on the same plant) and the Control treatment (i.e. open-pollinated raceme on an adjacent plant) (Table 3.1A). Individual seed weight followed a slightly different pattern. Bagged racemes had a lower seed weight than open-pollinated racemes on the same plant; however, when bagged racemes were compared with the control treatment, no significant difference was detected. When compared to bagged racemes, seed set in the control treatment increased by 23%, seed weight per pod by 29% and individual seed weight by 4% (Figure 3.3). Intra-plant (Bagged and Open-pollinated) differences were also detected with seed set increasing by 16%, seed weight per pod by 25% and individual seed weight by 7% in open-pollinated racemes (Figure 3.3).

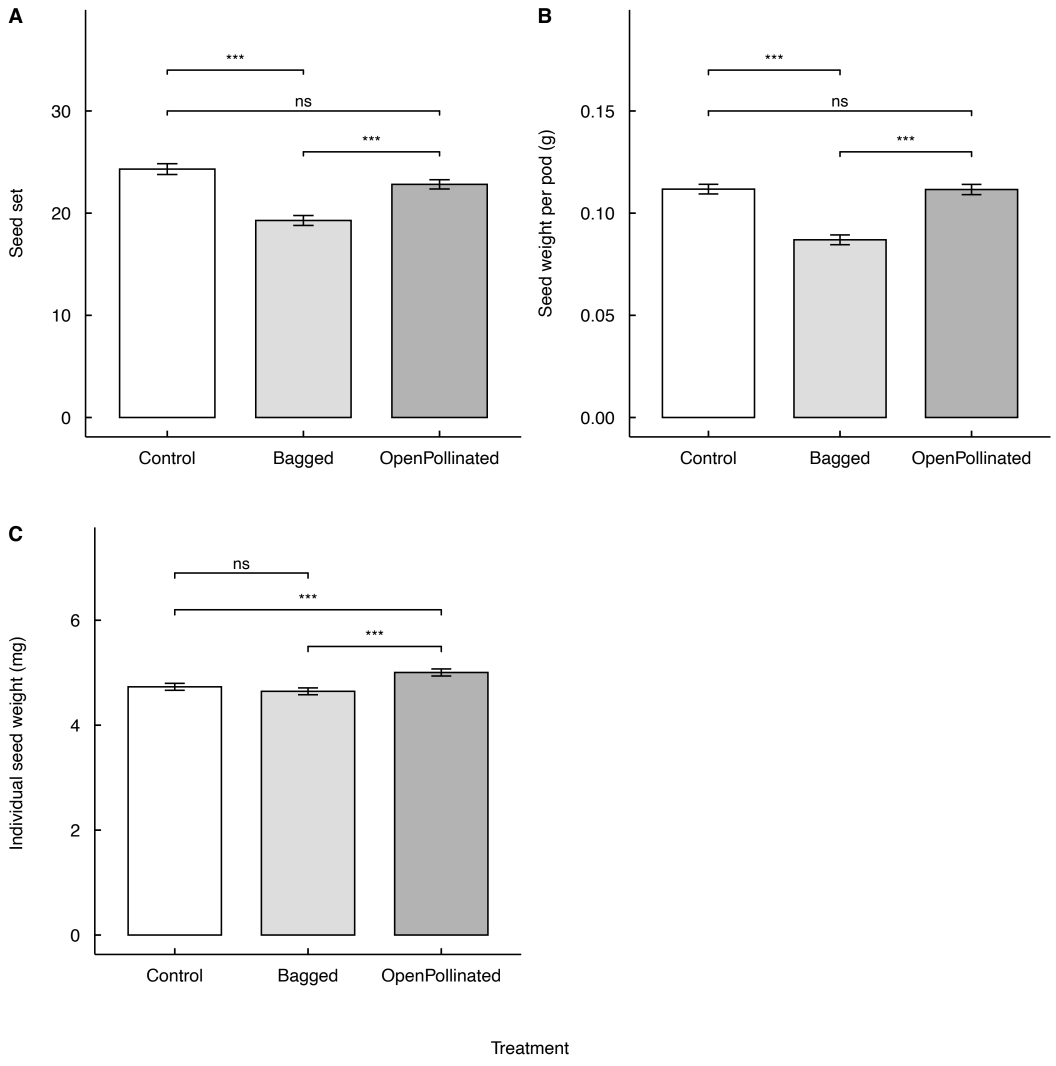

Figure 3.3 The effect of treatment on mean: **A** seed set per pod, **B** seed weight per pod and **C** individual seed weight, for one raceme per plant over two years. Bagged and Open Pollinated treatments were applied to the same plant. Error bars show standard error. Significance: ***P < 0.001; **P<0.01; *P<0.05.

Effects of year and treatment on seed set per pod were consistent across the two sampling years. However, a significant interaction was detected between 'treatment x year' for both seed weight metrics (Table 3.1). In 2016, insect pollination did not affect seed weight per pod or individual seed weight when comparing yield from Bagged and Control plants. However, intra-plant differences (between Open-pollinated and Bagged flowers on the same plant) were significant in both years (Figure 3.4). We also discovered seed weight per pod to be significantly lower for Bagged ($P < 0.05$) and Open-pollinated flowers ($P < 0.001$) in 2017, while individual seed weight of Control plants significantly increased ($P < 0.05$; Figure 3.4). No significant interactions were found between treatment and the location of experimental plots (centre of crop vs crop edge) or breeding system (conventional vs hybrid) of OSR varieties on any of the yield metrics.

Table 3.1 Results of mixed-effects models (F-values and probabilities indicated by *) for: **A** the effect of pollination on yield metrics and **B** the effect of pollinator abundance on pollinator contribution. Significant effects of crop location (i.e. crop centre verses crop edge) and OSR breeding system (i.e. hybrid verses conventional) were not detected and these fixed effects are thus omitted from the table. Significance: ***P < 0.001; **P<0.01; *P<0.05.

	Seed set	**Seed weight per pod**	**Individual seed weight**
(A) Effect of pollination		**F-values**	
Treatment	37.12 ***	49.59 ***	11.55 ***
Treatment x Year	-----	19.07 ***	11.22 ***
(B) Effect on pollinator contribution			
Pollinator abundance	0.42	0.13	0.48

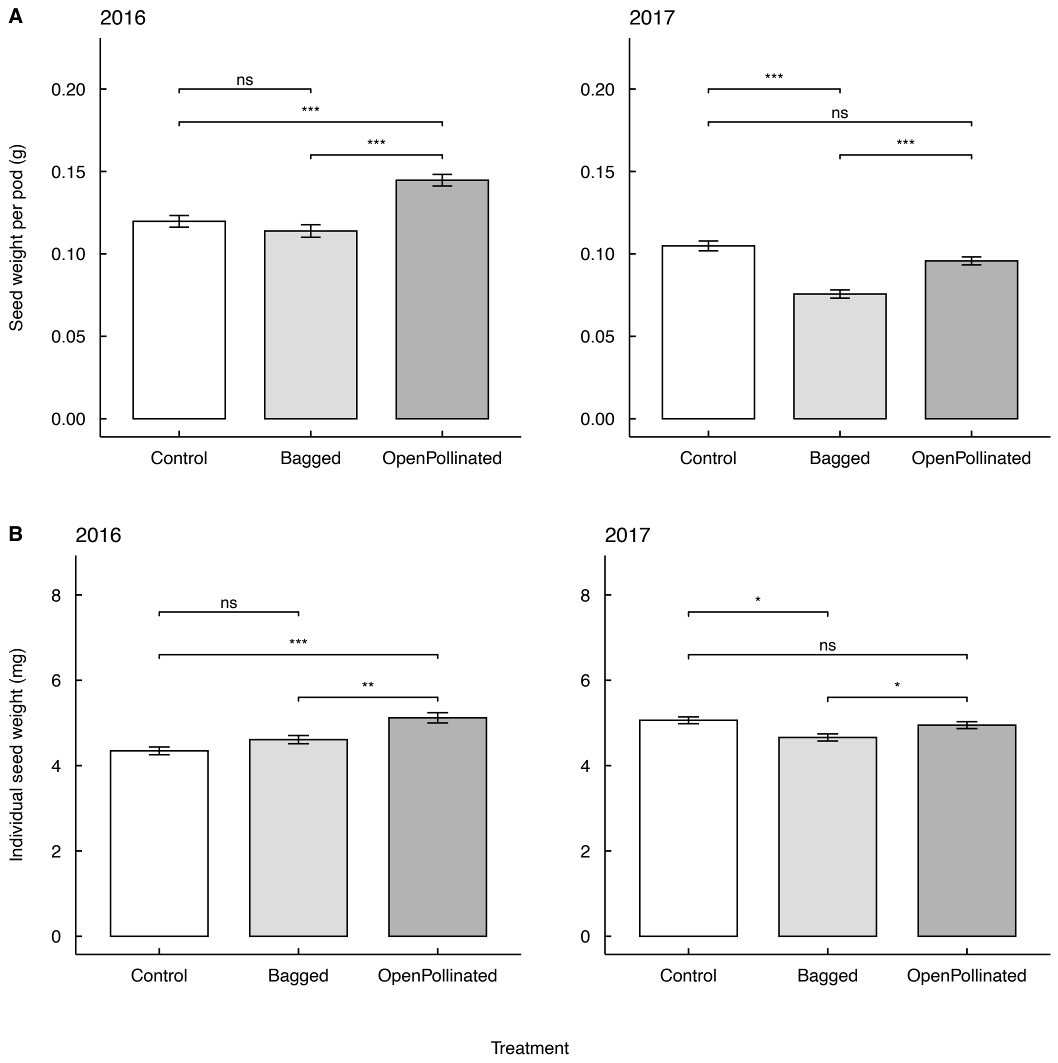

Figure 3.4 The effect of treatment x year interaction on mean: **A** seed weight per pod and **B** individual seed weight for one raceme per plant over two years. Bagged and Open Pollinated treatments were applied to the same plant. Error bars show standard error. Significance: **P < 0.001; **P<0.01; *P<0.05.

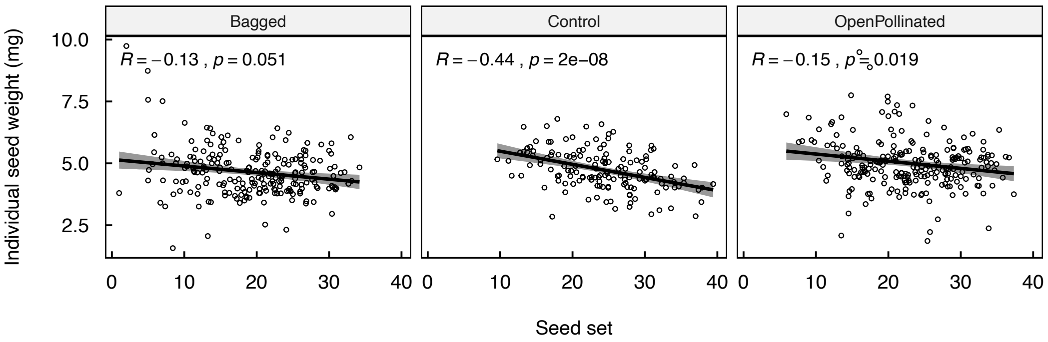

Figure 3.5 Relationship between individual seed weight and seed set for all treatments. Relationships for Control and Open-pollinated treated plants are statistically significant (Spearman correlations; *n* = 7907).

3.4.2. Allocation of resources

Resource allocation was evident in seed weight metrics in 2016 only. Flowers produced significantly heavier pods and seeds in Open-pollinated flowers than Control (Figure 3.4). In 2017, seed weight between Control and Open-pollinated plants were comparable for both metrics (Figure 3.4). We also found that individual seed weight was negatively correlated with the number of seeds produced per pod for all treatments. However, only Control and Open-pollinated plants were statistically significant ($P < 0.05$; Figure 3.5).

3.4.3 Effect of pollinator abundance on pollinator contribution

As resource reallocation was only observed between the treatments applied to the experimental plants in 2016, and to control for the variability between plants, the effect of pollinator abundance on pollinator contribution (i.e. the proportion of yield attributed to insect pollination) was tested on the experimental plants rather than untreated plants. The effect of pollinator abundance was minimal; increasing pollinator contribution for both seed weight metrics but reducing seed set (Figure 3.6). However, these effects were found to be non-significant (Table 3.1B).

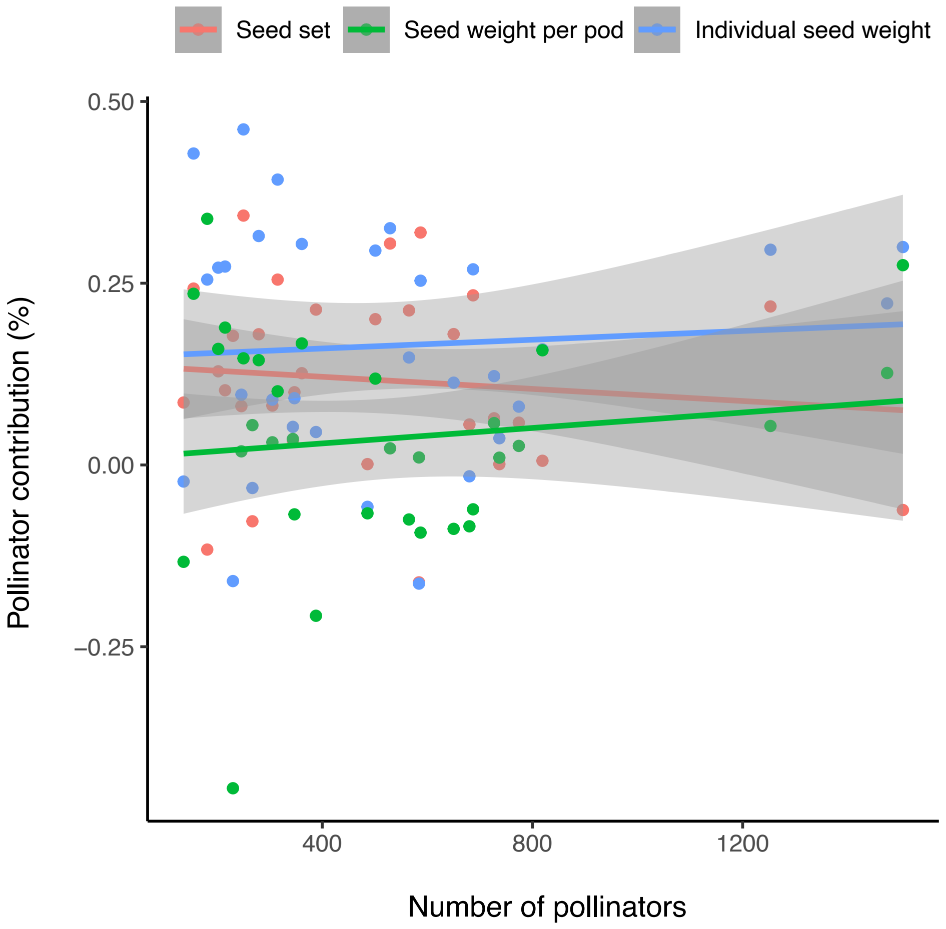

Figure 3.6 Changes in pollinator contribution for all yield metrics in response to pollinator abundance. Grey area shows 95% confidence intervals of the mean.

3.5 Discussion

3.5.1 Overview

This study builds upon similar research investigating the effect of insect pollination on oilseed rape. However, most pollination studies of this manner focus on either a single variety in the field or several varieties under controlled conditions. Investigating the effect of pollination across breeding systems, in an agricultural environment, allows for more real-world comparisons to be made. Although the effect of insect-pollination was inconsistent between the two years of this study, when combined, insect pollination has a positive effect on all yield metrics. It demonstrates the potential to redistribute resources when pollen is limited.

3.5.2 The effect of pollination on oilseed rape yield

Our study clearly shows that while oilseed rape is proficient at setting seed without insect visitation, insect pollination does result in higher yields. By allowing pollinator visitation, all yield metrics were positively affected, albeit at varying degrees. Insect pollinated racemes increased seed set by 23% compared to those where pollinators are excluded. These results are comparable with the increases in OSR seed set reported by Steffan-Dewenter (2003) and Bommarco *et al.* (2012b). Seed weight per pod also benefitted from insect pollination, with weight increasing by, on average, 29% as a result of insect pollination. Overall, individual seed weight increased marginally by 4%. The importance of seed weight should not be underestimated as it has previously been used as a proxy for seed quality in OSR. As heavier seeds possess a higher oil content (Bartomeus *et al.*, 2014), and with UK farmers receiving an oil bonus of 1.5% of their contract price for every 1% oil above 40% (Limagrain UK Ltd, 2018), seed quality can be as economically important as quantity. We did not test the seeds for oil content during this study and are unable to make assumptions about the effect of pollination on oil content.

For both seed weight metrics, the effect of pollination was inconsistent across years. In 2017 only, Open-pollinated racemes benefit from higher seed weights (at the pod and individual level) than racemes where pollinators have been excluded. The temporal inconsistencies of these results may be explained by the variability in the flowering period of the crops. In 2017, flowering started two weeks earlier than the previous year and may be responsible for fewer pollinators being recorded during sampling. This may have resulted in a greater dependency on unreliable wind-pollination.

These inconsistencies between years were unexpected, as seed set was not affected temporally. For there to be no difference in seed weight between insect-pollinated and pollinator-excluded flowers contradicts other studies (Bommarco *et al.*, 2012b, Stanley *et al.*, 2013, Bartomeus *et al.*, 2014). For insect- and wind-pollinated plants to receive equal pollination services suggests several things: highly efficient wind-pollination, a reduction in insect-pollination, an impact of treatment at the plant level or a combination of factors.

Overall, the yield was higher in 2016, as were pollinator abundance, although 2016 may have been a better growing season generally. Although the wind carries large amounts of pollen, the probability of it setting on a stigma is low, with as little as 2.5% of all pollen deposition attributed to the wind being reported (Langridge and Goodman, 1982, Ouvrard *et al.*, 2017). The bagging of plants gave us extreme results where pollinators were excluded. To find similar seed weight in bagged and control plants would suggest that insect pollination was irrelevant. However, significant intra-plant differences were found to exist in the same year. These flowers either received sufficient insect pollination to increase seed weight, or experimental plants reallocated resources from the bagged flowers to other parts of the plant. Studies have also suggested that inconsistencies in the effect of insect pollination on OSR seed production may be varietal (Kołtowski, 2005, Jauker *et al.*, 2012b, Hudewenz *et al.*, 2014). This was something that was not possible to test for during this study, as site selection protocols reduced the availability of a broader range of varieties.

Additionally, the importance of insect-pollination is often over-stated. For example, Klein *et al.* (2007) report that 70% of leading global crops benefit from animal pollination. Whilst this may be true, these crops account for only 35% of global food production. Other studies are also split as to the dependency of insect pollinators, with some claiming complete dependency and others estimating dependency to be very low (Delaplane *et al.*, 2000, Witter *et al.*, 2014, Ouvrard *et al.*, 2017). Comparisons between studies investigating the contribution of insect-pollinators to OSR yield are difficult. In an extensive review of the literature published between 1956 and 2018, Ouvrard and Jacquemart (2019) found several factors that influence pollinator dependency, including the type of cultivar (conventional or hybrid), geographical location, field conditions and plot size.

3.5.3. Breeding system differences

Contradictory to our prediction that the effect of insect pollination would be more significant in conventional varieties, we found that varieties across both breeding systems responded equally. Although conventional varieties have been found to benefit more from insect pollination because of a higher genetic diversity (Tommasini *et al.*, 2003), this may have been counteracted by hybrid varieties having more stable and effective yields under unfavourable conditions (Diepenbrock, 2000). The attraction of higher nectar quantity found in hybrid varieties may have increased pollinator visitation rates, thus counteracting the genetic diversity of conventional varieties (Carruthers *et al.*, 2017).

3.5.4 Resource allocation

Evidence of resource reallocation, for both seed weight metrics, was only present in 2016, where pods and seeds were heavier in open-pollinated flowers on the experimental plant than those on the untreated control plant. This dissimilarity suggests that in the absence of adequate pollination, and with flowers only exposed to self- and wind-pollination, plants reallocate resources to increase seed weight in other areas of the plant. As we only collected data from one raceme per plant during this study, it is difficult to confirm that resource allocation has occurred or, as Tayo and Morgan (1975) found, variability exists across racemes of the same plant. If indeed, plants are reallocating resources to other parts of the plant in the event of limited pollination, this may also occur in other instances such as increased herbivory or damaged racemes. Most studies attempt to quantify the effect of insect pollination on OSR yield parameters at the whole plant level; as far as we are aware, this is the first study that explores resource reallocation within a plant alongside a control plant. Another consideration is that variability exists in how resources are allocated for yield metrics between varieties (Grosse *et al.*, 1992). Our study area was relatively compact, with several fields handled by the same management company. Despite collecting data from sixteen fields, we only sampled ten varieties, so varietal differences may have influenced our results. Indeed, Ouvrard *et al.* (2017) report that autogamous self-pollination, in the absence of wind or insects, is becoming a feature of newer varieties of oilseed rape. As expected, we found that seed quantity and quality are negatively correlated in the plants exposed to insect pollination. Pods with a higher seed set produced lighter seeds, suggesting a reallocation of resources within the plant level. However, these correlations were weak.

3.5.5. Effect of pollinator abundance on pollinator contribution

Although oilseed rape is considered self-fertile (Free, 1993), pollination has been found to increase seed quantity and quality. Therefore, it was unexpected that we found pollinator abundance to have no effect on pollinator contribution for any of the yield metrics measured. Our results contradict other studies of oilseed rape which report enhanced yield metrics with increasing pollinator population densities (Steffan-Dewenter, 2003, Lindström *et al.*, 2016, Adamidis *et al.*, 2019, Woodcock *et al.*, 2019). In studies where honeybee abundance is increased by the placing of hives in OSR fields, increased yield contributions of between 22% and 46% were found (Manning and Wallis, 2005, Sabbahi *et al.*, 2005). It is possible that pollinator abundance was not large enough to produce a positive effect on yield, thereby providing inadequate pollination services.

However, pest abundance was also higher in the crop edge (Chapter 2). The tulle bags used during this study also excluded pest species from visiting the bagged flowers. Nevertheless, pests were still able to attack the exposed Control and Open-pollinated racemes. If increased insect pollination, caused by a higher abundance of pollinators, can have a positive effect on yield in these exposed flowers, it is also possible that increased pest activity has the opposite effect. This increased pest activity may have counteracted any positive effect caused by increased pollinator abundance.

In this study, we included all flies within our pollinator data, as the most commonly regarded pollinators (i.e. bees and hoverflies) were too scarce for statistical analysis. Wild bees are considered the most important pollinators of crops (Winfree *et al.*, 2008), and the value of hoverflies as pollinators is underestimated (Rader *et al.*, 2016). However, the majority of the flies included in our data were non-syrphid flies; the pollination services provided by these has been little studied.

One consideration for the absence of an effect between pollinator abundance on yield metrics could be experimental design. For us to model pollinator contribution with abundance, the insect data were aggregated to only 32 observations (centre and edge for each field), causing considerable limitations when using mixed-effect models. Also, pollinator abundance data were collected passively using pan trapping and averaged across all sampling dates. While pan traps are considered an effective method for collecting data simultaneously from multiple sites (Westphal *et al.*, 2008), this passive collection method does not necessarily provide a true reflection of the number of pollinators actively visiting crop flowers, despite often being used as a surrogate for it (Ricketts *et al.*, 2008a). This method has shown to exhibit bias, with efficiency affected by habitat and landscape context (Baum and Wallen, 2011). Standardised transect walks with aerial nets would have been more effective at surveying pollinators actively foraging on OSR flowers and the favoured method of several similar studies (Bommarco *et al.*, 2012b, Bartomeus *et al.*, 2014, Lindström *et al.*, 2016). Initially, attempts were made to survey insect visitation using 30-minute transects walks. However, with less than one insect per minute observed, the data generated was low in comparison to the sampling effort.

3.5.6 Conclusions

This study supports the body of literature that highlights the importance of insect pollinators to gain enhanced yields from entomophilous flowering crops, in this case, oilseed rape. We have shown that insect pollination can considerably increase yield in oilseed rape, although the stability of these effects differs temporally. Overall, we found evidence of a trade-off between seed quantity and quality, with an increase in the number of seeds resulting in a slight loss in seed weight. However, losses from the reduction in quality were outweighed by quantity. Although yield gains as a result of insect pollination are relatively modest, they can lead to considerable economic revenue increases for farmers when scaled up.

Confronted with localised pollen limitation, plants can reallocate resources to other racemes to increase seed weight. However, as a caveat, a trade-off exists between quantity and quality where resources are spent on more or heavier seeds. The redistribution of resources across racemes can potentially minimise yield losses as a result of limited pollination. In contrast to other studies, we found pollinator abundance to have no effect on pollination contribution. However, this is possibly attributed to the limitations of sample size and the collection method. For measuring visitation, transect walks are more effective.

Insect-pollination has an overall positive effect on oilseed rape yield. The economic benefits of insect-pollinators are highlighted by the overall increase in all yield parameters over the two years. However, their contribution is not to be misinterpreted as essential to obtaining good yields from oilseed rape in Scotland. There remains inconsistency in their overall contribution, particularly from season to season, where the differences in some yield metrics are negligible between wind- and insect-pollinated plants. Investing in pollinator management, such as agri-environment schemes may increase pollinator activity during favourable conditions, the benefits from these may not always be consistent.

Chapter 4

The effect of pollination on growth and reproduction of oilseed rape (*Brassica napus*)

4 The effect of pollination on growth and reproduction of oilseed rape (*Brassica napus*)

4.1 Abstract

Phenotypic plasticity is the ability of an organism to alter its development and life history in response to changes to its environment. For sessile plants, in particular, the distribution of available resources between the functional traits associated with growth and reproductive is a successful life-history strategy. A combination of abiotic and biotic factors facilitates responses in plants. One such biotic factor is insect pollination. Studies show that oilseed rape (*Brassica napus*) benefits significantly from wind and insect pollination in the form of enhanced yields. However, the impact of pollination on growth metrics is less known. To determine the effect of pollination on the phenotypic plasticity of oilseed rape, a crop that benefits greatly from it, we conducted controlled experiments with two methods of supplementary pollen deposition. Using simulated wind and insect pollination delivery, we assessed the impact of pollination on growth and functional reproductive traits. We found that plants receiving supplementary pollination were shorter with a reduced flowering period. Despite producing fewer flowers, these plants set a higher proportion of fruits compared to those not in receipt of supplementary pollination. Although untreated plants benefitted from higher individual seed weight, plants receiving pollination produced more seeds per plant, resulting in an overall higher yield per plant. The reallocation of resources from growth and flowering

metrics has the potential to stabilise yields, through increased seed production directly, or indirectly through shorter plants and a reduced flowering period. Through a combination of wind and insect pollination, oilseed rape can produce seeds in more significant quantities and of higher quality. The floral resources provided by mass-flowering crops and surrounding flower-rich habitat are essential for maintaining insect pollinator communities in agricultural environments. Investment in pollinator management allows growers to exploit the economic benefits of insect pollination, particularly during environmental conditions unsuitable for wind pollination.

4.2 Introduction

4.2.1 Adapting to environmental conditions

A successful strategy adopted by individual organisms is having the capability to alter their development and life history in response to their environment (Bradshaw, 1965, Schlichting, 1986, Sultan, 2000). These environment responses are driven by a combination of genetic traits, environmental conditions (e.g. temperature, humidity and soil pH) and resources (e.g. sunlight, water availability and nutrients). Distributing resources between the functional traits for growth and reproduction is considered a central theme in life-history strategy (Doust, 1989). Misallocation of resources can directly influence plant development during maturity (Kozłowski, 1992). For example, if plants redirect resources from traits that are essential to developmental growth to those responsible for reproduction too early in their life cycle, it may have a considerable effect on their chances of survival (Lacey, 1986).

Similarly, investing too many resources in flowering and growth may leave plants with insufficient resources for seed investment (Pyke, 1991).

4.2.3 Phenotypic plasticity

Phenotypes are the functional traits of an organism related to the fitness and success of an individual and often relate to competition and dominance within a community. They are expressed by a genotype and influenced by environmental factors. Phenotypic plasticity is a strategy used by individuals of a given genotype to adjust their phenotype according to their surroundings (West-Eberhard, 2003, Bradshaw, 2006). Plasticity can vary from little, or none, to high (Figure 4.1), and is fundamental for an organism to cope successfully with challenging environments and can be expressed through changes in behaviour, morphology and/or physiology (Price *et al.*, 2003).

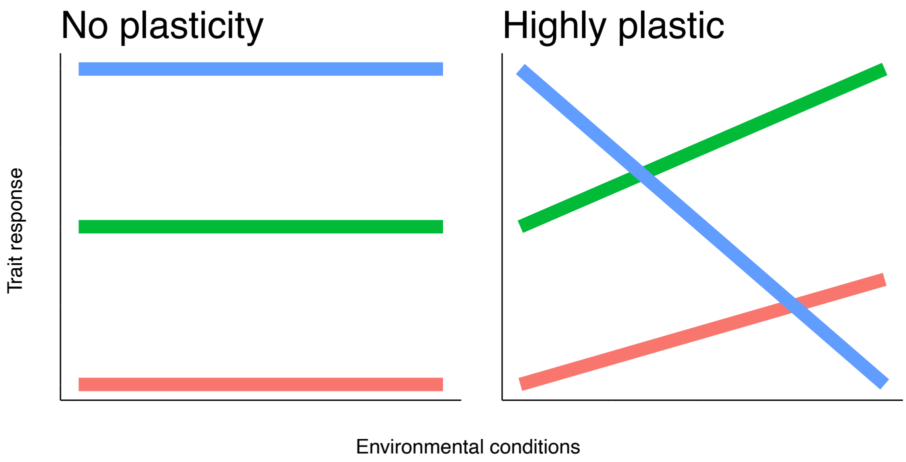

Figure 4.1 An example of trait responses to environmental conditions. When traits remain the same, no plasticity is evident. High plasticity causes traits to respond independently based on genotype.

While environmental changes initiate phenotypic plasticity; the ability to respond to these changes is genetic and therefore subject to change through natural selection in wild populations (Via, 1994). Phenotypic plasticity is common in plant species (Schlichting, 1986, Sultan, 1987, Dudley, 2004) with observations in response to many environmental conditions, including biomass allocation, morphological and architectural structure, physiology and phenology (Kozłowski and Wiegert, 1986, Chapin, 1991, Nicotra *et al.*, 2010, Freschet *et al.*, 2018). Once stimulated, these environmental responses may become permanent, for example, the thickening of tree branches in environments exposed to high winds (Watt *et al.*, 2005) and the onset of flowering after exposure to cold conditions (Filik *et al.*, 2007, Andrés and Coupland, 2012), or they may be short-lived such as the effect of light on photosynthetic chemistry (Pacini *et al.*, 2003). While studies addressing plant responses to environmental change have mostly focused on abiotic factors, such as soil nitrogen concentration and light limitation (Freschet *et al.*, 2018), biotic interactions, such as defence chemistry in response to herbivory (Baldwin, 1999), also offer essential insight into the phenotypic plasticity of plants. One biotic interaction with considerable importance, certainly in terms of crop production, is insect pollination, which can have an overwhelming effect on reproduction (Obeso, 2002, 2004). Despite this, research on pollination has focussed extensively on reproductive metrics (e.g. seed weight and number), with studies evaluating the impact on growth metrics less common (Bommarco *et al.*, 2012b, Bartomeus *et al.*, 2014).

4.2.4 Pollination

Insect pollination, as an ecosystem service, is vital to the reproduction of wild plants, trees and crops. It is under threat as a result of declines in species richness and abundance of wild pollinators (Allen-Wardell *et al.*, 1998, Biesmeijer *et al.*, 2006, Potts *et al.*, 2010a, Bommarco *et al.*, 2012a) and managed honeybees (Cox-Foster *et al.*, 2007, Potts *et al.*, 2010b). Agricultural intensification, resulting in larger field sizes and loss of semi-natural habitat, (Steffan-Dewenter *et al.*, 2002, Tscharntke *et al.*, 2005, Rundlöf *et al.*, 2008), alongside the increased use of agri-chemicals (Kevan, 1975, Tilman *et al.*, 2001, Robinson and Sutherland, 2002) is cited as a primary driver. With more than 87% of flowering plant species benefit from animal pollination (Ollerton *et al.*, 2011) and 70% of leading crops experiencing increased yields through insect visitation (Klein *et al.*, 2007), pollinator decline has serious consequences to the conservation of (semi)-natural habitats and global food production.

4.2.5 Oilseed rape and insect pollinators

Oilseed rape (*Brassica napus*; OSR) is one of the most economically important crops in the world and is the dominant mass-flowering crop in Europe, cultivated on 34.9 million hectares in 2019 (USDA, 2019). Primarily grown as a source of oil; crop cultivation is increasing to meet biofuel demands (Van Der Velde *et al.*, 2009). With such importance, seed companies continuously develop and bring to market new varieties, each with unique characteristics adapted to specific environmental conditions, including disease resistance. Varieties are bred as either conventional, by traditionally crossing the most desirable genotypes, or as restored hybrids, using selected inbred lines (Friedt and Snowdon, 2009). OSR plants are highly plastic, responding to abiotic and biotic interactions, particularly surrounding flowering. For example, oilseed rape plants can adjust flower production in response to planting density (Cresswell *et al.*, 2001) and insect visitation (Mesquida *et al.*, 1988b).

Although OSR can be cross-pollinated by utilising abiotic and biotic vectors such as the wind and insect visitors, it is also capable of self-pollination. Vectors assist with self-pollination, primarily through geitonogamy (fertilisation by pollen from another flower of the same plant). However, the flower structure of OSR, particularly the inward-facing anthers, also lends itself to autogamy (fertilisation by pollen from the same flower; Figure 4.2); therefore, some pollination is possible in OSR with very little external assistance (Eisikowitch, 1981).

Figure 4.2 Oilseed rape (*Brassica napus*) flower with petals removed. **1**: Male structures are arranged in two sets. Four long stamens **A** encircle the female style **C**, with a shorter pair located outside **B**. Anthers of the short stamens dehisce towards the centre of the flower and rely on insect visitors as a vector for pollen transfer. **2**: The anthers of the long stamens dehisce outwards, although the curvature of these anthers position themselves close to the stigma **D** allowing for pollen transfer when the style extends.

Nevertheless, OSR flowers offer high quantities of nutrient-rich floral resources, particularly sticky pollen grains, suggesting OSR is more suitable to direct insect pollination than indirect wind pollen deposition (Westcott and Nelson, 2001, Cresswell *et al.*, 2004). Indeed, studies indicate that insect visitation benefits OSR yield, in terms of both seed production (seed set) and seed quality (seed weight) (Sabbahi *et al.*, 2005, Bommarco *et al.*, 2012b, Bartomeus *et al.*, 2014, Hudewenz *et al.*, 2014, Lindström *et al.*, 2016). With the increased demand for cultivation, the economic implications caused by pollinator decline is a cause for concern to OSR growers.

Insect pollinators are continuing to decline in Europe despite the EU's Common Agricultural Policy's increasing commitment to environmental protection. Since current agri-environment schemes are failing to provide all resources pollinators require in sufficient quantities (Cole *et al.*, 2020), there is an urgent need to explore novel means of protecting insect pollinators. Mass-flowering crops such as OSR can provide a pulse of food at a critical point in the season, filling hunger gaps and complementing agri-environment measures such as floral-rich field margins (Carvell *et al.*, 2007, Stanley and Stout, 2013). The role that such crops can play in enhancing the temporal stability of food resources should, therefore, be considered within management frameworks targeted to mitigate pollinator declines and conserve pollination services.

4.2.6 Aims of this study

In this study, we aim to assess the 'pollination effect' on OSR vegetative growth and reproduction, and potential economic consequences, with a particular focus on breeding system differences. We will explore how OSR allocates resources to growth, flowering and yield metrics under pollen limitation, wind-simulated pollination and insect-simulated pollination to answer the following questions:

(1) Do OSR plants display phenotypic plasticity in response to different methods of pollination by changing the way they allocate resources toward reproduction and growth?

(2) Does pollination increase seed production, and if so, is there a trade-off with vegetative growth and flowering phenology?

(3) Does the method of pollen deposition (i.e. insect versus wind) affect the functional traits of OSR, equally?

(4) Does the effect of pollination remain constant across OSR breeding systems?

Through answering these questions, we will establish the effect of pollination on the phenotypic plasticity of resource allocation for eight varieties of OSR (four conventional; four hybrids) to determine the impact of pollen limitation and the relative importance of wind- versus insect-simulated pollination across breeding systems. Increasing our understanding of how OSR growth and yield metrics (i.e. quantity and quality) alter under different pollination treatments could assist growers to make informed decisions, concerning variety selection, based on their pollinator availability.

4.3 Methods

4.3.1 Plant material

Eight cultivars of commercially available oilseed rape (OSR; *Brassica napus*), comprising of four conventionally bred and four hybrid varieties were grown under insect-free, environmentally controlled, conditions in the glasshouses at Scotland’s Rural College, Edinburgh (55°55’18.3” N, 03°10’43.7” W). Cultivars were selected from varieties undergoing regional in-field testing for inclusion on the 2016/17 AHDB Recommended List for cereals and oilseeds (North UK region). To ensure a fair representation of available cultivars, selected cultivars covered a range of agronomic characteristics: gross output results (both high- and low-performers), and disease resistance scores obtained from previous Recommended List trial data (AHDB, 2019).

4.3.2 Plant growth

In August 2017, seeds were sown in modular trays containing a 50/50 mixture of peat (*Clover, N. Ireland*) and washed horticultural sand. Added to every 100 L of compost mixture was 187.5 g of garden lime (*William Sinclair Horticulture, Lincolnshire, UK*) and 75 g of 'programme released' fertiliser - Osmocote Exact 5-6 months (*ICL, Cleveland, UK*). At the 3-4 true leaf stage, seedlings were vernalised at 5° C for eight weeks. The lights for the vernalisation cabinets at SRUC are manually controlled. Therefore, lights remained on throughout the vernalisation period. Post-vernalisation, plants were re-potted into deep 4 L pots (215mm x 180mm) and moved to an insect-free glasshouse with a 16-hour light / 8-hour dark photoperiod. Minimum illuminance during the 'light' period was 15 kilolux, equivalent to full daylight (not direct sun) (Schlyter, 2009). Glasshouse heating maintained a daytime (07:00 – 19:00) mean temperature of 20.0°C (± 1.7°C) and night temperature of 15.9°C (± 1.7°C), and relative humidity was maintained at 52% (SD ± 7%). Plants were watered daily and organised into a 3-block, randomised block design, at a density of 8 pots m^{-2}.

4.3.3 Supplementary pollination treatments

On the onset of flowering, plants were allocated to one of the following three treatments:

1. Insect-pollination

   To simulate direct cross-pollination by insect pollinators flowers were hand-pollinated using a size 8, ‘Filbert-style’ artist’s paintbrush, with pollen collected from the anthers of another plant of the same cultivar. All open flowers were hand-pollinated until flowering ended.

2. Wind-pollination only

   Flowers were self-pollinated with pollen from their own flowers by gently shaking the plant stem to simulate the movement of plants and resulting indirect pollination by the wind. To prevent stem damage by excessive shaking, but to allow for sufficient pollen to dislodge, each plant was shaken for 10 seconds only. Before shaking, plants were carefully removed from the glasshouse to prevent filling the compartment with airborne pollen. Post-shaking, plants remained separated for 30 minutes before being returned.

3. Auto-pollination only

   Flowers were left untreated as a control. Auto-pollinated plants were not disturbed during the experiment. Particular care was taken to avoid any contact that could dislodge pollen, limiting flowers to autogamous pollination only.

As *Brassica* pollen retains some viability for at least 72 hours (Bots and Mariani, 2005, Rosa *et al.*, 2010), wind- and insect-pollination treatments were performed every other day during the duration of flowering. Treatments were applied at 09:00 hours, with the order of treatment alternated each time.

4.3.4 Harvesting

Flowering and maturing periods varied across cultivars. To control for early-flowering cultivars benefitting from an extended maturation period, or for fruits from late-flowering cultivars having insufficient time to mature, the harvest of plants was standardised. The date of harvest was calculated for each cultivar by allowing 56 days from the mean flowering end date for all plants within the cultivar. Plants were then cut at the soil surface and dried in the glasshouse for 14 days, after which all seed pods were removed.

4.3.5 Resource metrics

To distinguish between the allocation of resources towards growth and reproduction, metrics were broadly divided as follows:

- Growth and biomass: To determine the effect of pollination on vegetative growth, we measured the final height of each plant and total above-ground dry biomass (Table 4.1).

- Reproduction: To measure reproductive resource allocation, we recorded the length of the flowering period, the number of flowers that each plant produced, fruit set, number of seeds per pod and seed weight, as well as the overall number of seeds and yield for each plant (Table 4.1).

Table 4.1 Measurement methods for vegetative and reproductive metrics.

Metric	**Method of measurement**
Growth and biomass	
Plant height	Measured post-flowering
Biomass	Weight of all above-ground vegetation, including seeds
Flowering	
Flowering period	Number of days between the first and final day of flowering
Number of flowers	Sum of all seed pods and flower abscission scars
Reproduction	
Fruit set	Number of seed pods / (number of seed pods + abscission scars)
Number of seeds	Seeds were manually removed from pods and counted.
Seed weight	Seed biomass per pod

4.3.6 Data analysis

The data were analysed using linear mixed-effects models. For response variables involving count data, i.e. flowering period, number of flowers, number of seeds per plant, a generalised linear mixed-effects model (GLMM), drawn from a Poisson distribution, was used. For continuous data, (i.e. plant height, biomass and seed weight), general linear mixed-effects models (LMMs) were used. Reproductive success was determined by flowers developing into seed pods. A GLMM, drawn from a binomial distribution, was used with 'reproductive success' and 'reproductive failure' response variables. For all models, 'block' and 'variety', and their interaction, were included as random effects to take into account the hierarchical structure of the data. Fixed effects of 'pollination method' and 'breeding system' were used to determine if the effects of pollination method were consistent between breeding systems. The interaction between these fixed effects was also explored.

For each response variable, random effects were identified to produce the best model fit – i.e. those with the lowest Bayesian information criterion (BIC). As the effects of treatment and breeding system, including their interaction, were essential to our analysis, fixed effects remained constant. All models were fitted using Residual Maximum Likelihood (REML) with the lme4 package (Bates *et al.*, 2015). Pearson's correlation coefficients were calculated to explore the relationship between the number of seeds and seed weight. All analyses were undertaken using R version 3.6.1 (R Core Team, 2019).

4.4 Results

4.4.1 Effect of pollination on growth and biomass

The final height of oilseed rape plants was consistent between both breeding systems, but a significant effect of pollination treatment was detected (Table 4.2). Plants receiving supplementary pollination (i.e. both wind and insect simulated) were significantly shorter than untreated plants (Figure 4.3a) and these effects were consistent between breeding systems (Table 4.2). Dry biomass was not found to be significantly influenced by the breeding system or pollination treatment (Table 4.2; Figure 4.3b).

Table 4.2 Results of the mixed-effects models (*F* -values and probabilities indicated by asterisks) for the effects of pollination treatment and interaction with breeding system on vegetative, flowering and reproductive metrics of oilseed rape. There were no significant effects of breeding system. Significant results are highlighted in bold.

Response variable	**Treatment**	**Treatment x Breeding system interaction**
Growth and biomass metrics	*F*-values	*F*-values
Plant height	**21.44*****	1.69
Biomass	1.71	0.88
Flowering metrics		
Flowering period	**31.17*****	2.17
Number of flowers	**194.31*****	0.44
Reproductive metrics		
Fruit set	**452.49*****	0.28
Seeds per plant	**713.20*****	**113.59*****
Yield	**4.00***	2.60
Seeds per pod	**30.25*****	0.67
Pod weight	**10.47*****	2.01
Individual seed weight	**4.28***	0.83

***P < 0.001; *P<0.05

4.4.2 Effect of pollination on flowering

The length of the flowering period and the number of flowers per plant were consistent across the breeding systems but were significantly affected by pollination treatment (Table 4.2). Plants subject to supplementary pollination (i.e. both insect and wind simulated) flowered for a significantly shorter period (Figure 4.3c) and produced significantly fewer flowers (Figure 4.3d) than those plants left untreated. No significant difference was detected in either flowering metric between wind or insect simulated pollination. Breeding system effects were non-significant for both flowering metrics.

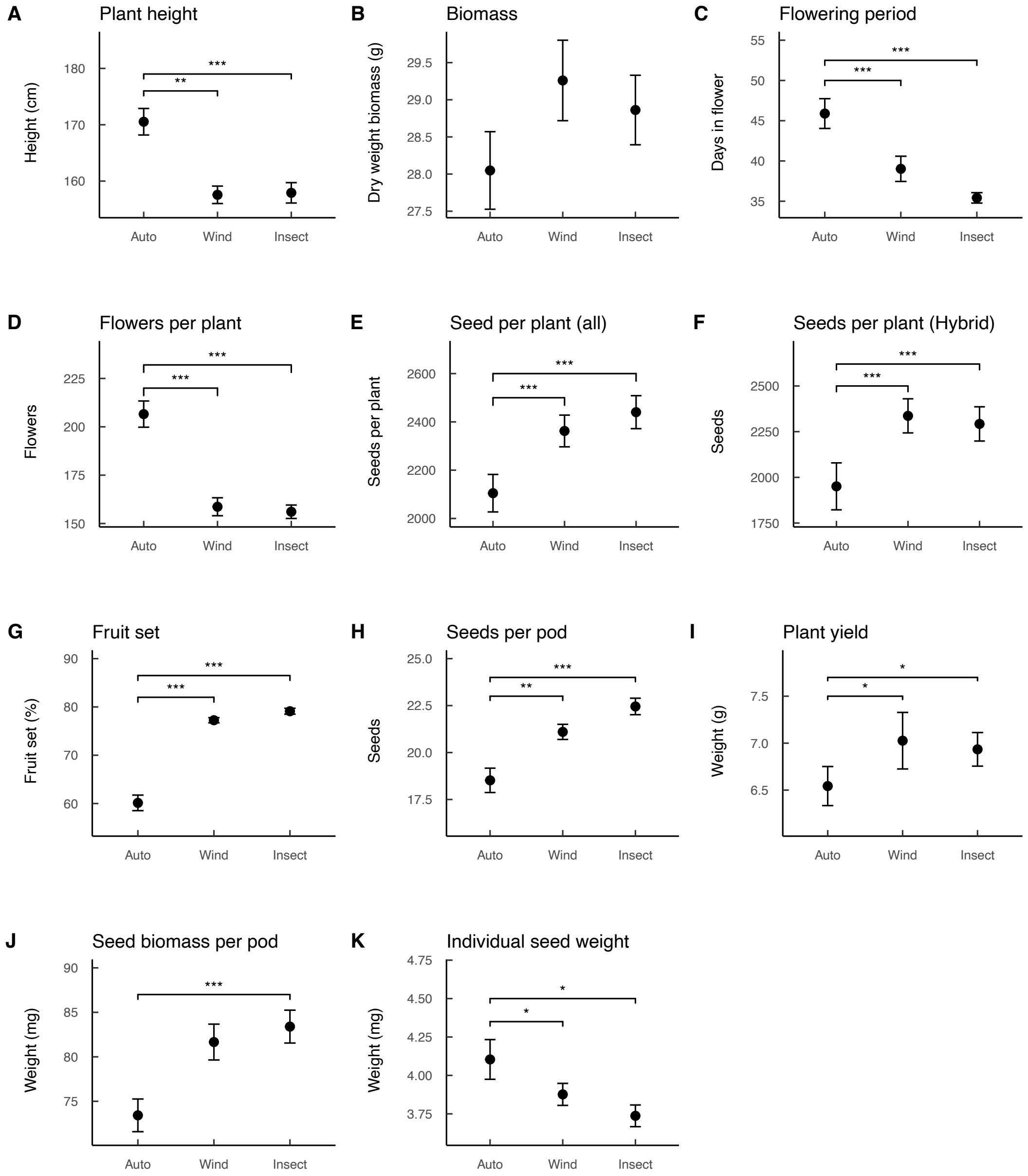

Figure 4.3 The effects of pollination treatment on vegetative growth, flowering and reproductive metrics (n = 8). Seeds per plant had a significant interaction between treatment and breeding system. Error bars ± SE. Significance: ***P < 0.001; **P<0.01; *P<0.05.

4.4.3 Effect of pollination on reproduction

The breeding system did not significantly influence any of the reproductive metrics under investigation. Significant effects of pollination treatment were, however, detected for all reproductive metrics and, while these effects were consistent between breeding systems, this was not the case for seeds per plant where a significant interaction between the pollination treatment and breeding system was detected ($P < 0.001$; Table 4.2). Supplementary pollination significantly increased fruit set (Figure 4.3g), the number of seeds produced per plant (Figure 4.3e) and seed weight (Table 4.2; Figures 4.3i – 4.3k). At the plant level, supplementary pollination in hybrid varieties produced significantly more seeds per plant (Figure 4.3f). However, this effect was not detected in conventional varieties. Plants produced significantly more seeds per pod (Figure 4.3h) and had a higher total yield (Figure 4.3i) with supplementary pollination; the effects of wind and insect simulated pollination being similar. Insect simulated pollination produced significantly heavier pods than wind-pollinated or untreated plants (Figure 4.3j). Untreated plants produced significantly heavier seeds than those receiving supplementary pollination (Figure 4.3k).

4.4.4 Relationship between seed weight and the number of seeds per pod

Seed weight was significantly correlated with the number of seeds per pod for untreated and plants pollinated by insect simulation (Figure 4.4), indicating that as the number of seeds in a pod increases, the weight of individual seeds decreases. This trend was not detected for wind-pollinated seeds.

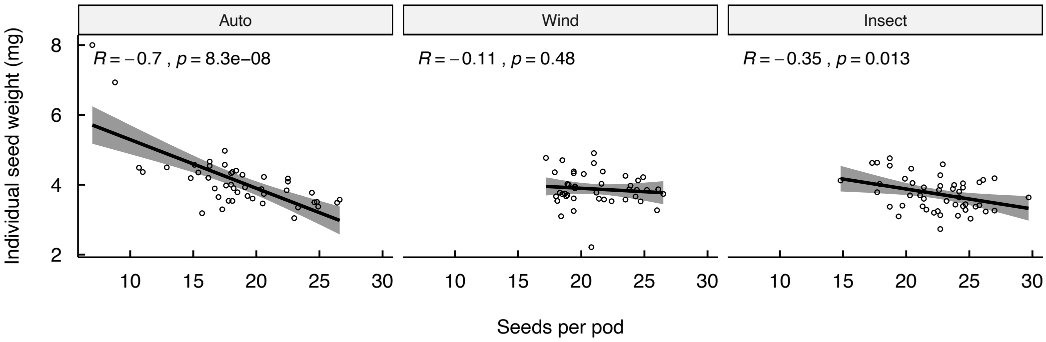

Figure 4.4 Relationship between the number of seeds per pod and individual seed weight, grouped by pollination treatment. Correlations for auto- and insect-pollination are significant. Pearson's correlation coefficients are shown (*n* = 120).

4.5 Discussion

4.5.1 Overview

This study considers the effects of pollination on OSR plant growth metrics and builds upon previous research where the focus has been constrained to reproductive metrics (Williams *et al.*, 1986, Steffan-Dewenter, 2003, Adamidis *et al.*, 2019). OSR plants showed phenotypic plasticity in response to pollination treatment, altering how they allocated energy towards plant growth, flowering, seed set and seed weight. Plants in receipt of supplementary pollination (i.e. both wind and insect simulated) were shorter and produced fewer flowers over fewer days, showed higher fruit set and yielded a significantly greater number of seeds per plant when compared to plants that received no supplementary pollination.

4.5.2 Vegetative growth and flowering

Oilseed rape plants exposed to supplementary pollination allocated fewer resources to plant growth and flowering, both in terms of the number of days in flower and the number of flowers produced. Instead, these resources were directed towards reproductive metrics (i.e. the number of seeds and yield). When pollination is limited, oilseed rape continues to grow and produce more flowers for a significantly extended period, producing, on average, 32% more flowers, and flowering, on average, nine days longer than pollinated plants. The difference in the flowering period between pollinated and pollen-limited plants might suggest that oilseed rape has a ‘maximum carrying capacity’, as Williams *et al.* (1986) refer to it. This ‘maximum carrying capacity’ may relate to the fulfilling of the plant’s pollination requirements through the number of ovules fertilised. The lack of fertilisation as a trigger to extend flowering in oilseed rape is supported by Sabbahi *et al.* (2006). They report that when open flowers are physically removed, the overall number of flowers produced per plant can increase two-fold. Lack of insect pollination has previously been found to prolong the duration of flowering (Herrera, 1995). Extending the flowering period and increasing the abundance of flowers will increase the probability of insect visitation and wind-pollination (Primack, 1985). Since foraging in many pollinating species is constrained by temperature (Kevan and Baker, 1983, Corbet *et al.*, 1993), phenotypic plasticity in flowering traits in response to pollen limitation is likely to help stabilise and maintain OSR yields in years where weather conditions are adverse. Adequately pollinated plants, with their shorter flowering period, are also likely to mature earlier. This allows seeds to ripen evenly and reduces the time the crop is in the ground, thus increasing flexibility within the crop rotation.

Furthermore, plants receiving supplementary pollination also appeared to direct fewer resources into growth, resulting in shorter plants, consistent with Adamidis *et al.* (2019). Shorter above-ground growth reduces the risk of lodging – the permanent displacement of the plant from its vertical position. Lodging affects up to 31% of OSR production, resulting in substantial yield losses estimated between £47 and £120 million per year, in the United Kingdom (Kendall *et al.*, 2017). Both reduced plant height and early maturation have the potential to positively impact yield, highlighting that pollination, both by wind and insect pollinators, may have additional, indirect, agronomic benefits.

4.5.3 Seed production

Phenotypic plasticity is evident in seed production in OSR. Contrary to the expectation that pollen-limited plants may produce seeds of lesser quality (Bommarco *et al.*, 2012b), seed weight was significantly higher when plants were pollen-limited, leading to heavier seeds. Seeds from auto-pollinated plants were 10% heavier than seeds from insect-pollinated plants and 6% heavier than seeds from wind-pollinated plants. Since auto-pollinated plants produced fewer seeds per pod, 21% and 14% fewer, when compared with insect-pollinated and wind-pollinated plants, respectively, additional resources were allocated to produce significantly fewer, but heavier seeds. As pollen-limited plants allocate excessive resources to growth and flowering, the remaining resources are limited and need to be conserved. Competition for these resources may be evident in OSR in the trade-off between allocating resources to seed weight rather than to the number of seeds.

Field-based studies have found insect pollination to increase the number of seeds (Steffan-Dewenter, 2003, Sabbahi *et al.*, 2005), and seed weight (Bommarco *et al.*, 2012b, Stanley *et al.*, 2013) when compared to wind-pollination. Although the results of this study also found the number of seeds to be greater in insect-pollinated plants, individual seed weight was heavier in plants left untreated. Infield experiments, comparisons tend to be made between pollinator excluded plants and those subject to wind and pollinators, with the latter having positive effects on yield. In this study, treatments were restricted, where possible, to either wind or insect pollination, whereas in field conditions, pollination is achieved by a combination of both. This suggests that complementarity of wind and insect pollination may be required to maximise yields under different conditions. In warm, still weather conditions, where wind pollination is limited, and insect activity is high, insect pollination would increase yields through increased seed production. Likewise, when conditions are not favourable for insect pollination, such as high winds and low temperatures, wind pollination makes up any shortfall.

Previous studies of oilseed rape confirm that fruit set is increased with pollination in controlled conditions (Williams *et al.*, 1986) by honeybees (Sabbahi *et al.*, 2005) wild bees and hoverflies (Jauker *et al.*, 2012a). In this study, auto-pollinated plants converted only 60% of flowers to fruit, compared to 79% and 77% in insect-and wind-pollinated plants, respectively. Despite supplementary pollination resulting in fewer flowers per plant, the higher fruit set and number of seeds per plant resulted in yield increases of 8% and 10% for insect- and wind-pollinated plants. This result could be considered modest in comparison to other studies where yield increased between 18% (Bommarco *et al.*, 2012b) and 46% (Sabbahi *et al.*, 2005). Nevertheless, from an economic standpoint, this is still a substantial increase for growers.

4.5.4 Differences between insect and wind simulated pollination

There was very little difference in vegetative or reproductive metrics between the plants that received wind- or insect-simulated pollination. The absence of any differences between wind- and insect-pollination indicate that winter-sown oilseed rape plants can still provide excellent yields without insect-pollination, provided there is adequate wind. In a similar study, Williams *et al.* (1986) also found no differences in yield between shaken (wind-simulated) and hand-pollinated (insect-simulated) plants. The absence of any significant differences between the supplementary pollination treatments may be explained by the efficiency of pollen delivery during this study. Thomson (1989) report that if pollen load is too large, clumping can occur, leading to stigma clogging and reduced yield. Indeed, Lankinen *et al.* (2018) found that increasing pollen load had a positive effect on OSR seed set initially, before levelling off, with an optimum pollen load ranging from 100 to 200 grains. Regular hand-pollination may have increased pollen deposition beyond this, and adversely affecting the yield.

Additionally, the supplementary pollination treatments undertaken during this study do not accurately represent those presented to oilseed rape plants under field conditions. Unlike those found in natural environments, pollination treatments during glasshouse experiments are controlled and regimented, temporally, and in intensity. Furthermore, the wind simulated pollination in this study focused on transferring pollen within a single plant. In contrast, in a field situation, wind pollination would result in both self- and cross-pollination. The treatments offered in this study represent what may be possible, and the results should be considered as ‘pollination potential’, rather than mimicking real-world situations.

Pollen limitation has a considerable impact to yield, although these impacts are somewhat mitigated by prolonging the flowering period and increasing seed quality. Increased deposition of pollen is more important than the delivery method. With the probability of windborne pollen finding a receptive stigma being low (Langridge and Goodman, 1982, Ouvrard *et al.*, 2017), there is potential for wind and insect pollination to complement each other to increase yield stability under different conditions. For example, lack of wind favours insect flight, but in periods of high winds and low temperature, wind satisfies the pollination demand.

4.5.6 Conclusion

This study demonstrates the impact of pollination on oilseed rape and how phenotypic plasticity alters the functional traits in response to pollination. The allocation of resources from growth and flowering metrics has the potential to increase and stabilise yields, either directly (i.e. through increased fruit set and the number of seeds), or indirectly (i.e. by producing shorter plants and reducing flowering time). With pollen limitation having a considerable effect on seed weight, this has economic effects. For oilseed rape to produce a more stable seed production, in terms of quantity and quality, a combination of wind- and insect-pollination, working together, offers the best opportunity. When environmental conditions affect the efficiency of one delivery system, the other can mitigate any potential pollen restrictions.

Alongside the floral resources and habitat provided by agri-environment schemes, mass-flowering crops are vital in the conservation of insect pollinators. With the effects of plasticity on the allocation of resources demonstrated in this study, pollinators have the potential to be equally crucial to growers of mass-flowering crops. By investing in pollinator management, growers can exploit the valuable ecosystem service offered by insect pollinators to increase economic output well into the future.

Chapter 5

Using functional plant traits to predict floral resources in oilseed rape (*Brassica napus*)

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5 Using functional plant traits to predict floral resources in oilseed rape (*Brassica napus*)

5.1 Abstract:

A key contributing factor to pollinator decline is the loss of floral resources. Nectar and pollen from mass-flowering crops, such as oilseed rape (OSR; *Brassica napus*) are important for colony establishment and growth of many yield-enhancing insect pollinators. OSR breeding programs release new higher-yielding varieties annually. During varietal in-field testing, agronomic traits are measured, but there is no testing of floral resource availability. We investigated if floral resource availability in OSR could be predicted using agronomic trait data from varietal recommendation testing. We used multiple regression analyses to investigate the relationships between agronomic traits and floral resources in 19 varieties of conventional and hybrid OSR cultivars, to build predictive models for resources in varieties across OSR conventional and hybrid breeding systems. Agronomic traits influence nectar sugar content and pollen quantity in OSR. Predictive models explain 44.3% and 51.2% of the variance in sugar content, and 24.5% and 38.6% of the variance in pollen quantity for conventional and hybrid varieties, respectively. We also found that short-term climatic changes explain 32% of the variance in sugar content, and this should be taken into account in future floral resource research. Our study shows that the floral resources of OSR can be predicted using agronomic trait data and that the manipulation of these traits by OSR breeders does directly

affect the floral resources that a plant produces. These models also allow us to predict floral resources in future varieties and allow growers to make informed decisions about varietal selection to take advantage of enhanced yields through insect pollination.

5.2 Introduction

5.2.1 Pollinator decline

There has been considerable concern over the decline of insect pollinators and the impact on our crops, wildflowers and trees (Biesmeijer *et al.*, 2006, Potts *et al.*, 2010a, Cameron *et al.*, 2011, Carvalheiro *et al.*, 2013, Vanbergen and The Insect Pollinators Initiative, 2013). More than 87% of flowering plant species globally profit from animal pollination (Ollerton *et al.*, 2011), and 70% of leading crops experience increased yields as a result of insect pollination (Klein *et al.*, 2007). Pollinator decline, therefore, seriously threatens biodiversity and food security. Causes for this decline include the destruction of semi-natural habitats, such as hedgerows; increased insecticide use (Robinson and Sutherland, 2002); climate change; invasive species and pathogens (Potts *et al.*, 2016b); and the loss of wildflower-rich habitats (Blackstock *et al.*, 1999). However, the key contributing factor is the decrease in floral resource availability linked with agricultural intensification (Klein *et al.*, 2007, Potts *et al.*, 2010a, Goulson *et al.*, 2015).

5.2.2 Importance of floral resources

Floral resources provide insects with a valuable nutrient-rich resource. Flowers produce nectar as a reward for insect-visitors in exchange for pollination services. In addition to essential amino acids and minerals, nectar provides an essential source of carbohydrate-rich sugars (sucrose, glucose and fructose), used to fuel flight for foraging, nesting activities, mating and other physiological processes. For some species, it is their only food source (Jervis and Boggs, 2005). Pollen provides a secondary reward, adding proteins, lipids and vitamins to the diet for longer-term benefits (Roulston and Cane, 2000). For example, bees directly invest foraged pollen in the next generation (Bowers, 1986, Albrecht *et al.*, 2007). A single European honeybee (*Apis mellifera*) can collect as much as 120 kg of nectar and 20 kg of pollen annually (Seeley, 2009). Baude *et al.* (2016) highlighted the scarcity of floral resources in agricultural landscapes relative to urban semi-natural habitats. This shortfall is despite the richness of mass-flowering crops, such as oilseed rape (OSR *Brassica napus)*, soybean and sunflower which offer large quantities of floral resources, albeit in concentrated time-periods (Stanley and Stout, 2013, Gill and O'Neal, 2015, Requier *et al.*, 2015). Although mass-flowering crops can negatively affect the pollination of native plants (Holzschuh *et al.*, 2011), they can have a positive effect on pollinator abundance by increasing the nest densities of wild pollinators (Westphal *et al.*, 2003, Knight *et al.*, 2009, Holzschuh *et al.*, 2013). Westphal *et al.* (2009) and Jauker *et al.* (2012b) report positive effects of OSR presence during early colony growth of bumblebees

(*Bombus terrestris*) and solitary bees (*Osmia bicornis*). While this growth did not translate to reproductive success (the presence of males and/or queens), this could be caused by 'hunger gaps' present later in the season (Timberlake *et al.*, 2019). Current strategies to enhance late-season floral resource availability include agri-environmental options such as nectar- and pollen-rich flower mixes (Carvell *et al.*, 2007). However, the value of their overall contribution is questionable (Baude *et al.*, 2016). Jauker *et al.* (2012b) suggest that the early-season benefits of colony establishment outweigh any late-season reproductive disadvantages, highlighting the importance of resources offered by mass-flowering crop such as OSR. By providing forage at different points in the season, mass flowering crops such as oilseed rape have the potential to complement agri-environment schemes in the resources they offer. Despite this, variety selection in OSR is primarily driven by agronomic factors and their potential to provide forage for pollinators remains unconsidered.

5.2.3 Oilseed rape breeding programmes

OSR is the third-largest source of vegetable oil worldwide. In Europe, it is the largest oilseed crop, cultivated on 34.9 million hectares in 2019 and producing 18 million metric tons of seed annually (USDA, 2019). While OSR is capable of self-pollination, insect visitation increases crop yield (in winter-sown varieties) and consequently increases economic value (Morandin and Winston, 2006, Bommarco *et al.*, 2012b, Bartomeus *et al.*, 2014). Since the estimated global commercial value is over $7.5 billion per annum (USDA, 2019), OSR is subject to an intensive commercial breeding programme. Oilseed rape varieties are bred in one of two ways. Conventional varieties are ‘open-pollinated’ using traditional line-breeding methods, selecting parent plants with favourable, heritable traits. Restored hybrid varieties (Ollerton *et al.*, 2011) are created by crossing male-sterile (female) plants with pollen-producing, fertile (male) plants to produce seed with restored male fertility (Friedt and Snowdon, 2009). New varieties are the result of lengthy research by breeding companies, creating thousands of lines before selecting the most competitive varieties. For example, in the UK, before new varieties reach the market, they must undergo two years of regional infield testing by the Agricultural and Horticultural Development Board (AHDB), be declared ‘morphologically distinct, uniform and stable’ and prove to have ‘value for cultivation and use’. Following testing, successful varieties gain Plant Breeder’s Rights, a prerequisite for the seed reaching market. They are added to the National Recommended List (RL) as a recommended variety for the region where it excelled (AHDB, 2016). Since new varieties compete to outperform currently listed ones, duration on the list is typically short. Of the 26 OSR varieties that constitute the 2019 list, 15 are first- or second-year recommendations (AHDB, 2019).

5.2.4 Agronomic traits of oilseed rape

It is widely known that varietal differences in floral resources exist in winter OSR (Pelletier *et al.*, 1987, Mesquida *et al.*, 1991). However, their value to pollinators is not currently a consideration in breeding or varietal recommendation programmes. Recent studies have focused on differences in floral resource availability across specific varieties (Bertazzini and Forlani, 2016) or breeding systems (Carruthers *et al.*, 2017, Ouvrard *et al.*, 2017). However, the literature does not extend to identifying if the agronomic traits displayed by OSR varieties influence resource availability. Fulfilling this knowledge gap may not influence breeders to develop ‘pollinator-friendly’ varieties over high-performing varieties. However, it may allow conscientious growers to make informed decisions about variety selection, to exploit local pollinator communities for increased yield. Evaluating floral resources involves sampling nectar and pollen for each OSR variety, which is time-consuming and requires specialist training and equipment.

Furthermore, due to the high turnover of OSR varieties, annual surveying would be required to quantify the resources of new varieties. An alternative, more cost-effective means of evaluating floral resources may be to explore the relationship between measured OSR agronomic traits and resource availability. During infield testing, varieties undergo assessments for a variety of agronomic traits, including yield performance and disease resistance. In the UK, these traits are compared across varieties and ranked for each trial site. Alongside yield performance results, they are used by agronomists to recommend specific varieties, dependant on growers’ needs. For example, varieties with a low lodging or stem stiffness score could suffer on a farm exposed to high winds. Plant traits have been used previously to predict nectar sugar productivity in different plant species (Baude *et al.*, 2016), and to quantify pollen in animal-pollinated plants (Cruden, 2000). By narrowing the focus to investigate differences across agronomic traits rather than varieties, it may be possible to identify if any specific traits or combination of traits contribute to enhanced floral resources. These 'favourable' trait combinations could then be used to predict floral resources in future varieties.

5.2.5 Aim of this study

In this study, we set out to answer the following question: can we predict floral resource availability using agronomical traits currently measured as part of a varietal recommendation testing? To provide answers, we quantified the floral resources for 19 varieties of winter OSR, currently undergoing varietal recommendation testing. Combined with agronomic trait data obtained from the AHDB, we used multiple regression analyses to investigate the relationships between agronomic traits and floral resources to build predictive models to allow resource prediction in future varieties. The ultimate aim would be the inclusion of 'floral resource value' in future OSR breeding programmes and varietal recommendation initiatives.

5.3 Methods

5.3.1 Study site

The study was conducted in 2019 at the Agriculture and Horticulture Development Board's (AHDB) North Region Oilseed Rape Recommended List (RL) field site in Midlothian, Scotland (NT 251659). Winter OSR varieties were replicated across three randomised blocks, with two plots per variety per block (i.e. a total of six plots per variety across the trial site). Each plot measured 10m x 4m, with a seed rate of 60 seeds/m^2. Double guard plots, of identical dimensions, provided separation between varieties of different breeding systems. All plots were subject to conventional agrochemical treatments to control for pests and pathogens.

5.3.2 Varieties and sample size

To provide a comprehensive database for oilseed rape traits across varieties, including annual variation in these traits, surveying focussed on nineteen established varieties with previous in-field testing history (ten conventional/open-pollinated, nine restored hybrids). Nectar was sampled from ten flowers per variety from block one and five flowers per variety from blocks two and three, totalling twenty flowers per variety. Nectar sampling took place on dry days during peak flowering (24 April – 2 May 2019), between 09:00 and 17:00 hours. Sample times were split into ‘early’ (09:00 hrs), ‘mid’ (12:00 hrs) and ‘late’ (15:00) collection periods, where possible. All blocks were sampled at least once during each collection period.

5.3.3 Environmental conditions

As environmental conditions may influence nectar production (Southwick, 1984, Búrquez and Corbet, 1998), temperature and relative humidity data were collected using a Delta-T WS-GP2 weather station (Delta-T Devices, Cambridge, UK), located in the adjacent field. These data were used to calculate the air vapour pressure deficit (VPD) during the three hours before sampling. VPD is the difference between the saturated and actual water vapour pressure or the 'drying power' of the air and may, therefore, affect the water content of standing crop nectar.

It is calculated using both temperature and relative humidity using the Tetens equation first to calculate saturated vapour pressure (SVP) (Allen *et al.*, 1998):

$$SVP = 0.61078\, exp\left(\frac{17.27T}{T + 237.3}\right)$$

Then:

$$VPD = SVP\ \times \left(\frac{1 - RH}{100}\right)$$

Where T is the temperature in °C and RH is relative humidity (%).

For pollen quantification, all three blocks were sampled. During a single day, anthers were collected from 5 plants per plot, totalling 15 plants per variety (24 April 2019).

5.3.4 Nectar collection and analysis

The standing crop of nectar present in unvisited, newly opened flowers, rather than secretion rate, was measured (Corbet, 2003). Terminal stems with at least ten unopened flowers were used. To eliminate flower-age related variability, the most recent flower to open on each raceme was marked. To restrict pollinator visitation, the buds above the marked flower were enclosed in 15 x 35 cm tulle net bags (1 x 1 mm mesh). Bags remained in place for 36 hours before sampling. Once bags were removed, sampling was restricted to recently opened flowers (those above the marked flower). Only flowers with dehisced anthers were sampled.

5.3.5 Sugar content

Nectar was collected using calibrated 1 μl glass capillary tubes (Drummond Scientific Co., Broomall, PA, USA). Nectar volume was calculated by dividing the length of the nectar column in the capillary tube by the total length of the capillary tube (Cruden and Hermann, 1983). Sugar concentration (°Brix) was measured in the field with a low-volume, temperature-calibrated refractometer (Bellingham & Stanley Ltd, Farnborough, Hants, UK). As the units of nectar volume (μl) and °Brix (g) differ, multiplying them introduces significant errors at high percentages. To correct for this, mg sugar mg^{-1} was converted to mg sugar μl^{-1}, using the polynomial model equation (Bolten *et al.*, 1979, Galetto and Bernardello, 2005):

$$\hat{y} = 0.00226 + (0.00937\,x) + (0.0000585\,x^2)$$

Where x represents the concentration (°Brix) and $\hat{y}$ the predicted quantity of sugar (mg sugar μl^{-1}). Total sugar per flower is calculated by multiplying mg sugar μl^{-1} by total nectar volume.

5.3.6 Pollen quantification

Pollen from fifteen flowers per variety was sampled (one flower per plant, five plants per plot, three blocks) giving a total of 285 flowers across 19 varieties. The stamens of *B. napus* flowers encircle the stigma in two sets: an inner ring of four, long, outwardly facing anthers and an outer ring of two, shorter, inwardly facing anthers. All anthers per flower were collected. To reduce pollen loss, anthers were collected shortly before anthesis and stored in a 1.5 ml Eppendorf tube until dehiscence. They were then preserved in 1 ml of 70% ethanol. Pollen was harvested using ultrasonication (Fisher Scientific, Loughborough, Leics, UK), and grains, dispersed into known volumes of 70% ethanol solution were counted on a haemocytometer (Weber Scientific, Hamilton, NJ, USA).

5.3.7 Statistical analysis

Before analyses, the effect of environmental factors (i.e. temperature, humidity and VPD) were investigated using simple linear regression. Following this, to determine if agronomic traits influence floral resources, a stepwise bidirectional-elimination selection approach using Akaike information criterion

(AIC) was implemented to find the most parsimonious model (see Table 5.1 for a summary of effects).

Table 5.1 Summary and description of agronomic traits measured during varietal in-field testing by the Agricultural and Horticultural Development Board. Traits were used as predictor variables in regression analyses.

Agronomic traits	Description
Gross output	Tonnes per hectare
Seed yield	Tonnes per hectare
Oil content	Oil content %
Early vigour	Competition with weeds 1 – 9 (1 very weak, 9 very strong)
Emergence	Date of full emergence 1 – 9 (1 very slow, 9 very fast)
Establishment	Number of plants per square meter following emergence 1 – 9 (1 very thin, 9 very thick)
Lodging	Resistance to lodging during flowering period 1 – 9 (1 all plants lodged, 9 no plants lodged)
Stem stiffness	Resistance to lodging during maturity (post-flowering) 1 – 9 (1 all plants lodged, 9 no lodging)
Height	Average plant height at end of flowering Measured in centimetres
Earliness of flowering	Start of flowering period 1 – 9 (1 latest flowering plot, 9 earliest flowering plot)
Earliness of maturity	Degree of canopy senescence before harvest 1 – 9 (1 very late, 9 very early)
Winter hardiness	Survival rates throughout winter 1 – 9 (1 complete loss, 9 no damage)

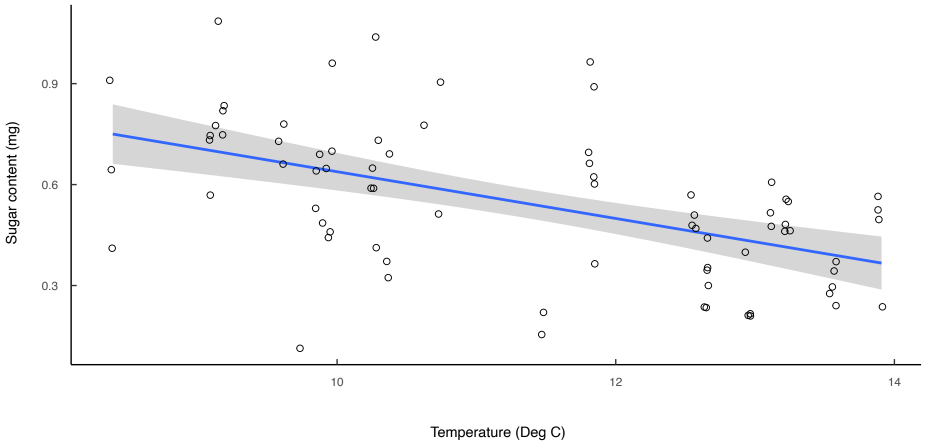
Temperature, humidity and VPD all had significant linear relationships with sugar per flower. To control for these climatic effects, VPD (variable with the highest R^2 value: see section 5.4.1) was therefore included as a predictor variable in our regression models for nectar. Bidirectional elimination stepwise multiple regression models for sugar per flower and pollen quantity were fitted separately for both breeding systems, using the agronomic traits in Table 1. Models with the lowest Akaike information criterion (AIC) were selected. All models were fitted using Residual Maximum Likelihood (REML) with the LME4 package (Bates *et al.*, 2015) in R version 3.6.1 (R Core Team, 2019).

5.4 Results

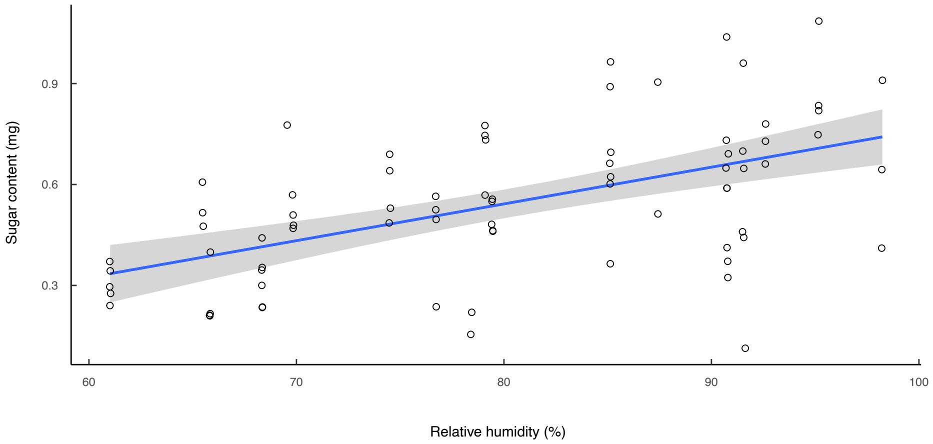
5.4.1 Environmental effects on sugar quantity

To determine the effects of environmental conditions on sugar quantity, simple linear regression showed that sugar content per flower declined significantly with increasing temperature ($F_{1,74}$ = 28.56, P <0.001; Fig. 1a.), increased with relative humidity ($F_{1,74}$ = 32.73, P <0.001; Fig. 1b.) and decreased with increasing vapour pressure deficit (VPD) ($F_{1,74}$ = 35.78, P <0.001; Fig. 1c.). These relationships were comparable for both breeding systems. Thus, to control for these effects, VPD was included as a predictor variable in the regression modelling.

(a) Temperature



(b) Humidity



(c) Vapour Pressure Deficit

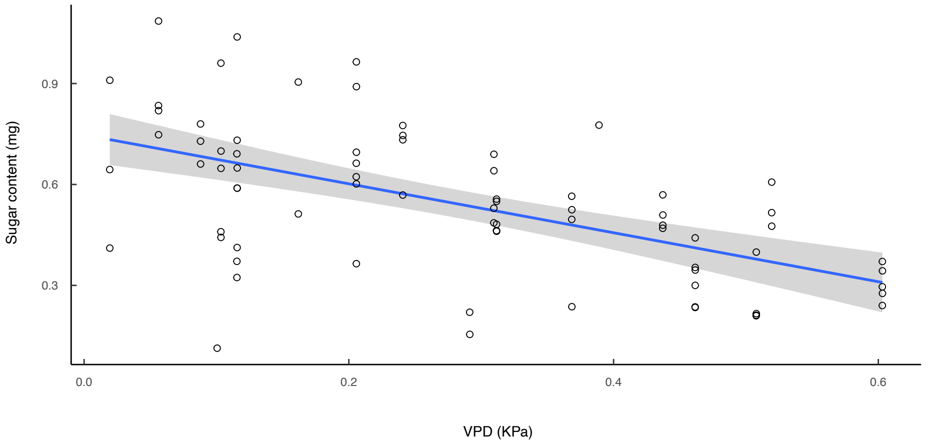


Figure 5.1 The effect of temperature ($R^2 = 0.27$, $n = 76$), relative humidity ($R^2 = 0.29$, $n = 76$), and vapour pressure deficit (VPD) ($R^2 = 0.32$, $n = 76$) on sugar per flower (mg) of oilseed rape. Grey area shows 95% confidence intervals of the mean.

5.4.2 Sugar per flower

All varieties of oilseed rape produced nectar. The mean sugar per flower across all varieties was 0.54 mg (± 0.03 SE). On average, hybrid varieties (mean 0.60 mg ± 0.03 SE) produced more sugar per flower than conventional varieties (mean 0.49 mg ± 0.04 SE; Figure 2a). However, inter-varietal differences in both breeding systems were similar in both breeding systems with both having a range of 0.85 mg of sugar per flower (Figure 2b).

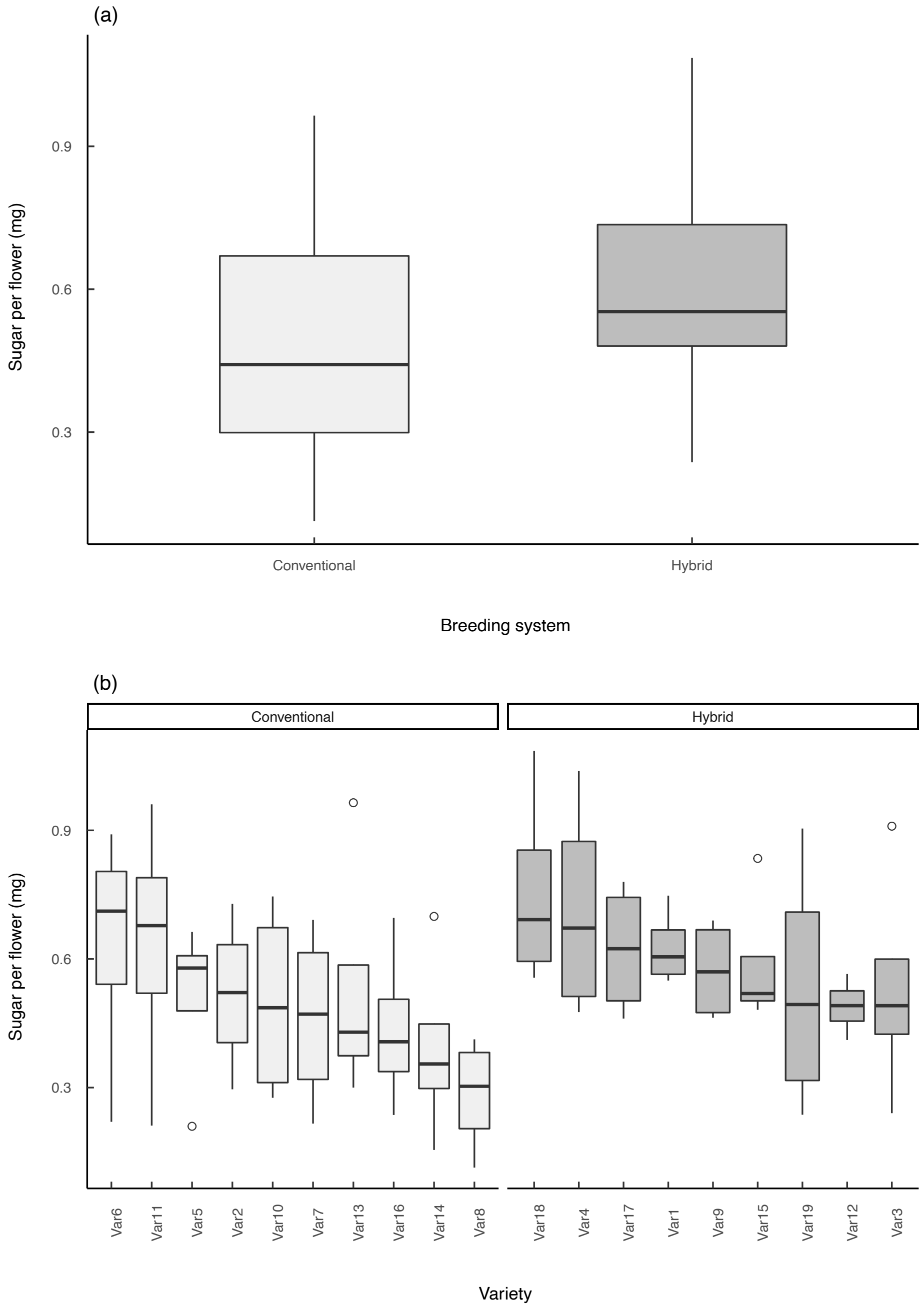


Figure 5.2 Boxplots showing the quantity of sugar per flower in (a) oilseed rape varieties grouped by the breeding system: conventional varieties (n = 10) and hybrid varieties (n = 9) and (b) 19 oilseed rape varieties (n = 4). Boxes around the median shows interquartile range. Whiskers extend to minimum and maximum values. Variety names have been anonymised by request.

5.4.3 Pollen grains per flower

The mean number of pollen grains across all varieties of OSR was 233, 421 (± 8, 060.91 SE) grains per flower. Hybrid varieties produced a greater number of pollen grains per flower (mean 237, 195 ± 11, 693.9 SE; range = 233, 616 grains) than conventional varieties (mean 230, 036 ± 11, 279.3 SE; range = 214, 063 grains; Figure 3a). Inter-varietal differences in the range were considerably high in both breeding systems (Figure 3b).

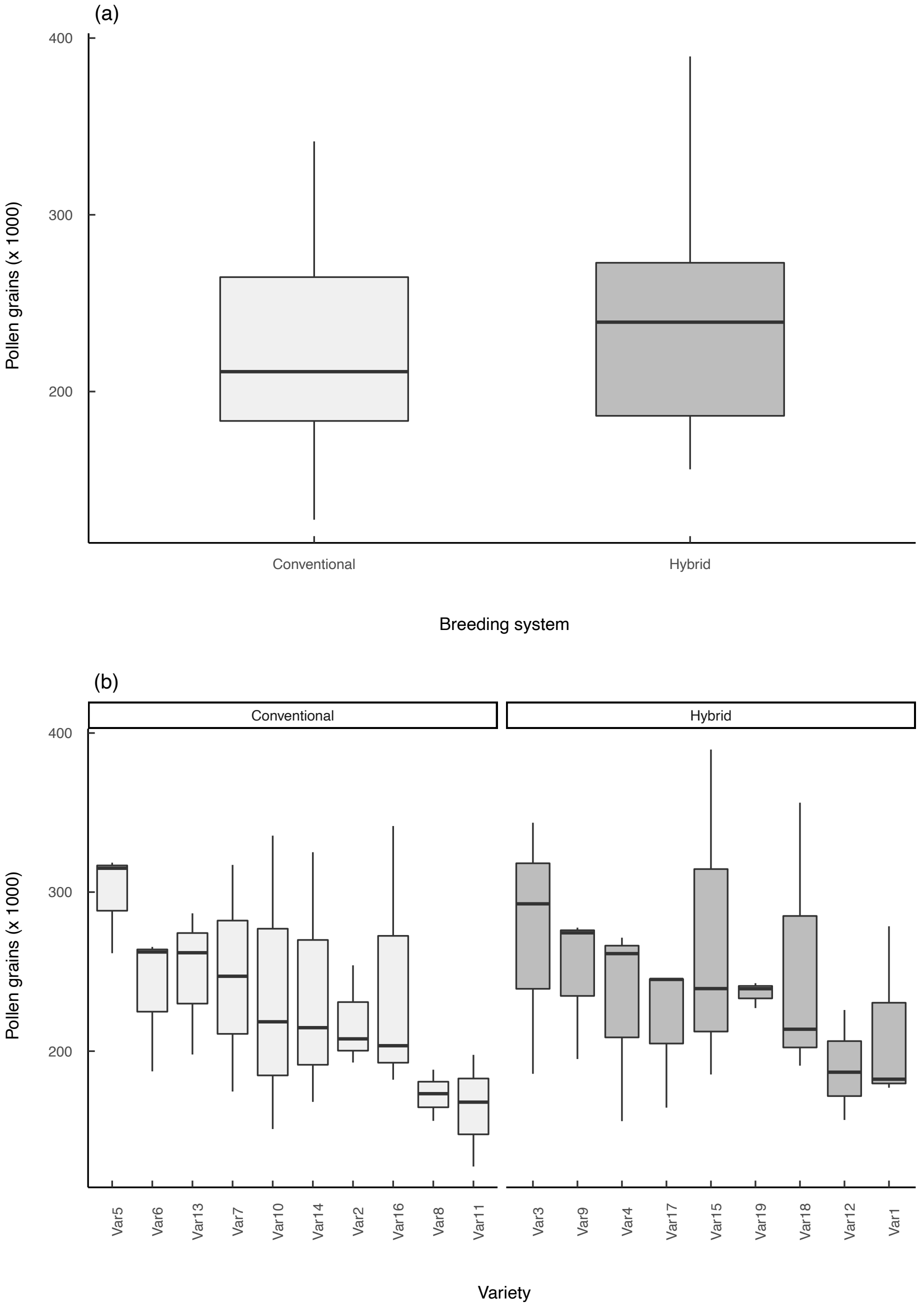


Figure 5.3 Boxplots showing the number of pollen grains per flower in (a) oilseed rape varieties grouped by breeding system: conventional varieties (n = 10) and hybrid varieties (n = 9). (b) 19 oilseed rape varieties (n = 3). Boxes around the median shows interquartile range. Whiskers extend to minimum and maximum values. Variety names have been anonymised by request.

5.4.4 Prediction models

As differences in pollen and sugar quantity were detected between hybrid and conventional varieties, multiple regression analyses were conducted independently for the two breeding systems. Multiple regression analyses examined the relationship between sugar quantity and pollen quantity per flower and the recorded agronomic trait values.

5.4.5 Sugar per flower

Stepwise multiple regression analyses were performed to investigate whether agronomic traits could predict the quantity of sugar per flower within each oilseed rape breeding system. For conventional oilseed rape varieties, regression analyses indicated that the most parsimonious model explained 44.3% of the variance and that the model was a significant predictor of sugar per flower ($F_{3,36} = 11.32$, $P < 0.001$). VPD ($\beta = - 0.82296$, $P < 0.001$) and early vigour ($\beta = 0.12628$, $P < 0.012$) contributed significantly to the model. Although winter hardiness had only a marginal effect, it was included in the model based on AIC comparisons ($\beta = 0.22494$, $P < 0.082$). The final predictive model was:

$$\text{Sugar (mg)} = -2.1754 + (-0.82 * VPD) + (0.13 * early\ vigour) + (0.22 * winter\ hardiness)$$

For hybrid varieties, the most parsimonious model explained 51.2% of the variance and was a significant predictor of sugar ($F_{2,33} = 19.37$, $P < 0.001$). Both VPD ($\beta = - 0.76427$, $P < 0.001$) and stem stiffness ($\beta = 0.09746$, $P = 0.011$) contributed significantly to the model.

The final predictive model was:

$$\text{Sugar (mg)} = 0.16517282 + (-0.76 * VPD) + (0.097 * stem\ stiffness)$$

5.4.6 Pollen per flower

Multiple linear regression was also performed to determine whether agronomic traits could be used to predict the number of pollen grains per flower within each oilseed rape breeding system. For conventional varieties, the most parsimonious regression model explained 24.5% of the variance and was a significant predictor of pollen quantity ($F_{1,28} = 10.41$, $P < 0.003$). Earliness of maturity contributed significantly to the model ($\beta = -24180.56$, $P = 0.003$). The final predictive model was:

$$\text{pollen quantity} = 358181.60 + (-24180.56 * earliness\ of\ maturity)$$

For hybrid varieties, the regression model explained 38.6% of the variance but was not a significant predictor of pollen quantity ($F_{1,25} = 2.044$, $P = 0.165$). The best fit model using AIC was:

$$\text{pollen quantity} = 160953.2 + (11779.8 * resistance\ to\ lodging)$$

5.5 Discussion

5.5.1 Overview

This study is the first to use readily available agronomic trait data to predict floral resource availability of winter-sown oilseed rape varieties successfully.

Results demonstrate that manipulation of agronomic traits by oilseed rape breeders does affect the quantity of sugar and pollen that a plant produces. The varietal differences in agronomic traits also make it possible to predict pollen quantity and sugar content in future conventionally bred and hybrid oilseed rape varieties without extensive sampling.

5.5.2 Impact of environmental factors on nectar resources

Short-term climatic changes before sampling significantly affect the sugar content of nectar. The amount of sugar per flower significantly reduces with increasing temperatures, a result also found by Villarreal and Freeman (1990) and Takkis *et al.* (2018). However, these studies focus on the effect of nectar production from long-term elevated temperature exposure to replicate climate change. In contrast to temperature, humidity has a positive relationship with sugar content. With changes in sugar content occurring three hours before sampling, it indicates that they are post-secretory rather than a physiological response of the plant. Corbet *et al.* (1979) found that low humidity caused water evaporation from post-secretary nectar resulting in a higher concentration of sugar, and low humidity caused dilution. Although this does not explain fluctuations in sugar per flower, it may suggest a sampling artefact. As humidity increases, nectar viscosity decreases. Using micropipettes with minute diameters to extract concentrated nectar may result in small amounts of sugar remaining in the flower. Mesquida *et al.* (1988a) compared nectar collection methods and, although centrifugation extracted four to six times more liquid than micro pipetting, differences in sugar content were unaffected by the sampling method. Vapour pressure deficit (VPD) is calculated using temperature and relative humidity. Although it affects sugar content similarly to temperature, it explains more of the variance (32%) than either temperature or humidity (27% and 29% respectively). While the impact of temperature is commonly considered when sampling plant nectar (Jakobsen and Kritjansson, 1994) this research indicates that VPD is a better predictor of sugar content. With VPD being easily calculated from temperature and humidity, it is recommended that it is taken into account in future research of floral resource availability.

5.5.3 Impact of breeding system on floral resources

Hybrid varieties produced, on average, 22% more sugar per flower than conventional varieties, a result consistent with a glasshouse study by Carruthers *et al.* (2017). However, these results do not support other studies undertaken in the field where hybrid and conventional varieties were found to produce similar sugar content (Pernal and Currie, 1997, Pierre *et al.*, 1999). There may be several reasons to explain these differences. Firstly, Pernal and Currie (1997) sampled at various times a day (08:00, 11:00, 14:00 and 16:00 hours) over four weeks, and although their analysis included sampling time and day, it did not account for variable environmental conditions. The glasshouse study, under controlled conditions by Carruthers *et al.* (2017), would be less affected by temperature and humidity. As previously mentioned, VPD has a significant effect on sugar content in oilseed rape and may explain the conflicting results between the studies.

Conventional and hybrid varieties of OSR produce a similar number of mean pollen grains per flower, with a difference of only 2% across breeding systems. However, within breeding systems, inter-varietal differences are high. In conventional varieties, the lowest-performing variety, in terms of mean pollen grains per flower, produces only 55% of the highest-performing variety. Hybrid varieties showed less variability than conventional varieties in pollen grains per flower, with the lowest-performing variety producing 78% of the highest-performing hybrid variety.

5.5.4 Impact of agronomic traits on floral resources

Mass-flowering crops, such as OSR, provide a vital source of food for insect pollinators, particularly in the early stages of their lifecycle when colony establishment is underway (Westphal *et al.*, 2003, Holzschuh *et al.*, 2013). Floral resource availability in OSR differs considerably across varieties. Breeders produce varieties based on the heritable traits of the parent plants and focus primarily on increasing yield potential, seed quality, disease and pest resistance and agronomic traits (Christen and Friedt, 2007). Varietal differences in floral resources have previously been reported for OSR (Pernal and Currie, 1997, Ouvrard *et al.*, 2017) as well as differences between breeding systems (Pierre *et al.*, 1999, Carruthers *et al.*, 2017). To breed varieties with traits that are considered ‘desirable’ to growers, such as those that increase seed production, oil content and resistance to lodging, could result in a trade-off at the expense to floral resources.

In contrast, some traits may affect floral resources positively. Floral resource availability is not currently included as a measurable factor in varietal selection. Gathering floral resource data during field trials would require specialist equipment and training and involve considerable work by trials managers. The ability to predict floral resources using already available agronomic traits would allow inclusion into varietal recommendation lists and provide growers with the opportunity to make an informed varietal selection.

5.5.5 Model application

Using agronomic traits obtained during national testing and floral resource data extracted from field sampling, we were able to create models to predict the availability of floral resources across OSR varieties. In models for predicting sugar content, VPD had a more substantial effect on sugar per flower than individual agronomic traits in both breeding systems, highlighting the importance of environmental factors when sampling nectar. For sugar content in conventional varieties, early vigour and winter hardiness were the main agronomic trait predictors. This suggests that varieties with a higher tolerance to stressful environmental factors during the early season allocated more resources to pollinators by offering increased sugar per flower. Varieties that show higher early vigour may also benefit from increased soil moisture later in the season. In water-limiting environments, early leaf development accelerates canopy closure and reduces water evaporation of soil (Ludlow and Muchow, 1990, Passioura and Angus, 2010). This reserve of water may benefit later season plant development, such as sugar production. With winter hardiness and early vigour being 'desirable' OSR traits to growers, the addition of increased floral resources is a bonus. Growers who select these varieties, therefore, benefit in two ways. In addition to having a more robust, healthier crop, the increase in food availability will encourage pollinators, potentially increasing yield through enhanced pollination.

If early season growth contributes to increased sugar content, varieties that mature early have the opposite effect on pollen quantity. Conventional varieties that matured earlier produced fewer pollen grains per flower, suggesting that resources are allocated into growth rather than pollen production. Early maturity is considered a 'desirable' trait by growers as it increases the likelihood of getting the following on crop sown immediately after harvest. Harvesting schedules do not allow for much leeway, so early maturity is less likely to result in the harvesting of under-ripe seed pods. Immature seeds may weigh less and suffer from reduced oil content – the two metrics that contribute to the economic value of OSR seed.

For hybrid varieties, the strength of the stem during maturity is important to sugar availability and a resistance to lodging during the flowering stage is an important predictor for pollen quantity. From agronomic data used in this study, these agronomic traits were highly correlated and may be a proxy for the overall health of the plant.

The models created to predict sugar content explained more of the variance than those created for pollen, although the influence of VPD may explain this. To increase the accuracy of the predictions, the method in which trial managers collect the agronomic trait data may need revision. During national testing in the UK, these traits are given a value as a comparison against other varieties, consequently generating ordinal rather than interval data. Baude *et al.* (2016) predicted nectar productivity for plant species using plant trait data collected from an online database and included physical measurements such as plant height, breeding system and length of the flowering period. By basing regression models on measurement data rather than comparison data from agronomic traits, it may be possible to build models with stronger predictive power. However, models based on actual field measurements collected in an interval scale would require the lengthy collection of agronomic trait data to enable prediction in future varieties.

5.5.6 Conclusions

We have demonstrated that sugar and pollen quantity can be predicted using agronomic trait data collected during national testing. These models are future proof, as they can be used to predict floral resources in new varieties. The benefits of being able to predict floral resources are not only good for the maintenance of pollinator populations but also arable farmers. It is widely accepted that oilseed rape yields increase through pollinator visitation (Bartomeus *et al.*, 2014, Lindström *et al.*, 2016). Growers with access to a large and diverse pollinator community, whether from managed honeybees or abundant natural habitat for wild pollinators, will benefit the most (Bommarco *et al.*, 2012b, Stanley *et al.*, 2013). With information about the floral resources available to them, growers will be able to make informed choices about the selection of varieties and take advantage of this valuable ecosystem service.

Chapter 6

General Discussion

6.1 General discussion

In agricultural environments, the nutritional demands of insect pollinators are often supported by mass-flowering crops. With pollination increasing yields in an estimated 70% of leading crop species (Klein *et al.*, 2007), recent declines in pollinator abundance are a cause for concern for global food production (Potts *et al.*, 2016b). Oilseed rape (*Brassica napus*; OSR) is the dominant mass-flowering crop in Europe and the third-largest source of vegetable oil worldwide (USDA, 2019). Although capable of self-pollination, OSR benefits from increases in yield from insect pollination (Bommarco *et al.*, 2012b). Subject to intensive commercial breeding programmes, new varieties, with a focus on yield and favourable agronomic traits, are released annually. Each year, growers are offered a frequently changing selection of varieties to choose from, typically highlighting yield and agronomic trait characteristics. At a critical time for pollinators, little is known about the impact of varietal selection on resource provisioning. This thesis examines the inter-dependence between pollinators and OSR growers, with an emphasis on cultivar breeding systems.

Variety selection plays a vital role in pollinator resource availability and the reproductive success of OSR. To explore the relationship between growers and pollinators of OSR, the following broad questions were asked:

1. How important is the relationship between oilseed rape and insect pollinators, with respect to resource provisioning?

2. What role do insect pollinators play in the pollination of oilseed rape?
3. How does oilseed rape distribute resources between growth and reproductive metrics under different pollination treatments, and what are the implications to quantity and quality of yield?
4. Can oilseed rape growers increase the pollination services of oilseed rape through varietal selection and management practices?

These questions were addressed through field-based and controlled experiments. Chapter 2 focused on the value of OSR cultivation to insect community composition and the effect of proximity to semi-natural habitat on invertebrate populations within the OSR crop. To achieve this, flower-visiting invertebrate populations were compared in the crop centre (i.e. a distance of 75 m from semi-natural habitat) and the crop edge (i.e. a distance of approximately 2 m from semi-natural habitat). Proximity to semi-natural habitat (i.e. regenerated grassland field margins adjacent to hedgerows) affected most taxonomic groups, with higher abundances of insects in the crop edge than the centre of the field. Trends in overall insect abundance were driven by the most abundant taxa, pollen beetles and parasitic wasps. Insect surveying was timed to coincide with peak flowering in oilseed rape which was approximately two weeks earlier in 2017. Insect abundance differed considerably across the two sampling years, with lower numbers found during the earlier flowering period of 2017. There was no effect of breeding system on insect communities for either year.

Irrespective of study years, commonly recognised pollinators (e.g. bees, hoverflies and butterflies) were scarce. Although marginally higher in the centre of the field, this was influenced by an isolated and atypically high bumblebee population in one sample. Although commonly underestimated, non-syrphid *Diptera* are also regular flower visitors and effective pollinators (Rader *et al.*, 2016) and were found in large numbers, suggesting that non-syrphid flies play an important role in pollination services in Scotland. Thus, to explore the contribution of insect pollination to OSR yield in chapter 3, they were included alongside key pollinators.

In chapter 3, pollinator exclusion bags were used to estimate the effect of insect pollination on three yield metrics covering both quantity and quality of yield. To measure yield quantity seed set and seed weight per pod were calculated, and individual seed weight was calculated as a measure of quality. Resource allocation, when pollen is limited, was also tested by testing for differences within plants. To explore the impact of pollinator abundance on yield, we also explored the relationship between pollinator abundance and the proportional change in yield from pollinator visitation. Contrary to the findings of Sabbahi *et al.* (2005), no relationship between pollinator abundance and pollinator contribution was found. This discrepancy could be the result of the abundance of insects in pan traps not being an appropriate proxy for crop visitation rates or other confounding factors (i.e. pest exposure or position in the crop).

Despite a lack of correlation between insect abundance and the proportional change in yield from pollinator visitation, apparent differences were observed between open-pollinated racemes and pollinator excluded racemes. Exposure to insect pollination increased seed set and seed weight per pod. A trade-off in seed weight and seed set was found indicating investment is split between seed quantity or quality. However, increases in yield quantity metrics as a result of insect pollination did not appear come at an expense to yield quality, with seed weight in racemes exposed to insect pollination increasing marginally by 4%.

When faced with pollen limitation, plants were observed to reallocate resources from pollinator-excluded racemes to those available to pollinators, resulting in a higher seed weight in comparison to plants not subject to bagging. Resource reallocation was also present at the pod level, where resources were invested in either more or heavier seeds.

In chapter 4, this redistribution of resources was explored further by investigating the impact of pollination on growth and additional reproductive metrics. In controlled experiments, using two methods of supplementary pollen deposition to simulate wind and insect pollination, the effect of supplementary pollination on growth, flowering and seed production of eight commercially available OSR cultivars (four hybrid and four conventional cultivars) was assessed. Plants receiving supplementary pollen delivery were shorter with a reduced flowering period. They also produced fewer flowers and set a higher proportion of fruit compared to untreated plants. As with chapter 3, evidence of a resource trade-off between seed number and seed weight was found. Since insect pollination has a positive effect on OSR yield, maintaining populations within the crop has economic benefits.

In chapter 5, the relationship between floral resource availability and functional agronomic traits for nineteen varieties of OSR was investigated. Predictive models found that functional agronomic traits influence sugar content and pollen quantity across OSR breeding systems. We also found significant effects of short-term climatic change in sugar content.

In this final chapter, key findings for each of the experimental chapters are addressed to answer the overarching questions. Limitations in experimental design and methodological considerations are identified, and areas for further research are suggested.

6.2 How important is the relationship between oilseed rape and insect pollinators with respect to resource provisioning?

6.2.1 Agricultural habitats

Although agricultural habitats are generally unsuitable for pollinators because of modern farming practices (Robinson and Sutherland, 2002), uncultivated field margins, particularly those that contain hedgerows, and wildflower meadows are rich in biodiversity (Landis, 2017). These habitats provide valuable resources, in terms of nesting and overwintering sites, shelter and forage for many insect taxa (Öckinger and Smith, 2007). Resources provided by this habitat plays a fundamental role in maintaining healthy populations (Kells and Goulson, 2003, Jönsson *et al.*, 2015, Campbell *et al.*, 2017). In chapter 2, proximity to these habitats affected the abundance and diversity of insect populations in oilseed rape. For most taxa, more individuals were found in the crop edge, adjacent to the field margin, than in the centre. This suggests that field margins act as a 'sink' for a diverse group of visiting insects to OSR. The implications of this are that although flower visitors show a willingness to move from the refuge provided by semi-natural habitat, they do not disperse too far into the crop. This is understandable considering the high densities of floral resources available in a mass-flowering crop. Foraging is a question of energetics. When resources are plentiful locally, there is no need to spend valuable energy unnecessarily. Under different weather conditions, the complementarity of wind and insect pollination is required to maximise yields. This is both directly, through increased seed production, and indirectly, by minimising losses caused by immature seeds or lodging. With pollinators being more abundant closer to crop edges, plants towards the centre of the field may not be adequately pollinated when conditions are favourable for insects. This potentially leads to an inconsistent harvest. For a more stable yield, smaller fields, with a larger percentage of crop edge, may solve this problem.

Key pollinators (e.g. bees and hoverflies) were found in very low densities, whereas flies outnumbered them considerably (i.e. a total of 17,554 non-syrphid Diptera were trapped by plan traps compared with 123 bumblebees, 34 honeybees, 69 solitary bees and 18 hoverflies). Fly abundance was also similar in both field locations (centre and edge) indicating that they provide consistent pollination services throughout the field. Jauker *et al.* (2012a) estimate that hoverfly densities are required to be five-fold of bees to achieve similar yields. Assuming that our pan traps provide an accurate estimation of visitation rates in the crop, and the estimation of efficiency for hoverflies is transferrable to non-syrphid *Diptera,* it is possible to provide a rough estimation of the relative value of bees versus non-syrphid *Diptera.* We estimate that the pollination service value of non-syrphid *Diptera* approximately 15.5 times greater than bees (i.e. 226 for bees compared with 3,510 (17,554 divided by 5) for non-syrphid *Diptera*). If our assumptions are correct, our research indicates that *Diptera*, and not customarily perceived key pollinators, are delivering the majority of pollination services in OSR in central Scotland.

Although the focus of this thesis is on the pollination of OSR, pest and beneficial taxa abundance was higher in the crop edge. Pest abundance is particularly important as any yield enhancements from increased pollinator activity may be outweighed by pest damage. Particularly of relevance is that pollination exclusion via mesh bags would also exclude pests such as pollen beetle during flowering. Thus, yield increases observed in pollinator exclusion experiments may actually be higher than estimated as plants exposed to insect pollinators are also exposed to pests.

6.2.2 Floral resources

Although oilseed rape (OSR) provides important forage for key pollinating insects (Westphal *et al.*, 2008), very few bees and hoverflies were trapped in either sampling year. Low insect abundances observed in OSR in Scotland, are not representative of OSR in general, but possibly a combination of poor climatic conditions for pollinators and phenological variation in both OSR flowering period and insect emergence and population growth. Although intensively managed, the emergence and development of crops are still affected by their environment. Meteorological differences during crucial periods within the lifecycle of a plant can have severe effects. Indeed, in our two survey years, peak flowering time deviated by two weeks, with farmers suggesting that this was driven by higher temperatures and reduced rainfall over the winter. In chapter 5, it was discovered that even short-term climatic changes influence the sugar concentration of nectar. Since total sugar content provides almost all of the energetic value of nectar, visiting flowers with low sugar concentrations is incredibly costly to flower visitors.

6.3 What role do insect pollinators play in the pollination of oilseed rape?

6.3.1 Reproductive metrics

Many studies highlight the importance of insect pollination to enhance OSR seed production, typically reporting increases in the number of seeds and seed weight (Sabbahi *et al.*, 2005, Araneda Durán *et al.*, 2010, Bommarco *et al.*, 2012b, Woodcock *et al.*, 2019). Seed weight is often used as a proxy for quality and represented by a higher oil content (Bartomeus *et al.*, 2014). In the UK, the economic value of oilseed rape is a combination of quantity and quality, with farmers receiving an 'oil bonus' of 1.5% for every 1% oil above 40% (Limagrain UK Ltd, 2018).

Exposure to insect pollination increased seed set by an average of 23% and seed weight per pod by an average of 29%; comparable to increased detected in previous studies (Steffan-Dewenter, 2003, Bommarco *et al.*, 2012b). In glasshouse experiments, little difference was detected in yield metrics between wind and insect simulated pollination, indicating that the method of pollen transfer was of little importance. In extreme conditions, where pollination was limited to autogamy (transfer of pollen within a flower), individual seeds were heavier than those from plants subject to supplementary pollination. This highlights a trade-off between distributing resources from one reproductive metric to another, with improved quantity coming at a cost to quality. Under controlled conditions, wind pollination was found to produce better quality seeds than insect pollination. However, insect-pollinated plants produced seeds in greater quantity. This could suggest that a combination of wind and insect pollination, acting together under different conditions, is required to maintain stable and optimal yields. Integrating results from our glasshouse and field experiments highlights that wind and insect pollination may complement each other, resulting in more stable yields under a range of environmental conditions. For example, in wet, windy weather when pollinators are unable to fly, the wind is likely to play an important role in pollination, whereas, in warm, still conditions, insect pollination is likely to be more important. Since wind is an unpredictable vector for pollen dispersal, increasing pollination services through field margin management is the best option for farmers to increase pollination services.

6.3.2 Growth metrics

Although pollination studies are numerous, the focus is primarily on reproductive metrics (i.e. seed set, seed weight) due to economic implications. However, while pollination increases OSR yield (Bommarco *et al.*, 2012b, Bartomeus *et al.*, 2014), it is just as important to address the factors that may negatively impact it. In chapter 4, plants suffering pollen limitation grew taller and flowered for longer, although converted fewer flowers to seed. Tall plants are particularly prone to lodging, resulting in lost yield when occurring in high numbers. Similarly, extended flowering can have adverse effects financially. With relatively small windows for harvest, pollen limitation may result in yield losses from under-ripe seeds. This highlights that lack of insect pollination may not merely reduce yields directly (i.e. through lower seed set and fewer seeds per pod) but also indirectly through increased risk of lodging and lengthening the growing season.

6.4 How can oilseed rape growers exploit the pollination services of oilseed rape through varietal selection and management practices?

One of the implications of insects being more abundant in the crop edge rather than the centre is that pollination services, and indeed predation by natural enemies, are not consistent throughout the crop. This has the potential to create unstable yields, with economic consequences. How can growers increase pollinator numbers throughout the crop? An obvious suggestion may be the reduction in field size and creating more a favourable field margin area. Field enlargement has already identified as a driver of pollinator decline (Senapathi *et al.*, 2015a). This does have impracticalities for farmers, with the cultivation of many small fields requiring an increase in effort when compared to larger fields. Another suggestion may be the adoption of modified ‘beetle banks (Collins *et al.*, 2002, MacLeod *et al.*, 2004). Initially created to assist with pest control, these mid-field grassy ridges provide overwintering sites for beneficial invertebrates. Adoption of this strategy has three advantages. Firstly, by considered plant selection, these ‘pollinator banks’ can provide valuable floral resource availability before and after OSR flowering, particularly during hunger gaps (Timberlake *et al.*, 2019). More importantly, for improving pollination services spatially, they provide overwintering habitat in the centre of the field enabling more rapid colonisation of the crop. Finally, they can be used for their original intention of biological control. Our findings indicate that field margin habitats not only benefit beneficial insects (e.g. solitary bees and parasitic wasps) but also pest species (e.g. pollen beetles). Selecting plants favourable to the natural enemies of pest species, but not the pests themselves, offers economic and environmental value by reducing yield loss and pesticide use (Naylor and Ehrlich, 1997).

The provision of floral resources is not currently included in OSR breeding or varietal recommendation programmes, despite variability across cultivars (Bertazzini and Forlani, 2016). In pursuit of new, more productive varieties, genetic adjustments during breeding may put floral resource availability under threat. The production of nectar is energetically costly. From a breeder's perspective, redistributing this energy towards seed production would be economically beneficial. However, this does nothing to mitigate pollinator declines where floral resources are already under threat. The predictive models in chapter 5 assess the impact of functional trait selection on floral resources. Traits driving nectar and pollen quality were typically desirable traits to growers indicating that by selecting varieties with such traits, they would benefit in both a more robust, healthier crop and increased insect pollination services. Adoption of these models would allow breeders to include floral resource provision in OSR breeding programmes. With the value of insect pollination estimated between €213 and €523 billion (Lautenbach *et al.*, 2012, LWEC and Insect Pollinators Initiative, 2014, Potts *et al.*, 2016b), allowing growers to make informed choices about varietal selection will reward them with the financial benefits associated with increased yield from insect pollination.

6.5 Limitations of approach

Limitations in experimental design and the methods used have been identified. These methods were chosen to maximise data collection subject to time and funding constraints.

6.5.1 Experimental design

For chapters 2 and 3, references to semi-natural habitat were made. Although field margins contained mixed hedgerow (hawthorn and gorse) and regenerated grass, we did not evaluate margin quality (e.g. with respect to availability of forage, shelter or nesting sites). In future, it is suggested that vegetation assessments are conducted to explore the relationship between field margin quality and infield insect populations.

When selecting sites for the fieldwork chapters, an emphasis was placed on breeding system differences. To control for landscape context and ensure independency between survey sites, we focussed on finding paired fields with conventional and hybrid varieties close to one another with each pair of fields being at least 4 km from adjacent pairs. As a result, sites were spread over a geographical area that involved significant travel time. As modelling did not identify ‘pair’ as having any significant effects on either insect abundance or yield metrics, fields within closer proximity to each other would free up time for further experimental purposes.

6.5.2 Methodological considerations

In chapter 2, to assess the abundance and diversity of insect visitors to oilseeds rape, pan traps were used. Pan traps are a widely used, passive trapping method, capable of collecting large amounts of data from multiple sites, simultaneously (Westphal *et al.*, 2008). However, these data do not accurately reflect visitation, and exhibit sampling biases across species, particularly with bees (Roulston *et al.*, 2007). Although net sampling was attempted several times, using 30-minute transect walks when conditions were favourable, this yielded very little data on key pollinators (i.e. bees and hoverflies). For future work, particularly in times when key pollinators are scarce, increased sampling effort and expanding taxa to include all insects observed on flowers may remedy this.

In chapter 4, pollen deposition by wind and insects were simulated by shaking the plant and hand pollination, respectively. While these methods successfully transferred pollen, they are not accurately representative of natural conditions. When pollen load is too large, as may have been the case for hand-pollination, clumps can form and clog the stigma, resulting in reduced yield (Thomson, 1989). This may explain inconsistencies in our results where the effects of wind- and insect-pollination were mostly indistinguishable.

6.6 Suggestions for further research

Further work is still required to improve our understanding of the pollination requirements of oilseed rape. Some key areas for future research are highlighted.

- *How far into the crop does insect abundance remain higher than the field centre?*

  Significant differences were found between insect abundance in the crop edge and the centre of the field. The implications of this affect the interactions between insects providing beneficial services (e.g. insect pollinators and natural enemies) and those delivering disservices (e.g. pests). A greater understanding of how far into the crop the benefits of field margin proximity has on both beneficial and pest species may identify the need for mid-field pollinator refuges.

- *Is resource allocation in oilseed rape consistent across pollinator species?*

  With pollen deposition having considerable effects on growth and reproductive metrics under simulated treatments, is this consistent with various species of insect pollinator. Using caged experiments comparing plants pollinated by bees and hoverflies will increase or understanding into pollinator effectiveness and allow for target species management.

- *Does the amino acid profile increase a flower's attractiveness to pollinators?*

  Amino acids are identified as influencing the taste of nectar (Gardener and Gillman, 2002). Given this, is taste a driver of flower attractiveness and does it vary between OSR cultivars? Identifying factors considered favourable by pollinators increases our understanding of insect foraging behaviour.

6.7 Conclusion

Overall, results from this thesis increase our understanding of the interdependency between pollinators and growers of oilseed rape. The inter-relationship between oilseed rape and pollinators is complex but has the potential to be mutually beneficial. Insect pollinators undoubtedly benefit yield in oilseed rape directly (e.g. through increased seed set and the number of seeds per pod). Our study also identifies that adequate pollination may also impact other growth parameters resulting in indirect benefits to yield (i.e. decreasing the risk of lodging and shortening the growing season). Field margins adjacent to oilseed rape fields provide essential habitat, suitable as foraging, nesting and overwintering sites. The floral rewards offered by oilseed rape attracts a diverse range of insects during a challenging period of resource scarcity. In return for this resource provision, beneficial insects have positive effects on plant development and seed production, in terms of both quality and quantity. Furthermore, by making considered varietal choices, oilseed rape growers can financially benefit from this mutualistic relationship by exploiting this valuable ecosystem service.

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Appendices

Appendix 1. Author Contribution Statement for Chapter 5:

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SMF conceived the idea.

SMF, LJC, GJ designed methodology.

SMF, GJ, TK, CJM collected the data

SMF, LJC, GJ contributed to data analysis and interpretation of results.

SMF led the writing of the manuscript.

All authors contributed critically to the drafts and gave final approval for publication.