Sustainable production of sweet cherry: maximising benefits from ecosystem services

Zeus Mateos-Fierro

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Abstract

To meet rising food demand, agricultural production has increased dramatically in the past 50 years. This has involved a greater proportion of land being converted to agriculture, combined with the use of inorganic fertilisers and extensive use of Plant Protection Products (PPPs). However, this has caused habitat and biodiversity loss, soil degradation, and land fragmentation and, as a result, pollinators and natural enemies of crop pests, on which many economically important crops depend, have also been negatively impacted. Sweet cherry is an economically important pollinator-dependent crop with a global annual production of around 2.56 million tonnes; an increasing demand has been met through new intensive production systems. If a greater reliance is to be placed on beneficial arthropods as part of more sustainable cherry intensification, their abundance and diversity must be supported by meeting their requirements such as alternative resources and shelter. Wildflower habitats are an approach that can enhance wild pollinators and natural enemies throughout the growing season supporting Conservation Biological Control as part of Integrated Pest Management programmes.

In this PhD, to enhance the sustainability of sweet cherry production, native perennial wildflower strips (1 x 95 m) were established in alleyways in ten sweet cherry protected orchards in the West Midlands, UK. The effect of wildflower strips on natural enemies and pollinators and pest regulation and pollination services were investigated over a three-year period (2017 to 2019). The effects on abiotic factors, and fungal disease incidence were also considered. In each orchard, two different management treatments of sown wildflowers were compared; a Standard Wildflower Strip (SWS) managed with a single cut in September; and an Actively Managed Wildflower Strip (AMWS) managed with regular cutting to 20 cm height. These treatments were compared with unsown Control Strips (CS).

Wildflower establishment and development over the three-year period was successful, with a cover of 75.7% (± 6.1) by year three. Both wildflower strip treatments increased the number of floral units by over 300% compared to CS, increasing the potential nectar and pollen resources for beneficial arthropods. Wildflower habitats were associated with an increased abundance of natural enemies in the alleyways (73.9% increase) and adjacent cherry trees (12.9% increase) compared to the CS. Resulting pest regulation services were also greater with 25.3% more aphids being depleted from baited cards in wildflower strips. Pollinating insects underpin cherry yields, with 30.2% fruit set in the presence of insects compared to only 1.4% when excluded. Pollinating insects also responded positively to wildflower strips with increased abundance. However, during the cherry blossom period only abundance was greater in AMWS with an associated 6.1% increase in fruit set. No differences between treatments were recorded with regards to humidity and temperature under protective covers, and the incidence of fungal disease was not increased. Supplementary pollination experiments indicated pollination deficits in the study orchards with the value of pollinating insects to sweet cherry in the UK estimated at £11.3 million (£14.7K ha^{-1}). Although increases to £25.6K ha^{-1} could be achieved if pollination was optimised.

In conclusion, this study has shown that wildflower strips can be effective in enhancing ecosystem services delivered by natural enemies and pollinators in intensive sweet cherry orchards under protective covers. The establishment of wildflower strips in alleyways between rows is therefore recommended for cherry growers, with greater benefits being delivered with regular cutting to a height of 20 cm (AMWS). The adoption of wildflower strips could allow growers to reduce PPP inputs and still increase cherry yields and profitability.

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Chapter 1. Introduction

1.1 Food production and food security

The global population is increasing with nearly 8 billion people at present and is predicted to reach 9.6 billion by 2050 (Tripathi *et al.,* 2019). Food production is also increasing to meet current demands for food but more will be needed to meet future demands (Tripathi *et al.,* 2019). As a consequence, agricultural production has substantially increased in the past decades (McKenzie & Williams, 2015), made possible through agricultural intensification (Ramankutty *et al.,* 2018), the implementation of new technologies (Woodcock *et al.,* 2016), and the increased use of Plant Protection Products (PPPs) (Bonner & Alavanja, 2017) and fertilizers (Altieri *et al.,* 2017). However, key challenges such as climate change (Campbell *et al.,* 2016), including more frequent extreme events, water scarcity, soil deterioration (McKenzie & Williams, 2015; Campbell *et al.,* 2016), coupled with a reduced availability of land (Nooghabi *et al.,* 2018) may pose additional threats for food growing.

Agricultural systems represent over a third of the total land mass of the world (Ramankutty *et al.,* 2018), which has led to habitat loss, soil degradation, land fragmentation, and associated losses of biodiversity (Ramankutty *et al.,* 2018), including beneficial arthropods. These provide essential services to food production, such as pollination and pest regulation services (Woodcock *et al.,* 2016). Therefore, expanding agricultural areas would cause more environmental repercussions and require an increase demand on water (Nooghabi *et al.,* 2018). In addition, PPPs and fertilizers are associated with greater risks to human health (Lamichhane, 2017), disruption of soils (Prashar & Shah, 2016), and greater declines of beneficial arthropods (Woodcock *et al.,* 2016). Detrimental effects on soil are directly related to nutrient cycling and soil fertility (i.e. soil quality) (Prashar & Shah, 2016).

In order to guarantee food security and protect the environment and biodiversity, food production should move towards a sustainable agriculture rather than the conventional approach (DeLonge *et al.,* 2016). Sustainable agricultural intensification is an alternative aiming to produce greater yields in the same harvested area, whilst reducing resource inputs, such as water and PPPs (DeLonge *et al.,* 2016; Nooghabi *et al.,* 2018). This would allow an increase in food production and therefore for food demands to be met in the long-term (McKenzie & Williams, 2015).

Better management of food supply chains is another option for improving sustainability (Nooghabi *et al.,* 2018). A third of food produced is lost or wasted (Govindan, 2018), which is directly linked to undernourishment (Munesue *et al.,* 2015). It is estimated that 868 million people are undernourished and about two billion have micro-nutrient deficiencies (Ramankutty *et al.,* 2018; Tripathi *et al.,* 2019). Fruit losses are primarily attributed to losses at harvest and during food processing, however food waste can occur during any phase of the supply chain, including producers, intermediaries, and consumers (Govindan, 2018). Improvements in supply chain management would therefore reduce food loss and food waste (Govindan, 2018). Undernourishment is also caused by inadequate food distribution (Nooghabi *et al.,* 2018). The calorific content of the food currently produced is sufficient to feed global population (McKenzie & Williams, 2015). Moreover, food production also depends on dietary choice and sustainable diets, for example a move towards vegan-based diets would increase the availability of global calories (Ramankutty *et al.,* 2018). Meat, sugar, refined fats, and oils are associated with a lower efficiency of calorie production, and more energy and resources, including land, are required for their production (McKenzie & Williams, 2015; Ramankutty *et al.,* 2018).

Food production and food security are therefore a challenge for a growing global population. Sustainable food production and consumption may be essential to ensure sufficient quantity of food for everyone (Govindan, 2018; Nooghabi *et al.,* 2018). Hence, improvements in the supply chain from the first stages (in the field) are important. For example, the adoption of sustainable approaches that enhance wild pollinators and reduce of crop losses through pest regulation services delivered by natural enemies.

1.2 British food and fruit production

Food production in the UK is important with an annual income of £4.7 billion utilizing 71% of the land mass (17.4 million hectares) (DEFRA, 2019a). However, only 53% of the total food consumed in the UK is produced on this land, whilst 12% is exported (DEFRA, 2019a). As a result, 47% of all the food that is consumed in the UK is imported. The EU is a major importer at 27% (DEFRA, 2019a). Fruit production accounted for 718,900 tonnes worth £769 million in 2018 (DEFRA, 2019b), of which 545,300 tonnes were orchard fruit and 173,606 tonnes were soft fruit (DEFRA, 2019c). Apple is the major fruit produced with 502,661 tonnes produced on 16,163 ha of land in 2018 (DEFRA, 2019c). Strawberry is the second major fruit (top soft fruit) produced, accounting for 131,639 tonnes over a harvested area of 4,731 ha. The second most important orchard fruit in the UK is pear accounting for 26,317 tonnes per year. 15,699 tonnes of blackcurrants and 15,073 tonnes of raspberries were produced in 2018. Plums are also important in the UK with 8,680 tonnes being produced. Whilst cherry annual production accounts for 3,568 tonnes, which makes cherries an important fruit in the UK.

Fruit imports accounted for 3.7 million tonnes worth £3,788 million in 2018, whilst exports only were 157,000 tonnes with a value of £156 million (DEFRA, 2019b). Spain is the main exporter to the UK accounting for 21% of the total fruit imported, followed by 11% from South Africa and 6.3% from the Netherlands (DEFRA, 2019b). In 2018, grapes and citrus fruit were the most imported fruit from Spain and South Africa, but bananas, grapes, and apples were the three key fruits imported to the UK, which includes imports from other countries such as Colombia, Costa Rica, and France (DEFRA, 2019b). Fruit production is therefore not sufficient to meet British consumption. Hence, in order to achieve a greater reliance on British fruit, sustainable production should be increased.

1.3 Sweet cherry (*Prunus avium* L.) production

Cherry is a deciduous stone fruit tree, belonging to the family Rosaceae. The genus *Prunus* is composed of around 200 species; the majority are from temperate regions, but some are tropical and subtropical (Lee & Wen, 2001). The major commercial importance of cherry trees is their edible fruit, although they are also valued for ornamental use, and their oil and timber (Lee & Wen, 2001). Two species are cultivated for fruit production, *Prunus avium* (sweet cherry) and *Prunus cerasus* (sour cherry) (Bujdoso & Hrotko, 2017) but only *P. avium* is native to the UK (Leather & Bland, 1999). There is greater demand for sweet cherry and consequently it is cultivated globally (Figure 1.1) (FAO, 2020), principally for fresh fruit. Sour cherries are normally processed, being grown primarily in the northern hemisphere (Bujdoso & Hrotko, 2017). Depending on cultivar and environmental conditions, cherry trees in the northern hemisphere typically blossom in April (Lech *et al.,* 2008), whilst the fruit ripens from June to August (Bujdoso & Hrotko, 2017). The cherry ripening window is short compared to other tree fruit such as apples (Lang & Ophardt, 2000).

Sweet cherries are a highly valuable crop (Lang, 2013) and the annual worldwide production of sweet cherry is over 2.56 million tonnes from an area of approximately 441,953 hectares (FAO, 2020). Both figures have increased in recent years due to increased demand (Table 1.1). Turkey is the principal producer of sweet cherry, with

639,564 tonnes produced in 2018 (Table 1.1), followed by the USA (Figure 1.1). Uzbekistan, Chile and Iran have increased their production over the last two decades, becoming very important countries for sweet cherry production.

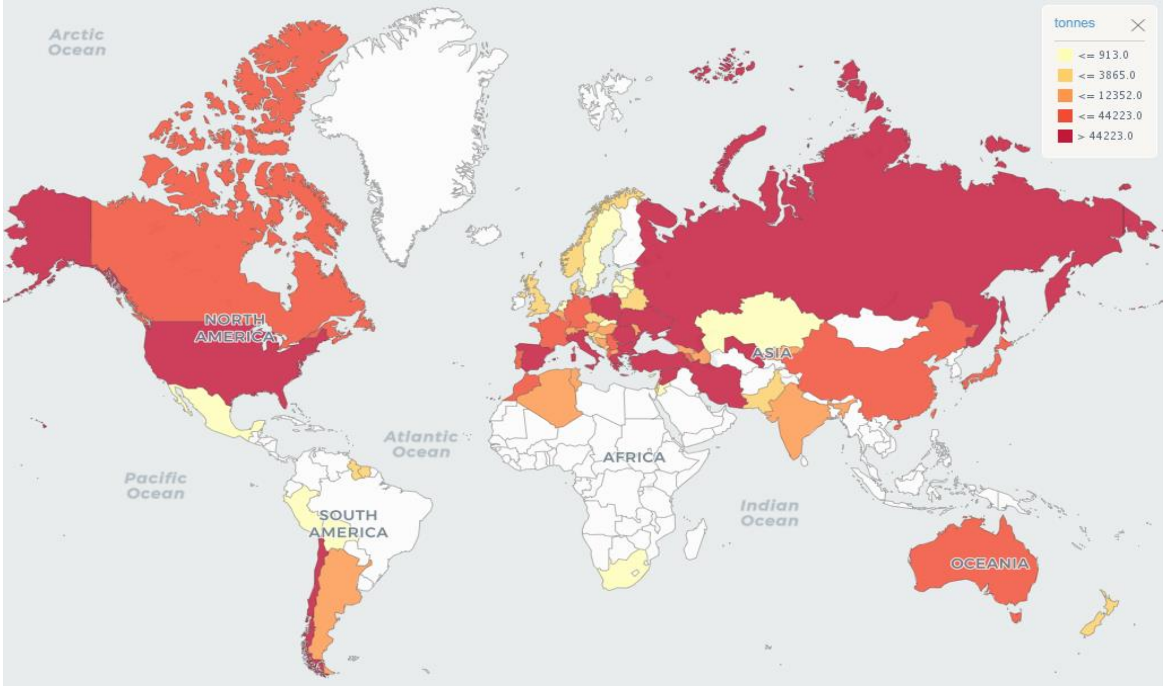

Figure 1.1. The main producing countries of sweet cherry (shown in red; darker shades indicate higher levels of sweet cherry production) (FAO, 2020).

In the UK, sweet cherry production (Figure 1.2; Table 1.1), harvested area (Figure 1.2; Table 1.1) and incomes (Figure 1.3) have fluctuated over time (DEFRA, 2019c; FAO, 2020). Traditional cherry orchards consisted of large trees planted at low density, which required greater areas and had lower productivity compared to modern cherry orchards (Major, 1997). In 1951, cherry orchards occupied 5,193 ha in Kent alone (Major, 1997) compared to 756 ha in the UK in 2018 (DEFRA, 2019c). New approaches such as dwarfing rootstocks, protective covers and planting systems have allowed high-density orchards to develop, reducing losses caused by scarce pollination, poor fruit retention, and damage due to frost, cracking and birds (Cahn *et al.,* 2001). Yet, in the UK, yields have varied in recent years (Figure 1.2), mainly due to weather conditions. In 2018, the annual production in the UK halved to £11.7 million compared to 2017 due to the cold weather during the blossom period whilst the long dry summer caused the development of smaller cherries (DEFRA, 2019a).

Table 1.1. Main producing countries of sweet cherry including the UK according to year from 1980 to 2018, including production (tonnes) and harvested area (ha) (FAO, 2020). – No data available; [a] Unofficial figure; [b] FAO data based on imputation methodology; [c] FAO estimate.

Country	Metric unit	1980	1985	1990	1995	2000	2005	2010	2015	2018
Turkey	Tonnes	96,000	130,000	143,000	186,000	230,000	280,000	417,905	535,600	639,564
	Ha	13,743	14,417	16,413	22,383	29,000	43,000	67,046	81,409	84,087
USA	Tonnes	155,760	120,200	142,180	150,140	185,070	227,522	284,148	306,991	312,430
	Ha	19,020	18,090	19,910	21,075	24,869	32,027	34,411	36,353	34,398
Uzbekistan	Tonnes	-	-	-	18,000[a]	19,800[a]	22,000[a]	75,000[a]	90,000[a]	172,035
	Ha	-	-	-	4,685[b]	3,500[a]	3,000[a]	8,300[a]	8,298[b]	12,161
Chile	Tonnes	5,303	8,900	13,700	20,000	31,050	32,000	60,356	105,109[b]	155,935[b]
	Ha	1,820	2,800	2,970	3,265	5,832	7,100	13,143	20,591	30,179
Iran	Tonnes	53,000[c]	65,000[a]	85,411	156,755	213,251	224,892	228,093	136,000	137,268[b]
	Ha	7,600[c]	8,000[a]	9,209	17,918	24,929	27,815	27,817	3,470	17,024
Italy	Tonnes	119,500	157,100	100,470	120,167[a]	145,672[a]	101,295	115,476	111,119	114,798
	Ha	28,000[a]	23,126	23,168	24,771[a]	26,958[a]	27,888	30,020	30,123	29,156
Spain	Tonnes	79,700	79,579	54,900	55,500[a]	112,900[a]	92,600[a]	85,192	94,145	106,584
	Ha	13,400	22,100[a]	24,500[a]	27,800	28,777	23,515[a]	24,290	26,492	27,368
UK	Tonnes	7,100	4,788	1,582	3,500	400	1,100	1,200	4,700	3,568
	Ha	1,600[c]	830[c]	872	800	500	400	500	711	756

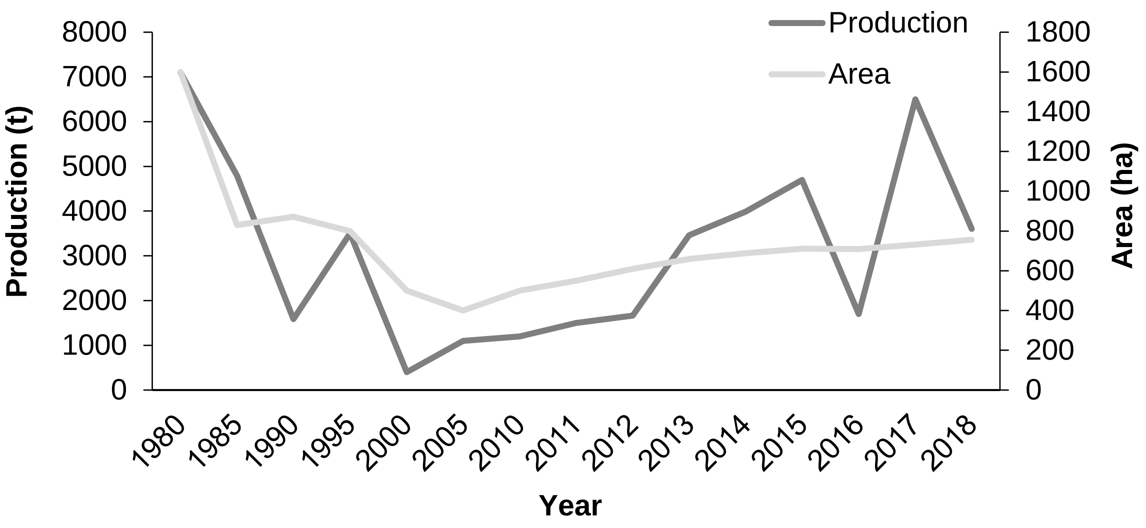

Figure 1.2. Sweet cherry production (tonnes) and harvested area (hectares) in the UK from 1980 to 2018 (DEFRA, 2019c; FAO, 2020).

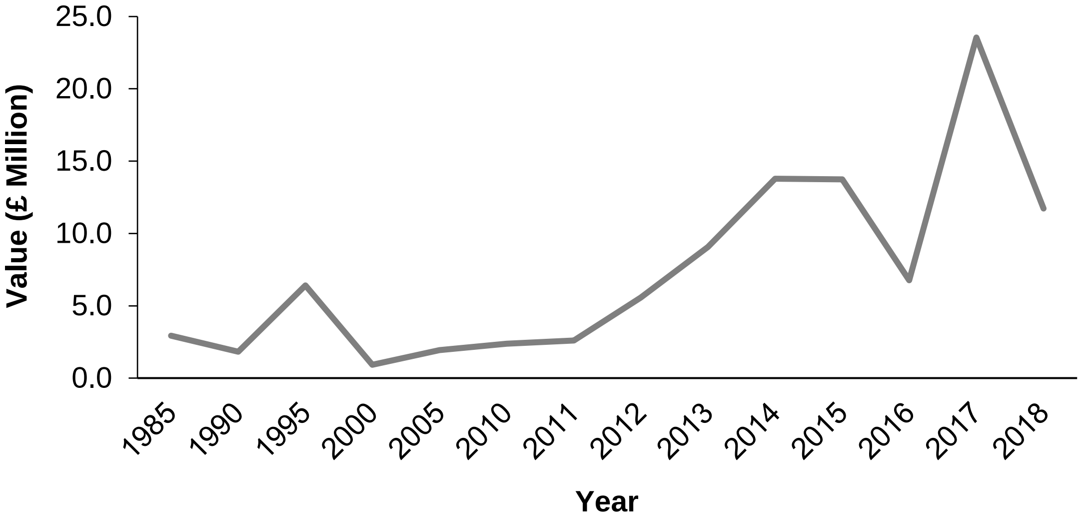

Figure 1.3. Value (£ million) of sweet cherry production in the UK from 1985 to 2018 (DEFRA, 2019c).

Sweet cherry is a valuable fruit to the UK market, but British production is highly variable, and consumption greatly exceeds supply (Wermund *et al.,* 2005; DEFRA, 2019c) (Figure 1.4). In 2015, 19 thousand tonnes of sweet cherries were imported, ranking the UK as the seventh country worldwide, with the largest cherry imports (IndexBox, 2017), and the third of stone fruit in Europe (CBI, 2017). Cherry imports into the UK within the European Union come primarily from Spain, with 7,359 tonnes imported in 2018 (Forte, 2019), whilst Turkey is the Non-European main supplier (CBI, 2017). However, during the off-season months in the Northern Hemisphere, most of the cherries are imported from Chile (CBI, 2017). The USA and Canada also export cherries into Europe in summer (CBI, 2017).

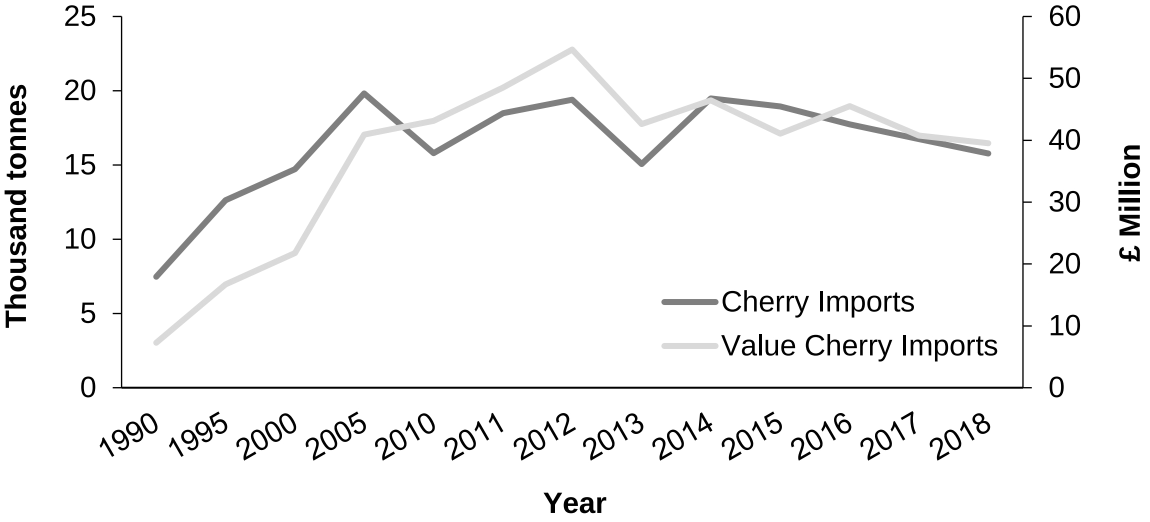

Figure 1.4. Sweet cherry importation (thousands tonnes) and its value (£ million) in the UK from 1990 to 2018 (DEFRA, 2019c).

1.3.1 Traditional cherry orchards

Cherry production in the UK began in Kent in the sixteenth century at large farms with deep, well-drained soils (Hunt & Folley, 1964). Cherry trees, which could measure 12 m height, were grafted onto vigorous rootstocks at low densities (Figure 1.5A) (Lang, 2005) and were poorly managed (Hunt & Folley, 1964). Pruning was conducted in winter (Webster, 1998) to remove dead or crossing branches during the first years (Grubb, 1949; Hunt & Folley, 1964). Cherry trees become fully mature after 15 – 20 years of establishment and could be productive for 20 – 40 years (Hunt & Folley, 1964). Outcomes were therefore protracted after plantation (Lang, 2000). In addition, weather conditions, including frosts and rain, and bird damage directly affected production (Hunt & Folley, 1964). Management and harvest were demanding due to the size of the trees (e.g. use of ladders to reach the top of trees (Figure 1.5B)) (Lang, 2005). As a consequence, yields were low and inconsistent (Cahn *et al.,* 2001). Therefore, due to the increased demand for sweet cherry (Wani *et al.,* 2014), production could not be sustained with the traditional cherry orchard systems (Lang, 2000) and new approaches were needed to maximize yields on smaller area of land (Bujdoso & Hrotko, 2017).

Figure 1.5. A) Traditional cherry orchard: Church lane cherry orchard, Stockbury (Kent, UK) (Kentorchards, 2020). B) Labourers at harvest in a traditional cherry orchard (Kentorchards, 2020).

1.3.2 Modern orchard design

Cherry production is now highly dependent on a number of improvements to management, which began in the 1980s, and ensured maximum yields based on an increase of yield per hectare (Bujdoso & Hrotko, 2017). However, to maximize yields in high-density, modern, orchards, improvements needed to be considered together (e.g. matching dwarfing rootstock with an adequate training system) (Hrotkó & Rozpara, 2017). As a result, modern orchard systems (Figure 1.6) have increased sweet cherry production globally (Bujdoso & Hrotko, 2017) (Table 1.1).

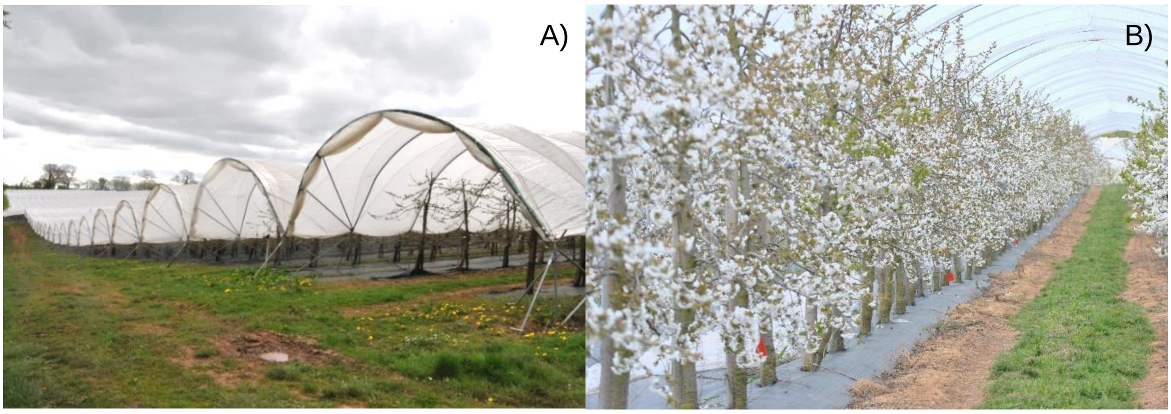

Figure 1.6. Modern sweet cherry orchards: A) Sidnall Farm, Pencombe (Herefordshire, UK) and B) Billington Farm, Stafford (Staffordshire, UK).

1.3.2.1 Rootstocks

Rootstocks started to be developed for cherries about a century ago, although this began much earlier on apples and pears (Webster, 2002). The introduction of new dwarfing and semi-dwarfing rootstocks from the 1980s entailed a great improvement associated to cherry modern orchards (Lang, 2000). The foremost advantage for orchard cultivation of grafting onto dwarfing rootstocks is the evident control on the scion (canopy) size (Hrotkó & Rozpara, 2017), which also allowed the use of protective covers over the trees, improved spray coverage and ceased the use of high ladders (Webster, 2002). However, the precocious and constancy of yield, the attainment of better fruit quality and size and resistance to pests and diseases, and environmental factors are also important qualities for a rootstock (Webster, 2002; Hrotkó & Rozpara, 2017). For instance, using precocious rootstocks, commercial yields can be expected by the second or third year after establishment (Lang, 2000).

Two rootstocks widely used in the UK during the twentieth century were Mazzard F12/1 (*P. avium*) and Colt (*P. pseudocerasus* x *P. avium*). Both were produced at East Malling Research Station (Kent, UK) (Hrotkó & Rozpara, 2017). However, the scion vigour grafted onto those rootstocks were not sufficiently dwarfed to allow intensive sweet cherry commercial production (Webster *et al.,* 2000). As a result, new dwarfing rootstock programmes were developed in Europe and the USA. GiSelA$^{®}$5, GiSelA$^{®}$6, Weiroot 10, Weiroot 13, Edabriz, and P-HL-A are considered the best dwarfing rootstock for sweet cherry in Europe (Wertheim *et al.,* 1998; Webster *et al.,* 2000; Sansavini & Lugli, 2014). Whilst, in the UK, GiSelA$^{®}$5, GiSelA$^{®}$6 and Colt are currently the most popular rootstocks used.

1.3.2.2 Planting system

Traditionally, in Kentish cherry orchards, trees were spaced at least 9 m apart (normally 11 – 12 m) (Grubb, 1949). On soils where cherry trees could grow more, a distance of 15 m was recommended (Grubb, 1949), which would permit approximately 44 trees per hectare. After the introduction of dwarfing and semi-dwarfing rootstocks, and the subsequent reduction in tree vigour, tree density in cherry orchards increased (Lang, 2000) up to 6,670 trees per hectare (Koumanov & Long, 2017), and new planting systems were therefore developed to maximize cherry production (Cahn *et al.,* 2001).

Planting system refers to the number of trees planted per hectare according to distances between trees and rows. In Europe, the system 8 x 8 m was used for cherry on Mazzard rootstock but in the mid-1970s the Zahn system (2 – 3 m (between trees) x 4 – 5 m (between the rows)) was introduced initiating high-density cherry orchards (Robinson, 2005). In modern high-density cherry orchards, tree density varied from approximately 1,240 trees / ha (1.8 x 4.5 m) to 6,670 trees / ha (0.5 x 3.0 m) (Koumanov & Long, 2017). The placement of trees in the orchard was on single row system. In this type of systems, the distance between trees within rows is shorter than the distance between rows, which is wider and remains constant throughout the plantation (e.g. 3 x 2 m). However, double (the distance between rows is wider every two rows; e.g. 3 + 2 x 2 m) and triple (the distance between rows is wider every three rows; e.g. 3 + 2 x 2 x 2 m) row systems were introduced to increase tree density and therefore productivity (Cahn *et al.,* 2001). Overall, the double row system is most commonly used due to the pragmatic management and lower investment (Cahn *et al.,* 2001; Sitarek *et al.,* 2008).

1.3.2.3 Cultivars

Orchard design is also highly dependent on cherry cultivar. The introduction of new cherry cultivars has contributed to an increase of high-density orchards (Kappel, 2002). Most sweet cherry cultivars are self-incompatible and some cultivar pairs are cross-incompatible (Zhou *et al.,* 2002). In addition, as cultivars differ in phenology (Radičević *et al.,* 2011) a combination of cross-compatible cultivars and flowering timing overlap is essential (Radičević *et al.,* 2011). Consequently, cultivars with the same phenology and compatible pollen are used as pollinizers (Brown *et al.,* 1989). Three compatible cultivars are recommended in commercial orchards. Cultivars are typically placed in solid single rows interspersing with other cultivars, avoiding placing single cultivars in more than three consecutive rows (Brown *et al.,* 1989). However, self-fertile cultivars can also be used in modern orchards to avoid the use of pollinizers (Radičević *et al.,* 2011), although they still benefit from cross-pollination (Granger, 2004). The ability to self-fertilise can compensate for low pollination services (Brown *et al.,* 1989), and cultivating trees in solid blocks without pollinizers makes harvesting more efficient (Choi & Andersen, 2001).

Hundreds of cultivars have now been developed in breeding programmes and yet, in many countries, most cherry production is achieved by only a few (e.g. Bing) (Quero-García *et al.*, 2017). Quero-García *et al.* (2017) defined 119 commercially important cultivars such as Kordia, Lapins, Regina and Skeena, which are all cultivated in the UK. In addition to marketable demands of cherry quality, including flavour, the importance of cultivars also relies on resistance to pests, diseases and viruses, spring frosts, fruit cracking, tree structure, etc. (Blažková, 2004; Lang *et al.*, 2011; Quero-García *et al.*, 2017). Therefore, when establishing a new plantation, cultivars are carefully selected. Moreover, cultivars influence and, in turn, are influenced by the rootstock and training and pruning systems (Whiting *et al.,* 2005; Usenik *et al.,* 2008), so that their management (Bound *et al.,* 2014), and yield and fruit quality (Lang, 2014) will differ.

1.3.2.3.1 Sweet cherry cultivar Kordia

Kordia is a sweet cherry cultivar created by a chance seedling. It was bred at the Research and Breeding Institute of Pomology, Holovousy, in the Czech Republic, and registered in 1981 (Blažková, 2004). Kordia is self-incompatible, and a pollinizer is needed to achieve an adequate fruit set (Lech *et al.,* 2008; Radičević *et al.,* 2011). This cultivar is of global importance (Quero-García *et al.*, 2017) and it is cultivated all over the world including Germany (Stehr, 2005), Turkey (Demirsoy *et al.,* 2017), the USA (Long *et al.,* 2008), Chile (Sagredo *et al.,* 2017), and Australia (Granger, 2004).

The bloom time is mid to late season (Long *et al.,* 2008). And in the UK (West Midlands), it is typically from early/mid-April to early/mid-May. Harvest is then from mid- to late-July, although this can be extended to early August, depending on weather conditions. The productivity of Kordia is high with large heart-shaped fruit (Long *et al.*, 2008; Quero-García *et al.*, 2017). It is also firm and very dark with red flesh. The flavour is strong and sweet (18% sugar) (Long *et al.,* 2008). Kordia also has good resistance to fruit splitting and brown rot (*Monilinia* spp.). However, flower buds and blossoms are susceptible to frost and leaf spot (*Blumeriella jaapii*) (Blažková, 2004).

To underpin large commercial yields, Kordia needs to be grafted onto dwarfing or semi dwarfing rootstocks (Stochl *et al.,* 2008) such as Colt or GiSelA®5. Kordia grafted on GiSelA®5 (from the GiSelA® selection (hybrid of *P. cerasus* x *P. canescens*) bred in Germany (Callesen, 1998)) produce less vigorous trees and larger yields than grafted on to Colt (Stehr, 2005; Sitarek & Grzyb, 2010). Hence, in modern sweet cherry orchards, GiSelA®5 has replaced Colt in many countries (Stochl *et al.,* 2008). However, newer interstem combinations have shown promising results. GiSelA®5 interstem grafted on F12/1 showed higher cumulative yield than grafting Kordia directly on GiSelA®5 or Colt (Bielicki & Rozpara, 2010).

1.3.2.4 Training system

Tree training is a technique applied in orchards to control the scion growth and modulate tree architecture in order to increase cherry quality and yield (Long *et al.,* 2015). If not controlled, cherry trees grow naturally as a central leader with rapid development and strong apical dominance. Therefore, training systems modifies the cherry tree normal growth enhancing precocity, which is usually done in new plantations (Long *et al.,* 2015). Training systems also enhance photosynthesis and transpiration due to a better light distribution; consequently, tree growth is enhanced (Whiting *et al.,* 2005) and harvest efficiency is improved (Ampatzidis & Whiting, 2013).

There are numerous high density training systems for sweet cherry trees (Ampatzidis & Whiting, 2013; Long *et al.,* 2015). In modern cherry orchards two of the first systems, the Spanish bush and the V-shaped systems, were developed in Spain and Australia, respectively during the 1980s (Robinson, 2005). However, the need to improve precocity and productivity led to the development of other systems including steep leader, super slender axe and Vogel central leader (Long *et al.,* 2015). The adoption of a training system depends on factors such as region climate, soil type, rootstock, scion cultivar, orchard design, and management ability (Lauri & Claverie, 2008; Long *et al.,* 2015).

1.3.2.5 Pruning

Pruning is key to control the size of the scion (Lang, 2005), removal of old unproductive woody branches, selection of new shoots (Webster, 2002), and required to improve light diffusion throughout the orchard (Lang *et al.,* 2011). Photosynthesis and cherry productivity (i.e. fruit quality and yield) are therefore maximized (Lang & Ophardt, 2000; Lauri & Claverie, 2008). Pruning can be conducted in winter or summer (Ayala & Lang, 2017). When compared with the traditional winter pruning, summer pruning reduces the probability of bacterial canker (*Pseudomonas syringae*) infection and other diseases (Usenik *et al.,* 2008). It also enhances the number of floral buds leading to yield increases (Guimond *et al.,* 1998), and improves fruit quality (Usenik *et al.,* 2008). The reduction on tree vigour through dwarfing or semi-dwarfing rootstocks allowed improvements on orchard management such as harvest and PPP application but these are synergistically enhanced when pruning is applied.

Flower bud thinning, either manually or mechanically, is also a modern approach to cherry production, which is applied during the blossom period to manage crop loads and reduce the burden of excessive fruit on the trees (Lauri & Claverie, 2008). As a result, the remaining cherries grow bigger and fruit quality is enhanced (Spornberger *et al.,* 2014).

1.3.2.6 Covering systems

The adoption of cover systems (protective covers) such as plastic polytunnels has revolutionised cherry production in the UK by making it economically viable (Cahn *et al.,* 2001). Reduction in tree vigour though rootstocks and training and planting systems (e.g. two rows inter-connected to enhance cross-pollination) has facilitated the use of polytunnels. These are essential for cherry cultivation to protect the crop against environmental factors (e.g. rainfall), which are a key issue in temperate regions (Lang *et al.,* 2016). Sweet cherries are covered during the blossom and fruit development stages, before the blossom period begins (typically early April) until the end of summer (typically early September) (Lang *et al.,* 2016) and are uncovered in winter to ensure the cherry chilling period requirement (Fadón *et al.,* 2015).

Protective covers minimise fruit cracking damage, which is mainly caused by rainfall during ripening, they also protect the crop against rain related diseases and some pests; this ensures constancy in marketable production (Cahn *et al.,* 2001; Lang, 2014). However, the development of diseases and pests (e.g. *Tetranychus urticae* (two-spotted spider mite)) can be encouraged (Lang, 2014). Protective covers reduce wind speed, whilst air and soil temperatures and relative humidity are increased. Soil moisture and, finally, solar radiation are also affected (Blanke & Balmer, 2008; Lang *et al.,* 2016). This generates a microclimate within polytunnels which increase evapotranspiration, and subsequently tree growth rate and fruit development (Lang, 2013, 2014), but irrigation may be needed to compensate (Blanke & Balmer, 2008). Adequate irrigation and fertigation systems help produce better yields and higher fruit quality (Webster, 2002). Within tunnels, both blossom period and harvest time are expected about one to two weeks earlier, depending on varieties (Blanke & Balmer, 2008), whilst fruit quality and yield are enhanced (Blanke & Balmer, 2008; Lang, 2014). In addition, under cover systems, persistence of PPPs is increased, reducing inputs (Shaw *et al.,* 2019). However, frosts during spring can still damage cherry blossoms and reduce yields as temperatures at night are similar to outside the tunnel (Blanke & Balmer, 2008). In addition, bee behaviour is affected by some brands of plastic interfering in light spectrum, which can deter honeybee navigation (Lang, 2014). In addition, towards the centre of the tunnels, bee visits are reduced probably due to increased temperature and reduced wind speed among other factors (Hatt *et al.,* 2017).

A number of covering support systems have been developed (Figure 1.7) including pole-and-wire tent-like structures (e.g. Voen), high tunnels (e.g. Haygrove), and automated greenhouse-like systems with retractable roofs (e.g. Cravo) (Lang *et al.,* 2016; Lang, 2019). These structures can be combined with different types of film (polythene) (e.g. Luminance and Voen mesh) (Lang, 2014). High tunnels can be actively managed to control conditions to enhance cherry production; sidewalls are raised to ventilate and release excess heat (Lang, 2014). Also, vented polythene plastic covers can be used, which allow alleyways to receive rainfall as does Voen systems. Netting, during fruit development, can be used on pole-and-wire and high tunnel systems to minimise fruit damage by birds (Lang, 2014).

Figure 1.7. A) Pole-and-wire structure with Voen mesh covers, B) high tunnel with Luminance polythene plastic and bird netting, and C) high tunnel with vented Luminance polythene plastic polytunnels.

1.4 Ecosystem services

Costanza *et al.* (2014) estimated the annual global value of ecosystem services at $125 trillion in 2011. A number of ecosystem services regulate agricultural systems (Zhang *et al.,* 2007; Power, 2010), in which food production relies (Zhang *et al.,* 2007). This highlights the importance of ecosystem services to ensure food security and human well-being. In turn, agricultural systems also provide ecosystem services such as food, fibre, bioenergy, forage, and pharmaceuticals (Power, 2010; Ramankutty *et al.,* 2018). Agriculture has increased in recent decades (Ramankutty *et al.,* 2018) and so has the use of PPPs (Woodcock *et al.,* 2016), which is directly linked to negative effects on ecosystem services (Woodcock *et al.,* 2016). This puts at risk the required growing rates of food production (McKenzie & Williams, 2015). To ensure the availability of ecosystem services and subsequently food security and human well-being, more sustainable agricultural approaches are therefore needed (McKenzie & Williams, 2015).

Ecosystem services are categorized into four broad sections: provisioning, regulating, supporting and cultural services (MA, 2005), each of them with a number of subcategories, which are updated by the Common International Classification of Ecosystem Services (CICES). Pollination, pest regulation, soil fertility, nutrient cycling, and water resources are ecosystem services intimately related to agricultural systems (Power, 2010) and can be enhanced in crops as part of sustainable agriculture. For instance, pollination and pest regulation services are classified under regulating services (Haines-Young & Potschin, 2018) and directly affect crop production and yield, and therefore food availability (Woodcock *et al.,* 2016; Ramankutty *et al.,* 2018). These two ecosystem services are delivered primarily by arthropods but also other organisms (e. g. pollination delivered by birds or pest regulation delivered by entomopathogenic fungi) (Zhang *et al.,* 2007). Therefore, to increase these services, the arthropods that deliver them must be enhanced. Moreover, they need to be spatially distributed to deliver services evenly throughout cropped areas (Woodcock *et al.,* 2016; McKerchar *et al.*, 2020). This is particularly challenging in large cropped areas as invertebrates usually have to disperse from surrounding semi-natural areas (Woodcock *et al.,* 2016), which have been reduced due to land use change into agricultural or urban areas (Foley *et al.,* 2005). To benefit from pollination and regulating ecosystem services, agricultural systems have to be managed sustainably (Shackelford *et al.,* 2013). Many studies have shown compatible approaches to enhance the delivery of multiple ecosystem services into crops including the provision of wildflower habitats for pollination and pest regulation services (Blake *et al.,* 2012; Shackelford *et al.,* 2013; Woodcock *et al.,* 2016; Campbell *et al.,* 2017). However, responses are likely to be dependent on crop type and the environmental context (Campbell *et al.,* 2017). Clearly, more research is required to maximize the benefits obtained from ecosystem services as part of sustainable agriculture.

1.5 Pollinators and pollination services

Pollinators deliver pollination services, transferring pollen from anthers to stigmas within or different plant individuals, providing therefore a major role in plant sexual reproduction

(Woodcock, 2012; Potts *et al.,* 2016). This key service is delivered to approximately three-quarters of all flowering plants in nature (Klein *et al.,* 2007; Ollerton *et al.,* 2011), and also to about three-quarters of global crop species for fruit or seed development (Ollerton *et al.,* 2011; Potts *et al.,* 2016). The global value of pollination services was estimated to be worth $361 billion US in 2009 (Lautenbach, 2019). In fact, without pollination services, approximately 5 – 8% of the total worldwide crop production would not be produced (Potts *et al.,* 2016), accounting for 153 billion EUR/year (Klein *et al.,* 2018). In the UK, this service is estimated at £430 million annually (Vanbergen *et al.,* 2014). In addition, global pollinator-dependant crops have increased in the past decades, resulting in an agriculture more dependent on pollination services (Potts *et al.,* 2016). Sweet cherry, for instance, is a pollinator-dependent crop (Holzschuh *et al.,* 2012), where demand has increased in recent years (Bujdoso & Hrotko, 2017). To underpin commercial yields, pollination services are therefore required (Holzschuh *et al.,* 2012).

The global number of pollinator species is estimated to be over 350,000 (Ollerton, 2017). Insects are the main pollinators, although some vertebrates can provide this service such as bats and birds (Woodcock, 2012). Within insects, Hymenoptera (bees), Diptera (flies), Lepidoptera (butterflies and moths), and Coleoptera (beetles) are the most important insect pollinators (Woodcock, 2012). Lepidoptera is the most diverse group of pollinators but its impact on pollination is considered of lesser importance (Ollerton, 2017). Diptera, particularly Syrphidae (hoverflies), are important insects for the delivery of pollination services to a number crops (Rader *et al.,* 2015; Hodgkiss *et al.,* 2018; Wotton *et al.,* 2019). However, the most dominant group of pollinators are bees (Ollerton, 2017). Globally, with over 20,000 described species (Nieto *et al.,* 2015), bees are visitors of over 90% of the main 107 crops (Potts *et al.,* 2016).

However, pollinators are declining globally, which directly threatens both natural and agricultural systems and subsequently plant diversity and human sustenance, respectively (Lebuhn *et al.,* 2013). This decline is particularly well documented in north-western Europe and eastern North America (Potts *et al.,* 2016). In Europe, 9.2% of bee species are threatened with extinction, although this figure is expected to be greater due to insufficient data for 56.7% of species (Nieto *et al.,* 2015). The key driver for this decline is landscape change, through agricultural intensification and habitat loss (Ollerton, 2017; Senapathi *et al.,* 2017).

Landscape change results in loss, reduction and fragmentation of natural or semi-natural habitats, in addition to the increase of both monocultures and active farm management

(Potts *et al.,* 2016). As a result, floral and nesting resources for pollinating insects are limited and disturbed, which directly impact on pollinator stress levels and immune systems (Potts *et al.,* 2016). Moreover, pollinators require a varied and balanced diet consisting of pollen and nectar from a range of plant species; not available in monoculture landscapes (Goulson *et al.*, 2015), nor in absence of semi-natural habitats (Vaudo *et al.,* 2015). The landscape context therefore influences abundance and richness of wild pollinators, for example solitary bees, as they can be affected by landscape factors up to 1 km (Holzschuh *et al.,* 2012).

Other important drivers of decline are the continued use of PPPs, invasive species, pathogens, and climate change (Goulson *et al.*, 2015; Potts *et al.,* 2016; Ollerton, 2017). PPPs, including some neonicotinoids (which are systemically uptaken in plants) and pyrethroids, can be detrimental to pollinators even if this effect depends largely on PPP toxicity and exposure (Nieto *et al.,* 2015; Potts *et al.,* 2016). For example, the exposure of the insecticide Elado which contains the neonicotinoid clothianidin and the pyrethroid b-cyfluthrin can reduce densities of wild bees, affect nesting success, and alter colony growth and reproduction of bumblebees (Rundlöf *et al*., 2015), although other groups of neonicotinoids are less toxic to pollinators, e.g. cyano-neonicotinoids (Walters, 2013; Ulziibayar & Jung, 2019). In addition, herbicides decrease plant abundance and diversity and, as a result, the availability of wildflowers for pollinators is reduced (Goulson *et al.*, 2015; Nieto *et al.,* 2015).

1.5.1 Managed commercial pollinators

Food demands have increased in recent years and therefore the need for sufficient pollination services to meet crop production requirements has led to the increased use of managed pollinators (Mallinger *et al.,* 2017). These can supplement wild pollinator deficits in large cropped areas, since wild pollinator abundance is often insufficient to achieve commercial yields (Trillo *et al.,* 2019). However, flowering periods of crops and the activity of wild pollinators may not overlap leading to poor pollination (Le Féon *et al.*, 2013; Fountain *et al.*, 2019). Crops grown in greenhouses are also poorly pollinated by wild species due to the restricted access resulting in the use managed pollinators (Trillo *et al.,* 2019).

Apis mellifera (the western honeybee) is the most widespread and used managed bee globally (Goulson *et al.*, 2015; Potts *et al.,* 2016). It was firstly used to produce honey and wax but at present, honeybees are mainly used for crop pollination (vanEngelsdorp & Meixner, 2010). The honeybee is a generalist species which can pollinate a great range of crops, including apple, cucumber, and almond (Klein *et al.,* 2018). As a result, its demand surpasses supply (Ollerton, 2017). However, in North America and many countries in Europe, the number of hives have decreased in the past years attributed to colony collapse disorder (CCD) (Goulson *et al.*, 2015). CCD is a not fully understood phenomenon, which is suggested being the result of a number of combined factors including the use of PPPs, spread of parasites such as *Varroa destructor*, and alteration in beekeeping practices (vanEngelsdorp *et al*., 2009). Yet, in some countries such as Argentina or China, honeybee colonies have increased; so much so that the number of managed hives in the world have increased by 45% (Potts *et al.,* 2016).

Despite the global use of honeybees and their contribution to crop production, their ability to support maximum yields compared to other pollinators in certain crops (e.g. blueberries) is questioned (Sedivy & Dorn, 2014; Mallinger & Gratton, 2015). In part, this is due to their limitation to pollinate in adverse weather conditions (cold and wet) (Földesi *et al.,* 2016). Therefore, bumblebees (e.g. *Bombus terrestris*) and solitary bees (e.g. *Osmia* spp.) are also managed to pollinate crops (Klein *et al.,* 2018). Globally, up to 50 bee species are managed for different purposes, whilst 12 bee species are specially managed to provide crop pollination services (Potts *et al.,* 2016).

Management of bumblebees began in the 19th century, when queens were collected to start new colonies, and it was in the 20th century when they began to be reared (Evans, 2017). Bumblebees ensured their importance in crop pollination due to their ability to pollinate plant species with poricidal anthers using buzz-pollination (Evans, 2017). Bumblebees started to be used in the 1980s to pollinate tomato in greenhouses in the Netherlands (Goulson, 2010). Since then, bumblebee commercial rearing increased, being exported worldwide (Evans, 2017). The use of bumblebee colonies have been extended from greenhouses to field crops that do not require buzz-pollination, including strawberry and apple (Trillo *et al.,* 2018). Moreover, bumblebees can be active at temperatures of 7ºC (Koumanov & Long, 2017), whilst honeybee activity is low at 12 – 14ºC (Vicens & Bosch, 2000) and minimal at 10ºC (Koumanov & Long, 2017). Bumblebees are often used in combination with honeybees to pollinate early spring flowering crops (Evans, 2017), including sweet cherry (Koumanov & Long, 2017). In addition, the irregular flying behaviour of bumblebees benefit cross pollination between rows of trees, which is also stimulated on honeybees when bumblebees are present (Koumanov & Long, 2017).

The use of solitary bees as managed pollinators began because of honeybee declines but also the poor performance of honeybees in certain crops (e.g. blueberries) (Boyle & Pitts-Singer, 2017; Sedivy & Dorn, 2014). Most solitary bee species are used to pollinate fruit tree and berry crops (e.g. cherry and blueberry) (Pinilla-Gallego & Isaacs, 2018). The majority of managed species belong to the genus *Osmia* such as *O. bicornis* (syn. *O. rufa*) for apple (Gruber *et al.,* 2011) and cherry (Ryder *et al.,* 2019), *O. cornuta* for pear (Fountain *et al.,* 2019) and cherry (Eeraerts *et al.,* 2020), or *O. lignaria* for blueberry (Pinilla-Gallego & Isaacs, 2018) and cherry (Boyle & Pitts-Singer, 2017). However, other crops are also pollinated; for instance *Megachile rotundata* is widely used to pollinate alfalfa fields (Pitts-Singer & Cane, 2011).

The emergence of adult solitary bees can also be regulated by temperature-controlled processes (Giejdasz & Wasielewski, 2017). This enables growers to synchronize pollinator activity with crop blossom phenology, which is particularly important when flowering periods are early and short (Boyle & Pitts-Singer, 2017; Giejdasz & Wasielewski, 2017). As for bumblebees, managed solitary bees can be used in combination with honeybees (Boyle & Pitts-Singer, 2017), enhancing cross-pollination (Brittain *et al.*, 2013).

However, the approach of using managed bees may negatively impact native wild bee communities (Graystock *et al.,* 2016; Klein *et al.,* 2018), leading to their extinction (Ollerton, 2017). For instance, *B. terrestris*, are exported worldwide, including to countries where it is not native (Evans, 2017). *Bombus terrestris* is a generalist species and spills-over from target crops to natural / semi-natural habitats in search of other floral resources, but also nesting sites, directly competing with native pollinators (Evans, 2017; Trillo *et al.,* 2019). Introduced pollinators can also increase the spread of pathogens and parasites causing declines in native populations (Evans, 2017; Mallinger *et al.,* 2017), which are readily transmitted in mass rearing facilities, where they can become more virulent (Evans, 2017). For example, the recent decline of *B. dahlbomii*, a native species in South America, is attributed to the invasive species *B. terrestris*, which compete for resources and transfer pathogens (Geslin & Morales, 2015).

Furthermore, plant communities can also be affected as non-native bees can have a preference for non-native plants, supporting their spread, and directly impacting native ecosystems (Mallinger *et al.,* 2017). As a consequence, the introduction of non-native pollinators can lead to native bee and plant declines (Mallinger *et al.,* 2017). Enhancing populations of native wild species rather than using managed pollinators to maximize pollination service delivery is therefore more sustainable.

1.6 Natural enemies and pest regulation service

Natural enemies are antagonist organisms of pests, and have the potential to regulate pests and the damage caused to crops (Hajek, 2004). Natural enemies can either target a range of prey species (generalist) or only one/few (specialist) (Hajek, 2004). However, both generalist and specialist natural enemies rely on natural and semi-natural habitats surrounding cropped areas to deliver pest regulation services (Rusch *et al.,* 2016). The estimated pest regulation service in crops provided by natural enemies represents a minimum of 50% of pest control, which in the USA alone accounts for $13.6 billion annually (Losey & Vaughan, 2006).

Pest regulation services provided by natural enemies are delivered by three main groups, including predators, parasitoids, and pathogens and nematodes (Hajek, 2004).

I) Predators are vertebrate or invertebrate agents that attack other organisms to feed on them. The most important predators in crops are arthropods belonging to the classes Insecta and Arachnida (Solomon *et al.,* 2000). Hemiptera, Neuroptera, Coleoptera, Diptera, Dermaptera, and Hymenoptera are the principal orders of insects in which predators are included; likewise, Acarina and Araneae are for arachnids (Hajek, 2004; Solomon *et al.,* 2000).

II) Parasitoids are agents which develop (during the larva stage) at the expense of hosts, normally insects (Cross *et al.,* 1999a). Unlike parasites, parasitoids develop inside and always kill the host (Cross *et al.,* 1999a; Hajek, 2004). Although some parasitoids can use a range of hosts and are therefore classed as generalists, when compared to predators, parasitoids are considered more specialised since they have more restricted host range and consequently are less generalist than predators (van Lenteren, 2012). Parasitic wasps (Hymenoptera) are the largest group of parasitoids but Diptera also occur (Cross *et al.,* 1999a; Hajek, 2004).

III) Pathogens, including Bacteria, Virus, and Fungi, and Nematoda (Cross *et al.,* 1999b; Hajek, 2004) enter into host body usually causing disease and eventual death of the hosts. Their application in crop protection is normally through biopesticides (Copping & Menn, 2000).

Pest regulation services are threatened by continued declines in natural enemies (Woodcock *et al.,* 2016). The key drivers are landscape change, including agricultural intensification and natural habitat loss, and the use of PPPs (Geiger *et al.,* 2010; Woodcock *et al.,* 2016). Landscape change causes declines in natural enemies due to an insufficient resource availability, including shelter, and additional or alternative food such as preys or pollen (Hatt *et al.,* 2017). Landscape complexity (i.e. high density of different habitats) is also reduced and is an important factor influencing both the abundance and diversity of natural enemies (Dainese *et al.,* 2017). In addition, some life stages of natural enemies are not pest-dependant (e.g. adult hoverflies) and if adult requirements are not met, larvae are not produced to deliver pest regulation services (Hatt *et al.,* 2017).

1.6.1 Managed commercial natural enemies

Due to declines in natural enemies, coupled with monoculture landscapes, pest regulation services provided by natural enemies can be limited (Woodcock *et al.,* 2016). Hence, to increase natural enemy abundance in crops, predators, parasitoids, pathogens, and nematodes can be artificially reared (Hajek, 2004). The release of natural enemies dates from over 120 years ago (van Lenteren, 2012), with the introduction of the Vedalia beetle (*Rodolia cardinalis*) to control scale in orange groves in California (Bale *et al.,* 2008). Since then, mass production has expanded in both greenhouses and field crops (Michaud, 2018), increasing the number of species commercially available. By 2012, approximately 230 natural enemy species were commercially available (van Lenteren, 2012); more than half belonged to Hymenoptera (120 species), followed by Acari, Coleoptera, and Hemiptera with 30, 28, and 19 species, respectively. The total global value of natural enemy sales at end-user level were estimated at €300 million (van Lenteren, 2012). Managed commercial natural enemies are mainly mass-produced for augmentation releases (see section 1.8) and can be used to control efficiently some pests that multiply rapidly, such as *Frankliniella occidentalis* (Sampson, 2018). However, despite the benefits to control pests while reducing PPP inputs, this approach is not sustainable, since continued releases are needed (Michaud, 2018). Enhancing natural enemies naturally occurring would be an approach aiming to self-preservation and balanced ecosystem.

1.7 Pests in cherry orchards

The key reason for using PPPs in crops is to reduce damage caused by pests below the economic threshold and ensure food security (Sharma *et al.,* 2017). Pests are defined by FAO/WHO as “any species, strain or biotype of plant, animal or pathogenic agent injurious to plants and plant products, materials or environments and includes vectors of parasites or pathogens of human and animal disease and animals causing public health nuisance” (FAO/WHO, 2014). Agricultural intensification has increased over the decades, with increasing monocultures and reduced natural habitats directly affecting pest incidence. This and global markets of crop exportation to different regions (Bebber *et al.,* 2014) has resulted in higher risks of pest outbreaks (Hatt *et al.,* 2017). Pests can influence crop quality and reduce yields, leading to increased crop losses (Sharma *et al.,* 2017). This accounts for 20 - 40% of worldwide agricultural production and can occur during pre- and/or post-harvest (Sharma *et al.,* 2017). 18 - 20% of crop losses are attributed to arthropods, worth over US$ 470 billion (Sharma *et al.,* 2017).

1.7.1 Arthropod pests

Sweet cherry suffers from a number of key arthropod pests. Some are specific to cherry and other *Prunus* species (e.g. *Myzus cerasi*), whilst others are polyphagous and may also attack other crops (e.g. *Drosophila suzukii*) (Papadopoulos *et al.,* 2017). Crop damage caused by different pests is largely dependent on the environmental conditions (Tochen *et al.,* 2016), cultivar resistance, and fruit development stage (Lee *et al.,* 2011). Moreover, some pests are climate specific and may not cause any damage in certain countries, whilst in others are major pests (Papadopoulos *et al.,* 2017).

In the UK and many other countries (e.g. Germany, Turkey, USA), two major pests of sweet cherry are *M. cerasi* (cherry blackfly) (Stutz & Entling, 2011) and *D. suzukii* (spotted wing drosophila) (Beers *et al.,* 2011). The eggs of *M. cerasi* hatch in spring and develop colonies of wingless aphids, which feed and cause leaf curling (Danelski *et al.,* 2015), and malformations of shoots (Stutz & Entling, 2011). In turn, fruit and leaves are affected by the honeydew the aphids excrete, which also supports the development of sooty moulds (Stutz & Entling, 2011). *Drosophila suzukii* also causes significant damage in sweet cherry (Cini *et al.,* 2012; Haye *et al.,* 2016). Females lay eggs into the fruit before it is ripe, the larvae and pupae can develop either inside or outside of the fruit, from which the adults emerge (Cini *et al.,* 2012) making the fruit unmarketable (Tochen *et al.,* 2016).

Tetranychus urticae (two-spotted spider mite) is a particular problem in protective covered crops (Lang, 2014). This pest feeds on the underside of leaves reducing plant photosynthetic capacity, and fruit quality and yield are reduced. Fruit may also become unmarketable due to a covering of webbing produced by large mite colonies (Harris *et al.,* 2017).

Other pests, including sawflies, weevils, and some caterpillars of tortrix moths can also provoke economically important damage on sweet cherries (Papadopoulos *et al.,* 2017). *Rhagoletis cerasi* (the cherry fruit fly) and other related species from this genus are known to cause significant losses (up to 100% of crop affected) in North America and a number of European countries (Alford, 2007; Daniel & Grunder, 2012). In the UK, it has been unintentionally introduced via the import of cherries, but has not yet established (Leather & Bland, 1999; Alford, 2007).

1.7.2 Pathogenic pests

Sweet cherry production is affected by diseases caused by bacteria, fungi and viruses. These pathogens can cause losses of up to a quarter of total production (Fogle *et al.,* 1973). Bacterial canker may be the most widespread disease in the UK (Roberts & Elphinstone, 2017) and is considered the most severe disease in sweet cherry (Wani *et al.,* 2014), particularly in nurseries and young plantations (Puławska *et al.,* 2017). Two pathovars of the bacteria *Pseudomonas syringae (P. syringae* pv. *morsprunorum* and *P. syringae* pv. *syringae*) cause bacterial canker. Both pathovars can infect other crops but the pathovar *P. syringae syringae* is more frequent in cherries (Roberts & Elphinstone, 2017). The bacterium usually enters through wounds and spreads throughout the tree, making treatment difficult. Preventative measures to avoid infection are therefore good orchard practice (Puławska *et al.,* 2017). Once trees are infected, the removal of the infected parts is required to reduce further infection (Puławska *et al.,* 2017). Chemically, it can be treated with cooper-based PPPs (Puławska *et al.,* 2017; Roberts & Elphinstone, 2017). The damage caused by this pathogen ranges from the loss of branches to the death of the tree, and can cause up to 30% of fruit loss (Roberts & Elphinstone, 2017). The risk of infection is greater in warm and moist environments (Lillrose *et al.,* 2017).

Numerous fungal diseases affect sweet cherry causing direct damage to fruit (Børve *et al.,* 2017). They are therefore economically important in commercial production. Fungi of most concern include brown rot (*Monilinia fructicola*, *M. fructigena*, *M. laxa* and *M.*

polystroma), blossom blight (*M. fructicola* and *M. laxa*), *Mucor* rot (*Mucor piriformis*), *Rhizopus* rot (*Rhizopus stolonifer*), grey mould (*Botrytis cinerea*), and blue mould (*Penicillium expansum*) (Børve *et al.,* 2017). Other fungi affect cherry leaves such as cherry leaf spot (*Blumeriella jaapii*) and powdery mildew (*Podosphaera clandestina*), whilst some directly affect trunks and branches including constriction canker (*Phomopsis amygdali*) and *Verticillium* wilt (*Verticillium dahliae*) (Børve *et al.,* 2017).

Brown rot and blossom blight are significant diseases worldwide (Børve *et al.,* 2017), including the UK (Xu *et al.,* 2007). *Monilinia laxa* infects blossoms, spurs and fruit, whilst *Monilinia fructicola*, a recent species in Europe, causes damage on blossoms and fruit. In contrast, *Monilinia fructigena* only affects twigs and fruit. All three *Monilinia* species can therefore damage fruit (brown rot) (Miessner & Stammler, 2010) but only two causes blossom blight. *Monilinia polystroma* infects fruits but has not been reported in the UK. The threat of fungal disease also depends on temperature and moisture, and responses vary enormously between these fungal species (Børve *et al.,* 2017). Risk of infection is especially high during the blossom period and prior to harvest (Børve *et al.,* 2017).

The fungus can infect fruits directly; however, infection frequently occurs through lesions or cracks, so that cultivars susceptible to splitting are more prone to infection. Yet, the likelihood of infection also depends on the susceptibility of the cultivar to rotting (Berrie *et al.,* 2017), and the maturity of the fruit (Xu *et al.,* 2007). Preventive chemical treatments are essential for effective blossom blight disease control (Børve *et al.,* 2017), whilst good sanitation practice of removing mummified fruit remaining on the tree and blighted twigs is recommended (Holb & Schnabel, 2005; Børve *et al.,* 2017). As for fruit, chemical applications prior to harvest are also advised (Børve *et al.,* 2017), and postharvest treatments can be applied (Berrie *et al.,* 2017) because pathogens remain latent on the fruit and develop symptoms after harvest. Fruit decay post-harvest is therefore an important cause of crop loss during storage (Berrie *et al.,* 2017), which can affect up to a third of the crop (Xu *et al.,* 2007).

Grey mould is also an important fungal disease in sweet cherry caused by *Botrytis cinerea* (Adaskaveg *et al.,* 2000; Børve *et al.,* 2017). Along with *Monilinia* spp, *B. cinerea* is economically significant in commercial cherry production due to post-harvest fruit losses (Børve *et al.,* 2017). However, the severity is dependent on cultivar susceptibility and maturity of the fruit at harvest (Wani *et al.,* 2014). This pathogen usually infects trees during blossom and may remain latent until ripening (Børve *et al.,* 2017). However, fruit can be infected at any phase of development, primarily through fractures or weaknesses in the exocarp. Thus, fruits presenting wounds are discarded for storage to avoid the spread of the pathogen (Børve *et al.,* 2000; Williamson *et al.,* 2007).

Viral diseases are important for commercial production since yield and fruit quality are directly affected (James *et al.,* 2017). For instance, 18-30% of crop loss can be caused by Prune dwarf virus and Prune necrotic ringspot. Fruit size and sweetness can also be reduced resulting in unmarketable fruit. Viruses can affect tree growth which, in turn, influences yields in successive years (Posnette *et al.,* 1968). A range of viruses have been detected in the UK. Posnette *et al.* (1968) reported Little cherry virus-1, Prune dwarf virus, Prunus necrotic ringspot virus, Cherry rusty mottle virus, Cherry green ring mottle virus, and Cherry necrotic rusty mottle virus. In addition, Little cherry virus-2 (Rott & Jelkmann, 2001), Plum pox virus (Mumford, 2006), and Cherry leaf roll virus (James *et al.,* 2017) have all been recorded. However, due to the global expansion of pathogens, new viruses may spread to UK cherry orchards. For instance, Cherry virus A, discovered in Germany (James *et al.,* 2017), was first recorded in 2001 (Kirby *et al.,* 2001).

Approximately, 20% of cherry viruses are transmitted through pollen and/or seed. Some viruses can be vectored by insects including Little cherry virus-2, transmitted by two species of mealybugs but only *Phenacoccus aceris* occurs in the UK. Plum pox disease affects different *Prunus* species, but only two of the nine strains can infect cherries (James *et al.,* 2017). In the UK, therefore, this virus has higher concern in plums than cherries. Some aphid species vector this virus; however, this transmission is non-persistent (James *et al.,* 2017). Both viruses can also be transmitted by infected material (e.g. grafting, budding) (James *et al.,* 2017). Additionally, viruses can be transferred by soil or soil borne vectors such as nematodes (James *et al.,* 2017).

Overall, the impact of pathogens on cherry production is dependent on the cherry cultivar and its rootstock, their sensitivity to infection (Posnette *et al.,* 1968; Rott & Jelkmann, 2001), and pathogen density (Lillrose *et al.,* 2017).

1.8 Integrated Pest Management (IPM) and Conservation Biological Control (CBC)

Adequate crop protection is essential to ensure good yields of marketable fruit. In conventional agriculture, growers have employed PPPs to control pests. In cherries, most of the arthropod pests (McLaren & Fraser, 2002; Van Timmeren & Isaacs, 2013) and diseases (Bogush *et al.,* 1986; Kennelly *et al.,* 2007; Xu *et al.,* 2007; Miessner & Stammler, 2010) are treated with chemicals.

However, the continued use of PPPs (especially broad-spectrum products) can lead to a number of undesirable outcomes (Lamichhane, 2017). For example, non-target species including natural enemies can be negatively impacted (Hajek, 2004). Pests can also develop chemical resistance (Miessner & Stammler, 2010). Individuals that survive sub-lethal doses can produce offspring that are resistant (e.g. pyrethroid resistance in *T. urticae* (Van Leeuwen *et al.,* 2010)). Consequently, higher doses and/or new PPPs are needed (Hajek, 2004). PPPs can also negatively impact on human health and the environment, including beneficial arthropods such as pollinators (Woodcock *et al.,* 2016; Lamichhane, 2017). As a consequence, some PPPs have been withdrawn from use (Hillocks, 2012).

Due to the need to manage cropped areas using more sustainable approaches, alternatives to PPPs have been developed (Hillocks, 2012). Integrated Pest Management (IPM) is an approach to enhance sustainable pest control. IPM aims to minimise the use of PPPs and integrate natural practices that ensure pest populations do not cause economic damage (Dent, 1995; Hillocks, 2012). The success of an IPM programme relies on pest monitoring so that scientific decisions can be made. The applied methods (biological, cultural, genetic, mechanical, physical, and non-toxic chemical (e.g. pheromones) controls) should be the least hazardous for human health and the environment and yet effective enough to reduce pest density below economic threshold damage (Dreistadt *et al.,* 2016). For example, *D. suzukii* incidence in cherry orchards can be reduced though cultural control. Crops can be covered, before ripening, with nets excluding *D. suzukii* (Haye *et al.,* 2016). Biological control is one key approach used in IPM programmes (Lamichhane *et al.,* 2017), which aims to restore a balance between pests and natural enemies in cropped areas, leading to reductions in populations of pests (Hajek, 2004). The term biological control is defined by Eilenberg *et al.* (2001) as “The use of living organisms to suppress the population of a specific pest organism, making it less abundant or less damaging than it would otherwise be”. Four biological control strategies have been developed (Eilenberg *et al.,* 2001; Hajek, 2004):

1) Classical biological control

The aim of classical biological control is to introduce natural enemies leading to their permanent establishment. Pests can be therefore controlled on a long-term basis with no further intervention. The main application of this approach is to control accidentally introduced non-native organisms that become pests in new regions (invasive species), where native natural enemies were unable to provide effective control. Additionally, non-native (exotic) natural enemies can also be introduced to control native pests. In order to use classical biological control effectively, in depth knowledge of pests and their natural enemies is required to avoid environmental impacts such as non-target interactions (Arnett & Louda, 2002).

2) Inundative biological control

Inundative and inoculative biological control, are also known as augmentation. Both aim to control pests through the release of natural enemies when they are scarce or absent. Unlike classical biological control, there is no intention to perpetuate their permanent establishment. Inundative biological control specifically consists of the release of natural enemies to rapidly control pests with no expectations of the natural enemies reproducing and building established populations. Large numbers of natural enemies are therefore released. It is likely that repeated releases are required to maximize efficacy. For example, the predatory mite *Neoseiulus andersoni* to control the mite *T. urticae* in cherries (Fountain *et al.,* 2017). Biopesticides, including bacteria, fungi, viruses, nematodes and plant compounds are also inoculatively released. For instance, the entomopathogenic fungi *Beauveria bassiana* to control the aphid *M. cerasi* in cherries (Andreev *et al.,* 2008).

3) Inoculative biological control

Inoculative biological control also aims to control pest outbreaks in the short term but released natural enemies are expected to propagate for a short period. This strategy is normally applied when pest outbreaks are likely to be controlled by the released natural enemies, or when mass rearing is not feasible. However, the success is dependent on the availability of sufficient resources for natural enemies to multiply so that their offspring can continue providing pest control. For example, the lacewing *Chrysopa carnea* to control of cotton bollworms *Heliothis* spp. (Ridgway & Jones, 1969).

4) Conservation biological control

In contrast to the other pest control strategies, based on release of natural enemies, Conservation Biological Control (CBC) implements methods to manipulate the environment to support natural enemies (Begg *et al.,* 2016). The aim is to provide a range of pest regulation services by predators, parasitoids and pathogens on a long term basis (Holland *et al.,* 2016). CBC was first used to protect natural enemies against the use of PPPs until it was realised the effectiveness of pest regulation services delivered (van den Bosch & Telford, 1964). After which, approaches to CBC were also deployed to enhance natural enemies. A key approach is to establish suitable habitat near or within the cropped areas to provide shelter and alternative food sources (e.g. pollen, alternative prey) for natural enemies (Begg *et al.,* 2016). Minimising disturbance by providing a long-term habitats is essential to enhance natural enemies (Cross *et al.,* 2015; Jonsson *et al.,* 2015). The complexity of the CBC habitat also influences the diversity and abundance of natural enemies it supports; as complexity increases diversity also tends to increase (Shackelford *et al.,* 2013). However, in some cases, less complex habitats (intermediate landscape complexity) increases pest regulation response compared to complex systems where pest regulation services are saturated (Jonsson *et al.,* 2015).

1.8.1 Approaches to IPM in cherry orchards

Pests and pathogens attack cherries at all phenological growth stages of the blossoms (Murray & Jepson, 2018), from dormancy (principal growth stage 0) to postharvest (principal growth stage 9) (Fadón *et al.*, 2015). Consequently, a number of IPM strategies have been developed for cherry orchards (Kutinkova & Andreev, 2004; Çetin *et al.,* 2008; Murray & Jepson, 2018). Other IPM strategies have focused on controlling specific pests such as *D. suzukii* (Cini *et al.,* 2012; Shaw *et al.,* 2019), *R. cerasi* (Daniel & Grunder, 2012), and *M. cerasi* (Kepenekci *et al.,* 2014). These strategies include cultural, non-toxic chemical (i.e. pheromones), and biological controls as alternative approaches to PPPs. For example, nets are used in modern cherry orchards as physical barrier to exclude *D. suzukii* (Haye *et al.,* 2016) and *R. cerasi* (Papadopoulos *et al.,* 2017), and pheromone disruptors, as chemical control, are used to disrupt mating behaviour in tortrix moths (Papadopoulos *et al.,* 2017).

Biological control is integrated into IPM strategies. Predators such as spiders, hoverfly larvae, and ladybirds are known to provide pest regulation services (de Roincé *et al.,* 2013; Holland *et al.,* 2016). In cherries, hoverfly larvae and ladybirds are particularly important to control *M. cerasi* (Wojciechowicz-Żytko, 2011), although their efficacy depends on ant density, which afford protection to aphids (Stutz & Entling, 2011). To maximize biological control, sticky bands can be applied to the base of trees to deter ant colonization and increase natural enemy abundance in the tree canopy (Papadopoulos *et al.,* 2017), although this approach can also deter the movement of other natural enemies (e.g. earwigs).

The entomopathogenic fungus *B. bassiana* has some efficacy on *M. cerasi* (Andreev *et al.,* 2008). *Isaria fumosorosea* in combination with plant compounds and *B. bassiana* can also be effective (Andreev *et al.,* 2008), including on *R. cerasi* (Daniel & Grunder, 2012), whilst, application of rapeseed oil reduced *M. cerasi* eggs in winter (Jaastad, 2007). Bacterial canker can be treated with antibiotics (Lillrose *et al.,* 2017) and the incidence of blight blossom and brown rot is reduced significantly by the fungi *Aureobasidium pullulans*. Although the control of the fungal diseases is not as effective as synthetic fungicides (Wittig *et al.,* 1997), the phytotoxicity levels detected on cherries are lower (Holb & Kunz, 2013). *Bacillus subtilis* is a bacterium known to be an antagonist of many fungi, thus it has been used to control different fungal diseases on a number of crops (Sharma *et al.,* 2009). In cherry, *B. subtilis* can contribute to the control of grey mould, blue rot and brown rot (Utkhede & Sholberg, 1986).

However, some pests cannot be managed with biological control alone and PPPs are needed. For instance, *D. suzukii* is host of several parasitoid wasp species but due to the high reproduction rate, it is unlikely that parasitoid wasps can reduce *D. suzukii* below the economic level (Cini *et al.,* 2012). Moreover, in accordance with Bebber *et al.* (2014), *D. suzukii* is a worldwide generalist pest, which has reached areas where the native antagonists do not provide effective control (Chabert *et al.,* 2012). Also, better control of some fungal diseases is achieved when combining microbial antagonists with low doses of fungicide, resulting in a reduction of fungicide residues, e.g. the yeast *Cryptococcus infirmo-miniatus* and the fungicide propiconazole on brown rot (Spotts *et al.,* 2002).

To improve the sustainable production of sweet cherry in the UK, the dependence of growers on PPPs needs to be addressed by gaining a thorough understanding of the role of key pests and natural enemies to control them. CBC is a sustainable addition to PPPs, which can be used as part of IPM programmes (Cini *et al.,* 2012; Danelski *et al.,* 2015; Begg *et al.,* 2016). However, suitable habitats for natural enemies are essential to achieve CBC (Begg *et al.,* 2016; Holland *et al.,* 2016). It is possible that wildflower interventions may enhance populations of beneficial arthropods to deliver pest regulation services to sweet cherry crops.

1.8.1.1 Wildflower interventions for CBC in cherry orchards

In the UK, alleyways in commercial modern sweet cherry orchards are dominated by grass species (e.g. *Poa trivialis* and *Holcus lanatus*), although flowering forbs (e.g. *Trifolium repens*, *Taraxacum officinale* and *Ranunculus repens*) are frequently found. Alleyways are frequently mown to a height of approximately 10 cm throughout the growing season. As a result, the support of arthropod functional groups is limited. The introduction of new habitats, especially those consisting of wildflower species, is an approach commonly used in cropped areas to enhance both pollinator and natural enemy communities and the associated pollination and pest regulation services (Holland *et al.,* 2016; Potts *et al.,* 2016; Campbell *et al.,* 2017; McKerchar *et al.*, 2020). Wildflower interventions can be established as patches or strips adjacent to or within cropped areas (Blake *et al.,* 2012; Westbury *et al.,* 2017). For example, strips of wildflowers have been established between rows of trees in apple orchards (Campbell *et al.,* 2017; McKerchar *et al.*, 2020)). These interventions influence arthropod behaviour, richness and abundance in orchards (McKerchar *et al.*, 2020) but arthropods are also influenced by edge effects (Nguyen & Nansen, 2018).

To create suitable habitats for a range of pollinators and natural enemies, seed mixes have to be designed accordingly (Campbell *et al.,* 2017). Species of pollinators and natural enemies have preferences for wildflower resources (Carrié *et al.,* 2012). For instance, long-tongued bees (e.g. *B. hortorum*) prefer legume species with deep corollas, whilst composite flowers with open and short corollas (e.g. *Achillea millefolium*) are preferred by adult hoverflies (e.g. *Eristalis tenax*) and short-tongued bees (e.g. *Halictus rubicundus*) (Willmer, 2011). Plant species are also selected by pollinators according to their provision of pollen and/or nectar. Pollinators can also benefit from a varied diet provided by diverse plant communities (Wood *et al.,* 2016). Wildflower habitats also provide shelter, especially if they can contain species as tussock-forming grass species (e.g. *Dactylis glomerata*) (Hajek, 2004). The species richness and diversity of wildflower areas therefore directly influences pollinator and natural enemy diversity (Blaauw & Isaacs, 2012; 2014).

When establishing wildflower habitats the use of perennial species rather than annuals and biennials is advantageous as it provides greater consistency in floral resources between years (McKerchar *et al.*, 2020) and throughout the year, including winter (Isaacs *et al.,* 2009), leading to positive impacts on pollinator and natural enemy communities (Begg *et al.,* 2016). It also means that wildflower strips do not have to be sown on an annual basis, saving time and cost. It is recommended that seed mixes are based on native species. These are adapted to the local environment and are more likely to propagate than non-native species (Isaacs *et al.,* 2009). Furthermore, native plants can increase beneficial arthropod abundance, including native arthropods (Isaacs *et al.,* 2009). Overlapping bloom periods is also important to provide resources throughout the season, which can be extended when including early- and late-season native wildflowers (Isaacs *et al.,* 2009). In addition, native plant communities can be increased, which has favourable impacts on plant conservation (Isaacs *et al.,* 2009).

Wildflower strips in the alleyways between rows of cherry trees are therefore likely to enhance pollinating insects and natural enemies to deliver pollination and pest regulation services in sweet cherry orchards. However, how to manage these strips, except for a recommended annual cutting (Haaland *et al.,* 2011), and how establishment is affected under protective covers are not well-documented. Standard management of wildflower strips aims to create a complex sward structure for beneficial arthropods e.g. alternative pollen and nectar sources for pollinators and shelter and alternative prey for natural enemies. However, Marliac *et al.* (2015) investigated height management for the control of *Cydia pomonella* in apple orchards and found that tall or medium (20 cm height) compared to short (5 cm height) vegetation can attract natural enemies from trees and decrease pest regulation. Wildflower strips may have other adverse outcomes if management is not adequate. For instance, tall vegetation in alleyways may impede worker movement and increase levels of humidity, which could be associated with an increased incidence of fungal disease (Børve *et al.,* 2017). In addition, mowing can help to reduce the incidence of *D. suzukii* (Knapp *et al.,* 2019).

Nonetheless, impacts of wildflower height management on pollinator and natural enemy comunities and their ecosystem service delivery in sweet cherry orchards are still not known. Many wildflowers can develop flower heads at heights of 20 cm (Fitter *et al.*, 1978), which could therefore enhance beneficial arthropods and subsequently ecosystem services whilst reducing potential detrimental issues compared to an untreated alleyway or tall wildflower strips. Active management of wildflower strips to a height of 20 cm is therefore a novel grower-friendly strategy that aims to maximize ecosystem service delivery. Compared to conventional, grass dominated, regularly mown alleyways, wildflower strips in sweet cherry orchards are expected to enhance pollinators and natural enemies and subsequently pollination and pest regulation services. Whilst, the new actively management of wildflower strips to a height of 20 cm might enhance beneficial arthropods, and subsequently ecosystem services, similarly to the standard management of wildflower strips with a single cut at the end of the growing season.

1.9 Aim and objectives

Globally, sweet cherry is a highly valuable commercial crop. Yet, to date, no research has investigated how wild pollination and pest regulation services can be enhanced in protected sweet cherry orchards by introducing wildflower habitat between rows of trees and under protective covers. Furthermore, given the importance of habitat management on interactions between plant species and beneficial species (Woodcock *et al.,* 2016), there is also a need to investigate how these services can be maximised through targeted management of wildflower habitats.

The main aim of this study was to enhance ecosystem services provided by pollinators and natural enemies towards a more sustainable production of commercial sweet cherry. The research was focused on a single sweet cherry cultivar, Kordia, which is of commercial importance. The objectives of the study were to:

1. Identify the key insect pollinators and their effectiveness in commercial cherry orchards, and to determine the dependence of cherry yields on insect pollination.
2. Investigate the effect of wildflower interventions on pollinating insect foraging preferences and whether this leads to an improvement in sweet cherry pollination.
3. Investigate the effect of wildflower interventions on the abundance and activity of natural enemies and how the former influences pest regulation and therefore pest incidence.
4. Maximise the benefits of wildflower interventions by investigating the most appropriate cutting management regime to enhance services, whilst minimising costs and inconvenience to growers.
5. Examine the effect of wildflower strips on humidity levels under tunnels and, subsequently the occurrence of related fungal diseases.

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Chapter 2. Experimental design

2.1 Abstract

A three-year study was carried out in ten sweet cherry (cultivar Kordia) orchard blocks at five sites in the West Midlands, UK. The landscape context of the sites was dominated by improved grasslands (46%) and arable and horticulture areas (45%); broadleaved woodlands were also present, but typically occupied less than 6%. The ten study orchard blocks were managed conventionally for sweet cherry production, but different combinations of cover structures and film covers were used, being high tunnels covered with luminance polythene plastic the most frequent combination. Alleyways between rows of trees received one of three different treatments to investigate their influence on the ecosystem services provided by pollinators and natural enemies. In each orchard block, two wildflower strips measuring 1 m wide x 95 m long were established in non-adjacent, separate alleyways. One wildflower strip received the standard cutting regime with a single cut at the end of the growing season, whilst the second wildflower strip was managed with a novel cutting regime to a height of 20 cm throughout the growing season. These two wildflower strips were compared to an untreated control alleyway, managed conventionally with regular cutting.

2.2 Location of the study

In the UK, sweet cherry production has generally increased over the past two decades (see Chapter 1) due to the introduction of modern orchard systems (Bujdoso & Hrotko, 2017). These are associated with high-density planting, protective covers, enhanced fruit quality and greater yields per hectare. Data provided by Berry Gardens Ltd. (one of the project partners) indicated that in the UK, cherry production is divided between the South East (Kent and East Anglia), the West Midlands, and Scotland, accounting for 46.0%, 45.5% and 8.5%, respectively. This study was carried out in the West Midlands, at three sites in Herefordshire and two in Staffordshire (Figure 2.1; Table 2.1). Consequently, distances between sites varied. The two closest sites were located in Staffordshire with a distance of 2.8 km apart, whilst the greatest distance between the sites in Herefordshire and Staffordshire was 92.9 km. The five study sites were selected based on the partnership of growers with Berry Gardens Ltd. Data were collected from April 2017 (year one) to August 2019 (year three).

Table 2.1. County, altitude and coordinate location of the study sites in the West Midlands, UK.

Site	Orchard blocks	County	Altitude (m)	Coordinates
1	1 and 2	Herefordshire	220	52°10'46.7"N, 3°05'22.2"W
2	3 and 4	Herefordshire	110	52°11'25.6"N, 2°56'53.2"W
3	5 and 6	Herefordshire	180	52°09'37.1"N, 2°35'38.2"W
4	7 and 8	Staffordshire	88	52°47'30.6"N, 2°09'35.7"W
5	9 and 10	Staffordshire	102	52°45'59.9"N, 2°09'48.3"W

At each site, two commercial orchard blocks (defined as a separate parcel of land), were selected for study consisting in each replicate. Orchard blocks included the cultivar Kordia.

Figure 2.1. Location of the five study sites within A) the United Kingdom and B) the West Midlands. Three sites located in Herefordshire and two in Staffordshire (Google Maps, 2020).

2.3 Landscape context and geology of the sites

To investigate the landscape context of the study sites, the percentage cover of different land use types within a 1 km radius of each site was determined using ArcGIS and R (R Core Team, 2019). Land cover broad habitat classes were defined using Land Cover Map 2015 (Rowland *et al.,* 2017), which provided dominant land classes for 25 m^2 areas. The average landscape context was dominated by improved grasslands (46.1%) and arable and horticulture areas (44.9%), but also broadleaved woodlands (5.5%), suburban areas (1.9%), and others (1.5%) were present. Percentage land covers are shown in Table 2. Table 2.3 shows underlying geology and soil type along with the additional crops that surrounded the study orchard blocks.

Table 2.2. Percentage cover of land use within 1 km radius of each orchard block according to Land Cover Map 2015 broad habitat classes.

Broad habitats class	**Orchard blocks**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
Improved grassland	60.7	58.3	24.2	23.9	40.9	53.9	37.1	34.7	54.8	72.8	**46.1**
Arable & horticulture	22.4	22.9	71.1	72.5	47.0	37.0	49.6	58.3	42.5	25.7	**44.9**
Broadleaved woodlands	13.4	15.5	2.9	3.2	11.1	4.2	2.4	0.5	1.6	0.4	**5.5**
Suburban	0.6	0.9	1.8	0.4	0.0	4.3	7.7	3.8	0.0	0.0	**1.9**
Urban	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.5	1.0	1.0	**0.7**
Inland rock	1.5	1.3	0.0	0.0	0.8	0.4	0.0	0.0	0.0	0.0	**0.4**
Coniferous woodland	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.2**
Acid grassland	0.5	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.1**
Freshwater	0.0	0.0	0.0	0.0	0.3	0.3	0.2	0.2	0.2	0.2	**0.1**

Table 2.3. Underlying geology and soil type of the study sites and additional crops surrounding the study orchard blocks.

Site	Underlying geology (BGS, 2020)	Soil (Cranfield University, 2020)	Additional crops surrounding the study orchard blocks
1	Sedimentary bedrock formed of interbedded siltstone and mudstone	Freely draining slightly acid loamy soil	Cherry and blueberry orchards
2	Sedimentary bedrock formed of interbedded siltstone and mudstone	Slowly permeable seasonally wet acid loamy and clayey soil	Cherry and apple orchards, and oilseed rape fields
3	Sedimentary bedrock formed of interbedded argillaceous rocks and sandstone	Slightly acid loamy and clayey soil with impeded drainage	Cherry and apple orchards, and raspberry fields
4	Sedimentary bedrock formed of halite-stone and mudstone	Slightly acid loamy and clayey soil with impeded drainage	Strawberry fields
5	Sedimentary bedrock formed of halite-stone and mudstone	Slightly acid loamy and clayey soil with impeded drainage	Cherry orchards and strawberry fields

2.4 Experimental design of the orchard study sites

2.4.1 Orchard management

All ten orchard blocks were managed intensively for the commercial production of sweet cherries. However, the design of orchards was not always consistent within and between sites (Table 2.4). The size of the orchard blocks varied from 1.3 to 7.5 ha, and three different combinations of cover structures and film covers were used (henceforth protective covers as a general term unless otherwise specified). Two orchard blocks had pole-and-wire systems with polythene Voen mesh covers, six orchard blocks were high tunnels covered with luminance polythene plastic, and two blocks were high tunnels coved by vented luminance polythene plastic (Table 2.4). In eight of the orchard blocks, cherry trees were covered before the blossom period (early April) until the end of summer (early September). However, at Site 3 the two orchard blocks were covered only after the blossom period. In addition, at Site 4, only in year three, protective covers were used after the blossom period.

Table 2.4. Summary of the orchard design and tree information for cultivar Kordia.

Site	Orchard block	Size (ha)	Covering structure	Covering polythene film	Tunnel width (m)	Date planted	Kordia rootstock
1	1	3.50	High tunnel	Vented luminance	8.7	2011	Gisela 5
	2	3.60	High tunnel	Vented luminance	8.7	2010	Gisela 5
2	3	1.33	Voen	Voen mesh	-	2006	Gisela 5
	4	2.35	High tunnel	Luminance	8.3	2011	Gisela 5
3	5	2.80	High tunnel	Luminance	8.0	2012	Gisela 5
	6	1.30	Voen	Voen mesh	-	2007	Gisela 5
4	7	2.50	High tunnel	Luminance	7.2	2011	Gisela 6
	8	4.20	High tunnel	Luminance	7.2	2012	Gisela 6
5	9	7.50	High tunnel	Luminance	8.3	2011	Gisela 5
	10	3.70	High tunnel	Luminance	8.3	2013	Gisela 5

The eight high tunnel orchard blocks with polythene covers (vented or not) received a similar management of raising the sidewalls to ventilate and release the excess heat (Lang, 2014). The two orchard blocks with Voen mesh covers were not able to regulate environmental factors. In addition, to minimise bird damage of fruit (Lang, 2014), all sites but Site 3 used bird nets on the front and sides of the tunnels. Bird scarers were used outside the orchard blocks at Site 3, whilst Site 5 used both bird deterring methods. When used, nets were set up at the beginning of July, when the fruit started to mature until after harvest.

All orchard blocks under polythene covers were planted in a double row system (Cahn *et al.,* 2001) to enhance cross-pollination (Lech *et al.,* 2008). The cherry orchards were planted in different years (Table 2.4). However, as commercial yields are reached when cherry trees are four to five years old (Lang, 2000), all blocks contained trees classed as fully mature.

Table 2.5. The pollinizer cultivars that occurred with cv. Kordia in the ten orchard blocks.

Site	Orchard block	Cultivars (used as pollinizers) planted with Kordia
1	1	Regina
	2	Regina
2	3	Karina, Lapins, Regina
	4	Karina, Regina
3	5	Penny, Regina
	6	Lapins, Karina, Regina, Skeena
4	7	Regina
	8	Karina, Regina
5	9	Karina, Lapins, Regina, Sweetheart
	10	Lapins, Penny, Regina, Sweetheart

Three of the orchard blocks had only one different cultivar (used as pollinizer) planted with Kordia, whilst the rest varied from two to four. The cultivars that occurred with Kordia in the ten orchard blocks are shown in Table 2.5. Eight orchard blocks maintained a different cultivar in the adjacent row, whilst Orchard blocks 9 and 10 had, respectively, two and three rows in succession with the same cultivar. These two orchard blocks and the two orchard blocks at Site 4 included single pollinizer trees in the studied Kordia row. In nine of the orchard blocks, Kordia trees were grafted onto Gisela 5 rootstock, whilst in one orchard block trees were grafted onto Gisela 6 (Table 2.4).

At all sites, cherry trees were thinned and pruned once a year. Thinning was carried out during the blossom period (typically mid-April) to reduce crop load, whilst pruning was done in late summer after harvest (Lauri & Claverie, 2008) to increase yield and improve fruit quality (Guimond *et al.,* 1998; Usenik *et al.,* 2008).

To enhance pollination, nine of the ten orchard blocks used a combination of managed honeybees (*Apis mellifera*) and buff-tailed bumblebees (*Bombus terrestris*) (Table 2.6). At Site 3, in Orchard block 5 only honeybees were used throughout the three years. However, in Orchard block 6 at the same site, bumblebees were not introduced in year one.

Table 2.6. Number of honeybee hives and bumblebee boxes per hectare used in each orchard block.

Site	Orchard block	Honeybee hives (number per ha)	Bumblebee boxes (number per ha)
1	1	2.6	10.0
	2	2.6	10.0
2	3	1.9	6.0
	4	1.9	6.0
3	5	3.6	-
	6	3.6	3.9
4	7	1.6	6.0
	8	1.6	6.7
5	9	2.2	4.0
	10	2.2	6.0

To protect the crop against arthropod and pathogen pests, spray programmes were applied at all sites. Spray records were provided by growers for all the three years. Spray programmes started in March, prior to the cherry blossom period until July/August before harvest each year. A total of three acaricides (which percentage from the total PPPs used was 4.8%), ten insecticides (34.1%), two bactericides (2.4%), and eight fungicides (58.7%) were used against the main cherry pests over this time. Within acaricides, spirodiclofen accounted for 75% of the total acaricides used to control *Tetranychus urticae* (two-spotted spider mite). Indoxacarb, spirotetramat, acetamiprid, and cyazypyr were the main insecticides applied to control insect pests, and accounted for 19.5%, 15.9%, 14.2% and 14.2% respectively, of the total 100% of insecticides used. The major targets were *Drosophila suzukii* (spotted winged drosophila), Lepidoptera (caterpillars), and *Myzus cerasi* (cherry blackfly). The single bactericidal active ingredient was copper oxychloride, to protect against *Pseudomonas syringae* (bacterial canker). The most common fungicides comprised fenhexamid (which was applied 32.3% within the total fungicides), cyprodinil and fludioxonil (27.2%), and pyraclostrobin and boscalid (24.6%) aimed at controlling *Monolinia* spp. (blossom wilt and brown rot) and *Botrytis cinerea* (grey mould). The number of PPP applications ranged from eight to 25 per orchard block and year, with a mean of 13.8 (± 0.9).

2.5 Alleyway treatment design

In each orchard block, three alleyways adjacent to the cherry cultivar Kordia were selected for study. Two random alleyways per orchard block were established with wildflower interventions and were subjected to two different management treatments. The third alleyway was an untreated control, consisting of the original orchard vegetation (Figure 2.2). The control alleyways were managed conventionally with regular cutting throughout the growing season to a height of approximately 10 cm. For the wildflower treatments, one strip in each orchard block was managed with annual cutting in September to a height of 8 cm, whilst the other was actively managed with regular cutting to a height of 20 cm. The three treatments investigated were therefore:

i) **Control Strips (CS)**. Conventionally managed alleyways not sown with wildflowers that were cut regularly to a height of 10 cm from May to September, and then to a height of 8 cm in late September.

ii) **Standard Wildflower Strips (SWS)**. Cut annually in late September to a height of 8 cm.

iii) **Actively Managed Wildflower Strips (AMWS)**. Cut regularly (twice/three times per month) to a height of 20 cm from May to September, and then to a height of 8 cm in late September.

Figure 2.2. An example of the experimental design deployed at Site 1 with the two separate orchard blocks and three 95 m alleyway treatments in each. Control Strips (blue), Actively Managed Wildflower Strips (green), Standard Wildflower Strips (red). Created using Google maps (Google Maps, 2020).

Across all sites, the average width of alleyways between rows of cherry trees was 2 m. To avoid frequent damage and soil compaction from vehicle movement, wildflower interventions were established in the central 1 m strip between tree rows. At all sites, wildflower strips measuring 95 m long were created at the beginning of tree rows towards the centre of the orchard block (Figure 2.2; Figure 2.3). The length of strips used enabled standardization between orchard blocks, which varied between and within sites.

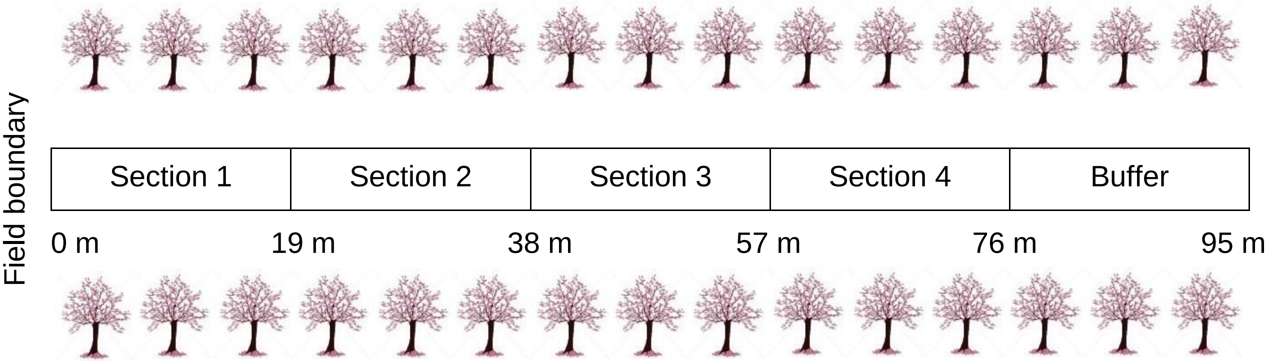

Figure 2.3. Representation of the 95 m strip in the alleyway from the orchard block edge (m), including the four sections for sampling and the buffer which was not assessed. Orchard block edge (boundary) is located at 0 m.

To investigate the influence of edge effects on arthropod distribution along the cherry tree rows, the 95 m long treatment strips were divided into five sections (Figure 2.3). Due to five of the alleyway treatments only measuring 95 m long in their entirety, the fifth section (76 m - 95 m) acted as a buffer and was not assessed.

The distance between alleyway treatments varied from 26 to 48 m, depending on where Kordia trees were situated in the orchard blocks. Distances between orchard blocks also varied within each site. At four sites the distance was between 250 and 975 m, whereas at Site 2, the distance between blocks was 30 m. Within each alleyway treatment, there was variation in the number of adjacent Kordia trees, ranging from 34 to 82 trees (Table 2.7).

Table 2.7. Number of Kordia trees adjacent to the 95 m strip for each orchard block and alleyway treatment distribution at the five studied sites.

Site	**Orchard block**	**Number of Kordia trees**	**Distance between alleyway treatments (m)**	**Distance between orchard blocks (m)**
1	1	39	43.5	250
	2	60	43.5	
2	3	42	32.0	30
	4	42	32.0	
3	5	44	48.0	975
	6	44	26.0	
4	7	41	36.0	310
	8	82	43.2	
5	9	34	41.5	825
	10	39	33.2	

2.6 Statistical analysis

For all datasets in all chapters, data distribution was tested to investigate whether data were parametric or non-parametric with the Shapiro–Wilk test, and analysed as specified in each chapter accordingly. For ordinal or proportional data (non-parametric data), datasets were also plotted using histograms to confirm normality. Where the response variable was normally-distributed, parametric analyses were used. Where the response variable was ordinal, models with Poisson error structures were used. Where the response variable was binomial, models with binomial error structures were used. Where the response variable was ordinal but consisted of many zero values, models with negative binomial error structures were used. Model dispersions were checked for over/under dispersion; and when confirmed, quasi-version of the error structures were used.

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Chapter 3. Wildflower strip management for the delivery of ecosystem services in sweet cherry orchards*

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In the paper, the establishment for the three sites located in Herefordshire over a one-year period was described. However, in this Chapter, all five sites (located in Herefordshire and Staffordshire) are included and the development of vegetation over the three years is presented.

3.1 Abstract

The practice of introducing wildflower habitats in cropped areas is an approach that can be used to enhance ecosystem services. However, the efficacy of this approach can be affected by the establishment success of the sown species. To investigate this, 20 alleyway strips (1 x 95 m) between rows of cherry trees (cultivar Kordia) under protective covers, were sown with a bespoke wildflower mix at five sites in the West Midlands (UK). After wildflower strip establishment in year one, responses to two cutting regimes were investigated for two consecutive years. Ten wildflower strips were managed with an annual single cut in September (SWS) and ten were regularly cut to a height of 20 cm throughout the growing season (May - September) (AMWS). These treatments were compared to ten untreated (unsown) control strips (CS), managed conventionally with regular cutting (10 cm height). The wildflower strips were established by sowing nine native perennial species, consisting of eight forbs and one grass species. The successful establishment and contribution to alleyway vegetation was assessed using percentage cover in replicate 0.5 x 0.5 m quadrats. Habitat quality was determined by counting the number of floral units in quadrats throughout the growing season. Humidity and temperature were also recorded due to the potential for wildflower strips to influence these variables and therefore fungal incidence. The local climate was recorded in all three

alleyway treatments at two sites and the incidence of fungal disease on cherry fruit was evaluated at all five sites.

Floral species richness (number of species) and Shannon diversity were greater in both sown alleyway treatments compared to the unsown CS. Values of percentage bare ground decreased over time in the sown strips due to continued development of the sown wildflower species. Percentage cover of sown species did not differ between the two sown strip treatments and increased equally until the end of the study (year three). The mean sown species coverage was 75.7% (± 6.1). *Dactylis glomerata* established most consistently and with greater cover at 28.3% followed by *Leucanthemum vulgare* (10.6%), *Prunella vulgaris* (9.9%), *Achillea millefolium* (8.6%) and *Centaurea nigra* (7.9%). Both wildflower strip treatments were associated with over a 300% increase in the number of floral units compared to CS. Ambient humidity was 76.4% (± 16.0) in CS, 0.6% (± 0.3) significantly greater than wildflower strips, and temperature was 0.1ºC (± 0.1) higher in SWS compared to AMWS and CS. There was no difference in disease development on cherries after harvest between the alleyway treatments. These results indicate that the use of managed wildflower strips in protected orchards has potential to support the sustainable production of sweet cherry without increasing the incidence of fungal disease.

3.2 Introduction

The ability of farmland to support beneficial species is negatively affected by agricultural intensification and landscape change due to habitat loss and fragmentation (Blackmore & Goulson, 2014). As a consequence, the delivery of pollination and pest regulation services has been directly affected (Chaplin-Kramer *et al.,* 2011; Potts *et al.,* 2016). Many crops, including sweet cherry, are highly dependent on pollination services to maintain yields and fruit quality (Holzschuh *et al.,* 2012). Cherry production in the UK has increased (from 400 tonnes in 2000 to 3,568 tonnes in 2018) (see Chapter 1) due to intensive cropping practices (e.g. increased yield per ha) (Schoen & Lang, 2016), supported by the adoption of modern orchard systems (e.g. protective covers) (Cahn *et al.,* 2001). To improve yield, growers rely on the use of managed pollinating insects, mainly bees (Hansted *et al.,* 2015). However, evidence suggests that higher visitation rates and greater pollen transfer (Garibaldi *et al.,* 2013) may be better provided by a diverse range of wild pollinators (Holzschuh *et al.,* 2012).

If cherry production is increasingly reliant on wild pollinators to deliver pollination services, their resource demands need to be met outside the cherry flowering period; typically April/May in the UK. By providing wildflower habitat in alleyways between rows of cherry trees, wild pollinators are likely to benefit from the extended provision of nectar and pollen, potentially sustaining and building populations over time leading to enhanced delivery of pollination services during the cherry blossom period (Holzschuh *et al.,* 2012).

Sweet cherry is damaged by arthropod pests leading to the use of a number of different Plant Protection Products (PPPs) to protect crops. Two major pests of cherry are *Myzus cerasi* (cherry blackfly) (Stutz & Entling, 2011) and *Drosophila suzukii* (spotted wing drosophila) (Beers *et al.,* 2011). It is standard practice to control these pests using PPPs (McLaren & Fraser, 2002; Shaw *et al.,* 2019), which can have negative consequences for beneficial arthropods and the environment (Beers *et al.,* 2011). Increasingly, growers are adopting Integrated Pest Management strategies, but the number of options available to cherry growers is limited (Kutinkova & Andreev, 2004; Çetin *et al.,* 2008). To improve the sustainable production of sweet cherry, there is potential for Conservation Biological Control measures to be implemented (Begg *et al.,* 2016), and the deployment of floristically diverse wildflower habitats is one strategy (Campbell *et al.,* 2017).

Fungal diseases can cause up to a third of crop losses in cherry (Børve *et al.,* 2017). In UK sweet cherry orchards, brown rot and blossom wilt (*Monolinia* spp.), grey mould (*Botrytis cinerea*), blue mould (*Penicillium expansum*) and *Mucor* rot (*Mucor piriformis*) are among the most important fungal diseases (Berrie *et al.,* 2017). Principally, *Monolinia* species are the main fungi which cause damage to stored cherries in the UK (Xu *et al.,* 2007; Berrie *et al.,* 2017). Most fungi develop more rapidly under warm and moist conditions (Børve *et al.,* 2017) and potentially these conditions could be enhanced by high understory vegetation in cover protected orchards because of evapotranspiration (Schlesinger & Jasechko, 2014), increasing relative humidity. This is particularly important in enclosed environments such as crops under protective covers (Fountain *et al.,* 2017; Hall *et al.,* 2019). An increase in above-ground biomass in cherry orchards through the provision of wildflower strips might therefore be expected to increase humidity levels under these systems. Protected crops experience higher peaks in air temperature (Lang, 2014) and changes in climatic conditions due to the presence of wildflower strips could impact on fungal development and cherry disease incidence and subsequently affect yields and fruit quality.

Most cherry production in the UK is underpinned under protective covers and establishment and continuing management of wildflower strips has not been studied. Hence, this study compared three treatments in protected sweet cherry orchard alleyways; 1) unsown, grower managed alleyways cut regularity (once / twice per month) and maintained at a height of 10 cm (May – September), classed as Control Strips; 2) sown wildflower strips with a single cut at the end of the growing season (September), classed as Standard Wildflower Strips; and 3) sown wildflower strips with regular cutting (once / twice per month) to a height of 20 cm throughout the growing season (May – September), classed as Actively Managed Wildflower Strips. All three treatments were cut to a height of 8 cm at the end of the growing season in late September each year. The aims of this study were to determine i) the establishment and development of sown wildflower alleyways, ii) the influence of habitat management on floral composition, and iii) whether alleyway management regimes differentially affected abiotic factors (humidity and temperature), and iv) subsequent disease incidence in harvested fruit.

3.3 Material and methods

3.3.1 Alleyway treatment design

The study was done at three sites in Herefordshire and two in Staffordshire (West Midlands, UK), with two orchard blocks at each site. In each orchard block (defined as a separate parcel of land), three alleyways adjacent to tree rows of the sweet cherry cultivar Kordia were selected to be studied. Alleyway strips measured 1 m wide and 95 m long, starting at the beginning of the tree row towards the centre of the orchard. The length of strips used enabled standardization between orchard blocks, which varied in size from 1.3 – 7.5 ha (see Chapter 2). Two alleyways per orchard block were randomly selected to be established with wildflower interventions subjected to two different cutting management regimes. The third alleyway was an untreated control, which consisted of the original unsown alleyway vegetation (Figure 3.1).

The control strips were managed conventionally with regular cutting throughout the growing season (May – September). For the wildflower treatments, one strip in each orchard block was managed with annual cutting in September to a height of 8 cm, whilst the other was actively managed with regular cutting to a height of 20 cm throughout the growing season. The three alleyway treatments investigated were therefore:

i) **Control Strips (CS)**. Conventionally managed alleyways not sown with wildflowers that were cut regularly to a height of 10 cm from May to September, and then to a height of 8 cm in late September.

ii) **Standard Wildflower Strips (SWS)**. Cut annually in late September to a height of 8 cm.

iii) **Actively Managed Wildflower Strips (AMWS)**. Cut regularly (twice/three times per month) to a height of 20 cm from May to September, and then to a height of 8 cm in late September.

Figure 3.1. A) Control Strip, B) Standard Wildflower Strip and C) Actively Managed Wildflower Strip in year three.

3.3.2 Wildflower selection

The wildflower mixture (Table 3.1) was designed specifically to support a range of beneficial arthropod functional groups. The forb species were included to provide a range of forage resources and shelter for pollinators and natural enemies, and included plant species with different flowering morphologies and phenologies, whilst *Dactylis glomerata* (a tussock forming grass) was chosen to provide refuges for natural enemies (Hajek, 2004; Pywell *et al.,* 2005). All species were perennial and native to the UK to maximize benefits from ecosystem services delivered by native pollinators and natural enemies (McKerchar *et al*., 2020). Perennial species also reduce variation in floral resource availability between years (Carvell *et al*., 2007). Wild types of *Lotus corniculatus, Trifolium pratense, and D. glomerata* were used rather than agricultural forage varieties due to their greater longevity (Woodcock *et al.*, 2014).

Table 3.1. Beneficial arthropods positively associated with species sown in the wildflower interventions.

Plant species	**Beneficial arthropods**	**Source**
Achillea millefolium	Lacewings, ladybirds, hoverflies, solitary bees, parasitoid wasps, beetles, butterflies	Carrié *et al.,* 2010; Carrié *et al.,* 2012; Wood *et al.,* 2016; Hatt *et al.,* 2017
Centaurea nigra	Honeybees, solitary bees, bumblebees, hoverflies	Blake *et al.,* 2012; García & Miñarro, 2014; Wood *et al.,* 2015; Wood *et al.,* 2016
Leucanthemum vulgare	Parasitoid wasps, hoverflies, solitary bees, butterflies, beetles, spiders	Haaland *et al.,* 2011; Carrié *et al.,* 2012; Wood *et al.,* 2016; Hatt *et al.,* 2017
Leontodon hispidus	Hoverflies, solitary bees, butterflies, beetles	Carvell *et al.,* 2004; Wood *et al.,* 2016
Lotus corniculatus	Parasitoid wasps, honeybees, bumblebees, butterflies	Carrié *et al.,* 2010; Blake *et al.,* 2012; García & Miñarro, 2014
Prunella vulgaris	Bumblebees	Carvell *et al.,* 2004; Blackmore & Goulson, 2014
Silene dioica	Hoverflies, bumblebees, butterflies	Goulson, 2009; Blackmore & Goulson, 2014
Trifolium pratense	Spiders, hoverflies, honeybees, bumblebees, butterflies	Blake *et al.,* 2012; Blackmore & Goulson, 2014; Sutter *et al.,* 2017
Dactylis glomerata	Carabids, staphylinids, spiders	Hajek, 2004; Pywell *et al.,* 2005

3.3.3 Wildflower establishment and development

In autumn (September/October) 2016, across all five sites, a total of 20 randomly selected alleyway strips were sprayed with the broad-spectrum, systemic herbicide Roundup™ (glyphosate) to kill existing vegetation in preparation for sowing (McKerchar *et al*., 2020).

At least six days after spraying, the strips were cultivated to create a fine seed bed and sown with the wildflower mix within 24 hours. The forb mix was sown at a rate of 2.0 g m^2 and the grass at 1.0 g m^2 (Table 3.2). Seed was purchased from Emorsgate Seeds (https://wildseed.co.uk) and Yellow Flag Wildflowers (www.wildflowersuk.com). Prior to hand sowing, seeds were mixed with sand to ensure a more even distribution. After sowing, the strips were rolled to ensure contact of seed with the soil. Due to poor establishment, probably owing to the late autumn sowing, all sites were re-sown the following year (2017) after light cultivation. The three sites in Herefordshire were re-sown in March/April 2017, whilst the two sites in Staffordshire were re-sown in September 2017. Despite re-sowing the wildflower strips at Site 4, establishment was still very poor and this site was discarded from the study in year three.

Table 3.2. Seed mix composition and sowing rate used to establish the wildflower strips. w.t. (wild type).

Scientific name	**Common name**	**Sowing rate (seeds m^2)**	**Sowing rate (g m^2)**	**% by weight**
Achillea millefolium	Yarrow	200	0.033	1.69
Centaurea nigra	Knapweed	200	0.444	22.60
Dactylis glomerata (w.t.)	Cock's-foot	100	0.100	5.10
Leontodon hispidus	Rough hawkbit	200	0.222	11.30
Leucanthemum vulgare	Ox-eye daisy	200	0.100	5.08
Lotus corniculatus (w.t.)	Bird’s-foot trefoil	200	0.400	20.34
Prunella vulgaris	Selfheal	200	0.200	10.17
Silene dioica	Red campion	200	0.200	10.17
Trifolium pratense (w.t.)	Red clover	200	0.267	13.56

Sowing took place when the protective covers were not in place, but from April to September, alleyways were covered and did not receive water directly from rainfall. However, some rainfall was still able to reach the alleyways in four orchard blocks. Orchard blocks 1 and 2 used vented high tunnels and Orchard blocks 3 and 6 used Voen mesh covers on pole-and-wire systems.

During the establishment year (2017 to May 2018), the wildflower strips, along with the unsown CS, were cut regularly to a height of approximately 10 cm to promote the establishment and development of the sown species (Aldrich, 2002). The different alleyway treatments were applied from May 2018 (year two). In comparison to the annual cutting in September of the SWS, AMWS were mown two to three times per month to a height of 20 cm from May to September in years two and three. All cuttings were left *in situ*.

3.3.4 Vegetation sampling

3.3.4.1 Plant species diversity and percentage cover

In August 2017 (year one), July 2018 (year two), and July 2019 (year three), quadrat sampling (Figure 3.2) was carried out to determine the contribution of each sown and unsown species in the alleyways for all treatments. Ten quadrats (0.5 x 0.5 m) were randomly distributed and assessed in each alleyway. All plant species were identified and assigned a percentage cover value. Values of bare ground were also recorded. Percentage cover of some plants overlapped and more than 100% was therefore recorded in some cases. The percentage occurrence was also determined for each species dividing the total number of occurrences by the number of quadrats sampled.

Figure 3.2. 0.5 x 0.5 m quadrat deployed in a Standard Wildflower Strip.

3.3.4.2 Resource availability

Resource provision for pollinating insects and natural enemies was determined by recording floral units in years two and three. The number of floral units (single flower e.g. *S. dioica* or flower cluster e.g. *L. vulgare*) in ten random quadrats (0.5 x 0.5 m) for each plant species was recorded in each alleyway treatment (Carvell *et al*., 2015). All flower heads of forb species were considered clusters, except for *S. dioica*, for which flower heads were recorded as single flowers, along with *D. glomerata*. Assessments were conducted once per month from June to September in year two and from June to August in year three.

3.3.4.3 Vegetation height

To investigate the influence of alleyway management on sward height, measurements were taken using the drop-disc method (Stewart *et al.,* 2001) to provide an indication of habitat structure for natural enemies (Benton *et al.,* 2003). Twenty measurements were taken per alleyway treatment, 120 from each site per month from June to September in year two and from June to August in year three.

3.3.5 Environmental factors

Abiotic factors (humidity and temperature) were recorded at 30-minute intervals using twelve data loggers (EL-USB-2 dew point, humidity, temperature data logger, EasyLog) deployed at two of the sites (Site 3 in Herefordshire and Site 5 in Staffordshire). One data logger was deployed per alleyway treatment. These were attached to the middle trees for each alleyway treatment at a height of ~1 m above ground for the three years of the study. Records were from early April to early October when the orchard blocks were covered.

3.3.6 Fungal disease monitoring

To explore whether the treatments had an impact on disease incidence, two fungi (*Monolinia* spp. and *Botrytis cinerea*) were monitored on cherry flowers (blossom surveys)

(Tamm *et al.,* 1995) and post-harvest fruit (shelf-life tests) (Adaskaveg *et al.,* 2000) in years two and three. However, due to the lack of blossom wilt recorded in year two and the starting time of the wildflower strip management in mid-May, resulting in no difference in height between alleyway treatments during the blossom period, the blossom surveys were not repeated in year three.

To investigate blossoms, 50 cm of four different branches were studied in each alleyway treatment, randomly selected from ten different trees (Berrie *et al.,* 2017). The total number of blossoms and number of blossoms suffering blossom wilt were counted. Fruits were assessed at post-harvest using established shelf-life tests (Berrie *et al.,* 2017). Fifty cherries from each alleyway treatment were randomly picked in July and placed onto trays (40 x 30 x 10 cm) with no contact between fruits. Trays were placed and sealed in polythene plastic bags and sprayed with water to increase humidity to 100% in order to maximize fungal development. The fruits were kept at room temperature and assessed every day for seven days (sweet cherry shelf life (Wani *et al.*, 2014)) recording fungus coverage on each cherry. Humidity and temperature were recorded with two data loggers (30-minute intervals) placed in two random trays.

3.3.7 Statistical analysis

All statistical analysis were conducted in R (Version R-3.6.1) (R Core Team, 2019).

3.3.7.1 Species richness and Shannon diversity

Differences in values of species richness and Shannon diversity between alleyway treatments according to year were analysed using generalized linear mixed models (lme4 package, function = GLMER (Bates *et al.,* 2014)). Alleyway treatment and year were set as fixed factors, whilst quadrats nested within orchard blocks nested within sites were specified as random effects (*Species richness* ~ *Alleyway treatment* + *Year* + *(random: Site/Orchard/Quadrat); Shannon diversity* ~ *Alleyway treatment* + *Year* + *(random: Site/Orchard/Quadrat)*). Significant differences in alleyway treatments and years were subsequently tested with Tukey *post-hoc* tests (multcomp package (Hothorn *et al.,* 2008)).

3.3.7.2 Percentage cover

Percentage covers were analysed with one-way ANOVA Kruskal-Wallis test (package stats), and significant results were further tested with the *post-hoc* Pairwise-Wilcoxon test (package stats).

3.3.7.3 Resource availability and vegetation height

Floral units and vegetation height were analysed using generalized linear mixed models with negative binomial error structures (lme4 package, function = GLMER.NB (Bates *et al.,* 2014)). Alleyway treatment and year were set as fixed factors, whilst quadrats nested within orchard blocks nested within sites were defined as random effects as above (*Floral units ~ Alleyway treatment + Year + (random: Site/Orchard/Quadrat); Height ~ Alleyway treatment + Year + (random: Site/Orchard/Quadrat)*). Tukey *post-hoc* tests were used to investigate pair-wise differences between alleyway treatments and years (multcomp package (Hothorn *et al.,* 2008)).

3.3.7.4 Environmental factors

Generalized linear mixed models were used to analyse the humidity and temperature data (lme4 package, function = GLMER (Bates *et al.,* 2014)). Humidity and temperature were specified as the response variable in separate models, and alleyway treatment and year were set as fixed effects and orchard blocks nested within sites as random effects for both (*Humidity ~ Alleyway treatment + Year + (random: Site/Orchard); Temperature ~ Alleyway treatment + Year + (random: Site/Orchard)*). The same models were used to determine differences throughout the day in two time periods. One period included the coldest hours of the day, between 18:00 and 09:30 hrs and the second period included the warmest hours, between 10:00 and 17:30 hrs.

3.3.7.5 Fungal disease monitoring

No statistical tests were conducted for the blossom surveys due to only five cherry blossoms being recorded suffering from blossom wilt. Shelf-life tests were analysed using a generalized linear mixed model with a negative binomial error structure (lme4 package, function = GLMER.NB (Bates *et al.,* 2014)). The cumulative percentage cover of each individual cherry was calculated at day seven of assessment and was used as a variable. Alleyway treatment and year were set as fixed factors, and the 50 cherries analysed nested within orchard blocks nested within sites were defined as random effects (*Fungal percentage cover ~ Alleyway treatment* + *Year* + *(random: Site/Orchard/Cherries)*).

3.4 Results

3.4.1 Species richness and Shannon diversity

In total, 41 plant species were recorded in 840 quadrats sampled across all orchard blocks and three years of study (300 quadrats in years one and two and 240 in year three). Species richness was affected by alleyway treatment with more species found in Standard Wildflower Strips (SWS) (Tukey test: $Z = 17.88$, $P < 0.001$) and Actively Managed Wildflower Strips (AMWS) (Tukey test: $Z = 19.09$, $P < 0.001$) compared to Control Strips (CS), conventionally managed alleyways (Table 3.3). There was no difference between SWS and AMWS. On average, 3.0 (± 0.3) sown species and 3.3 (± 0.2) unsown species were recorded in the SWS, whilst 3.0 (± 0.3) sown species and 3.6 (± 0.2) unsown species were recorded in the AMWS (Table 3.3). None of the sown species were recorded in the CS, which on average contained 3.0 (± 0.2) unsown species.

Year affected species richness between year one and three (Tukey test: $Z = -4.23$, $P < 0.001$), and year two and three (Tukey test: $Z = -3.42$, $P < 0.01$). Species richness and Shannon diversity increased from year one to three. All sown wildflower species were recorded each year but *L. hispidus* was not recorded in year one. In the CS, 12 species were recorded in year one, 19 in year two and 15 in year three, compared to 21, 20 and 16 in the SWS and 22, 18 and 14 in the AMWS in years one, two and three, respectively.

Shannon diversity was affected by alleyway treatment but not by year and was greater in SWS (Tukey test: $Z = 7.93$, $P < 0.001$) and AMWS (Tukey test: $Z = 8.19$, $P < 0.001$) than in CS (Table 3.3) with average values of 1.4 (± 0.03), 1.5 (± 0.03) and 0.7 (± 0.03), respectively.

Table 3.3. Values of total species richness and Shannon diversity (± SE) per year according to strip type and whether sown or unsown components. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Alleyway treatment	**Year one**		**Year two**		**Year three**	
	Species richness	Shannon diversity	Species richness	Shannon diversity	Species richness	Shannon diversity
All Species						
CS	2.8 (± 0.3)	0.6 (± 0.1)	3.0 (± 0.2)	0.8 (± 0.1)	3.3 (± 0.2)	0.8 (± 0.1)
AMWS	7.4 (± 0.7)	1.4 (± 0.1)	6.3 (± 0.4)	1.5 (± 0.1)	6.0 (± 0.6)	1.5 (± 0.1)
SWS	6.3 (± 0.8)	1.3 (± 0.1)	6.7 (± 0.6)	1.5 (± 0.1)	6.0 (± 0.4)	1.5 (± 0.1)
Sown Species						
CS	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)
AMWS	2.8 (± 0.5)	0.6 (± 0.1)	3.2 (± 0.6)	0.9 (± 0.1)	3.1 (± 0.4)	1.0 (± 0.2)
SWS	2.4 (± 0.5)	0. 7 (± 0.1)	3.3 (± 0.5)	0.8 (± 0.2)	3.5 (± 0.5)	0.9 (± 0.1)
Unsown Species						
CS	2.8 (± 0.3)	0.6 (± 0.1)	3.0 (± 0.2)	0.8 (± 0.1)	3.3 (± 0.2)	0.8 (± 0.1)
AMWS	4.7 (± 0.3)	1.0 (± 0.1)	3.1 (± 0.2)	0.8 (± 0.1)	2.9 (± 0.2)	0.8 (± 0.1)
SWS	3.9 (± 0.4)	0.9 (± 0.1)	3.3 (± 0.2)	0.9 (± 0.1)	2.6 (± 0.3)	0.7 (± 0.1)

3.4.2 Percentage covers of sown and unsown wildflowers and bare ground

The percentage cover values of sown and unsown species and bare ground were not significantly different between SWS and AMWS. However, the percentage cover of sown species varied significantly between years one and two (Pairwise-Wilcoxon test: $F_2 = 25.6$, $P < 0.01$), one and three (Pairwise-Wilcoxon test: $F_2 = 25.6$, $P < 0.001$), and two and three (Pairwise-Wilcoxon test: $F_2 = 25.6$, $P < 0.05$); sown cover increased each year (Figure 3.3). Bare ground percentage cover also differed between years one and two (Pairwise-Wilcoxon test: $F_2 = 11.7$, $P < 0.05$) and one and three (Pairwise-Wilcoxon test: $F_2 = 11.7$, $P < 0.01$); however, this percentage decreased over time (Figure 3.3). In contrast, no differences between percentage cover of unsown species and year were found. Values of percentage cover in the CS were not influenced by time (year) and remained fairly consistent between years (Figure 3.3).

Due to the lack of significant differences between wildflower treatments (AMWS and SWS) (Figure 3.3) for values of species richness, Shannon diversity, percentage cover of sown and unsown wildflowers, and percentage of bare ground, data were combined for subsequent analyses. The average cover of sown species in both wildflower strips combined increased from 22.3% (± 3.7) in year one to 75.7% (± 6.1) in year three (Figure 3.3), compared to a constant contribution of around 38% from unsown forb species. Unsown grasses were ~26% cover throughout the study. In contrast, in the unsown CS, the unsown forb species were less dominant than grasses, 37% and 68%, respectively. Mean cover of bare ground in the wildflower strips decreased from year one to year three compared to cover values in the CS treatment, which remained stable over the study period at 7.3% (± 1.8).

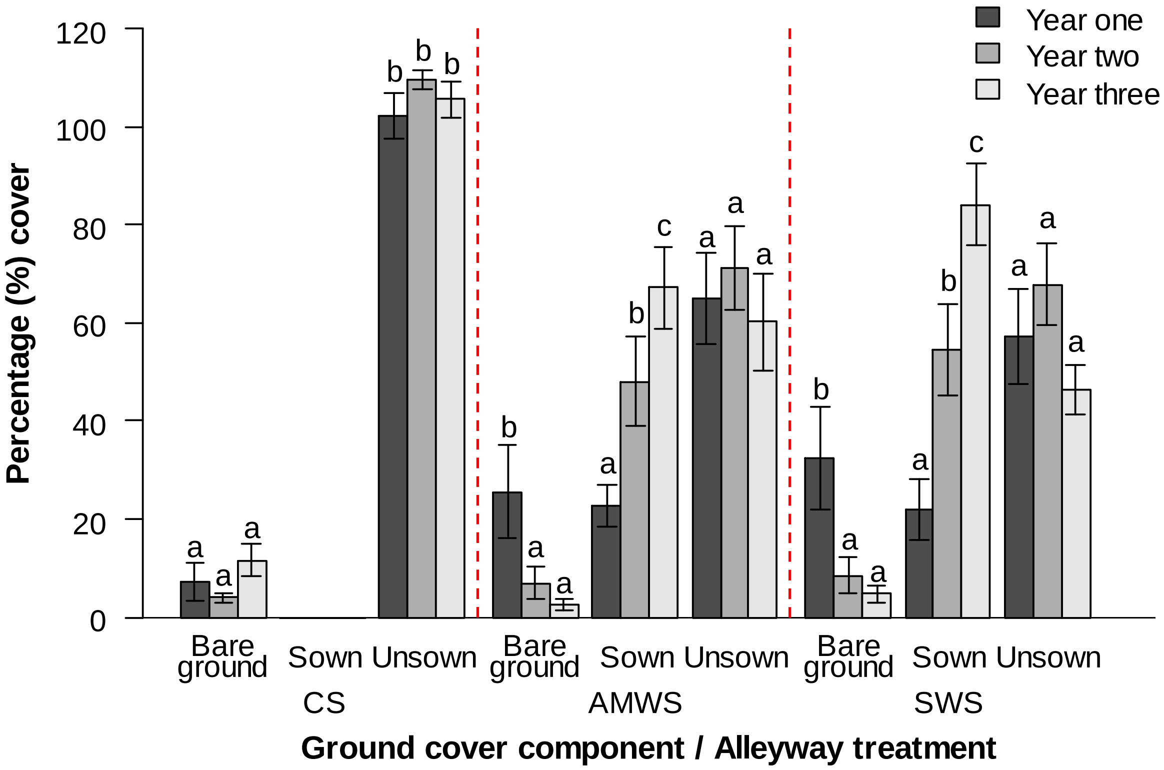

Figure 3.3. Percentage cover (± SE) of ground cover component: bare ground, sown and unsown wildflowers (forbs and grasses) according to alleyway treatment and year. The same superscript letters indicate no significant differences for each ground cover component (bare ground, sown and unsown) according to the Pairwise-Wilcoxon test (*P* > 0.05). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Of the sown species, the grass, *Dactylis glomerata,* had the highest percentage cover each year, with an average of 9.5% (± 2.7) in year one, 17.3% (± 2.9) in year two and 28.3% (± 3.9) in year three (Figure 3.4; Table 3.4), followed by *Leucanthemum vulgare*, *Prunella vulgaris* and *Achillea millefolium* with final values of 10.5% (± 2.0), 9.9% (± 2.2) and 8.5% (± 2.6), respectively. The percentage cover of *Centaurea nigra* was low in year one (0.3% (± 0.2)) but increased in years two and three to 6.4% (± 1.6) and 7.9% (± 1.6), respectively. *Lotus corniculatus* and *Trifolium pratense* had similar percentage covers of ~2.5% throughout the study. This percentage cover was reached by *Silene dioica* in year three, which had lower cover percentages the previous years (~0.4%). *Leontodon hispidus* had poor establishment with average cover values of 0.6% (± 0.2) in year two, and 0.5% (± 0.2) in year three. It was only recorded in 9% and 3.1% of quadrats surveyed, respectively. In contrast, *D. glomerata* had the greatest occurrence percentage, being present in 91.3% of the quadrats surveyed in year three (Table 3.4).

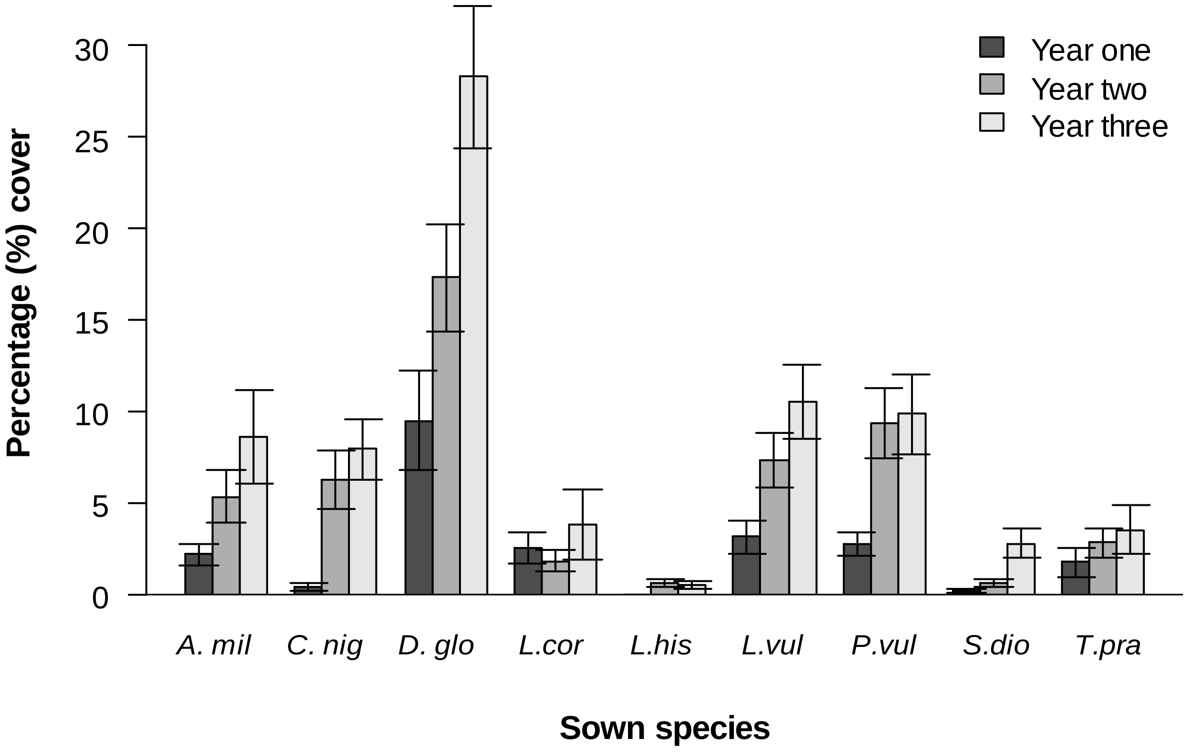

Figure 3.4. Average percentage cover (± SE) of sown wildflowers in both sown strips (Actively Managed Wildflower Strips and Standard Wildflower Strips) according to year*. A. mill* (*Achillea millefolium*), *C. nig* (*Centaurea nigra*), *D. glo* (*Dactylis glomerata*), *L. cor* (*Lotus corniculatus*), *L. his* (*Leontodon hispidus*), *L. vul* (*Leucanthemum vulgare*), *P. vul* (*Prunella vulgaris*), *S. dio* (*Silene dioica*), *T. pra* (*Trifolium pratense*).

Table 3.4. Average percentage cover values (± SE) and percentage occurrence (in 0.5 x 0.5 m quadrats) of the sown species in the Standard Wildflower Strips (SWS) and Actively Managed Wildflower Strips (AMWS) across all surveys for each year, plus the total coverage on sown, unsown species and bare ground.

	Year one				**Year two**				**Year three**			
Sown wildflower	SWS		AMWS		SWS		AMWS		SWS		AMWS	
	% cover	% occur	% cover	% occur	% cover	% occur	% cover	% occur	% cover	% occur	% cover	% occur
Achillea millefolium	1.9 (± 0.7)	34.0	2.4 (± 1.0)	41.0	5.0 (± 1.9)	36.0	5.7 (± 2.4)	34.0	9.6 (± 3.8)	38.8	7.5 (± 3.7)	30.0
Centaurea nigra	0.3 (± 0.2)	7.0	0.4 (± 0.4)	7.0	7.1 (± 2.5)	45.0	5.4 (± 2.1)	37.0	8.8 (± 2.3)	46.3	7.0 (± 2.4)	41.3
Dactylis glomerata	10.6 (± 4.5)	66.0	8.4 (± 3.2)	69.0	19.7 (± 4.3)	77.0	14.9 (± 4.1)	68.0	30.9 (± 4.9)	97.5	25.7 (± 6.3)	85.0
Leontodon hispidus	0.0 (± 0.0)	0.0	0.0 (± 0.0)	0.0	0.7 (± 0.3)	8.0	0.5 (± 0.3)	10.0	0.6 (± 0.4)	2.5	0.4 (± 0.2)	3.8
Leucanthemum vulgare	2.7 (± 1.3)	39.0	3.5 (± 1.2)	44.0	7.5 (± 1.8)	50.0	7.0 (± 2.4)	49.0	11.3 (± 2.9)	53.8	9.8 (± 3.0)	50.0
Lotus corniculatus	2.0 (± 1.0)	31.0	2.9 (± 1.4)	34.0	1.8 (± 0.9)	22.0	1.8 (± 0.8)	19.0	5.7 (± 3.7)	26.3	1.9 (± 0.9)	13.8
Prunella vulgaris	3.1 (± 1.0)	43.0	2.3 (± 0.7)	46.0	9.7 (± 3.1)	60.0	9.0 (± 2.4)	60.0	10.8 (± 3.7)	48.8	8.9 (± 2.5)	45.0
Silene dioica	0.1 (± 0.03)	3.0	0.3 (± 0.1)	7.0	0.2 (± 0.1)	4.0	1.0 (± 0.5)	14.0	1.6 (± 0.9)	10.0	3.9 (± 1.2)	20.0
Trifolium pratense	1.2 (± 0.8)	18.0	2.4 (± 1.4)	30.0	2.8 (± 1.2)	31.0	2.8 (± 1.1)	28.0	4.9 (± 2.6)	25.0	2.0 (± 0.7)	22.5
Total sown species	21.9 (± 6.2)	82.0	22.6 (± 4.3)	91.0	54.5 (± 9.4)	89.0	48.1 (± 9.1)	85.0	84.1 (± 8.2)	100	67.2 (± 8.3)	97.5
Total unsown species	56.8 (± 9.7)	100	64.6 (± 9.3)	100	67.9 (± 8.4)	100	71.2 (± 8.6)	99.0	46.5 (± 5.0)	100	60.3 (± 9.9)	98.8
Bare ground	32.5 (± 10.4)	78.0	25.6 (± 9.7)	73.0	8.5 (± 3.8)	47.0	6.9 (± 3.3)	42.0	4.7 (± 1.8)	33.8	2.4 (± 1.2)	23.8

The most abundant unsown forb species in the wildflower strips were *Trifolium repens*, *Ranunculus repens*, *Taraxacum officinale*, and *Rumex obtusifolius*, which accounted for 8.6% (± 1.5), 8.3% (± 2.1), 5.3% (± 1.0), and 4.7% (± 0.7), respectively. Whilst *Poa trivialis* was the most frequent unsown grass species with 11.6% (± 2.1) cover. The same forb species were also the most frequently recorded in the CS. *Taraxacum officinale* cover was 12.5% (± 2.9), compared to 9.2% (± 3.0) for *R. repens*, 9.2% (± 2.2) for *T. repens* and 3.3% (± 0.7) for *R. obtusifolius*. *Poa trivialis* was the most frequent unsown grass species in all three years (44.4% (± 7.0)).

3.4.3 The influence of alleyway treatment on resource availability

The mean number of floral units recorded per quadrat over years two and three was greater in the AMWS (Tukey test: $Z = 7.22$, $P < 0.001$) and SWS (Tukey test: $Z = 8.74$, $P < 0.001$) compared to CS (Figure 3.5A).

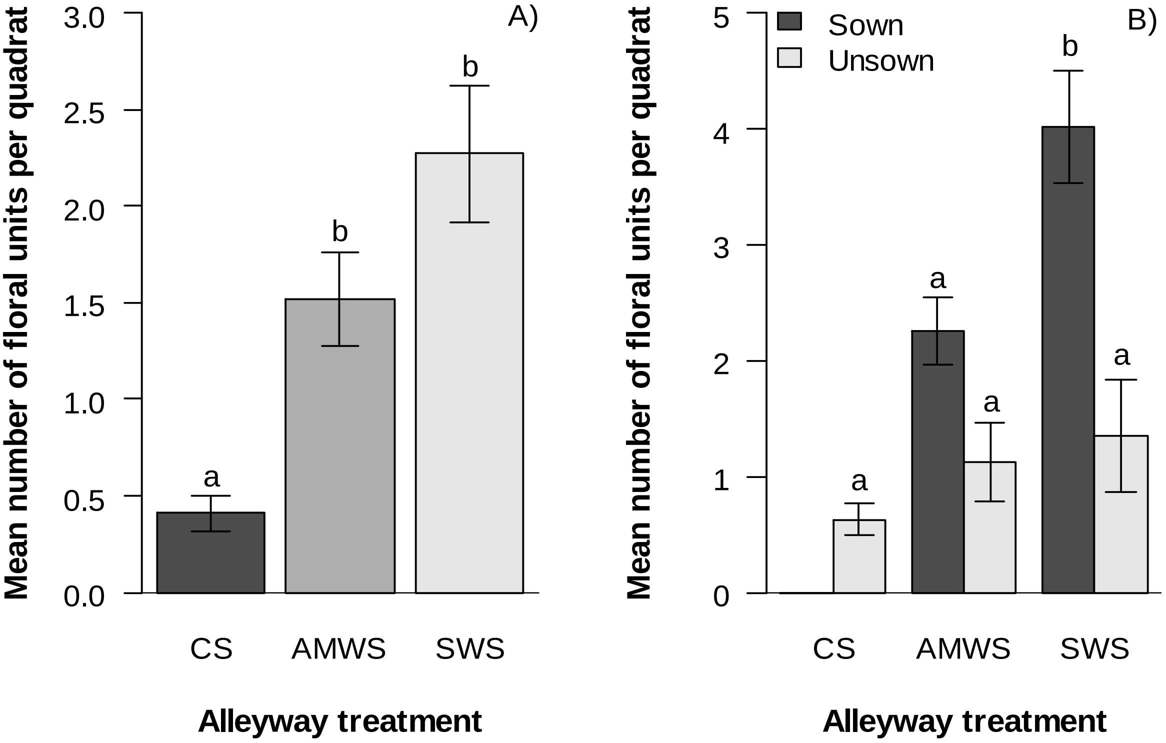

Figure 3.5. Mean number (± SE) of floral units per quadrat according to A) alleyway treatment across all surveys and years, and B) alleyway treatment and sown / unsown species across all surveys and years. The same superscript letters indicate no significant differences according to the Tukey test ($P > 0.05$); in B), for each series (sown and unsown). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

However, the number of floral units of unsown species in the three alleyway treatments did not differ significantly (Figure 3.5B). Floral units associated with SWS increased by 453.7% compared to CS, whilst the increase associated with AMWS was 270.7% compared to CS.

Between wildflower strips, SWS contained more floral units (mean 2.3 (± 0.4)) per quadrat compared to AMWS (1.5 (± 0.2)), but there was not significant difference (GLMER.NB: $Z = 1.70$, $P = 0.09$) (Figure 3.5A). The total number of floral units was consistent between years two and three. However, when unsown species were excluded, SWS contained 4.0 (± 0.5) sown floral units per quadrat compared to 2.3 (± 0.3) in AMWS resulting in significantly higher numbers of floral units in SWS compared to AMWS (GLMER.NB: $Z = 2.83$, $P < 0.01$) (Figure 3.5B). The mean number of sown floral units also varied between years (GLMER.NB: $Z = 4.38$, $P < 0.001$) with more recorded in year three (2.6 (± 0.3)) than in year two (1.5 (± 0.3)).

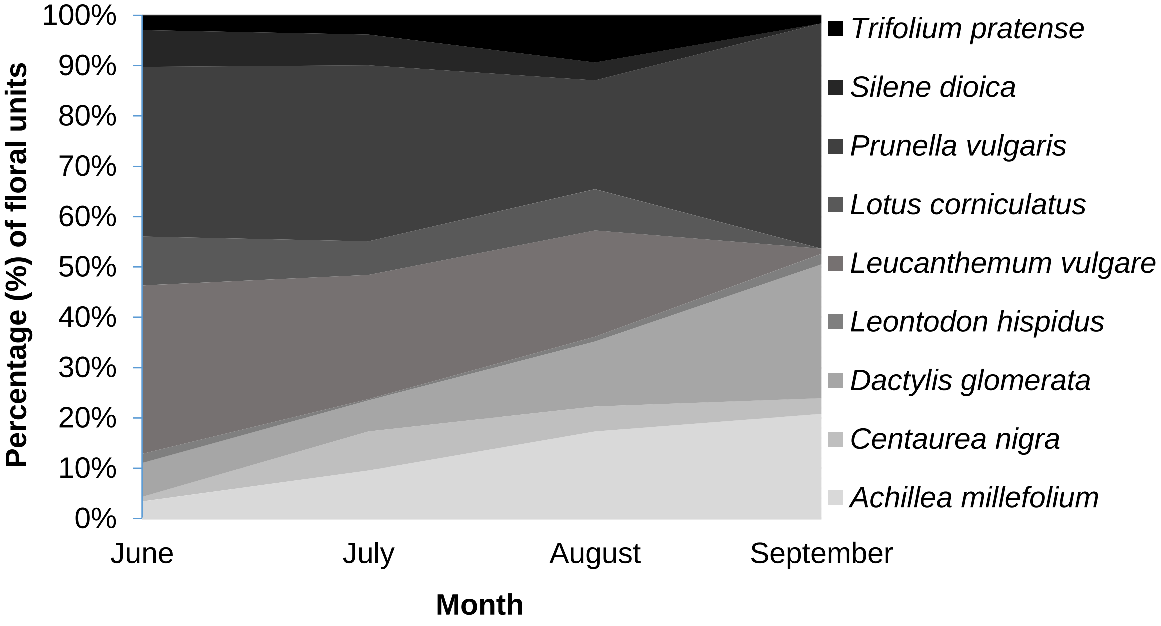

Figure 3.6. Percentage (%) of floral units of sown species recorded per month in both wildflower strips (Actively Managed Wildflower Strips and Standard Wildflower Strips) across all surveys and years.

The resources provided by the sown wildflowers varied throughout the growing season (Figure 3.6). Whilst the mean number of sown floral units varied between the nine species (Figure 3.7) and between years (GLMER.NB: $Z = 4.53$, $P < 0.001$). *Prunella vulgaris* and

L. vulgare had higher numbers of floral units recorded per quadrat. However, the most frequent floral units recorded in the strips were unsown *T. repens* (14.0 (± 4.7) floral units per quadrat) (Appendix 3.1). Overall, in year three, a mean of 5.0 (± 0.7) floral units from sown species per quadrat were recorded compared to 1.6 (± 0.2) in year two.

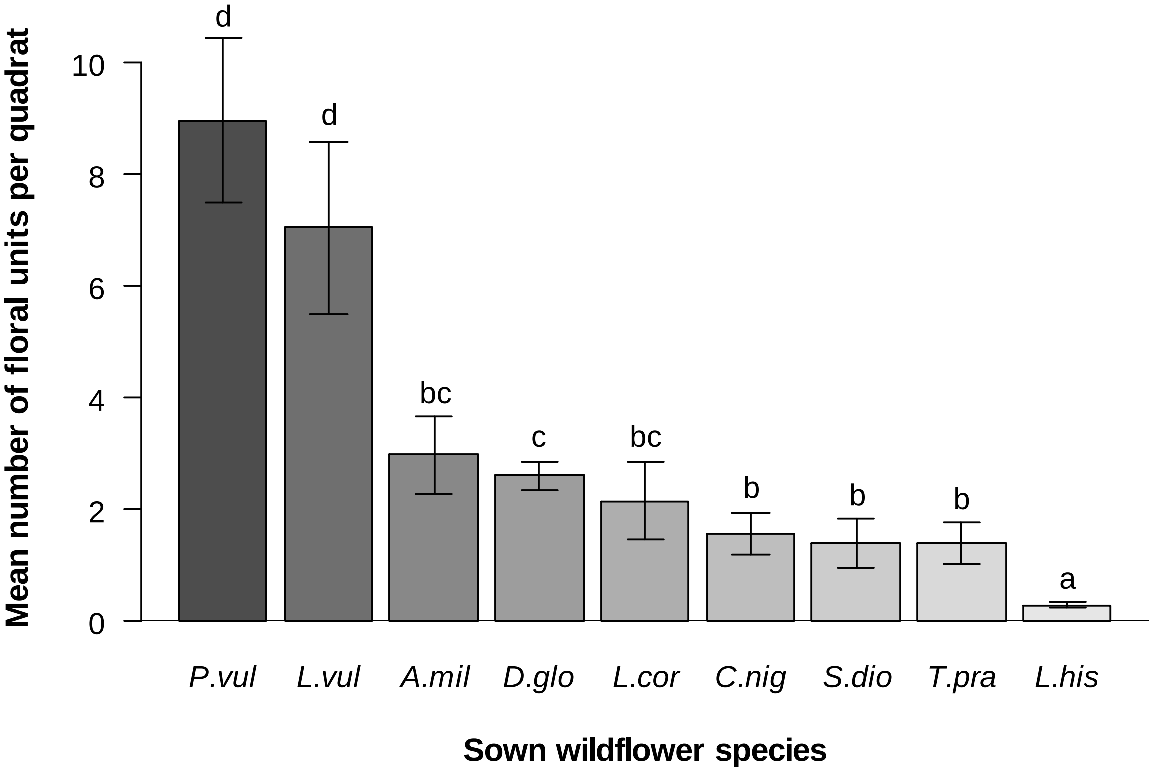

Figure 3.7. Mean number (± SE) of floral units in both wildflower strips (Actively Managed Wildflower Strips and Standard Wildflower Strips) of the nine sown species per quadrat across all surveys and years. The same superscript letters indicate no significant differences according to the Tukey test ($P > 0.05$). *A. mill* (*Achillea millefolium*), *C. nig* (*Centaurea nigra*), *D. glo* (*Dactylis glomerata*), *L. cor* (*Lotus corniculatus*), *L. his* (*Leontodon hispidus*), *L. vul* (*Leucanthemum vulgare*), *P. vul* (*Prunella vulgaris*), *S. dio* (*Silene dioica*), *T. pra* (*Trifolium pratense*).

3.4.4 The influence of alleyway treatment on vegetation height

Vegetation height was affected by alleyway treatment. Vegetation height in SWS, which was uncut until the end of the growing season, was 43.3 cm (± 1.0), 19.0 cm (± 0.8) taller than in AMWS (Tukey test: $Z = 28.31$, $P < 0.001$), which received a regular cutting regime to 20 cm height, and 28.0 cm (± 0.8) than CS (Tukey test: $Z = 51.84$, $P < 0.001$), which received standard management cuttings. AMWS also had higher vegetation than CS (Tukey test: *Z* = 24.41, *P* < 0.001) with a difference of 9.0 cm (± 0.003) (Figure 3.8). Year also affected vegetation height with taller vegetation recorded in year three compared to year two (GLMER.NB: *Z* = 19.58, *P* <0.001).

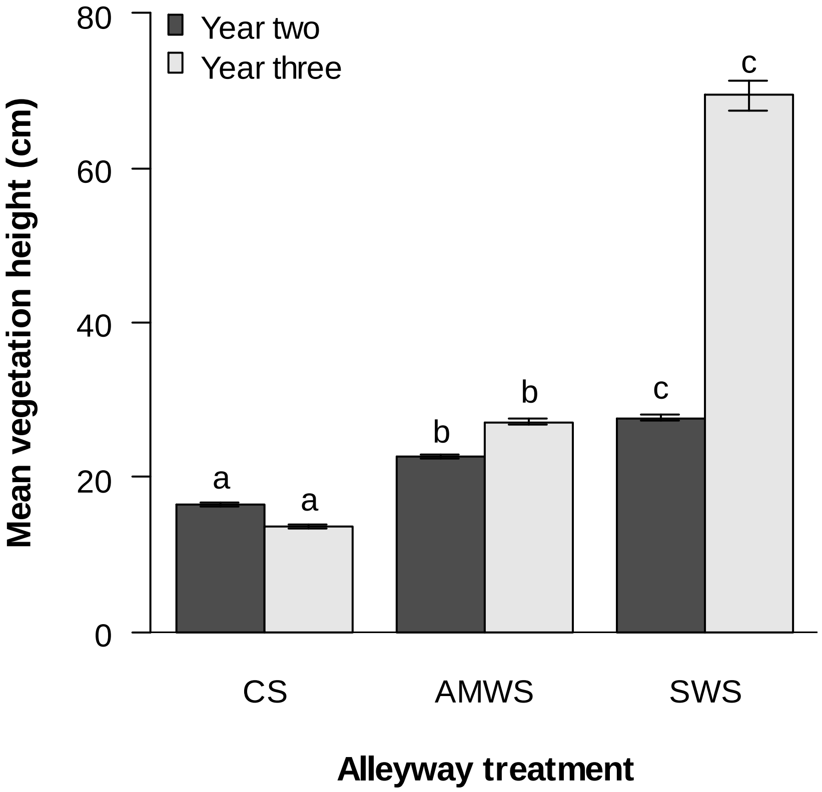

Figure 3.8. Mean height of vegetation (± SE) according to alleyway treatment and year across 20 measurements taken along each alleyway treatment. The same superscript letters indicate no significant differences for each series (year two and year three) according to the Tukey test (*P* > 0.05). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

3.4.5 The influence of alleyway treatment on environmental factors

Humidity was affected by alleyway treatment (Figure 3.9A). CS averaged 76.4% (± 16.0) humidity, 0.4% (± 0.3) higher than AMWS (Tukey test: *Z* = -8.35, *P* < 0.001) and 0.7% (± 0.2) than SWS (Tukey test: *Z* = -13.54, *P* < 0.001). SWS had 0.3% (± 0.1) lower humidity mean than AMWS (Tukey test: *Z* = -5.28, *P* < 0.001). Humidity was not affected by year.

During the coldest hours (between 18:00 and 09:30 hrs), the same trend was recorded (Figure 3.9B); CS was 0.7% (± 0.2) higher compared to AMWS (Tukey test: $Z = -12.36$, $P < 0.001$) and 0.8% (± 0.2) compared to SWS (Tukey test: $Z = -15.40$, $P < 0.001$). Humidity was also higher in AMWS than in SWS by 0.1% (± 0.04) (Tukey test: $Z = -3.10$, $P < 0.01$). However, during the warmest hours (between 10:00 and 17:30 hrs), the humidity in SWS was 0.5% (± 0.02) lower than CS (Tukey test: $Z = -6.97$, $P < 0.001$) and 0.6% (± 0.01) than AMWS (Tukey test: $Z = -5.76$, $P < 0.001$). No difference was recorded between CS and AMWS (Figure 3.9C).

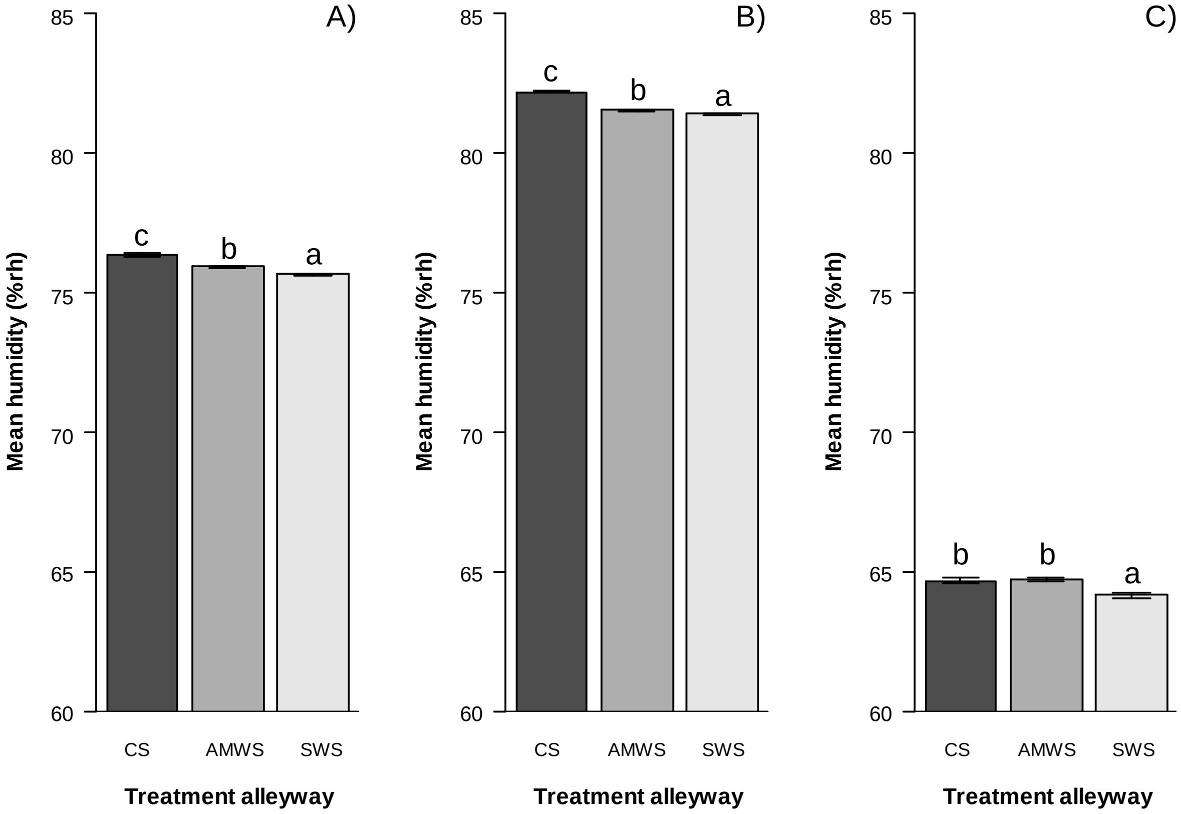

Figure 3.9. Mean ambient orchard humidity (%rh) (± SE) according to alleyway treatment recorded by data loggers with a frequency of 30 min across A) the three years of study, B) between 18:00 and 09:30 hrs, and C) between 10:00 and 17:30 hrs. The same superscript letters indicate no significant differences within each graph according to the Tukey test ($P > 0.05$). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Temperature was also affected by alleyway treatment (Figure 3.10A). The highest temperature was recorded in SWS at 15.3ºC (± 5.6), 0.1ºC (± 0.1) higher than CS (Tukey test: $Z = 4.72$, $P < 0.001$) and AMWS (Tukey test: $Z = 4.97$, $P < 0.001$), whilst it did not differ between AMWS and CS. Temperature was not affected by year. Temperature records between 18:00 and 09:30 hrs (Figure 3.10B) were 0.1ºC (± 0.1) lower in CS compared to AMWS (Tukey test: $Z = 2.76$, $P < 0.05$) and 0.2ºC (± 0.1) than SWS (Tukey test: $Z = 4.53$, $P < 0.001$) but similar between wildflower strips. In contrast, between 10:00 and 17:30 hrs, the lowest temperature recorded was in AMWS at 19.4ºC, 0.2ºC (± 0.1) lower compared to CS (Tukey test: $Z = -3.93$, $P < 0.001$) and 0.2ºC (± 0.2) than SWS (Tukey test: $Z = 7.37$, $P < 0.001$) (Figure 3.10C). There was also a significant difference during these hours between CS and SWS (Tukey test: $Z = 3.43$, $P < 0.01$), being higher in SWS by 0.1ºC (± 0.2).

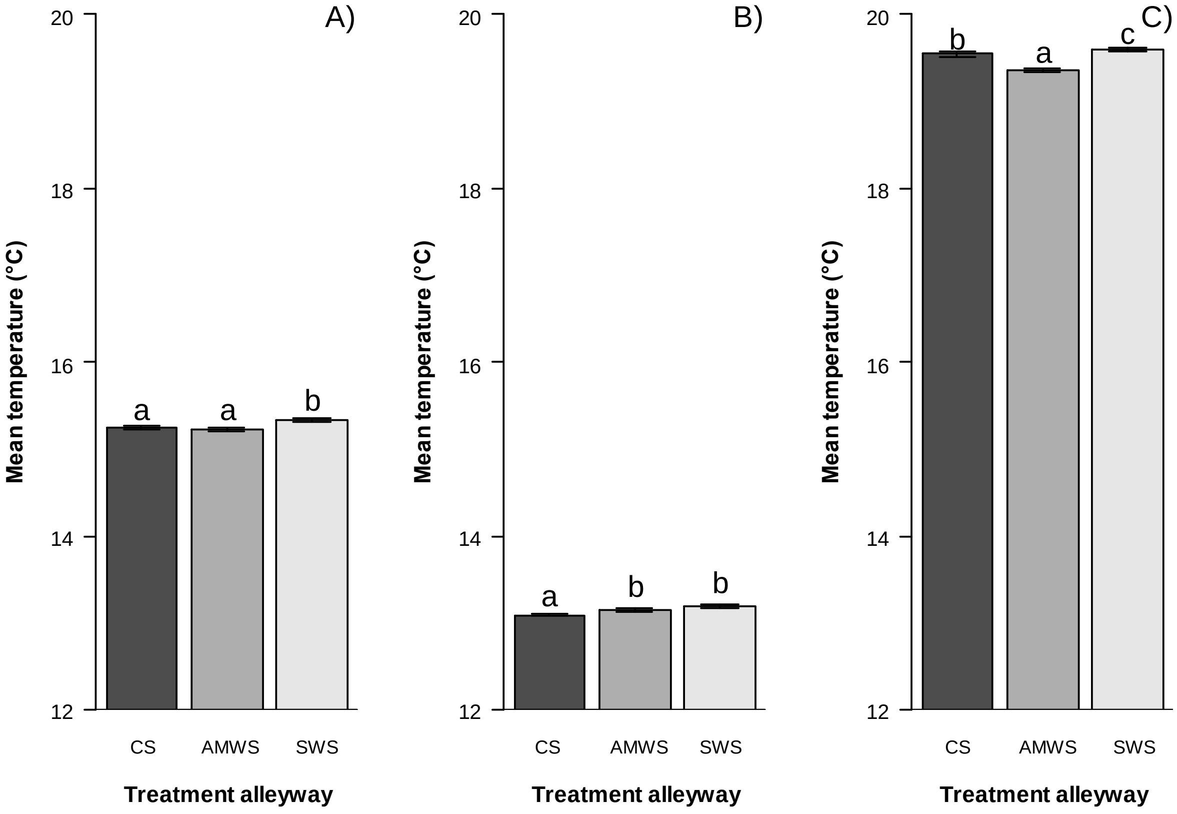

Figure 3.10. A) Mean ambient orchard temperature (ºC) (± SE) according to alleyway treatment recorded by data loggers with a frequency of 30 min across A) the three years of study, B) between 18:00 and 09:30 hrs, and C) between 10:00 and 17:30 hrs. The same superscript letters indicate no significant differences within each graph according to the Tukey test ($P > 0.05$). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

3.4.6 The influence of wildflower interventions on fungal disease incidence

In year two, blossom wilt was only recorded on five cherry blossoms during the blossom period. The percentage cover of fungal disease on cherries was not affected by alleyway treatment or year after seven-day assessments. AMWS (Tukey test: $Z = 0.83$, $P = 0.41$) and SWS (Tukey test: $Z = 0.08$, $P = 0.94$) recorded similar percentages at 1.9% (± 0.3) and 1.7% (± 0.3), respectively, compared to CS 1.7% (± 0.3). Humidity recorded with data loggers into the trays averaged 99.5% (± 0.1), whilst temperature averaged 27.0 ºC (± 0.03).

Up to six species of fungus were recorded. Across both years and the three alleyway treatments, *Monilinia laxa* was the most frequent species with 1.1% of cover followed by *Botrytis cinerea* (0.9%) and *Mucor / Rhizopus* (0.8%). *Penicillium expansum*, *Monilinia fructigena,* and *Cladosporium* spp. were present on 0.4%, 0.3%, and 0.004% of the total percentage cover, respectively.

3.5 Discussion

This study aimed to investigate the establishment and development of wildflower strips under protective covers; how these were affected by different management cutting practices; and whether such practices influenced environmental factors (humidity and temperature) and the subsequent impact of those factors on the incidence of fungal disease on harvested fruit.

3.5.1 The development of the wildflower strips

Assessments of vegetation cover, percentage occurrence, and floral unit counts have demonstrated that establishing and managing wildflower strips under protective covers in modern sweet cherry orchards is possible when compared to other established wildflower habitats (Carvell *et al.,* 2004; Blackmore & Goulson, 2014). In this study, a wildflower habitat was created, which increased pollen and nectar availability and diversity. In turn, this additional resource has potential to support pollinators and natural enemies compared to standard unsown alleyways. However, it is evident that sown wildflower species differed in their performance (establishment and development), and *L. hispidus* was poorly recorded throughout the study as demonstrated in other studies (Pywell *et al.,* 2003; Blackmore & Goulson, 2014). Establishment success is important when designing wildflower mixes and is influenced by a number of factors (Aldrich, 2002), and the persistence and frequency of species would be expected to change throughout the three year study (Blackmore & Goulson, 2014). In this study, the introduction of perennial species increased consistency in the sown wildflower species and reduced variability among unsown species over time. As a result, the variability recorded could have been greater if annual or biennial species were used (Campbell *et al.,* 2017). Of the sown forb species, *A. millefolium*, *C. nigra*, *L. vulgare* and *P. vulgaris*, established most consistently and increased in cover with time and similar findings were reported by Pywell *et al.* (2003). The good development of *Lotus corniculatus*, *T. pratense*, and *S. dioica* by year three increased the pool of resources delivered by sown species.

3.5.2 Resource availability for beneficial arthropods

The greater number of floral units in the wildflower strips compared to controls, irrespective of management, potentially provided a richer semi-natural habitat for beneficial arthropods (Blackmore & Goulson, 2014) (See also Chapters 4 and 5). The greatest number of floral units recorded for *P. vulgaris* and *L. vulgare* in wildflower strips, provided available resources for pollinators and natural enemies; *Prunella vulgaris* is mainly visited by bumblebees (Carvell *et al*., 2004; Blackmore & Goulson, 2014), whilst *L. vulgare* supports both pollinators (e.g. hoverflies and solitary bees) and natural enemies (e.g. parasitic wasps and spiders) (Haaland *et al.,* 2011; Carrié *et al.,* 2012; Wood *et al.,* 2016; Hatt *et al.,* 2017). *Achillea millefolium* had the third greatest number of floral units, and is also important for a range of pollinators and natural enemies (Carrié *et al.,* 2010; Carrié *et al.,* 2012; Wood *et al.,* 2016; Hatt *et al.,* 2017). *Achillea millefolium* and *P. vulgaris* can both reproduce vegetatively enabling them to spread over time once established (Allison, 2002; Macek & Lepš, 2003). This strategy can also increase their persistence in swards (Pywell *et al.,* 2003).

Despite being sown at a lower sowing rate than the forbs (100 vs 200 seeds m^2), *Dactylis glomerata* was the most frequently recorded sown species, in addition to being the sown species with the greatest average percentage cover (28.3% in year three), although this did not result in the greatest number of floral units, particularly in the AMWS. Flowering stems of *D. glomera*, which can grow to 140 cm (Hubbard, 1992), were frequently removed with cutting. However, shelter and pollen to some extent were provided in AMWS whilst in SWS, more flower heads were recorded as these were not cut during mowing. Hoverflies, especially *Platycheirus* spp. and *Melanostoma* spp. (Syrphinae: Bracchini) are often recorded feeding on anemophilous grass species (Inouye *et al.,* 2015). The enhancement of these genera is important in crops since these hoverflies provide multiple ecosystem services, larvae are voraciously aphidophagous (Solomon *et al.,* 2000), whilst adults are effective pollinators (Hodgkiss *et al.,* 2018).

It is evident that the preparation of the alleyways for the establishment of sown species also increased germination of unsown species in the soil seedbank including vegetative spread from stolon fragments. Some of these, including *T. repens, R. repens* and *T. officinale*, also have potential to enhance beneficial arthropods (Altieri *et al.,* 1977; Steinbach & Gottsberger, 1994). *Trifolium repens* and *R. repens* can spread vegetatively (Steinbach & Gottsberger, 1994; Willoughby & McDonald, 1999), whereas *T. officinale* establishes readily from seed and can rapidly take advantage of gaps created in grassland swards (Martinkova *et al.,* 2009). Annual unsown species are also able to respond rapidly to soil disturbance and are usually a legacy of the preceding land use (e.g. arable) (Gentili *et al.,* 2017). However, combined with the presence of sown species, the diversity and abundance of unsown species in the sown strips could provide a greater range of opportunities, for a greater range of beneficial species (Blaauw & Isaacs, 2012, 2014).

3.5.3 The influence of wildflower interventions on bare ground percentage cover

The higher values of bare ground during the establishment year in association with the wildflower strip treatments was expected following preparation of a seed bed prior to sowing (Westbury & Dunnett, 2008). However, with continued development of the sown wildflowers throughout years two and three, cover values of bare ground decreased. The reduction in bare ground was also associated with a reduction in the number and percentage cover of annual unsown species (Gentili *et al.,* 2017). However, unsown annual species were still recorded in year three, indicating that sward disturbance continued in the orchard alleyways, most likely due to farm vehicles and workers during harvest. For the long-term maintenance of the sown wildflower community, ongoing disturbance is important for further recruitment of the sown species from seed (Westbury *et al.,* 2017).

3.5.4 The influence of wildflower interventions on environmental factors and fungus incidence

Both humidity and temperature were affected by alleyway treatments, but differences were small. A difference of <1.0% in humidity between CS and wildflower strips and a mean increase of ~0.2ºC in temperature in SWS when compared to AMWS and CS suggests that tall vegetation in alleyways is unlikely to have significant biological impacts or affect cherry fruit development or cherry quality. This was supported by the absence of effects of alleyway treatments on the incidence of fungal disease on harvested cherries. However, further study is needed to determine whether a larger area of wildflower habitat in an orchard would have the same limited effect (e.g. a third or half of alleyways with wildflower strips).Contrary to expectations, higher humidity was recorded in the conventionally managed alleyways compared to both wildflower treatments. This could be a consequence of soil being less insulated by vegetation in CS and, as a result, heated sooner and released more moisture than the wildflower strips. In addition, higher sward vegetation in the SWS and AMWS could have retained greater values of soil moisture due to competition for water with the cherry trees, although other factors may have led to an increase in humidity in CS. The greater temperature in SWS could have been a result of greater complexity in the sward generating a microclimate (Jones, 2014). Whilst the vegetation in AMWS may have been tall enough to develop this overall, but during the coldest hours the temperature dropped slower that in the CS, which had shorter vegetation. The overall low incidence of fungi on cherries can be explained by the spray programmes applied to the study sweet cherry orchards (see Chapter 2).

3.5.5 Conclusions

This study has demonstrated for the first time that wildflower strips can be successfully established in alleyways under protective covers. Furthermore, the successful establishment and development of the wildflower strips led to a diverse range of sown and unsown floral species providing nectar, pollen and structure for natural enemies and pollinators. Bespoke wildflower strips have the potential to support sustainable production of sweet cherry through enhanced pollination and pest regulation services. In addition, contrary to expectations, the presence of wildflower strips were not associated with increased values of humidity and greater fungal disease incidence, which is of clear importance for the industry. However, further research is needed to confirm whether these findings hold for larger wildflower areas within crops.

3.6 References

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Chapter 4. The impact of wildflower interventions on natural enemies and pest regulation

4.1 Abstract

Sweet cherry production is affected by several pests, which can cause up to 100% crop losses. To protect against pests, Plant Protection Products (PPPs) are frequently used by growers, but some have adverse environmental impacts. However, pests can also be controlled by natural enemies through the delivery of pest regulation services. In turn, this can reduce the reliance of growers on PPPs. Yet, natural enemies are less abundant in intensive agricultural systems with less non-cropped habitat. Wildflower interventions (e.g. wildflower strips), established as part of a Conservation Biological Control strategy, can reinstate habitat in cropped areas and enhance naturally beneficial arthropods. Such an approach could be adopted by growers as part of an Integrated Pest Management (IPM) programme to control cherry pests. The use of wildflower strips to enhance natural enemies and their pest regulation services was investigated in ten protected sweet cherry orchards in the West Midlands (UK). Over a three-year period, the influence of wildflower strips on predators and parasitoid wasps and pest regulation services were examined. Wildflower strips were established in alleyways between rows of cherry trees in commercial sweet cherry orchards under two different alleyway cutting regimes Standard Wildflower Strips (SWS) and Actively Managed Wildflower Strips (AMWS). These were compared to unsown Control Strips (CS).

Vortis suction sampling was done in alleyway vegetation and beat sampling on cherry trees. Araneae (28.2%) and Formicidae (26.8%) were the most frequent natural enemies recorded in alleyways, whilst Anystidae (48.4%) and Araneae (22.9%) were on cherry trees. Both wildflower treatments were associated with a significantly greater abundance of natural enemies (7.7 (± 0.5) natural enemies per sampling in AMWS and 8.3 (± 0.5) in SWS compared to CS, which averaged 4.6 (± 0.3)). The abundance of natural enemies on cherry trees adjacent to AMWS was 3.6 (± 0.2), compared to 3.4 (± 0.1) next to SWS, and 3.1 (± 0.1) adjacent to CS. This was also associated with significantly greater pest control (depletion from baited cards). In the AMWS, on average 32.0% (± 2.4) of aphids were depleted, compared to 28.9% (± 2.5) for SWS, and 24.3% (± 2.5) for CS. No difference was recorded between the wildflower treatments with regards to natural enemy abundance, richness or pest control. These findings were apparent despite growers continuing to use PPPs in their orchards. The use of wildflower strips in sweet cherry

orchards could therefore be of great importance for the sweet cherry industry with IPM programmes, especially in conjunction with reduced PPP inputs enabling natural enemies to control pest further.

4.2 Introduction

Sweet cherry production is affected by a number of arthropod and pathogen pests (Børve *et al.,* 2017; Papadopoulos *et al.,* 2017; Puławska *et al.,* 2017). Some of these directly attack fruit causing economic losses on commercial yields, which may reach up to 100% if not treated (Daniel & Grunder, 2012). Other pests attack leaves and wood, which also has a negative impact on cherry production, causing significant losses (Danelski *et al.,* 2015). As a consequence, to ensure commercial yields, growers rely on Plant Protection Products (PPPs) (Shaw *et al.,* 2019b). However, despite the benefits of PPPs, there are a number of adverse impacts from their use (Geiger *et al.,* 2010; Bonner & Alavanja, 2017), particularly if application rates and the number of applications are neglected (Hillocks, 2012). Hence, legislation may restrict PPP application use (e.g. timing and frequency of applications) to minimize negative impacts (Nienstedt *et al*., 2012). Some PPPs may affect human health (Bonner & Alavanja, 2017), mainly through pesticide residue on fruits (Lozowicka, 2015). As a result, the maximum residue limit is adopted for the active ingredients, which is approved by the European Food Safety Authority (EFSA) in Europe (Medina-Pastor & Triacchini, 2020). In addition, damage to the environment can also occur, including the increased mortality of beneficial arthropods (e.g. natural enemies and pollinators) (Geiger *et al.,* 2010; Bonner & Alavanja, 2017). Arthropod pests can develop pesticide resistance due to the continued use of PPPs (Hajek, 2004). This could result in the use of more hazardous products, some of which are banned (Hillocks, 2012). Hence, for a more sustainable approach to sweet cherry protection, strategies not relying fully on PPPs should be implemented.

Instead of the exclusive and/or intensive use of PPPs to protect crops, Integrated Pest Management (IPM) programmes are recommended (Murray & Jepson, 2018). Practices such as biological, cultural, genetic, mechanical, physical, and non-toxic chemical (e.g. pheromones and lures) controls can reduce pest incidence whilst minimising PPP applications (Papadopoulos *et al.,* 2017). Among them, natural enemies can provide high levels of pest regulation services (biological control), which can reduce pest pressure in crops, and therefore fruit damage by pests (Papadopoulos *et al.,* 2017). Natural enemies include predators, parasitoids, pathogens (e.g. entomopathogenic fungi), and nematodes

(Hajek, 2004). Some of these can be naturally enhanced (with no human introduction) in crops such as predators and parasitoids (Fiedler *et al.,* 2008). A number of predators including Anthocoridae (pirate bugs), Anystidae (whirligig mites), Araneae (spiders), Coccinellidae (ladybirds), Forficulidae (earwigs), Formicidae (ants), Neuroptera (lacewings), Opiliones (harvestmen), and Syrphidae (hoverfly larvae), can control some tree fruit pests (Solomon *et al.,* 2000; Stutz & Entling, 2011). Parasitoid wasps within Hymenoptera can also be effective natural enemies of some pests (Cross *et al.,* 1999; Feraru & Mustată, 2005). All these natural enemies have the potential to control pests in sweet cherry IPM programmes as part of biological control strategies, particularly Conservation Biological Control (CBC). CBC is one of the four biological control strategies (Eilenberg *et al.,* 2001; Hajek, 2004). Classical, inundative and inoculative biological controls are based on the release of natural enemies (see Chapter 1). In contrast, CBC implements different methods to manipulate the environment to support natural enemies on a long term basis (Begg *et al.,* 2016; Holland *et al.,* 2016). Consequently, pest regulation services are deemed more sustainable and can lead to greater control (Holland *et al.,* 2016). A key approach for enhancing natural enemies is to establish suitable habitat adjacent to, or within the cropped area. The aim is to provide refuge and food resources (e.g. alternative prey and pollen) (Begg *et al.,* 2016) to eventually increase the abundance and species richness of natural enemies in the system (Fiedler *et al.,* 2008).

The implementation of CBC strategies is important because of continued declines of natural enemies on a global scale (Wilby & Thomas, 2002; Sánchez-Bayo & Wyckhuys, 2019). Landscape change, and the use of PPPs and fertilizers are among the key drivers (Woodcock *et al.,* 2016; Sánchez-Bayo & Wyckhuys, 2019). Expansion of both agricultural and rural areas, and deforestation are the main causes for landscape change, which subsequently leads to habitat loss and degradation (Foley *et al.,* 2005). Crop monocultures also contributes to the decline of natural enemies (Sánchez-Bayo & Wyckhuys, 2019). Moreover, a greater reliance on pest regulation services through CBC, requires reduced applications of PPPs to enable populations of natural enemies to increase (Woodcock *et al.,* 2016). Broad-spectrum insecticides, such as neonicotinoid, organophosphate, pyrethroid and carbamate insecticides, are the most harmful PPPs to natural enemies and other arthropods (Epstein *et al.,* 2000). In addition, herbicides target non-crop vegetation reducing plant diversity on which natural enemies rely (Sánchez-Bayo & Wyckhuys, 2019). The creation of suitable habitats such as wildflower interventions could help offset habitat loss in agricultural landscapes and support beneficial arthropods (Blake *et al.,* 2012).

The presence of wildflower interventions has been shown to increase the abundance of natural enemies and the associated pest regulation services in apple (Campbell *et al.,* 2017), blueberry (Blaauw & Isaacs, 2015), and wheat (Woodcock *et al.,* 2016; Hatt *et al.,* 2017). Native wildflowers are adapted to local environments and can increase beneficial arthropod abundance, including native arthropods (Isaacs *et al.,* 2009). Perennial wildflowers provide greater consistency in floral resources between years and throughout the year (Isaacs *et al.,* 2009). Consequently, the deployment of native perennial wildflower strips in sweet cherry orchards might be expected to support natural enemies and reduce the incidence of pests in adjacent cherry trees. This would reduce the need for PPPs, reducing pesticide residue on fruit (Lozowicka, 2015), and increasing in production (Poveda *et al.,* 2008). However, some studies have shown that the enhancement of natural enemies through wildflower interventions decreases toward the centre of the cropped area, particularly when patches or strips of wildflowers are created next to field boundaries (Blaauw & Isaacs, 2015; Woodcock *et al.,* 2016). In addition, arthropods are influenced by temperature and other environmental factors (Leather & Watt, 2005), which can have an impact in their predatory/parasitoid activity (Netherer & Schopf, 2010).

The aim of the study was to investigate the role of wildflower strips established in alleyways of protected sweet cherry orchards to enhance the abundance, richness, and diversity of predators and parasitoid wasps in sweet cherry trees. Whether this enhancement led to an increase in pest regulation services was also investigated. Moreover, this study examined the influence of orchard edge, time of day surveyed, and environmental factors on natural enemies, which could affect their ability to deliver pest regulation services.

4.3 Material and methods

4.3.1 Study site and experimental design

The study was carried out in the West Midlands, UK, at three sites in Herefordshire and two in Staffordshire. At each site, two orchard blocks (defined as a separate parcel of land) were investigated. In each orchard block, three alleyways adjacent to rows of the cherry cultivar Kordia were selected to be studied. Two alleyways in each orchard block were randomly selected to receive one of two wildflower treatments, whilst the third was an untreated control, which consisted of the original alleyway vegetation (see Chapter 3). The three alleyway treatments were therefore:

i) **Control Strips (CS)**. Conventionally managed alleyways not sown with wildflowers that were cut regularly to a height of 10 cm from May to September, and then to a height of 8 cm in late September.

ii) **Standard Wildflower Strips (SWS)**. Cut annually in late September to a height of 8 cm.

iii) **Actively Managed Wildflower Strips (AMWS)**. Cut regularly (twice/three times per month) to a height of 20 cm from May to September, and then to a height of 8 cm in late September.

The average width of the alleyways was 2 m and wildflower interventions were established in the central 1 m strip. The strips were 95 m long, beginning at the edge of orchard towards the centre. The length of strips used enabled standardization between orchard blocks, which varied in size from 1.3 to 7.5 ha (see Chapter 2).

To investigate the potential influence of orchard edge effects on the response of the natural enemies to the wildflower interventions (Nguyen & Nansen, 2018), alleyway treatments were divided into five sections for sampling (Figure 4.1).

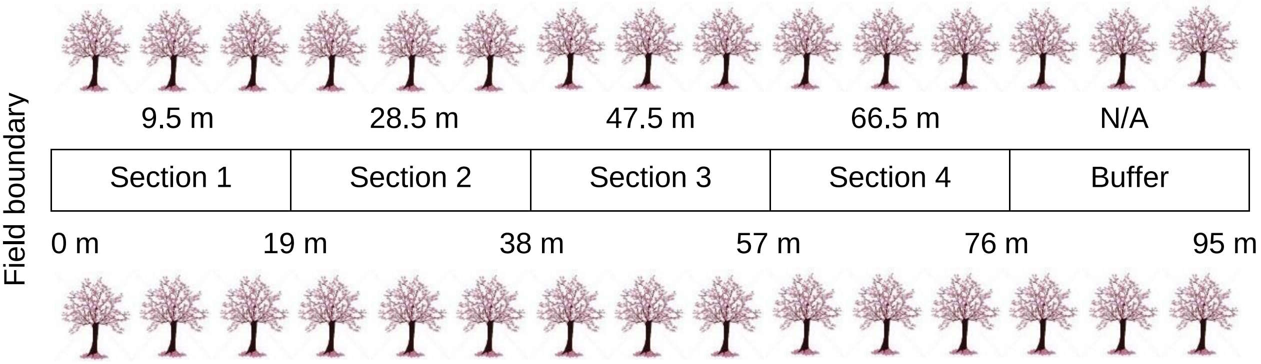


Figure 4.1. Division of the alleyways into four sections for sampling to investigate edge effects, including the buffer (not assessed). Distances of the alleyway sections from the orchard block edge (m); location of the mid-section points and Kordia cherry trees in which assessments in vegetation alleyways and trees were conducted, respectively.

As five out of the 30 alleyways investigated were 95 m in length, the fifth section acted as a buffer to the opposite edge of the orchard block and was not assessed. Moreover, the centre of the orchard blocks in the five 95-m alleyways was at 47.5 m. As a consequence, from the 47.5 m to the opposite end of the orchard block (at 95 m), distance decreased. Hence, half of section 3 and section 4 (from 47.5 to 76 m) were not included in the analysis. Assessments in alleyway vegetation and cherry trees were done in the centre of each of the four sections (Figure 4.1). Consequently, a sample was taken in the mid-point of each section for the vegetation sampling, whilst a sample was taken on the middle tree of each section for the cherry tree sampling. Each alleyway treatment was therefore sampled four times along the alleyway.

Assessments of natural enemies in the alleyways and trees took place over 12 time periods for each alleyway treatment, corresponding with the time of sampling of the day (10:30 - 11:00, 11:00 - 11:30, 11:30 - 12:00, 12:00 - 12:30, 12:30 - 13:00, 13:00 - 13:30, 14:00 - 14:30, 14:30 - 15:00, 15:00 - 15:30, 15:30 - 16:00, 16:00 - 16:30, 16:30 - 17:00). To avoid temporal bias, assessments were alternated between time of day sampled. For example, for the first assessments in the alleyway vegetation in Orchard block 1, CS was assessed between 10:30 - 11:00, SWS between 11:00 - 11:30, and AMWS between 11:30 - 12:00. The second assessment took place at three different time periods in a different day, e.g. SWS between 14:00 - 14:30, CS between 14:30 - 15:00, and AMWS between 15:00 - 15:30. To avoid further sampling bias, sites were grouped according to geographical location. Group one included the sites in Herefordshire (Sites 1, 2 and 3). Group two included the sites in Staffordshire (Sites 4 and 5).

4.3.2 Natural enemy studies in alleyway vegetation

To determine natural enemy abundance and richness in the orchard alleyways, direct search followed by Vortis suction sampling (Brook *et al.,* 2008) was conducted in the middle of each of the four alleyway sections for each alleyway treatment. Direct searches involved recording all natural enemies observed during a two-minute active search over a 0.5 x 0.5 m area. Vortis suction sampling consisted of 15, 10-second suction samples in alleyway sections one to four over the same area. In year one, this was done every two weeks from July to September and once per month in years two and three, from June to September and from May to August, respectively. In order to avoid predation in the sample collections between invertebrates, sample pots were frozen (Rebek *et al.,* 2005).

Subsequently, the pots were emptied over a white tray and the arthropods stored in 70% ethanol.

Ten taxonomic groups were identified: Araneae, Coleoptera, Formicidae, Hemiptera, Lithobiidae, Neuroptera, Opiliones, Panorpidae, parasitoid wasp (Hymenoptera), and Syrphidae. Specimens were identified to family, whilst parasitoid wasps were not identified further, only counted according to order (Hymenoptera). Only the predatory species within each taxonomic group were considered since some species are non-zoophagous (e.g. *Psyllobora vigintiduopunctata,* Coleoptera: Coccinellidae). Additionally, the species with different feeding behaviours on their life cycles were only recorded during the zoophagous stage (e.g. hoverfly larvae).

4.3.3 Natural enemy studies on cherry trees

To investigate the occurrence of natural enemies on cherry trees, two complimentary techniques were used, direct search and beat sampling. The canopy section from the base (~1 m above the ground) to a height of ~2 m was assessed on the side of tree that faced the alleyway treatment. Direct search assessments were carried out on the middle tree of each section for two minutes (Woodcock *et al.,* 2016). Following the direct search, beat sampling was used (Miliczky & Horton, 2005). A 1 m PVC stick was used to tap five different branches on each tree whilst holding a white plastic tray underneath (45 x 35 x 2.5 cm) (Miliczky & Horton, 2005). In year one, both methods were undertaken twice a month every fortnight from May to September and once in October. In years two and three, assessments were conducted once per month; from May to October in year two, and from May to August in year three.

The abundance of nocturnal natural enemies was also recorded with a focus on Forficulidae (earwigs). Earwigs often prey on aphids among other pests (Stutz & Entling, 2011) and therefore may provide an important pest regulation service in sweet cherry orchards. Night assessments were carried out at the end of June in year one, mid-August in year two and beginning of July in year three between 22:00 hrs and 01:00 hrs. Surveys in year two were postponed due to weather conditions (drier year). In year one, direct visual two-minute searches were conducted under torchlight coupled with beat sampling on five branches at different heights (1 to 2 m above the ground) holding a white tray underneath on the middle tree of each section. Due to very few earwigs being recorded in year one, sampling intensity was increased to 30 trees per alleyway in years two and three. Two-minute direct visual searches were performed, followed by beat sampling on two branches at different heights (1 to 2 m above the ground).

Individual arthropods recorded were categorized into 11 taxonomic groups, including nine of the groups considered for the studies in alleyway vegetation (except for Lithobiidae) and including Anystidae, and Forficulidae. Arthropods were identified to family except for parasitoid wasps, which were not identified further. Arthropods not readily identified *in situ* were photographed or collected for further identification in the laboratory.

4.3.4 Environmental factors

The environmental variables of temperature, humidity and wind speed were recorded using a Kestrel weather monitor (Kestrel 3500 weather meter) to investigate their influence on natural enemy abundance and richness. Environmental factors were measured in the middle of each of the four sections for each assessment conducted (direct search and Vortis sampling in the alleyways and direct search and beat sampling on cherry trees). In addition, an extra measurement was taken at the edge of the tunnel prior the start of the assessments. Readings were taken at an approximate height of 1.5 m above the ground.

4.3.5 Pest monitoring

4.3.5.1 Myzus cerasi

The number of *M. cerasi* (cherry blackfly) colonies on Kordia trees along all four sections of the row adjacent to the alleyway treatments were counted once per month from July to October in year one and from April to October in year two. Cumulative counting was done to determine the number of new colonies on each assessment.

4.3.5.2 Drosophila suzukii

Due to potential crop losses caused by *D. suzukii* (spotted winged drosophila) (up to 100% if untreated) (Tochen *et al.,* 2014; Gabarra *et al.,* 2015), growers applied insecticides throughout the study. Consequently, significant fruit damage (larvae in fruit) from *D. suzukii* was not expected. Furthermore, due to their high mobility, differences between alleyway treatments were neither expected. However, the presence of *D. suzukii* was investigated in year one.

Adult *D. suzukii* populations were monitored using Droso-traps® (Vaccari *et al.,* 2015; Rossi-Stacconi *et al.,* 2016), baited with Dros'Attract, purchased from Biobest (www.biobest.co.uk). In May, two traps were set up in each orchard block, irrespective of alleyway treatment. One was located at the orchard (field) boundary, and the other 50 m from the edge into the orchard. Traps were checked every two weeks and the attractant replaced. The number of male and female adult *D. suzukii* in traps was recorded on seven occasions between May and August.

Two sugar floatation tests were also carried out to detect larval presence in fruit (at the end of June and mid-July) (Shaw *et al.,* 2019a). 100 g of ripe cherries were sealed in a 19 x 19 cm polythene bag containing approximately 1-litre sugar solution (20%) enough to cover the quantity of fruit. The fruit was gently crushed to release the larvae into the solution. After 10 minutes, the content was mixed again and left for another 10 minutes. Then, the bags were checked and the number of *D. suzukii* larvae was counted.

4.3.5.3 Tetranychus urticae

To record populations of *T. urticae* (two-spotted spider mite), the ethanol washing method was applied (Harris *et al.,* 2017). In year two, a total of 50 cherry leaves were randomly collected from different heights above the ground (1 m to 2 m) from all Kordia trees adjacent to the alleyway treatments. At each site, a total of 300 leaves were collected once per month from May to September. The leaves were placed into sealed polyethylene bags in the field and transferred to polythene jars containing 70% ethanol. The jars were shaken vigorously to dislodge any mites from the leaves and left to soak for 24 h at room temperature. The samples were sieved though a 200 micron mesh sieve (Endecotts (Test Sieves) Limited), which retained any mites. The jars were refilled, shaken and sieved two more times to ensure that no mites were left in the jar or on the leaves. The sieve was examined under a microscope (ZEISS Stemi 305 Compact Greenough Stereo Microscope) and the number of *T. urticae* was determined (see Harris *et al.,* 2017 for detail). The number of predatory mites was also counted.

4.3.6 Bait cards

Due to the continued use of PPPs by growers in the experimental orchards during the study, interactions between pests and natural enemies could not be readily ascertained. From year two, aphid bait cards were therefore used to measure the predator/scavenger activity of natural enemies (Geiger *et al.*, 2010; Ximenez-Embun *et al.*, 2014; McKerchar *et al*., 2020). The primary aphid pest of sweet cherry is *M. cerasi*, but as this is not commercially available, *Acyrthosiphon pisum* (pea aphid) was used. A culture of *A. pisum* was purchased from Dartfrog (http://www.dartfrog.co.uk). Aphids were reared on *Pisum sativum* (pea plants) in cages (44.5 cm^3) with fine nylon mesh (160 μm mesh) in a laboratory at room temperature.

Experimental trials in year two were carried out to investigate the most suitable glue and type of card to attach the aphids. Three different glues (Vitalbond Cyanoacrylate Odourless Super Glue; Loctite® super glue; and translucent PVA glue, Pritt PVA Craft Glue) and two different types of card (5 x 5 cm red luggage card 120 gsm; and white PVC cards, 760 Micron, CR80) were tested (Geiger *et al*., 2010; McKerchar *et al*., 2020). Three rounds of bait cards were deployed. In round one, odourless super glue and red luggage cards; in round two, Loctite® super glue and red luggage cards; whilst in round three, PVA glue and white PVC cards were used. Because different approaches at different times of the year were used, comparisons could not be made and are not presented. However, PVA glue (Pritt PVA Craft Glue) and white PVC cards (McKerchar *et al*., 2020) (760 Micron, CR80) showed the highest rates of depletion and were used in year three. The three rounds of bait cards were based on the seasonal activity of predators (Ximenez-Embun *et al.,* 2014) and the life cycle of the *M. cerasi* (Wimshurst, 1925), corresponding with April, July, and October. *Myzus cerasi* eggs hatch in spring, typically in April and populations peak in summer between June and July, which corresponds to the aphids flying to secondary hosts, although some aphids remain on the trees. In autumn, around October, females return to the cherry trees to lay their eggs.

In year three, bait card assessments were carried out in eight out of ten orchard blocks (excluding the orchard blocks at Site 4 due to poor establishment of the wildflower treatments). To examine the influence of edge effects on depletion, eight trees were selected adjacent to each alleyway treatment at approximately 5, 14, 24, 33, 43, 52, 62, and 71 m from the orchard edge. Hence, two trees were sampled in each alleyway section. Bait cards were attached to the inner part of the tree with 2 mm wide, black, cable ties at a height of approximately 2 m above the ground (Figure 4.2A). Eight cards (one card per tree) were deployed on the selected trees per alleyway treatment.

Cards were freshly prepared on the day of deployment (around 12:00 hrs). Aphids were frozen before being glued. Ten adults and late stage nymphs (third and fourth) were glued to cards by their rear legs or abdominal sternum (Figure 4.2B). Care was taken to ensure aphids were not covered in glue. Three rounds of bait cards were deployed in the summer (June, July, and August), corresponding with the highest predicted arthropod activity (Bradshaw & Holzapfel, 2010). A total of 192 bait cards were used per round. The number of aphids depleted was determined on each assessment every 24 h for five days (Figure 4.2C). Aphids dried and shrunken and therefore not available to natural enemies were also recorded.

Figure 4.2. A) Bait card deployed at a height of ~ 2 m above the ground (highlighted with a red circle). B) Detail of a bait card (PVC card) with the ten aphids glued (PVA glue). C) Detail of a bait cards where all ten aphids were depleted.

To investigate whether depletion was affected by environmental factors, temperature and humidity were recorded (30-minute intervals) using one data logger (EL-USB-2 dew point, humidity, temperature data logger, EasyLog) deployed at each site. Each data logger was attached to a tree located at ~47.5 m from the orchard block edge in the middle alleyway of the three alleyway treatments (irrespectively of alleyway treatment) at a height of ~1 m above ground. Data loggers were set on the day of bait card deployment until the day of bait card collection for each month.

4.3.7 Spray records

Spray records were provided by growers across the three-year study. Average values of applications and the percentage of PPP type (insecticide, acaricide, bactericide, and fungicide) were calculated. The cumulative toxicity was also calculated to evaluate the level of exposure to PPPs (McKerchar *et al*., 2020). Only insecticides and acaricides were included in this analysis as these PPPs are the most hazardous to beneficial arthropods (Pekár, 2012; Kodandaram *et al.,* 2016). A scale with four classes was used to determine level of toxicity, based on mortality percentage, of the PPP side-effects according to the International Organisation for Biological Control (IOBC) guidelines (Table 4.1). Due to these data are not publicly available, data published by a commercial company were used (Biobest, 2019), consequently, results may be influenced by business interests. The persistence (residual effect on natural enemies) of the PPP was also calculated in days (Biobest, 2019). The natural enemy groups tested were Coleoptera, Hemiptera: Anthocoridae, Mesostigmata: Phytoseiidae (predatory mites), Neuroptera: Chrysopidae, and parasitoid wasps. An average value was calculated for each natural enemy group and for each active ingredient.

Table 4.1. Toxicity on natural enemies according to mortality percentages and assigned to a four-class value according to the International Organisation for Biological Control (IOBC) guidelines (Biobest, 2019).

Class	Toxicity	Mortality
1	Non-toxic	< 25%
2	Slightly toxic	25 – 50%
3	Moderately toxic	51 – 75%
4	Toxic	> 75%

4.3.8 Statistical analysis

For all datasets, the software R (version R-3.6.1) was used (R Core Team, 2019).

4.3.8.1 Abundance of natural enemies

To provide an overall response of natural enemies to alleyway treatments, data obtained from direct search and Vortis sampling in the alleyway treatments, and from direct search and beat sampling of cherry trees were combined for each section surveyed. Natural enemies were analysed all together (all taxonomic groups combined as total natural enemies) and individually (each natural enemy taxonomic group, except Panorpidae due to low individuals recorded). For all cases (total natural enemies and each of the natural enemy taxonomic groups), mean values were calculated for each section surveyed. Hence, to determine the influence of alleyway treatment and distance from the edge across all ten orchard blocks (eight in year three) on total natural enemy abundance, a global generalized linear mixed model with a negative binomial error structure (package lme4, function = GLMER.NB (Bates *et al.,* 2014)) was used. Total natural enemy abundance and the abundance of each of the natural enemy taxonomic group (nine groups on the studies of alleyway vegetation and ten on studies on cherry trees) were the response variable for individual models. Year was expected to influence natural enemy numbers, particularly as year one was a baseline year, when the wildflower treatments were maintained with regular cutting. Therefore, year was nested within alleyway treatment (interaction between alleyway treatment and year), and distance from the orchard block edge and time of day sampled were specified as fixed effects. Orchard blocks nested within sites were the random effects. However, to investigate whether there was a significant interaction between year and alleyway treatment, all the models were re-run with no interaction. All comparisons between models (interaction and no-interaction) were checked to test the relative importance of the interactions determined by the Akaike's Information Criterion (AIC). The model with the lowest AIC was taken as the most parsimonious model (Burnham & Anderson, 2002).

The relative implication of each of the fixed terms in the models were obtained using the AIC. Fixed factors were individually removed from the models and a difference of AIC was calculated (ΔAIC). AIC > 2 was accepted to empirically support significance (Burnham & Anderson, 2002). Tukey's *post-hoc* tests (multcomp package (Hothorn *et al.,* 2008)) were further used for pairwise comparisons between alleyway treatments and years for each model. $P < 0.05$ was considered significantly different.

No statistical tests were performed for night assessments due to only five earwigs being recorded across all sites and years.

4.3.8.2 Family richness and Shannon diversity of natural enemies

The mean family richness (number of families) and values of Shannon diversity based on the natural enemy families recorded were calculated for each alleyway treatment and year. To determine significant differences, generalized linear mixed models (package lme4, function = GLMER, family Poisson (Bates *et al.,* 2014)) were used. The family richness and Shannon diversity were specified as individual response variables in separate models, whilst the fixed and random effects remained the same as previous models. These models were also tested to investigate the possible interaction between alleyway treatment and year. The relative importance of the interactions in the models and pairwise comparisons were performed as above.

4.3.8.3 Environmental factors

Temperature, humidity, and wind speed recorded with Kestrel weather meters were the response variables for independent generalized mixed models (package lme4, function = LMER (Bates *et al.,* 2014)). Alleyway treatment, year, distance from the orchard block edge, and time of day sampled were set as fixed effects. Random effects included orchard blocks nested within sites. Models were also tested for the interaction between alleyway treatment and year. The relative implication of each of the fixed terms in the models were obtained using the AIC. Fixed factors were individually removed from the models and a difference of AIC was calculated (ΔAIC). AIC > 2 was accepted to empirically support significance (Burnham & Anderson, 2002), as in section 4.3.8.1. *Post-hoc* Tukey tests were used to investigate pair-wise differences between fixed factors (multcomp package (Hothorn *et al.,* 2008)). $P < 0.05$ was considered significantly different.

4.3.8.4 Myzus cerasi

The number of *M. cerasi* colonies were cumulatively recorded throughout each season. To analyse the influence of alleyway treatment on natural enemies and their ability to deliver pest regulation services, a generalized linear mixed model with a negative binomial error structure (package lme4, function = GLMER.NB (Bates *et al.,* 2014)) was used. The total number of colonies recorded for each treatment replicate was therefore specified as the response variable, whilst alleyway treatment was specified as a fixed factor. Year and the distance of cherry trees from the edge were included as fixed effects. Random effects were the orchard blocks nested within sites. The relative implication of each of the fixed terms in the model for was obtained using the AIC as above. The interaction between alleyway treatment and year was also studied.

4.3.8.5 Drosophila suzukii

To determine the impact of location (orchard block and field boundary) on abundance of adult *D. suzukii*, a generalized linear mixed model with a negative binomial error structure (package lme4, function = GLMER.NB (Bates *et al.,* 2014)) was used. Both male and female adults were combined and the total *D. suzukii* abundance was the response variable. The fixed effect was trap location whilst orchard blocks nested within sites were the random effects. No statistical tests were performed for *D. suzukii* larval extraction due to only one larva being recorded. The relative implication of each of the fixed terms in the model for was obtained using the AIC as above.

4.3.8.6 Tetranychus urticae

A generalized linear mixed model was used to investigate the effect of alleyway treatment on the number of *T. urticae* (package lme4, function = GLMER, family = Gaussian (Bates *et al.,* 2014)). *Tetranychus urticae* abundance was specified as the response variable and alleyway treatment was set as a fixed factor. Due to the variation in abundance according to the time of the year, month of survey was also considered as a fixed factor. The interaction between alleyway treatment and month was therefore analysed. Orchard blocks nested within sites were specified as random effects. The relative implication of each of the fixed terms in the model for was obtained using the AIC as above. The Tukey's *post-hoc* test (multcomp package (Hothorn *et al.,* 2008)) was further used for pairwise comparisons between alleyway treatments and months surveyed. $P < 0.05$ was considered significantly different. A Spearman's rank correlation test was also used to investigate the association between *T. urticae* and predatory mites.

4.3.8.7 Bait cards

To investigate differences in aphid depletion according to alleyway treatment in year three, a generalized linear mixed effect model with binomial error structure was used (package lme4, function = GLMER, and family = binomial (Bates *et al.,* 2014)). Aphids partially eaten or removed from bait cards were considered depleted. Values of depletion were calculated by subtracting the number of complete aphids remaining on the cards from their initial number. To investigate the influence of alleyway treatment, month surveyed, and distance from the edge, at which the bait cards were deployed, these factors were considered in the model as fixed effects. In addition, the interaction between alleyway treatments and month was also analysed. Orchard blocks nested within sites were set as random effects. The Tukey's *post-hoc* test was used as above.

Linear mixed-effect models (package lme4, function = LMER (Bates *et al.,* 2014)) were used to analyse the humidity and temperature data from data loggers. Humidity and temperature were specified as the response variable in separate models, and month was specified as a fixed effect, whilst sites as random effect for both models. The same models were used to determine differences in the warmest hours of the day, between 10:00 and 17:30 hrs, when diurnal natural enemies are likely to be more active.

4.4 Results

4.4.1 Natural enemy studies in alleyway vegetation

4.4.1.1 Abundance of natural enemies

10,033 arthropods were recorded during the direct search and Vortis sampling over the three-year period (Table 4.2). 11.2% of the arthropods were recorded by direct search whilst 88.8% through Vortis sampling. The most abundant groups were Araneae and Formicidae, followed by Coleoptera and parasitoid wasps. The sum of these four groups accounted for 87.2% of the total natural enemies recorded in the alleyway vegetation.

Three taxonomic groups were single families, in addition to parasitoid wasps, which was classed under a single group. Whilst five groups were non-monotypic. Of those, Araneae was the order that included more families; seven were identified. Linyphiidae had the greatest abundance with 85.1% of total Araneae. Theridiidae accounted for 8.6%, Lycosidae (Figure 4.3A) 4.2%, and Araneidae 1.1%. Clubionidae, Thomisidae, and Tetragnathidae were recorded sparsely, with 0.5%, 0.4%, and 0.1%, respectively. Three Coleoptera families were identified; Staphylinidae 64.1%, Carabidae (Figure 4.3B) 29.0%, and Coccinellidae 6.8%. Hemiptera, Neuroptera, and Opiliones included two families each. Anthocoridae was the highest percentage of Hemiptera (91.0%), whilst Nabidae accounted for 9.0%. Within Neuroptera, Hemerobiidae accounted for 95.3%, compared to only 4.7% for Chrysopidae. The majority of Opiliones recorded belonged to Phalangiidae (99.8%), and only 0.2% were Leiobunidae.

Table 4.2. Number of arthropod natural enemies recorded in the alleyway vegetation through both direct search and Vortis sampling, and on cherry trees through both direct search and beat sampling, and the percentage abundance over the three-year study.

Taxonomic group	**Alleyways**		**Cherry trees**	
	Number of individuals	**Percentage abundance (%)**	**Number of individuals**	**Percentage abundance (%)**
Anystidae	-	-	3948	48.4
Araneae	2827	28.2	1868	22.9
Formicidae	2686	26.8	239	2.9
Coleoptera	1623	16.2	146	1.8
Parasitoid wasps	1615	16.1	374	4.6
Opiliones	446	4.4	736	9.0
Hemiptera	310	3.1	455	5.6
Lithobiidae	256	2.6	-	-
Neuroptera	190	1.9	183	2.2
Syrphidae	77	0.8	197	2.4
Forficulidae	-	-	11	0.1
Panorpidae	3	0.03	6	0.1

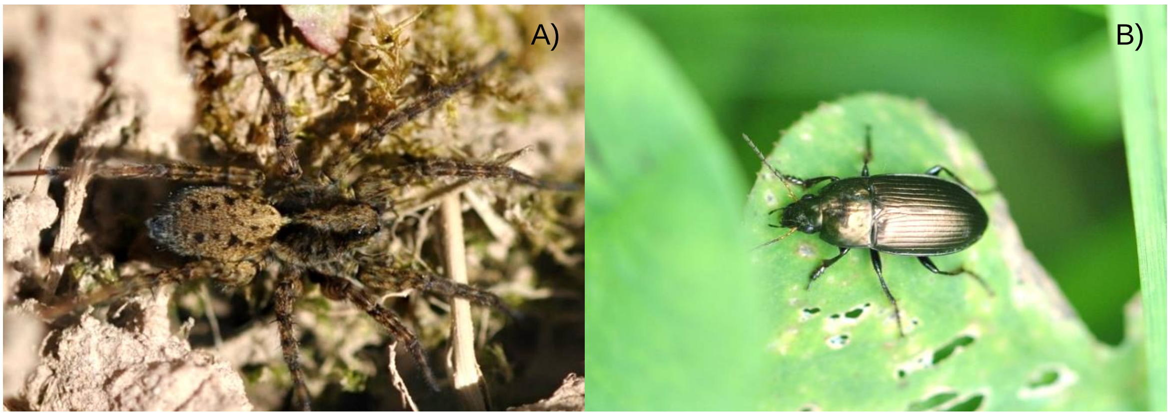


Figure 4.3. A) Lycosidae and B) Carabidae recorded during the natural enemy studies in alleyway vegetation.

4.4.1.2 *The influence of alleyway treatment on natural enemy abundance*

The interaction between alleyway treatment and year was significant, indicating that responses of total natural enemies to alleyway treatment were not consistent between years (Appendix 4.1). In year one, all three alleyway treatments had similar numbers of natural enemies, but both wildflower treatments, Actively Managed Wildflower Strip (AMWS) and Standard Wildflower Strip (SWS), were associated with a greater abundance compared to the Control Strip (CS) from year two (Figure 4.4B). In year three, this difference in abundance was even greater. The *post-hoc* test revealed that total natural enemy abundance was greater in AMWS (Tukey test: $Z = 7.64$, $P < 0.001$) and SWS (Tukey test: $Z = 7.53$, $P < 0.001$) compared to CS but there was no significant difference between wildflower treatments (Figure 4.4A).

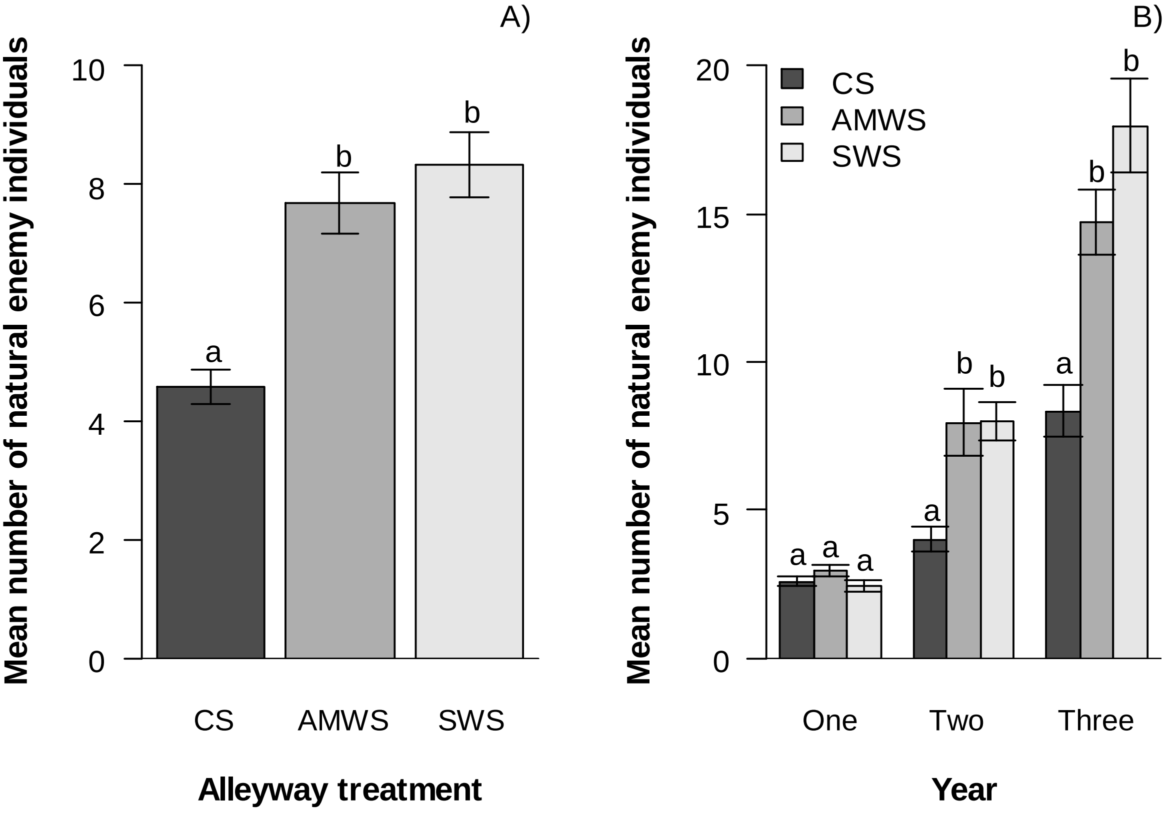


Figure 4.4. Mean numbers (± SE) of total natural enemy abundance per section sampled recorded though direct search and Vortis sampling throughout the three-year study A) according to alleyway treatment, and B) according to year and alleyway treatment. The same superscript letters indicate no significant differences according to the Tukey test ($P > 0.05$); for each category (year) in B). CS (Control Strips). AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Natural enemy abundance in alleyways clearly responded to alleyway treatments throughout the growing seasons (Figure 4.5). In year one, similar numbers of natural enemies were recorded for each alleyway treatment in each month. However, in year two and three, when wildflower management treatments were applied, AMWS and SWS were associated with greater natural enemy abundance compared to CS. This occurred for each monthly survey, and it was more noticeable in year three. In year two, natural enemy abundance peaked in July and decreased towards September. In contrast, in year three, more natural enemies were recorded towards the end of the growing season.

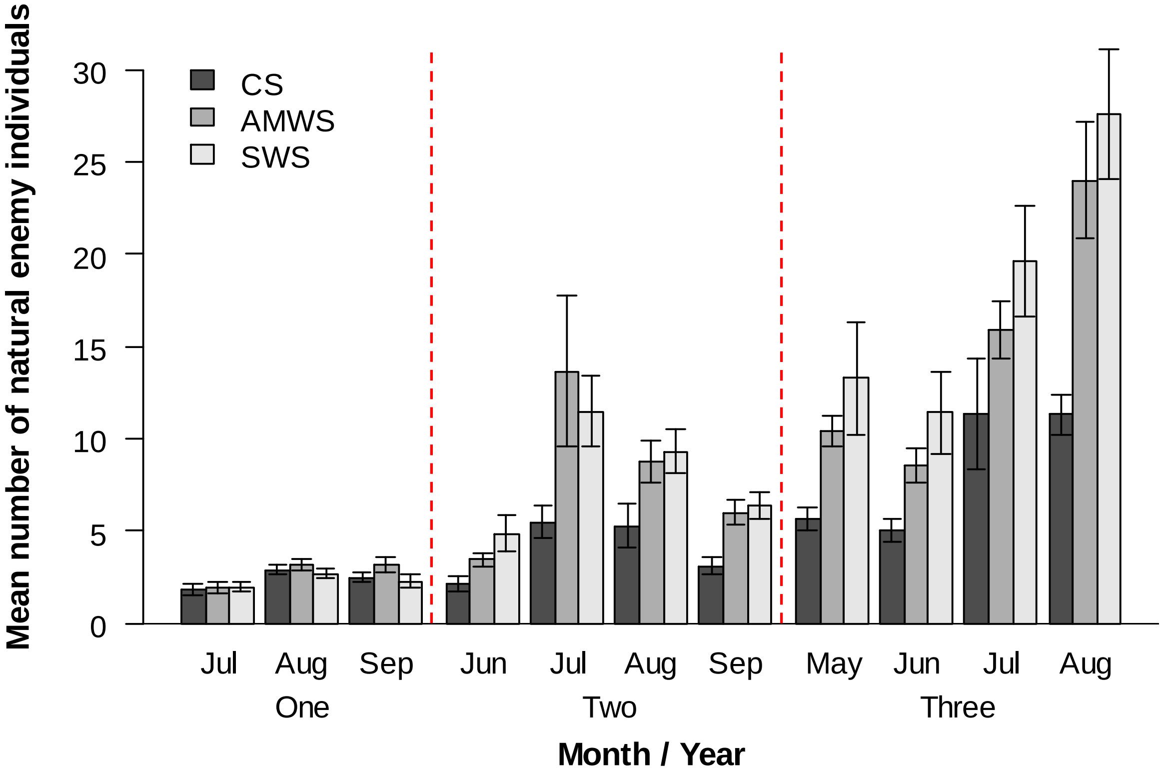
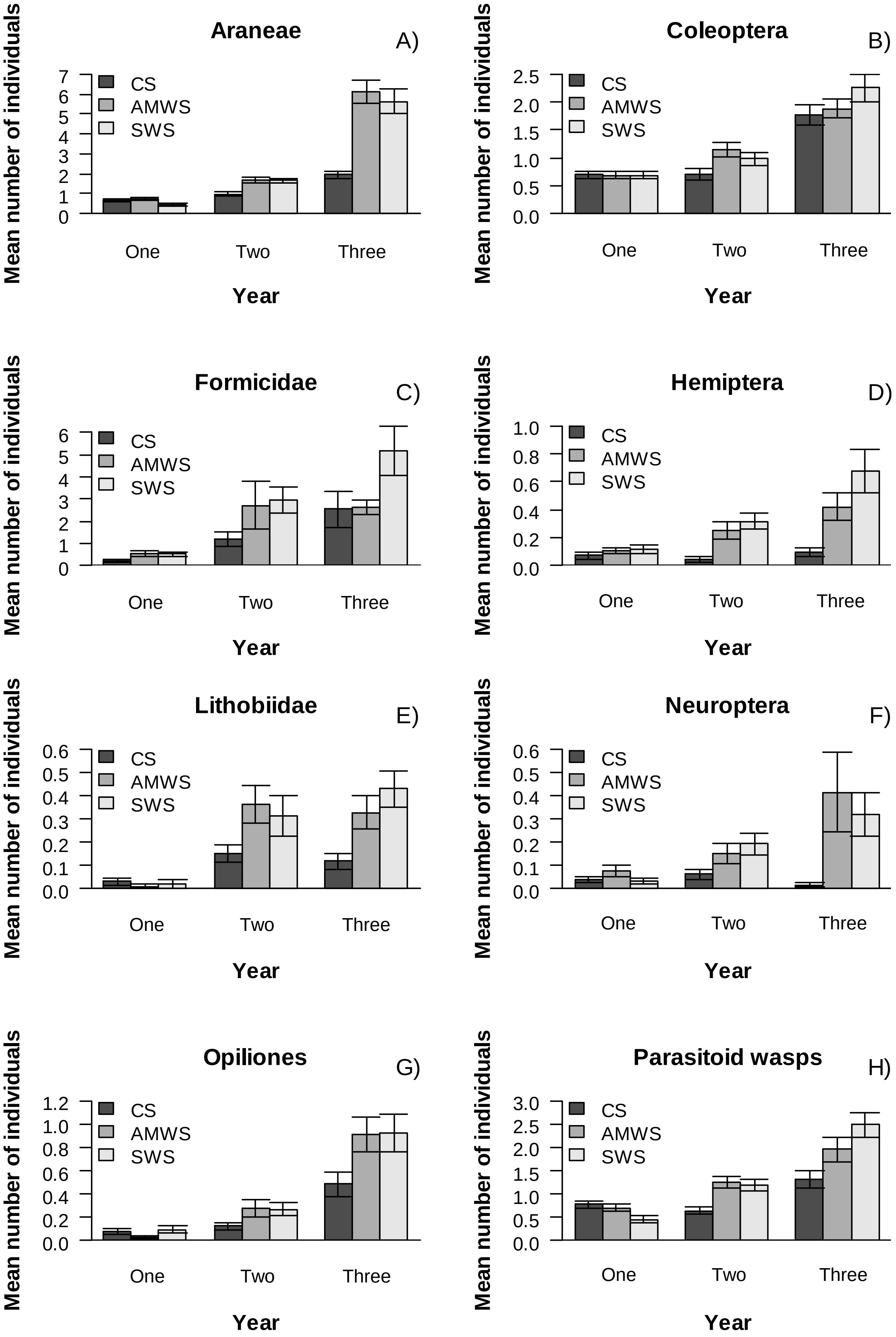


Figure 4.5. Mean numbers (± SE) of natural enemy individuals per section sampled throughout the three-year study recorded though direct search and Vortis sampling according to month, year, and alleyway treatment. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

For all taxonomic groups, except Syrphidae, models including the interaction term between alleyway treatment and year were the most parsimonious (Appendix 4.1). The abundances of Araneae, Formicidae, Hemiptera, Neuroptera, and parasitoid wasps were significantly greater in AMWS and SWS compared to CS in years two and three (Figure 4.6; Table 4.3). No differences between the wildflower treatments were found in these groups except for Formicidae, for which a greater abundance was associated with SWS compared to AMWS. The abundances of Coleoptera, Lithobiidae, Opiliones, and Syrphidae were not affected by alleyway treatment (Figure 4.6; Table 4.3). Only three Panorpidae were recorded and hence no statistical tests were conducted.

Table 4.3. Pair-wise comparisons between alleyway treatments using the *post-hoc* Tukey test for natural enemies, family richness, and Shannon diversity in vegetation alleyways. *P* < 0.05 was accepted to be significantly different. Values in bold are significant. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Factor	**AMWS - CS**	**SWS - CS**	**AMWS - SWS**
Formicidae	Z = 4.14, **P < 0.001**	Z = 6.77, **P < 0.001**	Z = 3.00, **P < 0.01**
Araneae	Z = 7.26, **P < 0.001**	Z = 4.92, **P < 0.001**	Z = -2.24, P = 0.06
Hemiptera	Z = 5.03, **P < 0.001**	Z = 6.30, **P < 0.001**	Z = 1.51, P = 0.29
Neuroptera	Z = 4.48, **P < 0.001**	Z = 3.45, **P < 0.01**	Z = -1.21, P = 0.44
Parasitoid wasps	Z = 3.04, **P < 0.01**	Z = 2.40, **P < 0.05**	Z = -0.60, P = 0.82
Coleoptera	Z = 2.03, P = 0.11	Z = 1.89, P = 0.14	Z = -0.14, P = 0.9
Lithobiidae	Z = 0.57, P = 0.83	Z = 1.73, P = 0.19	Z = 0.90, P = 0.64
Opiliones	Z = 0.37, P = 0.93	Z = 2.24, P = 0.06	Z = 1.66, P = 0.22
Syrphidae	Z = 0.50, P = 0.87	Z = -0.16, P = 0.97	Z = -0.65, P = 0.79
Family richness	Z = 6.45, **P < 0.001**	Z = 5.78, **P < 0.001**	Z = -0.65, P = 0.79
Shannon diversity	Z = 3.41, **P < 0.01**	Z = 2.69, **P < 0.05**	Z = -0.70, P = 0.76


Araneae
A)
Mean number of individuals
7
6
5
4
3
2
1
0
CS
AMWS
SWS
One
Two
Three
Year
Coleoptera
B)
Mean number of individuals
2.5
2.0
1.5
1.0
0.5
0.0
CS
AMWS
SWS
One
Two
Three
Year
Formicidae
C)
Mean number of individuals
6
5
4
3
2
1
0
CS
AMWS
SWS
One
Two
Three
Year
Hemiptera
D)
Mean number of individuals
1.0
0.8
0.6
0.4
0.2
0.0
CS
AMWS
SWS
One
Two
Three
Year
Lithobiidae
E)
Mean number of individuals
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CS
AMWS
SWS
One
Two
Three
Year
Neuroptera
F)
Mean number of individuals
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CS
AMWS
SWS
One
Two
Three
Year
Opiliones
G)
Mean number of individuals
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CS
AMWS
SWS
One
Two
Three
Year
Parasitoid wasps
H)
Mean number of individuals
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CS
AMWS
SWS
One
Two
Three
Year

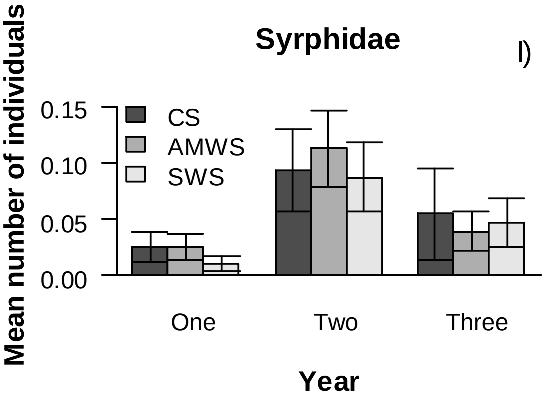


Figure 4.6. Mean numbers (± SE) of natural enemy individuals of A) Araneae, B) Coleoptera, C) Formicidae, D) Hemiptera, E) Lithobiidae, F), Neuroptera G) Opiliones, H) parasitoid wasps, and I) Syrphidae per section sampled according to year and alleyway treatment recorded through direct search and Vortis sampling throughout the three-year study. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

4.4.1.3 The influence of alleyway treatment on family richness and Shannon diversity of natural enemies

Family richness and Shannon diversity were also affected by the interaction between alleyway treatment and year, indicating that these responded differently to alleyway treatments between years (Appendix 4.1).

Table 4.4. Values (± SE) of total arthropod family richness and Shannon diversity per section through direct search and Vortis sampling in alleyway vegetation according to year and alleyway treatment. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Alleyway treatment	**Year one**		**Year two**		**Year three**	
	Family richness	Shannon diversity	Family richness	Shannon diversity	Family richness	Shannon diversity
CS	1.6 (± 0.2)	0.4 (± 0.1)	2.1 (± 0.3)	0.6 (± 0.1)	3.2 (± 0.2)	0.9 (± 0.1)
AMWS	1.7 (± 0.2)	0.4 (± 0.1)	3.1 (± 0.3)	0.9 (± 0.1)	4.5 (± 0.3)	1.2 (± 0.1)
SWS	1.5 (± 0.2)	0.4 (± 0.1)	3.1 (± 0.3)	0.8 (± 0.1)	4.7 (± 0.2)	1.2 (± 0.1)

A greater family richness and higher Shannon diversity were recorded in AMWS and SWS compared to CS in year two and three (Table 4.4), which was supported by the Tukey test (Table 4.3). No difference between wildflower treatments was found.

4.4.1.4 The influence of edge effect on natural enemies

The total abundance, family richness and Shannon diversity of the natural enemies in alleyways was not affected by the distance from the edge (Appendix 4.1). Accordingly, edge effect did not influence any of the taxonomic groups individually (Appendix 4.1).

4.4.1.5 The influence of survey time on natural enemies

Total natural enemy abundance and Shannon diversity were not affected by the time of day sampled (Appendix 4.1). However, the abundance of two individual taxonomic groups, Coleoptera (GLMER.NB: 0.02 ± 0.01, $Z = 2.34$, $P < 0.05$) and Lithobiidae (GLMER.NB: 0.07 ± 0.02, $Z = 2.73$, $P < 0.01$), was affected (Figure 4.7; Appendix 4.1). In both cases, more individuals were recorded towards the end of the survey period. Family richness was also affected by the survey time (Appendix 4.1) with more families being recorded in the afternoon surveys (GLMER: 0.01 ± 0.005, $Z = 2.63$, $P < 0.01$).

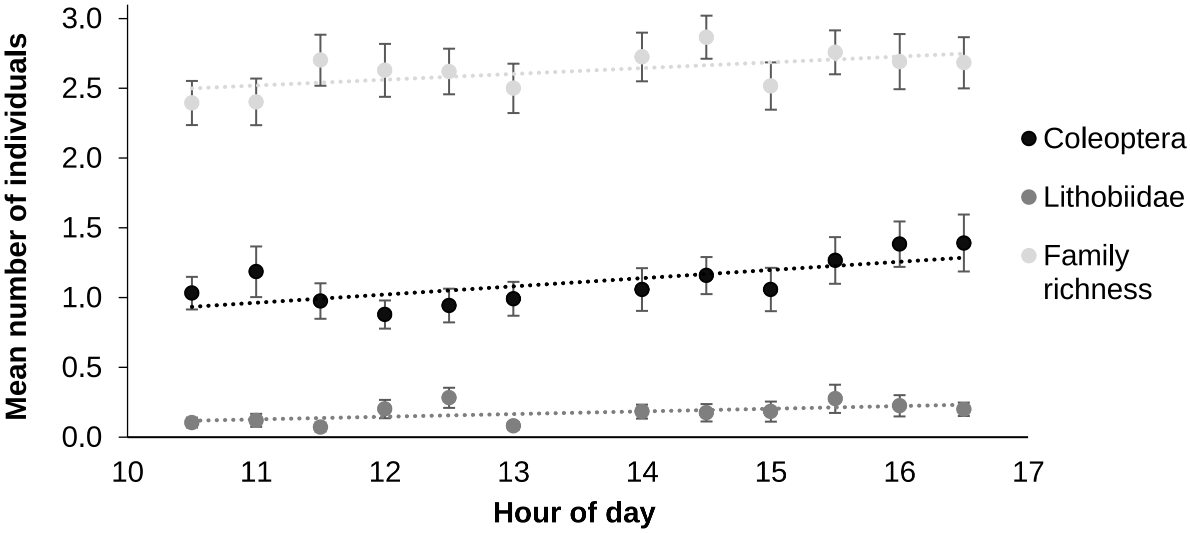


Figure 4.7. Response (± SE) of the Coleoptera, Lithobiidae, and family richness to survey time (hour of day the survey took place) recorded on direct search and Vortis sampling throughout the three-year study.

4.4.2 Natural enemy studies on cherry trees

4.4.2.1 Abundance of natural enemies

A total of 8,163 natural enemy individuals were recorded during direct search and beat sampling of cherry trees over the three-year period (Table 4.2). 7.8% of the arthropods were recorded by direct search whilst 92.2% through beat sampling. The most abundant groups were Anystidae and Araneae followed by Opiliones. Anystidae accounted for almost half of the total records, whilst these three groups summed 80.3% of the total natural enemy records.

Five of the taxonomic groups were single families, along with the group parasitoid wasps (Figure 4.8A) (classed as a single group). Araneae was represented by ten different families. The most frequent being Linyphiidae (35.6%), Theridiidae (33.9%), and Araneidae (22.4%). Together, they composed 92.0% of the total Araneae records. Thomisidae accounted for 4.7%, whilst Philodromidae and Tetragnathidae had 1.7% and 1.0%, respectively. Four families recorded less than 1% of Araneae abundance, including Clubionidae, Dictynidae, Metidae, and Salticidae, which accounted for 0.5%, 0.1%, 0.1%, and 0.1%, respectively. Three families were identified within Coleoptera; Coccinellidae was most frequent with 80.8% of the records, followed by Staphylinidae (17.1%). Carabidae accounted for 2.1%. Two families were identified for each of the three remaining groups. Most of the Hemiptera were Anthocoridae (99.8%) with only 0.2% Nabidae. Chrysopidae (Figure 4.8B) made up the majority of Neuroptera (96.7%) with only 3.3% Hemerobiidae. Likewise, Opiliones abundance was dominated by Phalangiidae (98.9%), with only 1.1% Leiobunidae.

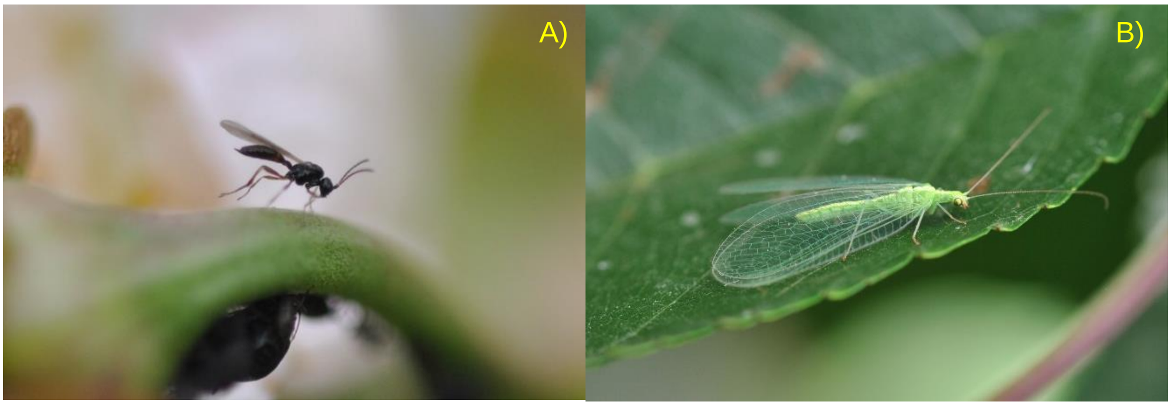


Figure 4.8. A) Parasitoid wasp and B) Chrysopidae recorded during the natural enemy studies on cherry trees.

4.4.2.2 The influence of alleyway treatment on natural enemy abundance

The interaction between alleyway treatment and year influenced total natural enemy abundance on cherry trees across all ten orchard blocks (eight orchard blocks in year three) (Appendix 4.2). Wildflower treatments were associated with a greater number of natural enemies in adjacent cherry trees. Total natural enemy abundance was also greater in years two and three under both wildflower treatments compared to CS (Figure 4.9B). AMWS (Tukey test: $Z = 3.21$, $P < 0.01$) and SWS (Tukey test: $Z = 2.47$, $P < 0.05$) were associated with significantly more natural enemies than CS (Figure 4.9A). The abundance of total natural enemies was similar between AMWS and SWS throughout the three-year study, and no significant differences were recorded.

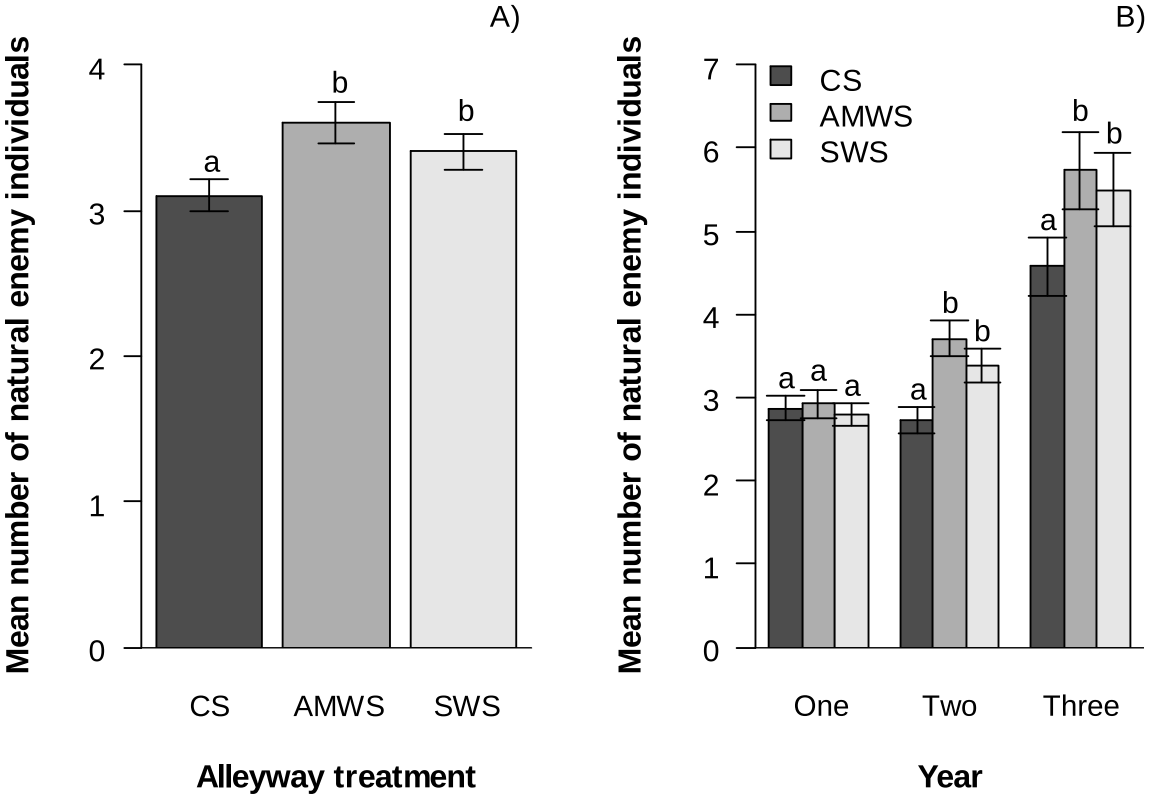


Figure 4.9. Mean numbers (± SE) of total natural enemy abundance per section sampled recorded though direct search and beat sampling throughout the three-year study A) according to alleyway treatment, and B) according to year and alleyway treatment. The same superscript letters indicate no significant differences according to the Tukey test (*P* > 0.05); for each category (year) in B). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Total natural enemy abundance was not consistent throughout the growing seasons (Figure 4.10). In year one, similar natural enemy abundance was recorded in cherry trees for all alleyway treatments, but in year two and three, greater abundance was recorded in AMWS and SWS compared to CS but only during some months. In year two, total natural enemy abundance was consistent between months, except for a drop in July. Whilst in year three, there was a peak in May and abundance decreased towards the end of the growing season.

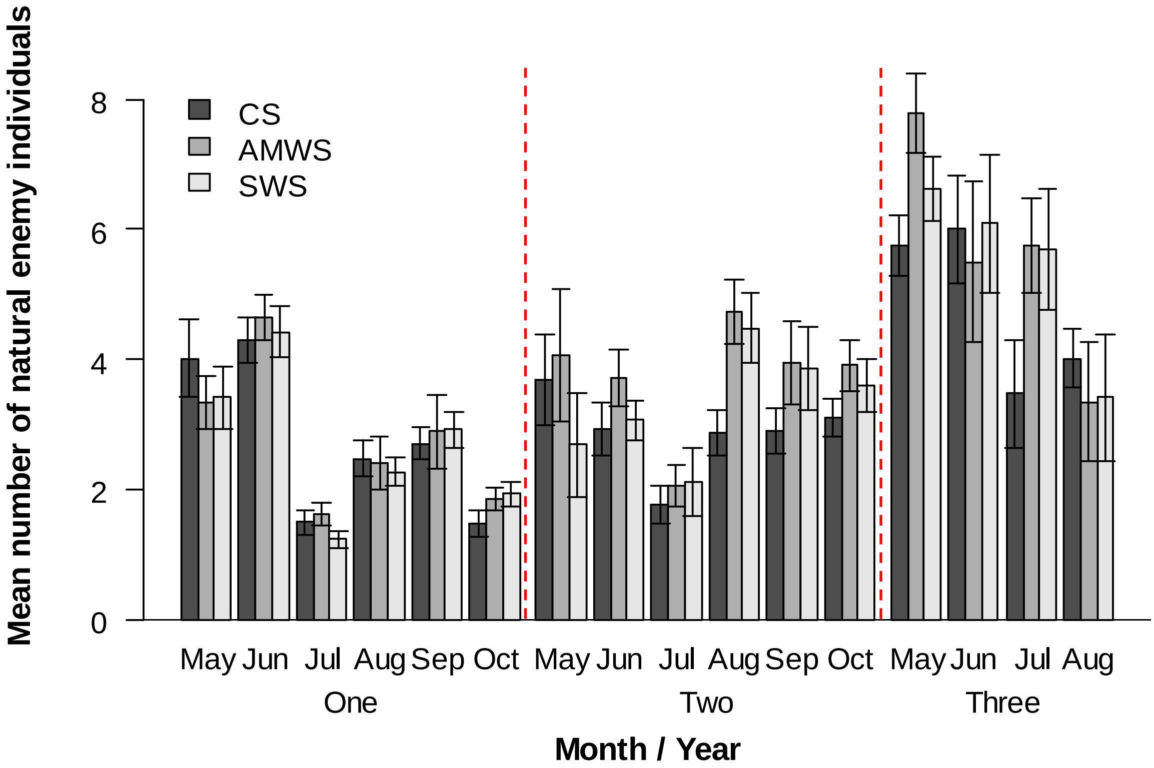


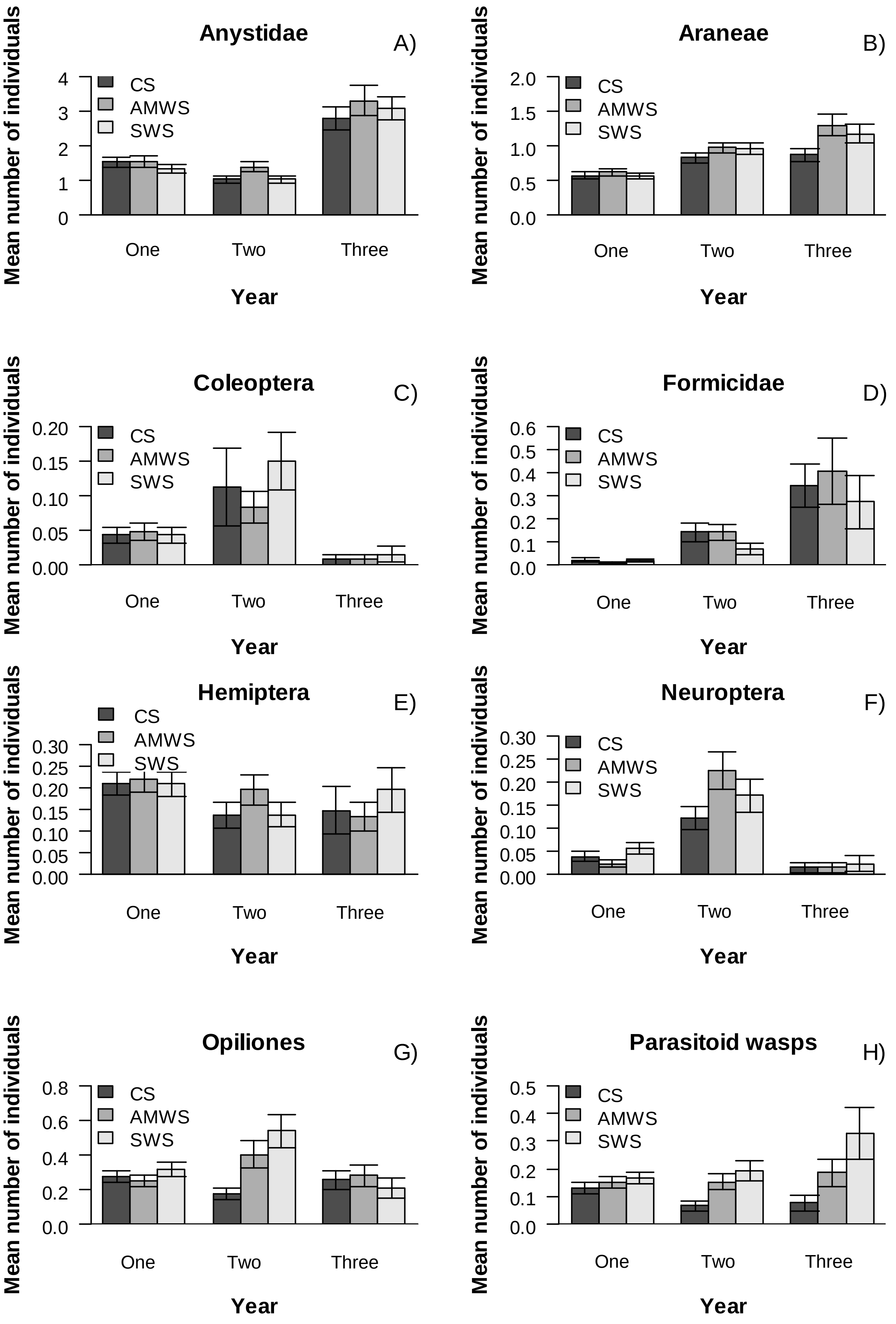
Figure 4.10. Mean numbers (± SE) of total natural enemy individuals per section sampled throughout the three-year study and recorded though direct search and beat sampling according to month, year, and alleyway treatment. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

For only three out of 11 taxonomic groups (Neuroptera, Opiliones, and parasitoid wasps), the most parsimonious model was defined by the interaction between alleyway treatment and year, indicating that the abundance of these groups was affected by alleyway treatment differently between years (Appendix 4.2). In contrast, for Anystidae, Araneae, Coleoptera, Forficulidae, Formicidae, Hemiptera, and Syrphidae, the most parsimonious model did not include this interaction, indicating that responses to alleyway treatment were consistent with time.

Araneae and parasitoid wasp abundance were affected by alleyway treatment. More parasitoid wasps were recorded in cherry trees adjacent to AMWS and SWS compared to CS but no differences were found between trees next to wildflower treatments (Figure 4.11; Table 4.5). However, only in the AMWS significantly more Araneae individuals were recorded compared to CS. Despite a tendency for some natural enemy groups to be more abundant in cherry trees adjacent to the wildflower treatments, no significant differences were found between treatments for Anystidae, Coleoptera, Forficulidae, Formicidae, Hemiptera, Neuroptera, Opiliones, and Syrphidae (Figure 4.11; Table 4.5).

Table 4.5. Pair-wise comparisons between alleyway treatments using the *post-hoc* Tukey test for natural enemies, family richness, and Shannon diversity on cherry trees. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Factor	**AMWS - CS**	**SWS - CS**	**AMWS - SWS**
Parasitoid wasps	Z = 3.24, **P < 0.01**	Z = 4.82, **P < 0.001**	Z = 1.76, P = 0.18
Araneae	Z = 2.38, **P < 0.05**	Z = 1.38, P = 0.35	Z = -1.01, P = 0.57
Anystidae	Z = 0.47, P = 0.89	Z = -0.60, P = 0.82	Z = -1.07, P = 0.53
Coleoptera	Z = -0.31, P = 0.95	Z = 0.84, P = 0.68	Z = 1.16, P = 0.48
Forficulidae	Z = 0.21, P = 0.98	Z = -0.76, P = 0.73	Z = -0.96, P = 0.60
Formicidae	Z = -0.28, P = 0.96	Z = -1.44, P = 0.32	Z = -1.18, P = 0.47
Hemiptera	Z = 0.97, P = 0.56	Z = 0.39, P = 0.92	Z = -0.58, P = 0.83
Neuroptera	Z = 0.01, P = 1.00	Z = 1.01, P = 0.57	Z = 1.00, P = 0.58
Opiliones	Z = 1.75, P = 0.19	Z = 2.15, P = 0.08	Z = 0.43, P = 0.90
Syrphidae	Z = 1.14, P = 0.49	Z = 2.30, P = 0.06	Z = 1.16, P = 0.47
Family richness	Z = 1.94, P = 0.13	Z = 1.73, P = 0.20	Z = -0.21, P = 0.98
Shannon diversity	Z = 0.96, P = 0.61	Z = 0.97, P = 0.60	Z = 0.02, P = 1.00


Anystidae
A)
Araneae
B)
Coleoptera
C)
Formicidae
D)
Hemiptera
E)
Neuroptera
F)
Opiliones
G)
Parasitoid wasps
H)
CS
AMWS
SWS
Mean number of individuals
Year
One
Two
Three
0
1
2
3
4
0.0
0.5
1.0
1.5
2.0
0.00
0.05
0.10
0.15
0.20
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.00
0.05
0.10
0.15
0.20
0.25
0.30
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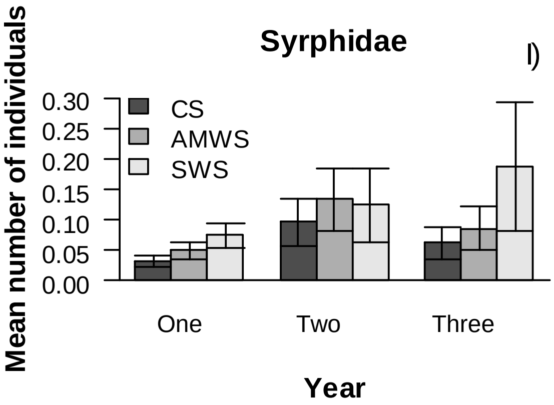

Figure 4.11. Mean numbers (± SE) of natural enemy individuals of A) Anystidae, B) Araneae, C) Coleoptera, D) Formicidae, E) Hemiptera, F) Neuroptera, G) Opiliones, H) parasitoid wasps, and I) Syrphidae per section according to year and alleyway treatment recorded through direct search and beat sampling throughout the three-year study. Forficulidae were excluded due to scarce records. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

4.4.2.3 The influence of alleyway treatment on family richness and Shannon diversity of natural enemies

The most parsimonious model for family richness and Shannon diversity did not include the interaction between alleyway treatment and year (Appendix 4.2). Both AMWS and SWS had greater mean family richness and higher Shannon diversity than CS in years two and three (Table 4.6) but this did not differ significantly (Table 4.5).

Table 4.6. Values (± SE) of total family richness and Shannon diversity per section according to year and alleyway treatment recorded through direct search and beat sampling in cherry trees. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Alleyway treatment	**Year one**		**Year two**		**Year three**	
	Family richness	Shannon diversity	Family richness	Shannon diversity	Family richness	Shannon diversity
CS	1.4 (± 0.1)	0.3 (± 0.0)	1.6 (± 0.2)	0.4 (± 0.1)	2.0 (± 0.2)	0.5 (± 0.1)
AMWS	1.4 (± 0.1)	0.3 (± 0.0)	2.0 (± 0.2)	0.5 (± 0.1)	2.2 (± 0.2)	0.6 (± 0.1)
SWS	1.4 (± 0.1)	0.3 (± 0.0)	1.9 (± 0.2)	0.5 (± 0.1)	2.1 (± 0.2)	0.6 (± 0.1)

4.4.2.4 The influence of edge effect on natural enemies

There was no significant effect of distance from the edge on total natural enemy abundance, family richness, and Shannon diversity (Appendix 4.2). However, specifically, Araneae (GLMER.NB: -0.003 ± 0.001, $Z = -2.22$, $P < 0.05$) and Anystidae (GLMER.NB: 0.01 ± 0.002, $Z = 3.21$, $P < 0.01$) were affected by the distance from the edge (Appendix 4.2). The abundance of Araneae decreased towards the centre of the orchard, in contrast to Anystidae, which increased (Figure 4.12).

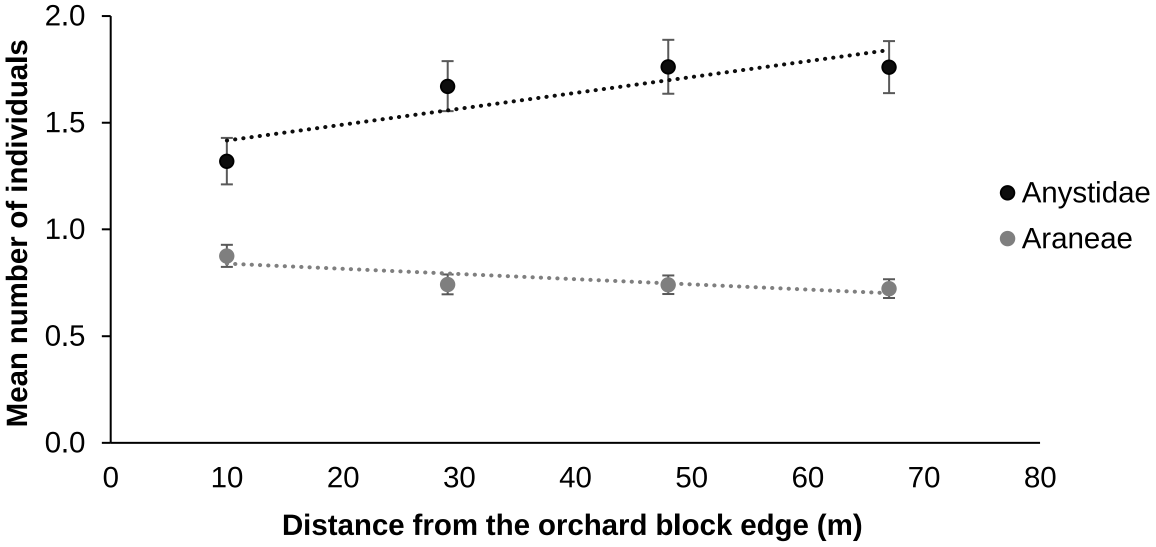

Figure 4.12. Response (± SE) of Anystidae and Araneae to distance from the orchard block edge (m) recorded on direct search and beat sampling in the three-year study.

4.4.2.5 The influence of survey time on natural enemies

The time of day sampled did not significantly affect the total abundance of natural enemies, family richness, and Shannon diversity (Appendix 4.2). However, the time when the surveys took place individually affected some natural enemy groups (Appendix 4.2); Araneae (GLMER.NB: -0.02 ± 0.01, $Z = -2.05$, $P < 0.05$), Neuroptera (GLMER.NB: 0.05 ± 0.02, $Z = 2.17$, $P < 0.05$), parasitoid wasps (GLMER.NB: -0.04 ± 0.02, $Z = -2.44$, $P < 0.05$), and Syrphidae (GLMER.NB: 0.11 ± 0.05, $Z = 2.123$, $P < 0.05$). Syrphidae and Neuroptera were recorded more frequently during afternoon surveys (Figure 4.13). In contrast, parasitoid wasps and Araneae were more frequently recorded in the morning.

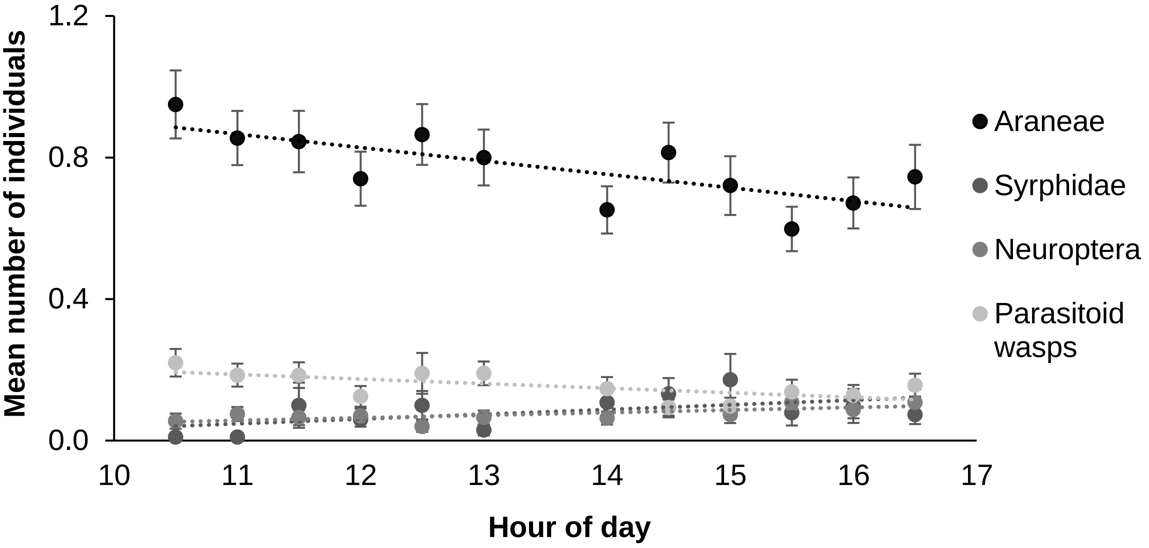

Figure 4.13. Response (± SE) of the Araneae, Syrphidae, Neuroptera, and parasitoid wasps to survey time (hour of day the survey took place) recorded on direct search and beat sampling throughout the three-year study.

4.4.3 Environmental factors

4.4.3.1 The influence of alleyway treatment on environmental factors

All three environmental factors measured were affected by year but not by alleyway treatment (Appendix 4.3).

Table 4.7. Pair-wise comparisons between years for the environmental factors (temperature, humidity, and wind speed) recorded with a Kestrel weather meter during assessments in alleyway vegetation and cherry trees using the *post-hoc* Tukey test. *P* < 0.05 was accepted to be significantly different. Values in bold are significant.

Environmental factor	**Year one – Year two**	**Year one - Year three**	**Year two - Year three**
Temperature	Z = 10.05, **P < 0.001**	Z = 12.00, **P < 0.001**	Z = 3.25, **P < 0.01**
Humidity	Z = 6.01, **P < 0.001**	*Z* = -7.91, **P < 0.001**	Z = -12.05, **P < 0.001**
Wind speed	Z = 7.79, **P < 0.001**	Z = -1.79, P = 0.17	Z = -7.76, **P < 0.001**

Year three was the warmest year with a mean of 22.6ºC (± 0.1), 0.5 ºC (± 0.01) warmer than year two and 1.8 ºC (± 0.02) than year one (Figure 4.14A; Table 4.7). Humidity was greater in year one with 63.6% (± 0.2), compared to 65.6% (± 0.2) in year two, and 60.6% (± 0.4) in year three (Figure 4.14B; Table 4.7). Wind speed in year two had a mean of 0.3 m/s (± 0.01), 0.1 m/s (± 0.01) greater than in years one and three (Figure 4.14C; Table 4.7).

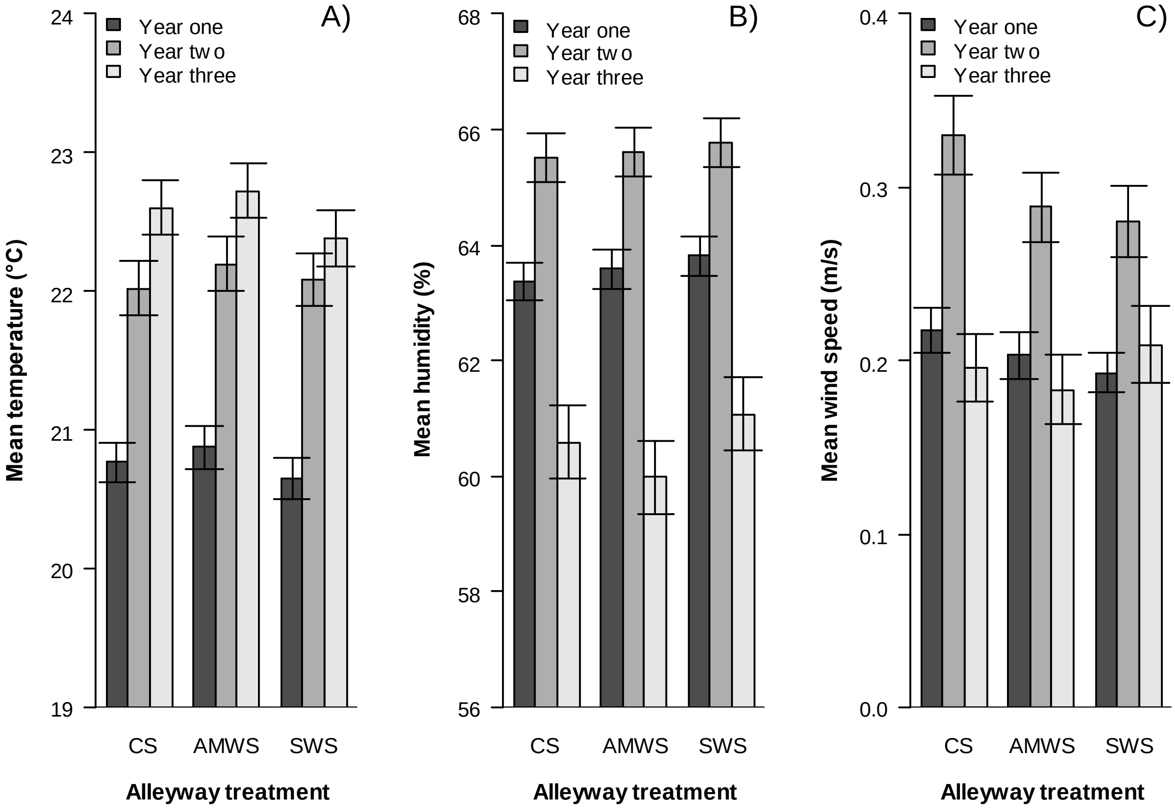

Figure 4.14. Mean (± SE) A) temperature (ºC), B) humidity (%rh), and C) wind speed (m/s) recorded (with a Kestrel weather meter) during assessments in alleyway vegetation and cherry trees according to alleyway treatment and year. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

4.4.3.2 The influence of edge effect on environmental factors

Distance from the orchard block edge affected temperature and wind speed, but not humidity (Appendix 4.3). Temperature increased towards the centre of the orchard (LMER: 0.01 ± 0.002, $T = 4.57$) by approximately 1ºC, contrary to wind speed (LMER: -0.004 ± 0.0002, $T = -18.29$), which was ~0.4 m/s higher at the edge of the orchard blocks.

4.4.3.3 The influence of survey time on environmental factors

Time of day surveyed affected temperature and humidity, but not wind speed (Appendix 4.3). Temperature increased ~1.5ºC in the afternoon assessments (LMER: 0.17 ± 0.03, *T* = 5.61) whilst humidity decreased (LMER: -0.75 ± 0.07, *T* = -10.36) by ~5%.

4.4.4 Pests Monitoring

4.4.4.1 Myzus cerasi

The number of *M. cerasi* colonies was consistent between alleyway treatments throughout the years, and were not affected by alleyway treatment or year (Table 4.8). A similar mean number of colonies was recorded in CS (2.1 (± 0.2)), AMWS (2.1 (± 0.2)), and SWS (1.9 (± 0.2)). However, a negative correlation between distance from the orchard block edge and the number of colonies was recorded (-0.01 ± 0.002, *Z* = -4.47, *P* < 0.001). More colonies were recorded on trees closer to the edge (Figure 4.15).

Table 4.8. Comparisons in the generalized linear mixed model with a negative binomial error structure using Akaike Information Criteria (AIC). Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Myzus cerasi *colonies ~ Alleyway treatment + Year + Distance from the edge + (random: Site/Orchard)*			
Global model		7656.1	0
Alleyway treatment	2	7652.4	-3.7
Year	1	7654.3	-1.8
Distance from the edge	1	7674.1	**18.0**

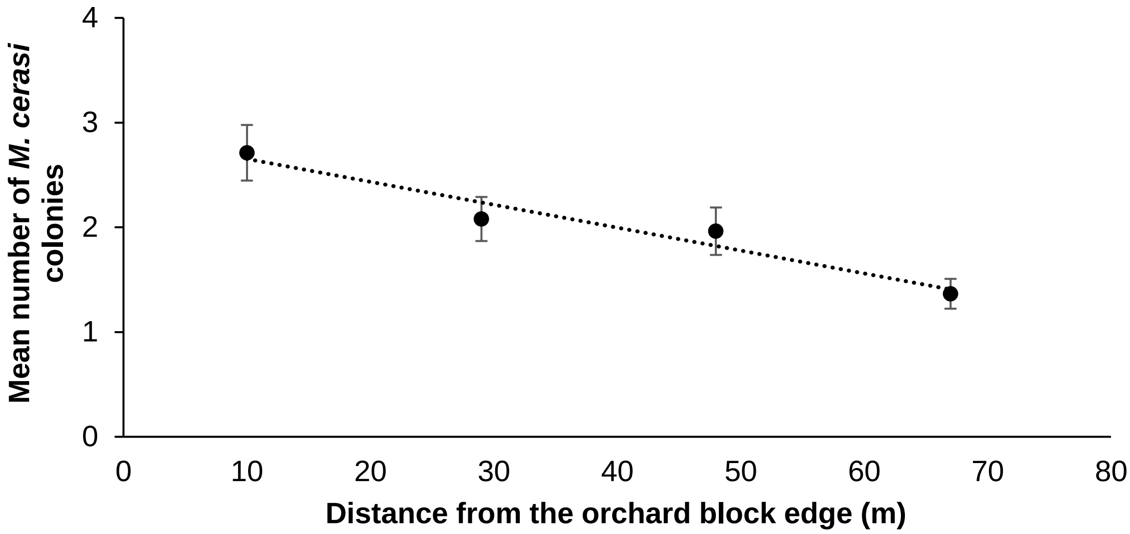

Figure 4.15. Mean number (± SE) of *Myzus cerasi* colonies per section across all five sites recorded in years one and two combined according to distance from the edge (m).

4.4.4.2 Drosophila suzukii

Adults of *D. suzukii* were monitored from May to August in year one. However, none were recorded in the two first months, and were therefore excluded from analysis. There was no significant difference in abundance of *D. suzukii* according to trap location (orchard block and boundary) (Drosophila suzukii *individuals ~ Trap location + (random: Site/Orchard)*; GLMER.NB: *Z* = -0.51, *P* = 0.61), which was very low for both locations throughout the summer. The trap located in the field boundary recorded a mean of 1.7 (± 1.1) *D. suzukii* adults, whilst a mean of 0.9 (± 0.3) was recorded in traps located within the orchard blocks.

4.4.4.3 Tetranychus urticae

The interaction between alleyway treatment and month surveyed was significant for *T. urticae* and predatory mite abundances, indicating that their abundances responded to alleyway treatment inconsistently between months. However, the number of *T. urticae* and predatory mites did not differ significantly between alleyway treatments (Table 4.9). Similar numbers of *T. urticae* were recorded in CS, AMWS, and SWS with 86.48 (± 26.71), 134.90 (± 44.13), and 104.35 (± 28.16), respectively. However, the numbers of *T. urticae* recorded was significantly influenced by survey month (Figure 4.16A). Populations of *T. urticae* peaked in August at 231.87 (± 67.80) mites per 50 leaves and decreased in October.

Table 4.9. Comparisons in the generalized linear mixed models with interaction between alleyway treatment and month surveyed for mean numbers of *Tetranychus urticae* and predatory mites using Akaike Information Criteria (AIC). Interaction between alleyway treatment and month surveyed represented by *Alleyway treatment: Month surveyed*. Models include degrees of freedom, the AIC value and the difference between models (ΔAIC). ΔAIC > 2 was considered significantly different.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
T. urticae			
T. urticae *individuals ~ Alleyway treatment: Month surveyed* + *(random: Site/Orchard)*			
Global model		2479.6	0
Alleyway treatment: Month surveyed	10	2464.0	-15.6
Predatory mites			
Predatory mite individuals ~ Alleyway treatment: Month surveyed + (random: Site/Orchard)			
Global model		1350.2	0
Alleyway treatment: Month surveyed	10	1332.2	-18.0

Similarly, predatory mites had comparable mean values between alleyway treatments at 11.5 (± 1.4) in CS, 13.0 (± 1.7) in AMWS, and 12.8 (± 1.6) in SWS, recording no differences between alleyway treatments (Table 4.9). Population of predatory mites also varied according to survey month (Figure 4.16B). Consistent with *T. urticae* populations, the abundance of predatory mites increased until August and decreased in autumn. This was supported by the Spearman's correlation test ($r = 0.2$, $P < 0.01$).

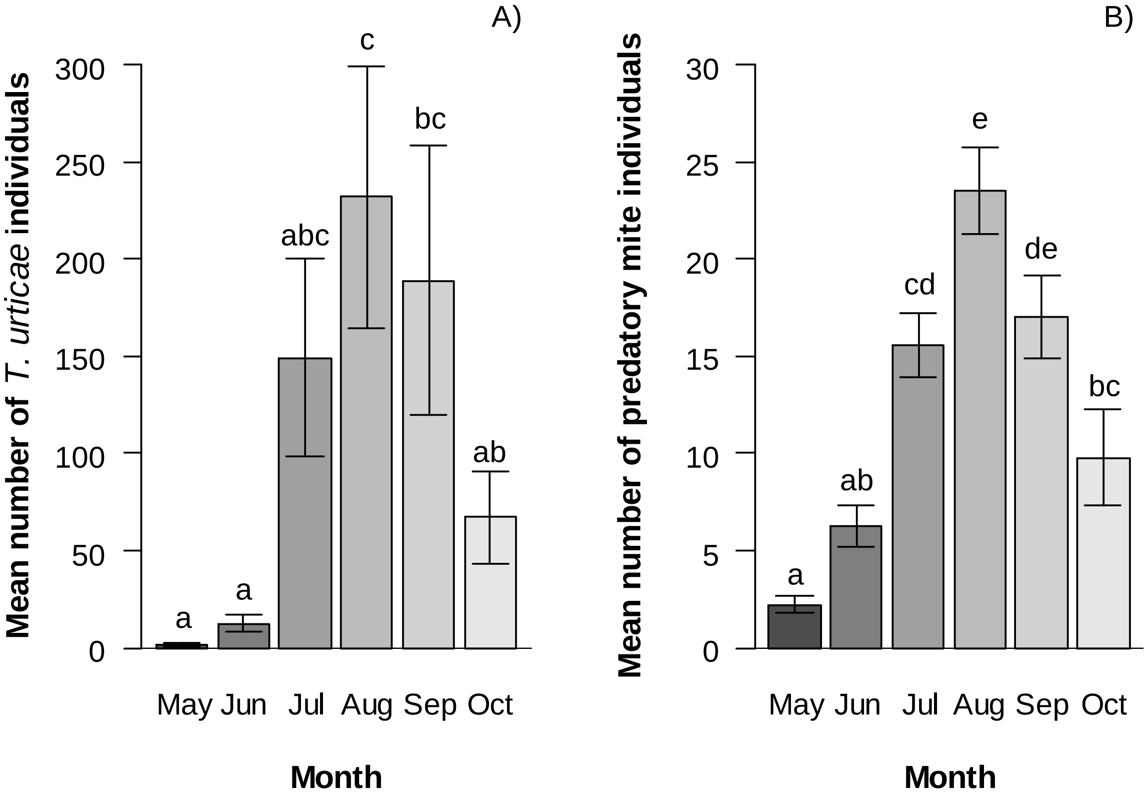

Figure 4.16. Mean number (± SE) of A) *Tetranychus urticae*, and B) predatory mites recorded from 50 leaves collected monthly from May to October in the three alleyway treatments in year two according to month. The same superscript letters indicate no significant differences according to the Tukey test ($P > 0.05$).

4.4.5 Bait cards

4.4.5.1 The influence of alleyway treatment on pest control

The model with an interaction between alleyway treatment and month was the most parsimonious model (lowest AIC), indicating that depletion response in alleyway treatments was inconsistent between months in year three (Table 4.10). Overall, across the three rounds of bait cards deployed, depletion was affected by alleyway treatment. A significantly greater depletion of aphids was recorded from cards deployed on trees adjacent to AMWS (Tukey test: $Z = 5.04$, $P < 0.001$) and SWS (Tukey test: $Z = 3.41$, $P < 0.01$) compared to CS (Figure 4.17), at 32.0 (± 2.4), 28.9 (± 2.5), and 24.3 (± 2.5), respectively. This means an increase of 31.9% in depletion in AMWS and 18.9% in SWS compared to CS.

Table 4.10. Comparisons in the generalized linear mixed model with interaction between alleyway treatment and month surveyed for mean numbers of aphids depleted from bait cards using Akaike Information Criteria (AIC). Interaction between alleyway treatment and month surveyed represented by *Alleyway treatment: Month surveyed*. Model include degrees of freedom, the AIC value and the difference between models (ΔAIC). ΔAIC > 2 was considered significantly different. Values in bold are significant.

Omitted terms in model	**Degrees of freedom**	**AIC**	**ΔAIC**
Number of aphids depleted ~ Alleyway treatment: Month surveyed + Distance from the edge + (random: Site/Orchard)			
Global model		2668.4	0
Alleyway treatment: Month surveyed	4	2693.7	**25.3**
Distance from the edge	1	2668.1	-0.3

However, this finding was not consistent between months and significant differences were found between June and August (Tukey test: $Z = -6.88$, $P < 0.001$) and July and August (Tukey test: $Z = -4.73$, $P < 0.001$) but not between June and July (Tukey test: $Z = -2.23$, $P = 0.07$) (Figure 4.17). A significantly greater number of aphids were depleted in AMWS and SWS compared to CS in June and July but not in August (Figure 4.17; Table 4.11).

Table 4.11. Pair-wise comparisons between alleyway treatments for each month using the *post-hoc* Tukey test for the most parsimonious binomial generalized linear mixed model for depletion on aphid bait cards. $P < 0.05$ was accepted to be significantly different. Values in bold are significant. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Month	**AMWS – CS**	**SWS – CS**	**AMWS – SWS**
June	Z = 5.44, **P < 0.001**	Z = 3.48, **P < 0.01**	Z = -2.07, P = 0.09
July	Z = 3.83, **P < 0.001**	Z = 2.72, **P < 0.05**	Z = -1.14, P = 0.49
August	Z = -1.05, P = 0.55	Z = -0.78, P = 0.72	Z = 0.27, P = 0.96

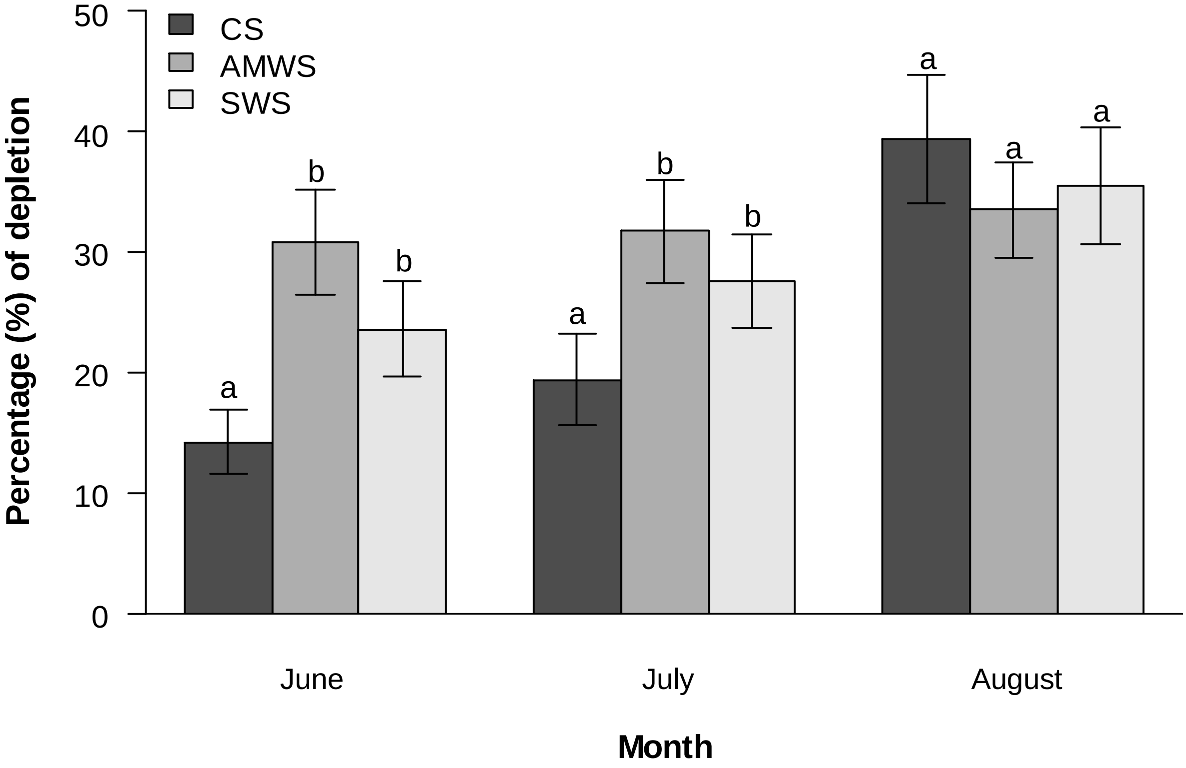

Figure 4.17. Mean percentage (± SE) of *Acyrthosiphon pisum* depleted from bait cards placed on sweet cherry trees according to month and alleyway treatment. The same superscript letters indicate no significant differences for each category (month) according to the Tukey test (*P* > 0.05). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

4.4.5.2 The influence of edge effect on pest control

Distance from the edge did not affect predatory/scavenger activity and similar numbers of aphids were depleted from cards on trees along the alleyways (Table 4.10).

4.4.5.3 Environmental factors

The bait card rounds conducted monthly in June, July, and August were affected by temperature (Figure 4.18A) and humidity (Figure 4.19A). In June, on average, the temperature over the five days the bait cards were out, was approximately 1.5ºC lower than in July and August (Table 4.12). Accordingly, temperature during the warmest hours of the day (mean of the five days) was ~2.5ºC lower in June (Figure 4.18B) compared to

July and August (Table 4.12). There was no difference in temperature between July and August.

Humidity was, also on mean of the five days, 10.2% (± 4.3) lower in July compared to June and 6.0% (± 3.8) compared to August (Table 4.12). Similarly, during the warmest hours (Figure 4.19B), humidity in July was 12.4% (± 0.8) lower than in June, and 5.3% (± 3.5) than in August (Table 4.12). Humidity was also higher in June compared to August by 7.2% (± 2.7).

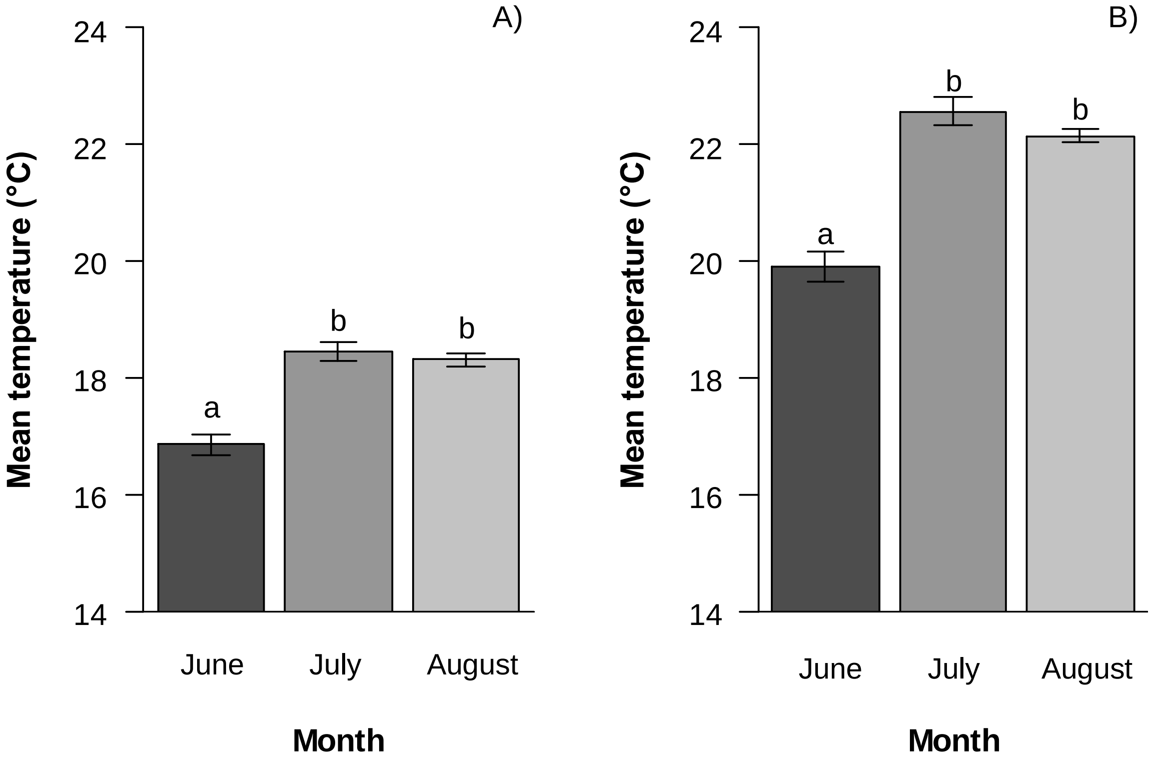

Figure 4.18. Mean (± SE) ambient orchard temperature (ºC) recorded (30-min interval with data loggers) during the aphid bait cards deployment according to the three rounds conducted in June, July, and August across A) the five days, and B) between 10:00 and 17:30 hrs. The same superscript letters indicate no significant differences according to the Tukey test (*P* > 0.05).

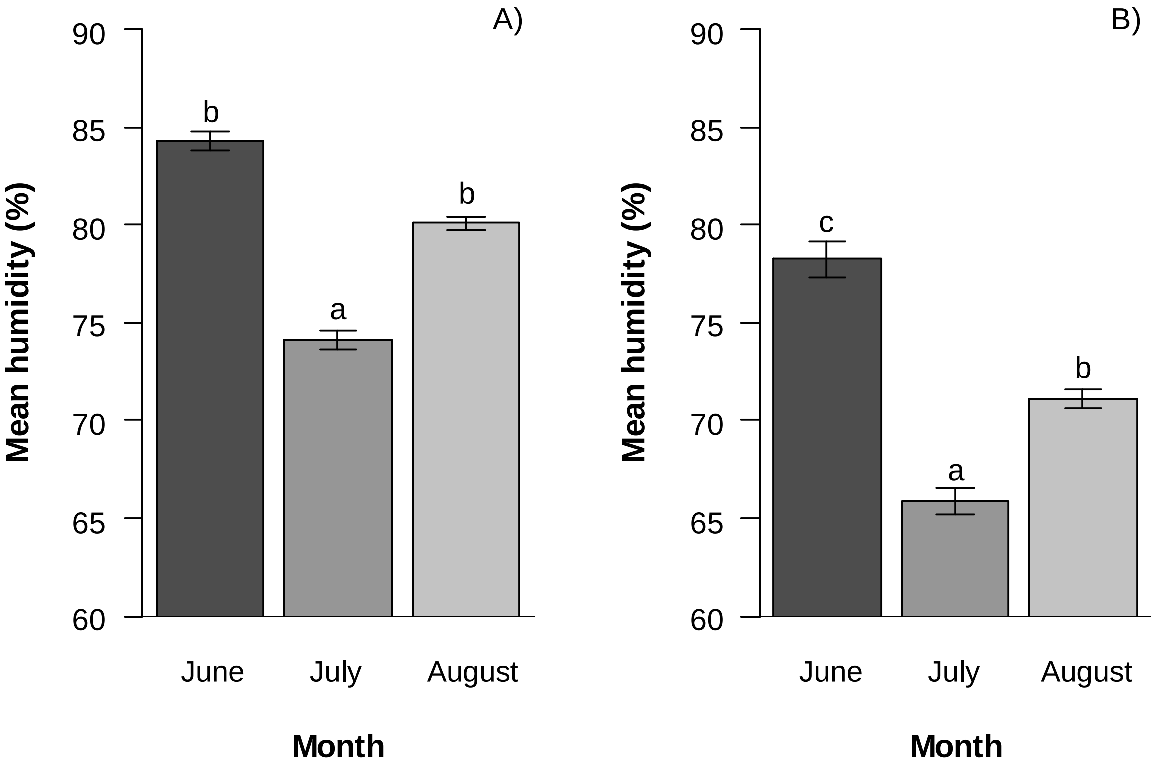

Figure 4.19. Mean (± SE) ambient orchard humidity (%rh) recorded (30-min interval with data loggers) during the aphid bait cards deployment according to the three rounds conducted in June, July, and august across A) the five days, B) between 10:00 and 17:30 hrs. The same superscript letters indicate no significant differences according to the Tukey test (*P* > 0.05).

Table 4.12. Pair-wise comparisons between months for the environmental factors (temperature and humidity) recorded with data loggers (30-minute intervals) during the five days the aphid bait cards were deployed using the *post-hoc* Tukey test. *P* < 0.05 was accepted to be significantly different. Values in bold are significant.

Environmental factor	**June – July**	**June – August**	**July – August**
Total hours			
Temperature	Z = -5.23, **P < 0.001**	Z = -4.58, **P < 0.001**	Z = 0.84, Z = 0.67
Humidity	Z = 10.14, **P < 0.001**	Z = 1.50, P = 0.29	Z = -11.28, **P < 0.001**
Warmest hours			
Temperature	Z = -5.59, **P < 0.001**	Z = -4.24, **P < 0.001**	Z = 1.78, Z = 0.18
Humidity	Z = 8.47, **P < 0.001**	Z = 3.52, **P < 0.01**	Z = -6.48, **P < 0.001**

4.4.6 Spray records

A total of three acaricides and ten insecticides were used against the main cherry arthropod pests over the three-year study. Within acaricides, spirodiclofen accounted for 75% of the total number of applications used to control *T. urticae*. Indoxacarb, spirotetramat, acetamiprid and cyazypyr (19.5%, 15.9%, 14.2% and 14.2%, respectively) were applied to control insect pests. The major targets were *D. suzukii*, Lepidoptera (caterpillars), and *M. cerasi*. The number of applications ranged from three to ten per orchard block and year, with a mean of 5.4 (± 0.4). Spray programmes started in March, prior to the cherry blossom period until July before harvest each year.

Table 4.13. Mean number (± SE) of spray applications of insecticides and acaricides per orchard block and year, percentage of the Plant Protection Product (PPP) type, the mean value of spraying toxicity from laboratory tests (1-4) (Biobest, 2019) according to the International Organisation for Biological Control (IOBC) guidelines, and persistence of the PPP (residual effect on natural enemies).

PPP type	Active ingredient	Mean number of applications	% application PPP type	Toxicity	Persistence (days)
Insecticide	Acetamiprid	0.7 (± 0.1)	14.2	3.1	15
Insecticide	Bifenthrin	0.3 (± 0.2)	5.3	4.0	56
Insecticide	Cyazypyr	0.7 (± 0.2)	14.2	-	-
Insecticide	Indoxacarb	0.9 (± 0.1)	19.5	1.3	21
Insecticide	Lambda-cyhalothrin	0.04 (± 0.04)	0.9	4.6	56
Insecticide	Pirimicarb	0.1 (± 0.1)	1.8	2.0	5
Insecticide	Pyrethrin	0.2 (± 0.1)	3.5	3.2	3
Insecticide	Spinosad	0.6 (± 0.1)	12.4	2.0	4
Insecticide	Spirotetramat	0.8 (± 0.2)	15.9	1.3	14
Insecticide	Thiacloprid	0.6 (± 0.1)	12.4	2.8	11
Acaricide	Etoxazole	0.1 (± 0.1)	12.5	-	-
Acaricide	Maltodextrin	0.5 (± 0.1)	12.5	-	-
Acaricide	Spirodiclofen	0.1 (± 0.1)	75	3.3	-

The mean toxicity value for all the natural enemy groups was 2.8 (± 0.3), whilst the mean persistence was 20.6 (± 1.5) days. Parasitoid wasps were the most vulnerable natural enemy group to PPPs. The active ingredients applied in the study orchards scored a toxicity level of 3.1 (± 0.7) for parasitoid wasps. In contrast, the lowest toxicity was 2.3 (± 0.4) for Chrysopidae. The most toxic active ingredients for natural enemies applied in the study orchards were the insecticides bifenthrin, lambda-cyhalothrin, and pyrethrin, which scored a toxicity class of four (> 75% of mortality), whilst the two former products had a persistence of eight weeks in laboratory tests (Biobest, 2019) (Table 4.13).

4.5 Discussion

This study aimed to enhance abundance and richness of predators and parasitoid wasps in sweet cherry orchards through the provision of wildflower habitat, and test whether this intervention could increase pest regulation services. In addition, this study examined the response of natural enemies, and their ability to provide pest regulation services, to wildflower management under protective covers comparing the standard approach of a single cut at the end of the growing season (September) to a novel approach based on regular cutting to a height of 20 cm from May to September. It also considered whether natural enemies were influenced by edge effects, time of day sampled, and/or environmental factors. This three-year study demonstrated that not only can natural enemies be increased in orchard alleyways by creating wildflower habitat, but this approach also boosts the numbers of natural enemies in the adjacent sweet cherry trees. As a consequence, this was coupled with increased pest regulation services in the trees even though growers continued to use insecticides.

4.5.1 The influence of wildflower interventions on total natural enemies

Sown alleyways with wildflowers creates more complex semi-natural habitats compared to unsown control alleyways (Balzan *et al.,* 2014) and it is likely that these semi-natural habitats provided alternative prey and shelter for natural enemies (Campbell *et al.,* 2017). Both wildflower treatments (AMWS and SWS) had a positive effect enhancing predator abundance and richness and parasitoid wasp abundance compared to alleyways managed conventionally (CS). Differences in natural enemy enhancement between wildflower strip treatments and CS were more apparent in alleyways than trees. This could be the result of a spill-over of natural enemies from the alleyways to cherry trees

(Woodcock *et al.,* 2016). Natural enemies could have been firstly enhanced in wildflower strips (natural habitats), and then, spilled over to cherry trees (cropped land) (Woodcock *et al.,* 2016). This indicates that non-cropped areas are important reservoirs for natural enemies (Wäckers & van Rijn, 2012). However, the enhanced response of natural enemies in the alleyway vegetation compared to the cherry trees could also be a sampling artefact due to two different sampling techniques being used to sample these habitats, even though similar taxonomic groups were recorded (Rodrigues *et al*., 2003), or greater and direct PPP exposure on trees compared to alleyways, which could have minimized negative pesticide effects on natural enemies (Pekár, 2012).

The impact of the wildflower strips on natural enemy abundance and richness was consistent with the wildflower management treatment being applied after the baseline year (year one). In year one, the alleyway treatments were not instigated, and all treatments were cut to a height of 10 cm. As a result, no difference in natural enemy abundance or richness between alleyway treatments was recorded. However, in year two, in the wildflower treatments, sown species dominated the alleyways and were able to flower (See Chapter 3). In addition, following soil disturbance in preparation for sowing in year one, unsown forb species were more abundant in wildflower treatments than in CS, which were dominated by grass species. The development of wildflowers, both sown and unsown, created a semi-natural habitat for natural enemies that provided a greater range of opportunities (Blaauw & Isaacs, 2012). Consequently, the abundance and richness of natural enemies in alleyways and cherry trees increased in AMWS and SWS compared to CS. This trend was repeated in year three, and greater natural enemy abundance and richness was recorded in wildflower strips than CS. However, the greater development of wildflowers in SWS compared to AMWS created a more complex sward (Chaplin-Kramer *et al.,* 2011), resulting in a trend of greater impact on natural enemy richness and abundance in SWS compared to AMWS.

4.5.2 The influence of wildflower interventions on pest regulation services

In cider apple orchards, it was found that the enhancement of natural enemies in orchard alleyways in association with wildflower treatments led to an improvement of 55% in pest regulation services, suggesting a relation between wildflowers and pest control (Campbell *et al.,* 2017), as found in this study. Increases in natural enemy abundance has been associated with greater pest regulation services (Blaauw & Isaacs, 2015; Campbell *et al.,* 2017), but greater natural enemy diversity also improves pest control, since different natural enemies can attack the same (Dainese *et al.,* 2017) or different pests (Marc & Canard, 1997). This highlights the importance of natural enemy abundance and richness to provide a more effective and resilient pest regulation service in sweet cherry orchards, which could result in lower PPP applications (Hatt *et al.,* 2017). Natural enemies were enhanced in wildflower treatments despite the continued use of PPPs in the study orchards. The PPPs scored a slight to moderately toxic class with a 2.8 mean value, indicating that some PPPs are harmful to natural enemies, which could have affected abundance and richness recorded throughout the three years (Beers *et al.,* 2016) and reduced predation on the aphid baited cards. However, these toxicity data were based on laboratory assays and may differ under field conditions, where length of PPP and timing of applications could be considered among other factors. Yet, it is probable that reducing PPP inputs could increase natural enemies and consequently, improve pest regulation services, bringing positive outcomes for IPM programmes.

During all three bait card rounds, similar percentages of aphid depletion on trees were recorded in AMWS and SWS, even though a trend to a greater abundance and richness of natural enemies was associated with SWS compared to AMWS. Natural enemy enhancement could have been resulted due to sward habitat complexity, being greater in SWS compared to AMWS (Begg *et al.,* 2016). However, pest abundance do not respond to habitat complexity (Chaplin-Kramer *et al.,* 2011), and therefore pest populations were similar in cherry trees. No difference between wildflower treatments suggests that wildflower strips can be actively managed without affecting pest regulation services. This could bring benefits to growers and workers through the improvement of movement along the alleyways and facilitation of management activities such as pruning.

The increase in depletion on bait cards throughout the growing season (in year three), from 22.4% in June to 25.7% in July, and to 35.7% in August was not completely related to temperature or natural enemy abundance on cherry trees. Temperature in July and August was similar but greater than in June, indicating that temperature may have played some role in predator/scavenger activity, but other factors may have also interacted. Greater depletion rate when the temperature was higher was consistent with Ximenez-Embun *et al.* (2014), where a 32% of depletion in cards deployed in summer was reached.

Despite the overall pest regulation increase in wildflower treatments compared to CS, depletion in bait cards was inconsistent between months, and no difference between alleyway treatments was recorded in August. Prior to cherry harvest, the use of PPPs to control pests had ceased, with no PPPs being applied from August, except for a post-harvest acaricide application in mid-August in Orchard blocks 7 and 8 in year one to control *T. urticae* (Murray & Jepson, 2018). However, in year three, the cessation of PPPs did not result in an increase of natural enemies on cherry trees, and contrary to expectations, abundance and richness decreased compared to previous months. In contrast, natural enemy abundance and richness in the alleyways increased in August compared to June and July, probably due to the lack of prey on trees. The scarce prey availability on trees in August resulted in sentinel aphids being a more convenient alternative prey to predators and scavengers, and may have been more intensively consumed. This would also explain the greater depletion occurred for all alleyway treatments during this month compared to June and July.

4.5.3 Response of natural enemy taxonomic groups to the wildflower treatments

Pest regulation services (depletion from bait cards) could have been mostly provided by Anystidae (Figure 4.20), the most frequent natural enemy recorded on trees, which accounted for about half of the total natural enemy records.

Figure 4.20. Detail of a bait card where four aphids were depleted and two were being consumed by Anystidae (whirligig mites).

The species most abundant of this family was *Anystis baccarum*, a cosmopolitan generalist predatory mite which can provide important pest control in UK apple orchards (Cuthbertson *et al.,* 2014). Species within this genus are active predators and can prey on pests such as *T. urticae*, reducing populations below threshold, which could reduce PPP applications (Iskra *et al.,* 2019). In addition, *A. baccarum* has tolerance to some PPPs including acetamiprid and spinosad (Cuthbertson *et al.,* 2014), which makes this mite an important predator in IPM programmes.

Araneae (spiders) may have benefited most from wildflowers, since they were the second most abundant natural enemy group on trees, the most frequent in alleyways and the most diverse overall. Abundance and richness of Araneae depend on landscape complexity and are enhanced when natural habitats are present (Schmidt & Tscharntke, 2005; Schüepp *et al.,* 2014). Araneae are heterogeneous generalist predators with a great range of hunting behaviours, which remain similar within each family (Bogya, 1999; Solomon *et al.,* 2000). Of the total 11 families identified in alleyways and trees, Linyphiidae, Theridiidae, and Araneidae were the most abundant on trees, which is consistent with previous studies in UK apple orchards (Chant, 1956; McKerchar *et al*., 2020). Individuals of these families use webs to catch prey; whilst Lycosidae, a ground-dwelling spider only recorded in alleyway vegetation, is an active predator (Solomon *et al.,* 2000). Other less abundant families such as Philodromidae, Clubionidae, and Salticidae are also active predators (Solomon *et al.,* 2000). Consequently, spiders may not have had an important effect on bait card depletion. In addition, spiders do not usually scavenge (Harwood & Obrycki, 2005). However, they are important natural enemies to control pests (e.g. aphids (Aphididae), beetles (Coleoptera), moths (Lepidoptera)) in other crops, such as apple orchards, which have been more extensively studied (Chant, 1956; Wyss *et al.,* 1995; Marc & Canard, 1997; Markó *et al.,* 2009; de Roincé *et al.,* 2013; McKerchar *et al*., 2020). In sweet cherry, spiders can feed on pests such as aphids (*M. cerasi* (Cichocka, 2007)), and beetles (*Phyllobius* spp. and *Phyllopertha* spp. (Schüepp *et al.,* 2014)), and may provide significant pest regulation services. However, PPP usage and prey availability also affect Araneae abundance (Markó *et al.,* 2009) and could have affected spider efficacy and movement between trees and alleyways. Swards could have acted as refuges from PPP sprays (Pekár, 2012) and provided alternative prey (Wyss *et al.,* 1995).

Opiliones, particularly individuals within Phalangiidae were also abundant on trees and alleyway vegetation, as found by Chant (1956) in UK apple orchards. Some species of Opiliones are generalist predators such as *Phalangium opilio*, which is common in cropped areas, and can provide important pest regulation services (Drummond *et al.,* 2010). Opiliones can also be scavengers (Harwood & Obrycki, 2005) and therefore may have consumed aphids on bait cards.

Formicidae were mostly often recorded in alleyway vegetation accounting for more than a quarter of the total natural enemy records, particularly in SWS compared to AMWS, since Formicidae activity is greater in tall vegetation, which provide a more shaded habitat (Holec *et al*., 2006). However, not many individuals were recorded on trees, probably due to the lack of prey and aphids to collect honeydew from. Although Formicidae are predators, they can also deter other predators when affording protection aphids to collect honeydew (Stutz & Entling, 2011).

The Coleoptera identified, Carabidae, Staphylinidae, and Coccinellidae, are active generalist predators, although Carabidae, and Coccinellidae can be scavengers (Harwood & Obrycki, 2005). Carabidae and Staphylinidae were common in alleyway vegetation rather than on trees, which is consistent with their ground-dwelling habits, as found in UK apple orchards (Cross *et al.,* 2015). In contrast, the greater abundance of Coccinellidae on trees can be explained by the preference for woody (trees and shrubs) habitats of some species such as *Adalia bipunctata* compared to herbaceous habitats (Sloggett & Majerus, 2000). Some species of Coccinellidae recorded, including *A. bipunctata*, *Coccinella septempunctata*, *Harmonia axyridis*, and *Propylaea quatuordecimpunctata*, can prey on *M. cerasi* (Stutz & Entling, 2011; Wojciechowicz-Żytko, 2011) and could have fed on the aphids from the bait cards. Coccinellidae could provide a significant pest regulation service to cherry orchards if this pest were more abundant (Stutz & Entling, 2011; Wojciechowicz-Żytko, 2011).

Other natural enemies were less frequent such as Hemiptera, Neuroptera, and parasitoid wasps, suggesting a minimal role in cherry pest control. However, low pest populations could have affected their abundance, although the use of PPPs could have also reduced their numbers (Dib *et al.,* 2016). Particularly, parasitoid wasps could been more affected by PPP toxicity, as this natural enemy group was the most vulnerable to PPPs. In contrast, the lowest toxicity recorded for Chrysopidae indicates that the scarce prey availability was more likely to limit Neuroptera populations. Nonetheless, these taxonomic groups responded positively to the wildflower treatments most likely because of the provision of sugar in nectar to meet their energy requirements (Wäckers & van Rijn, 2012). Feeding on nectar and pollen from a range of wildflowers also increases survival rate, longevity, reproduction, and oviposition of these taxonomic groups (Wäckers & van

Rijn, 2012) and nectar and pollen can be used as an alternative source of food when prey is scarce (Wäckers & van Rijn, 2012). Consequently, wildflower strips can enhance populations of Hemiptera, Neuroptera, and parasitoid wasps.

Despite Syrphidae adults being more abundant in AMWS and SWS compared to CS (see Chapter 5), the abundance of Syrphidae larvae in alleyway vegetation and on cherry trees was not affected by alleyway treatment. However, when *M. cerasi* populations peaked, Syrphidae larvae were often recorded on trees (Figure 4.21). About 40% of all British Syrphidae species have aphidophagous larvae or feed on other soft-bodied insects, including species within Syrphini, such as *Epysirphus balteatus* (Ball & Morris, 2015). Species with zoophagous larvae were dominant in alleyways accounting for 86.7% of the total Syrphidae recorded. However, probably due to the lack of aphids (the main prey of Syrphini (Tenhumberg, 1995)) and other prey on trees and alleyway vegetation, hoverfly laying behaviour could have been affected and females laid eggs in the surrounding landscape where aphids were more abundant (Almohamad *et al.,* 2009). Adults are highly mobile and could have flown to the wildflower strips to collect nectar and pollen (Almohamad *et al.,* 2009).

Figure 4.21. Syrphini (Syrphidae) larva preying on *Myzus cerasi*, delivering pest regulation services.

Overall, the PPP applications kept pest populations low, resulting in scarce prey availability for natural enemies on cherry trees. As a result, generalist natural enemies (e.g. Araneae, Anystidae) were more abundant than specialist (e.g. parasitoid wasps, Syrphidae), as they could have survived feeding on alternative prey (Harwood & Obrycki, 2005). This highlights the importance of introducing wildflowers that can support non-crop prey and therefore natural enemy populations when pest populations are low (Wäckers & van Rijn, 2012). In addition, low pest populations due to PPPs, can also explain the inconsistency in natural enemy abundance on trees between months, which could have affected to a lesser degree natural enemy abundance in the alleyway vegetation.

4.5.4 Edge effect on natural enemies and pest regulation services

Abundance and family richness of natural enemies in the alleyway vegetation were not affected by the proximity to the orchard edge, suggesting that wildflower strips in the orchard can provide resources and shelter for natural enemies throughout the whole strip. This enabled natural enemies to spill-over to the cherry trees along the entire length of the row and provide pest regulation services without being affected by the edge. In contrast, with approaches such as field margins, hedgerow edges or wildflower patches near cropped areas, natural enemy enhancement decreases in the crop when the distance from natural habitats increases (Blaauw & Isaacs, 2015; Woodcock *et al.,* 2016), which limits the availability to deliver pest regulation to the centre of the crop (Woodcock et al., 2016). However, this may be less noticeable for natural enemy groups with high mobility such as hoverflies (Haaland *et al*., 2011). Wildflower strips may attract initially natural enemies by providing resources, and allow them to disperse to the centre of the orchards. The response of natural enemies and their ability to provide pest regulation services based on the introduction of wildflower strips on small scale has been positive, and could be greater on a larger scale, but edge effect should be investigated.

4.5.5 The influence of environmental factors and time of the day surveyed on natural enemies

Environmental factors may also be an important component of pest regulation services. In year three, when the greatest annual mean temperature, and lowest humidity were recorded, overall more natural enemies were found in both alleyways and trees (excluding the wildflower effect and comparing only CS). This suggests that natural enemies might have responded to environmental factors (Leather & Watt, 2005). Although time of day surveyed did not affect the overall natural enemy abundance, despite the variations in environmental factors throughout the day, some natural enemy groups were affected, particularly on trees. This suggest that environmental factors may affect natural enemy groups differently and their activity (pest control) can be therefore be more effective depending on temperature, humidity and wind speed (although the latter to a lesser extent). For instance, Syrphidae larvae were more often recorded on trees in the afternoon surveys when the temperature was greater than in the morning, whilst, Araneae was mainly recorded in the morning surveys. Thus, changes in environmental factors (climate) may be a key factor determining pest regulation control (Thomson *et al.,* 2010) and some natural enemies could perform more efficiently than others, although more research is needed.

4.5.6 Conclusions

This study has demonstrated that wildflower interventions in sweet cherry orchards can enhance natural enemies, leading to an increase in pest regulation services. Moreover, the novel approach of maintaining wildflower strips in alleyways to a height of 20 cm throughout the growing season resulted in similar pest regulation services to standard management with a single cut in September. In addition, the response of natural enemy abundance and richness and pest regulation services were not influenced by edge effects, indicating that pest control can be evenly distributed within the orchards. Hence, the introduction of wildflower strips in sweet cherry orchards provides the potential for growers to reduce the number of PPP applications to control pests. Future work should focus on the response of natural enemies and pest control in scaled up wildflower habitats, the effect of wildflower habitats on natural enemies with a reduced use of PPPs, and the role of different natural enemy species in controlling cherry pests.

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Chapter 5. Pollinating insects in UK sweet cherry orchards and their pollination efficacy

5.1. Abstract

Pollinating insects provide pollination services to many crop species, including sweet cherries. However, pollinators continue to decline worldwide which threatens food production. To overcome pollinator deficits, managed pollinators are used, but wild pollinators may still provide a better pollination service, yet they also rely on semi-natural habitats for shelter and alternative floral resources. Wild pollinators could be enhanced in orchards if their requirements are met, for example by creating wildflower habitats. In turn, the production of sweet cherry could be maximized. However, there is little knowledge of what wild insect pollinators occur in protected sweet cherry orchards in the UK and which are the most efficient at delivering pollination services. To enhance pollinators in sweet cherry, two different wildflower treatments were established in alleyways between rows of trees. One wildflower treatment was managed with a single cut in September; classed as a Standard Wildflower Strip (SWS), and one was actively managed with regular cutting to a height of 20 cm; classed as an Actively Managed Wildflower Strip (AMWS). These treatments were compared to conventional unsown Control Alleyways (CS) over a three-year period. Transect surveys were conducted to investigate pollinator abundance and richness, whilst visitation observations were used to investigate pollinator efficacy. Pollinator behaviour observations were conducted to explore the efficacy of different pollinating insect guilds.

During the cherry blossom period, AMWS was associated with an increased abundance of pollinators compared to CS and SWS. However, after the blossom period, both wildflower treatments greatly increased pollinator abundance compared to CS. Overall, a greater pollinator abundance was also recorded with SWS compared to AMWS, especially in year three. Accordingly, pollinator species richness and diversity were greater in SWS than in AMWS and CS, whilst values were greater in AMWS than in CS. Over the three years, 104 species were identified, but managed pollinators (*Apis mellifera* and *Bombus terrestris*) encompassed ~ 60% of all visits. After the cherry blossom period, hoverflies were the most frequent pollinator guild recorded. Solitary bees and wild bumblebees were the most efficient cherry pollinators based on behavioural observations. Pollinator foraging was also influenced by proximity to the orchard edge with greater abundance and species richness recorded near to the edges. Environmental conditions

also affected pollinator abundance, which can have impacts on fruit set. In conclusion, this study showed that wildflower strips are an effective approach to enhance pollinator abundance and species richness in protected sweet cherry orchards, but the benefits of wildflower strips were not fully realised until year three.

5.2. Introduction

Pollinating insects support food production by providing pollination services to many different crops (Potts *et al.*, 2016). Some crops, including sweet cherry, are pollinator-dependent (Lech *et al.*, 2008), which means production is strongly underpinned by insect pollination (Lebuhn *et al.*, 2013; Potts *et al.*, 2016) and would not be economically viable without their pollination (Majewski, 2014). In addition, as most sweet cherry cultivars are self-incompatible (Lech *et al.*, 2008), insect pollinators are required for cross-pollination (compatible pollen delivery) to underpin yields in sweet cherry (Eeraerts *et al.*, 2020).

Successful transfer of compatible pollen depends on the behaviour of pollinating insects including the time spent visiting flowers, stigma contact, visitation rate (flowers visited per minute) (Vicens & Bosch, 2000a), and movement between trees or rows for cross-pollination (Brittain *et al.*, 2013b). This can differ between guilds, for example, compared to solitary bees, the western honeybee (*Apis mellifera*) makes fewer stigma contacts in apple blossoms and as a consequence their pollination efficacy is lower (Vicens & Bosch, 2000a; Garratt *et al.*, 2016). However, this is compensated for by higher visitation rates (Vicens & Bosch, 2000a; Garratt *et al.*, 2016). In addition, wild bee pollinators move more frequently between tree rows than honeybees (Brittain *et al.*, 2013b; Eeraerts *et al.*, 2020).

However, wild pollinators continue to decline globally leading to potential pollination deficits (insufficient pollination services resulting in limited yields), which could impact food production (Potts *et al.*, 2016). Key drivers for this decline include: landscape change including agricultural expansion and habitat loss, the use of Plant Protection Products (PPPs), the arrival of invasive non-native species, the spread of pathogens and disease, and climate change (Goulson *et al.*, 2015; Senapathi *et al.*, 2017). To overcome potential shortfalls in pollination and maintain yields and fruit quality, the introduction of commercially available, managed pollinators is therefore common practice in commercial cherry orchards (Koumanov & Long, 2017; Ryder *et al.*, 2019). *Apis mellifera* is the most widespread and utilised managed pollinator (Koumanov & Long, 2017), but bumblebees such as *Bombus terrestris* and mason bees such as *Osmia bicornis* and *O. lignaria* are also used (Bosch *et al.*, 2006; Hansted *et al.*, 2015; Koumanov & Long, 2017; Ryder *et al.*, 2019). *Apis mellifera* and *B. terrestris* are generalist species that can visit numerous crop species (Rader *et al.*, 2014), however they might not be the most efficient pollinators of cherry.

Wild pollinators provide pollination services to sweet cherry and some species can be more efficient than managed bees, resulting in improved fruit set and greater yields (Holzschuh *et al.*, 2012; Eeraerts *et al.*, 2020). Moreover, a diverse wild pollinator community can ensure pollination since wild pollinators can be active in poor weather conditions compared to honeybees (Brittain *et al.*, 2013a; Földesi *et al.*, 2016), and more resilient to a changing climate (Bartomeus *et al.*, 2013). Moreover, environmental factors (e.g. temperature, humidity and wind speed) vary throughout the day affecting directly pollinator abundance and behaviour (Chang *et al.*, 2016; Sgolastra *et al.*, 2016), and indirectly since these can alter pollen, nectar, and water concentrations in the flower, influencing therefore the number of visits to blossoms (Corbet *et al.*, 1979; Kearns & Inouye, 1993). Consequently, environmental factors can affect fruit set (Tuell & Isaacs, 2010). This is particularly important for sweet cherry because the crop blooms early in the season (Fadón *et al.*, 2015) (typically mid-April in the UK depending on weather conditions), and for only a short period, typically two to five weeks (Christensen, 1996). Hence, phenological synchrony between efficient pollinators and the crop is essential (Bartomeus *et al.*, 2013). However, landscapes surrounding cropped areas do not always support populations of wild pollinators (Goulson *et al*., 2015), resulting in an ongoing threat to the economic viability of sweet cherry production and other pollinator-dependent crops. The abundance and richness of wild pollinators including solitary bees, bumblebees, and hoverflies is highly dependent on the availability of non-cropped habitat in the farmed landscape, particularly semi-natural habitats (Cole *et al.*, 2017). As a consequence, pollinator abundance and richness can be enhanced when wildflower habitat is provided (Blaauw & Isaacs, 2014; Feltham *et al.*, 2015). The provision of wildflower habitat is also likely to influence the behaviour of insect pollinators (Blaauw & Isaacs, 2014; Feltham *et al.*, 2015), and pollinator behaviour might be influenced by edge effects in the orchards (Nguyen & Nansen, 2018).

During the sweet cherry blossom period, numerous cherry blossoms are available as a resource of nectar and pollen for pollinating insects. However, after the cherry blossom period, resources are substantially reduced due to alleyways being regularly cut and dominated by grass species (see Chapter 3). Resources surrounding the orchards can also be limited (see Chapter 2). Following the cherry blossom period, the reduced abundance of resources could impact wild pollinators. To support wild pollinators beyond the cherry blossom period and maximise the potential for the delivery of pollination services in subsequent years to avoid pollination deficits, there is a need to provide additional resources in the farmed landscape to support them. The introduction of wildflower interventions in orchards to provide pollen and nectar is an approach that may enhance pollinator abundance and diversity and consequently, fruit quality and yield (Blaauw & Isaacs, 2014).

The aim of this study was to investigate the abundance, species richness, and diversity of insect pollinators in sweet cherry orchards and their foraging preferences in relation to the presence of two wildflower treatments over three consecutive years during and after the cherry blossom period. A further aim was to investigate pollinator behaviour and the efficacy of cherry blossom pollinators and how this is influenced by orchard edge effects, and survey time (time of day surveyed), which can be associated with changes in environmental factors.

5.3. Materials and methods

5.3.1 Study site and experimental design

The study was carried out in the West Midlands, UK, at three sites in Herefordshire and two in Staffordshire. At each site, two orchard blocks (defined as a separate parcel of land) were investigated. In each orchard block, three alleyways adjacent to rows of the cherry cultivar Kordia were selected to be studied. Two alleyways in each orchard block were randomly selected to receive one of two wildflower treatments, whilst the third was an untreated control, which consisted of the original alleyway vegetation (see Chapter 3). The three alleyway treatments were therefore:

i) **Control Strips (CS)**. Conventionally managed alleyways not sown with wildflowers that were cut regularly to a height of 10 cm from May to September, and then to a height of 8 cm in late September.

ii) **Standard Wildflower Strips (SWS)**. Cut annually in late September to a height of 8 cm.

iii) **Actively Managed Wildflower Strips (AMWS)**. Cut regularly (twice/three times per month) to a height of 20 cm from May to September, and then to a height of 8 cm in late September.

The length of strips was 95 m, starting from the orchard block edge towards the centre of the orchard. This allowed standardization among orchard blocks due to the length of five out of 30 alleyways studied was 95 m, and the other edge was therefore at that distance. The distance between alleyway treatments varied from 26 to 48 m depending on the availability of Kordia in the orchard blocks. The distance between orchard blocks also varied. At four sites, the distance was between 250 and 975 m. At Site 2, the distance between blocks was 30 m (see Chapter 2). Consequently, due to the close proximity between alleyway treatments and the high mobility of pollinators (Zurbuchen *et al.*, 2010), the study was designed to assess the presence and foraging preferences of pollinators rather than impacts on orchard, farm or landscape scale abundance.

The wildflower strips were sown in autumn 2016, but establishment was poor (see Chapter 3). Consequently, alleyways were re-sown in year one (2017) at Sites 1, 2, and 3 in April and at Sites 4, and 5 in September. To promote the establishment of wildflowers, all alleyway treatments were cut regularly to a height of 10 cm throughout year one (baseline year) (see Chapter 3). During the establishment year, baseline data were collected to gain an understanding of the key pollinators that occurred in sweet cherry orchards. The different alleyway treatments were applied from May in year two (2018). All cuttings were left *in situ*. Due to the poor establishment of the wildflower strips at Site 4, both orchard blocks were dropped from data collection on transect surveys of orchard alleyways in year three.

5.3.2 Cherry floral abundance surveys

Cherry flower abundance was recorded to determine the number of blossoms available to pollinators, which is important to determine pollination efficiency and quantify pollination (Howlett *et al.*, 2018). The number of branches on four trees per alleyway treatment were recorded at the beginning of the cherry blossom period (after thinning; see Chapter 2) each year. Trees were selected based on distance from the orchard block edge (located at 9.5, 28.5, 47.5, and 66.5 m). The number of cherry blossoms was counted on five different branches on each tree, randomly chosen each time. This was done for every cherry floral abundance survey. These surveys were conducted prior to transect surveys of cherry blossoms (see section 5.3.3.1) in all three years and stationary timed visitation surveys (see section 5.3.4.1) in years two and three. Average values were obtained by multiplying the number of total blossoms recoded on the five branches by the total number of branches on the tree.

5.3.3 Pollinating insect abundance, richness and diversity

5.3.3.1 Transect surveys of cherry blossoms during blossom period

Pollinator abundance and richness (number of species) were recorded by visual observation with transect surveys of cherry blossoms in years one (2017), two (2018), and three (2019). Values of Shannon diversity were also calculated. A 16-minute walking transect was carried out along each alleyway treatment (Popic *et al.*, 2013; Kleijn *et al.*, 2015). In year one, two transect surveys were performed per alleyway treatment per day at two different sites. The second transect survey was carried out 90 minutes after the first survey. In years two and three, survey intensity was halved, transect surveys were conducted once per day also at two sites. The abundance of all pollinating insects was recorded, according to four different activities: i) visiting cherry blossoms, ii) visiting wildflowers, iii) flying (including flying along the alleyway, flying around cherry trees, and flying over ground), and iv) resting (including resting on cherry trees, and resting on ground). When pollinating insects were observed foraging on wildflowers in the alleyways, the plant species was identified.

Transect surveys were undertaken by two people once the cherry blossoms started to open (balloon stage; stage 59 in the BBCH scale (Fadón *et al*., 2015)) until the end of the cherry blossom period (stage 69 in the BBCH scale (Fadón *et al*., 2015)). Approximately from early/mid-April to early/mid-May. To maximize data collection during the short blossom period, surveys were conducted every day (except when raining or when temperatures were below 8ºC). To investigate whether time of day (Pisanty *et al.*, 2016) and environmental factors (Güler & Dikmen, 2013) affected abundance and richness of pollinating insects, 12 time periods during the day were designated to perform the transects per alleyway treatment (10:30-11:00, 11:00-11:30, 11:30-12:00, 12:00-12:30, 12:30-13:00, 13:00-13:30, 14:00-14:30, 14:30-15:00, 15:00-15:30, 15:30-16:00, 16:00-16:30, 16:30-17:00 hrs). Surveys were done alternating between time periods to avoid temporal bias as detailed in Chapter 4 (section 4.3.1). To avoid further temporal bias, each alleyway treatment was assessed at least three times each year covering all different times for surveys. Sites were grouped according to geographical location. Group one included the sites located in Herefordshire (Sites 1, 2 and 3). Whilst group two included the sites in Staffordshire (Sites 4 and 5).

5.3.3.2 Transect surveys of orchard alleyways post blossom period

After the cherry blossom period, the use of the different alleyway treatments by pollinators was investigated using 16-minute transect surveys of orchard alleyways (Blaauw & Isaacs, 2014). According to each of the treatments, abundance and species richness was recorded and values of Shannon diversity calculated. Surveys took place each month from May to September in year one, from June to September in year two (due to the late blossom period), and from May to August in year three. Two sites per day were assessed by one person. Transect surveys were also conducted considering time of day and environmental factors whilst sites were grouped according to geographical location as above.

Pollinator abundance and richness were recorded according to the activities described above except for ‘visiting cherry blossoms’. Towards the end of the cherry blossom period, extrafloral nectaries (leaf nectaries) started to develop at the base of cherry leaves, which were visited by pollinators. Therefore, the activity ‘visiting nectaries’ was included. In total, four activity categories were recorded: i) visiting wildflowers, ii) visiting nectaries, iii) flying (in which records of pollinators flying along the alleyways, flying around cherry trees, and flying over ground were combined), and iv) resting (sum of pollinators resting on cherry trees and resting on the ground). When pollinators were recorded visiting wildflowers, the plant species was identified.

5.3.4 Pollinating insect behaviour

5.3.4.1 Pollinating insect efficacy to pollinate cherry blossoms (stationary timed visitation surveys)

To investigate the efficacy of pollinating insects to pollinate cherry blossoms, stationary timed visitation surveys were conducted (Garratt *et al.*, 2016) in years two and three. Four Kordia cherry trees on each alleyway treatment were observed for four minutes. Stationary timed visitation surveys were conducted as described for transect surveys of cherry blossoms. Pollinator efficacy was determined by recording visitation time (duration of pollinator visit per flower), flowers visited per tree (the number of flowers visited during the visit), visitation rate (flowers visited per minute), visit duration (time spent during the visit), and stigma contact (Vicens & Bosch, 2000a). Due to the importance of cross-pollination in sweet cherry, the location of the tree subsequently visited (flying behaviour for cross-pollination) was recorded according to one of four categories: i) the pollinator was caught for identification or remained on the tree after the four-minute period; ii) the pollinator moved to a tree in the same row; iii) the pollinator moved to the adjacent row; and iv) the pollinator flew away. The part of the pollinator (head, sternum and legs) that made contact with the stigma and what substance (pollen and/or nectar) the insect was feeding on (pollinator feeding) were also recorded (Vicens & Bosch, 2000a). Percentages of body parts making contact with the stigma were calculated from the total of pollinators making stigma contact for each pollinator guild. Due to pollinators making contact with more than one body part during the same visit, total values can exceed 100%.

5.3.4.2 Edge effect

Pollinator behaviour, abundance, and richness might be influenced by edge effects in the orchards (Nguyen & Nansen, 2018). Hence, to investigate whether pollinator behaviour, abundance, and richness were influenced by sampling position in the orchard block, alleyway treatments were divided into five sections (Figure 5.1). However, the last 76 - 95 m section acted as a buffer and was not assessed. Moreover, the second half of the five alleyways which total length was 95 m was not included in the analysis because the centre of the orchard block was at 47.5 m, and from that point, sections would become closer to the other block edge (as in Chapter 4).

In order to gain a more detailed insight into the effect of edge effect on responses, the four alleyway sections were subdivided into 12 sub sections (three subsections per section) (Figure 5.1).

During the 16-minute transect surveys, four minutes were spent on each section, whilst during the stationary timed visitation surveys, each of the four trees observed were located in the middle of each section.

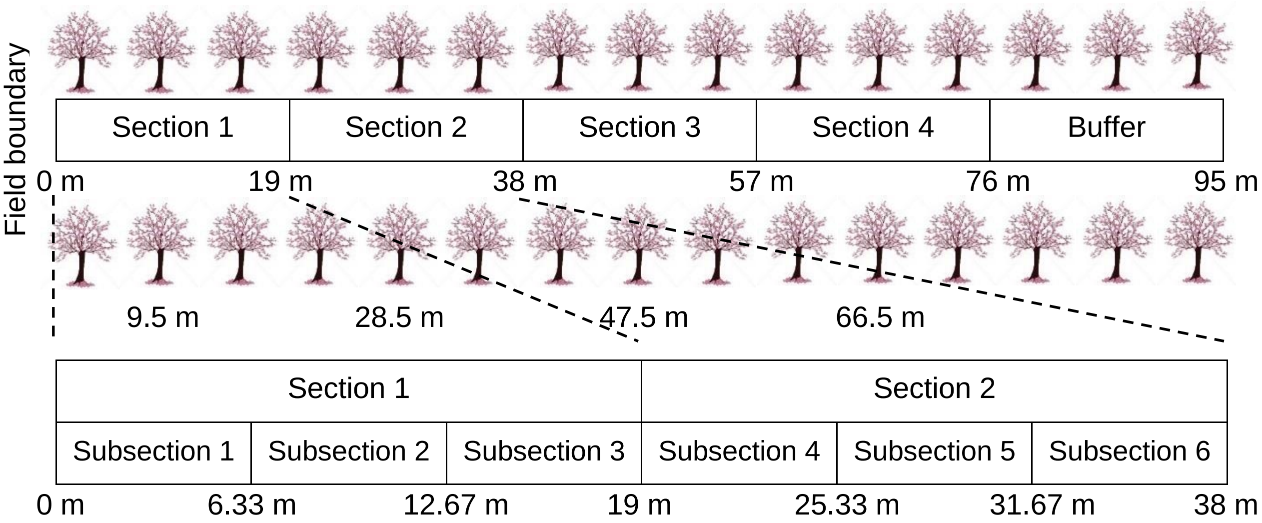

Figure 5.1. Representation of the four alleyway sections plus the buffer in which the alleyway strips were divided, including distances from the orchard block edge (m), the location of the mid Kordia cherry trees per alleyway section in which stationary visitation surveys were conducted. An example of the two first alleyway sections which were subdivided into three subsections each (m) is included. Subsections 7-12 are not included.

5.3.5 Pollinating insect guilds and species identification

Pollinators recorded during the cherry blossom period (transect surveys of cherry blossoms and stationary timed visitation surveys) were grouped into six pollinator guilds: 1) honeybees (*Apis mellifera*), 2) buff-tailed bumblebees (*Bombus terrestris*), 3) wild bumblebees, 4) solitary bees, 5) hoverflies, and 6) butterflies. Honeybees and buff-tailed bumblebees were used by the growers as commercial managed pollinators, whilst wild bumblebees, solitary bees, hoverflies, and butterflies were specified as wild pollinator guilds. As the cherry blossom period occurring in early spring, all wild bumblebees (henceforth bumblebees) recorded were queens, except some *Bombus pratorum* (early bumblebee). Therefore, it was assumed all buff-tailed bumblebee workers belonged to the commercial bumblebee boxes and were included into the category ‘buff-tailed bumblebees’ and not into the ‘bumblebees’ category. Flies, beetles, and other potential pollinators were also recorded and combined under the category ‘others’. However, due to the very low numbers recorded (particularly visiting cherry blossoms); this guild was excluded from analysis. Butterflies were also very scarce during the blossoms period and were only included in figures after the blossom period.

Pollinators recorded during the pollinator transect surveys of orchard alleyways (post-blossom) were allocated to one of five guilds: 1) honeybees, 2) bumblebees, 3) solitary bees, 4) hoverflies, and 5) butterflies. Buff-tailed bumblebees were no longer recorded as a separate guild due to wild workers being present from mid-May onwards. Although some individuals from managed bumblebee boxes would still be recorded, most boxes were removed by growers at the end of the blossom period. Workers of buff-tailed bumblebees (*Bombus terrestris*) and white-tailed bumblebees (*B. lucorum*) were grouped under the genus *Bombus* as their differentiation is highly recommended to be confirmed with DNA (Wolf *et al.*, 2010). However, queens and males of these species were still identified to species level.

An insect net (fourfold net 50 cm frame with telescopic handle; Watkins and Doncaster) was used to catch individuals (Popic *et al.*, 2013) not readily identified in the field and transferred into lidded plastic vials (Sterilin™ 7 ml polystyrene) for all three survey methods. The timer was stopped each time while catching and transferring pollinators. Pollinators caught and identified on completion of the surveys were released, whilst species not identified were retained for identification in the laboratory. Pollinators out of reach to be identified or caught were only identified to genus level if possible, otherwise, they were ignored. Species caught were put into vials and transferred to a freezer until pining. Insects were pinned using continental pins (stainless steel with nylon heads; Watkins and Doncaster) numbers 00, 0, 1, and 3, depending on pollinator size. Pollinators were stored in wooden store boxes (Watkins and Doncaster). A microscope (ZEISS Stemi 305 Compact Greenough Stereo Microscope) was used to identify the species.

5.3.6 Environmental factors

The environmental variables of temperature, humidity and wind speed were recorded using a Kestrel weather monitor during every survey (Kestrel 3500 weather meter) to investigate their influence on pollinator abundance, richness, diversity, and behaviour (Güler & Dikmen, 2013). Values were measured in the middle of each of the four alleyway sections for each survey conducted (transect surveys during and post-blossom and stationary timed visitation surveys). The timer was stopped each time to take the readings. In addition, an extra measurement was taken at the edge of the orchard block prior to the start of the surveys. Readings were taken at an approximate height of 1.5 m above the ground.

5.3.7 Statistical analysis

All data were analysed using the software R (version R-3.6.1) (R Core Team, 2019).

5.3.7.1 Cherry floral abundance

A negative binomial generalized linear mixed model (package lme4, function = GLMER.NB (Bates *et al.*, 2014)) was used to investigate differences between the number of blossoms according to tree. The number of blossoms per tree was specified as the response variable, whilst year and tree location along the alleyways (distance from the orchard block edge) were the fixed effects. Alleyway treatment nested within orchard blocks nested within sites were the random effects. However, the model was also run with alleyway treatment as a fixed factor to ensure there were not significant differences between alleyway treatments. The models were compared with the Akaike's Information Criterion (AIC) (Burnham & Anderson, 2002), and the model with the lowest AIC was chosen as the most parsimonious (Burnham & Anderson, 2002).

5.3.7.2 Pollinating insect abundance on transect surveys

To investigate whether alleyway treatment influenced total pollinator abundance during and after the cherry blossom period, two generalized linear mixed models with negative binomial error structures were used (package lme4, function = GLMER.NB (Bates *et al.,* 2014)). One model included total pollinator abundance during the blossom period whilst the second model included total pollinator abundance after the blossom period. Alleyway treatments were applied from May in year two; consequently, an interaction between alleyway treatment and year was expected. Total pollinator abundance was the response variable, which was the sum of all pollinators within each of the 12 subsections surveyed. Alleyway treatment, year, distance from the orchard block edge, and survey time (time when the surveys were conducted) were specified as fixed effects. Random effects were orchard blocks nested within sites.

Each model was run twice, with and without the interaction term and compared to test the relative importance of the interaction determined by the AIC (Burnham & Anderson, 2002). The model with the lowest AIC in each case was chosen as the most parsimonious model (Burnham & Anderson, 2002). The relative implication of each of the fixed terms in the models were obtained using the AIC. For each model, the relative significance of the model terms was calculated by taking an information theoretic approach using the AIC. Each of the fixed effects were individually removed from the global model and the difference in AIC values was calculated for the reduced model (ΔAIC). The AIC was used to select the most parsimonious model in each case; AIC > 2 was considered to have a substantial level of empirical support (Burnham & Anderson, 2002). Tukey's *post-hoc* tests (multcomp package (Hothorn *et al.,* 2008)) were subsequently used for pairwise comparisons between alleyway treatments and years for each model. $P < 0.05$ was considered significantly different.

The same model structure was used to determine which explanatory factors affected the abundance of each of the pollinator guilds during the cherry blossom period (honeybees, buff-tailed bumblebees, bumblebees, solitary bees, hoverflies, and butterflies) and after (honeybees, bumblebees, solitary bees, hoverflies, and butterflies). Each pollinator guild was the response variable for its model (11 models). This model was also used to investigate the effects of these factors on the abundance of total pollinators that were performing the four pollinator activities during the cherry blossom period (visiting cherry blossom, visiting wildflower, flying, and resting) and after (visiting wildflower, visiting extrafloral nectaries, flying, and resting). However, only the analysis of the total pollinator abundance visiting cherry blossoms during blossom and the total pollinator abundance visiting wildflowers post-blossom are presented, as these activities were considered the most important.

5.3.7.3 Pollinating insect richness and Shannon diversity

The mean number of pollinator species (species richness) and Shannon diversity per subsection were determined for each alleyway treatment, during and after blossom period according to year. To investigate significant differences, a generalized linear mixed model with Poisson error structure (package lme4, function = GLMER, family Poisson (Bates *et al.*, 2014)) was used. Species richness and Shannon diversity were specified as individual response variables in separate models, whilst the fixed and random effects remained the same as previous models (section 5.3.7.2). The relative implication of each of the fixed terms in the models were also obtained using the AIC, whilst models were tested to determine the most parsimonious model according to an interaction between alleyway treatment and year, and explored further using Tukey's *post-hoc* tests (multcomp package (Hothorn *et al.*, 2008)) for pairwise comparisons.

5.3.7.4 Pollinating insect efficacy to pollinate cherry blossoms

Generalized linear mixed models with negative binomial error structures (package lme4, function = GLMER.NB (Bates *et al.*, 2014)) were used to analyse visitation time, flowers visited per tree, visitation rate, and visit duration. Whilst stigma contact, flying behaviour for cross-pollination, and pollinator feeding were analysed using generalized linear mixed models with binomial error structures (package lme4, function = GLMER, and family = binomial (Bates *et al.*, 2014)). Models were re-run to study the interaction between alleyway treatment and year. The response variables were tested in separate models using the AIC and further studied with Tukey's *post-hoc* tests (as in section 5.3.7.2).

5.3.7.5 Environmental factors

To investigate the influence of wildflower interventions on environmental factors and the potential influence on pollinator abundance, the three environmental factors (temperature, humidity, and wind speed) were specified as response variables for three independent models. Each environmental factor was analysed using a generalized mixed model with negative binomial error structures (package lme4, function = GLMER.NB (Bates *et al.*, 2014)). For all three global models, alleyway treatment, year, distance from the orchard block edge, and time of day sampled were specified as fixed effects. Random effects included orchard blocks nested within sites. The three models were also tested to investigate whether there was an interaction between alleyway treatment and year. The relative significance of the model terms for each global model was calculated using the AIC as above. *Post-hoc* Tukey tests (multcomp package (Hothorn *et al.*, 2008)) were also used to investigate pairwise comparisons between fixed effects (alleyway treatments and years).

5.4 Results

5.4.1 Cherry floral abundance

The number of cherry blossoms did not vary significantly between alleyway treatments, distance from the orchard block edge or according to year (*Number of blossoms ~ Alleyway treatment + Year + Distance from the edge + (random: Site/Orchard/Alleyway treatment)*). On average, 4,350.3 (± 111.4) blossoms were recorded per tree in year one, compared to 3,707.8 (± 91.3) in year two and 3,904.8 (± 83.9) in year three. The cherry blossom period started in early April in years one and three, extending until early May. Whilst, in year two, the blossom period began in mid to late April and ended in mid-May (Figure 5.2).

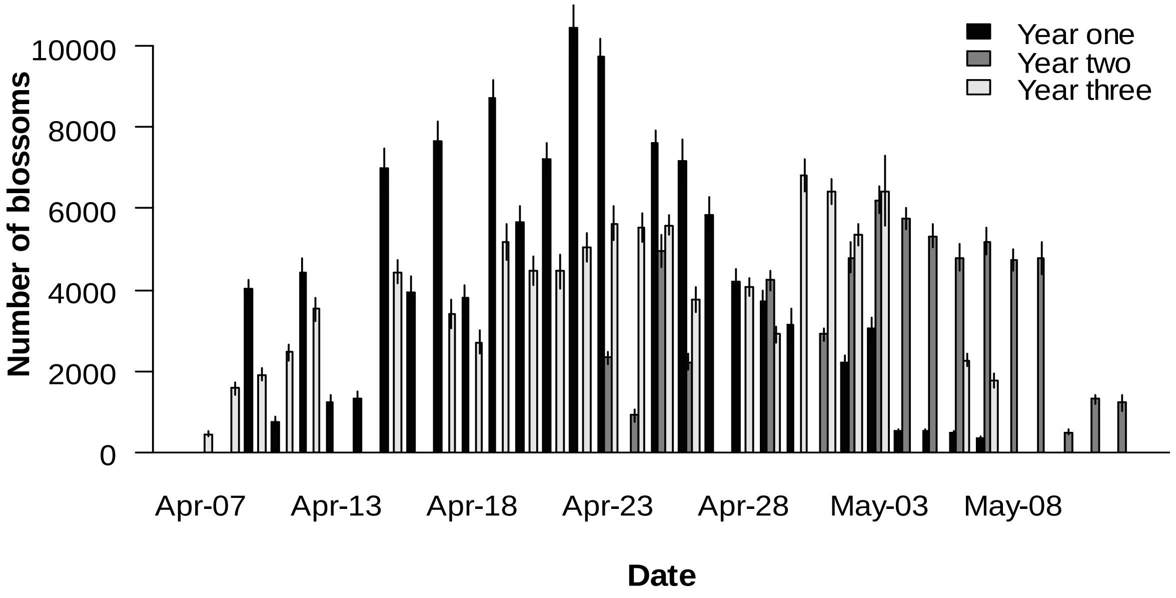

Figure 5.2. Mean number (± SE) of blossoms per tree according to date and year.

5.4.2 Pollinating insect abundance

5.4.2.1 During cherry blossom period

A total of 14,724 pollinators were recorded on the transect surveys conducted during the sweet cherry blossom period across the three-year study. Of those, 10,578 (72%) were managed pollinators (5,282 honeybees and 5,296 buff-tailed bumblebees) (Figure 5.3). Wild pollinators included 2,011 hoverflies, 1,119 bumblebees, 969 solitary bees, and 47 butterflies.

The most frequent visitors in sweet cherry orchards were managed pollinators (honeybees and buff-tailed bumblebees) (Figure 5.4). Similar numbers of honeybees and buff-tailed bumblebees were recorded with an average of 0.47 (± 0.01) individuals per subsection sampled. Frequencies of managed pollinators were much greater than for all wild pollinator guilds. Variation in pollinator abundance was also shown between sites. The highest number of managed pollinators was recorded at Site 1; a reflection of the use of buff-tailed bumblebees (see Chapter 2), which averaged 1.3 (± 0.03) buff-tailed bumblebee per subsection. In contrast, honeybees were the main pollinator guild recorded at all other sites. The most abundant wild pollinator guild was hoverflies with an average of 0.2 (± 0.005) pollinators per subsection, followed by bumblebees (0.1 (± 0.003)) and solitary bees (0.1 ± (0.004)), whilst butterflies were the scarcest pollinator guild (0.004 (± 0.001)). Wild pollinators were most abundant at Site 4 with 0.5 (± 0.02) individuals recorded; due to the greater numbers of hoverflies (0.3 (± 0.01)).

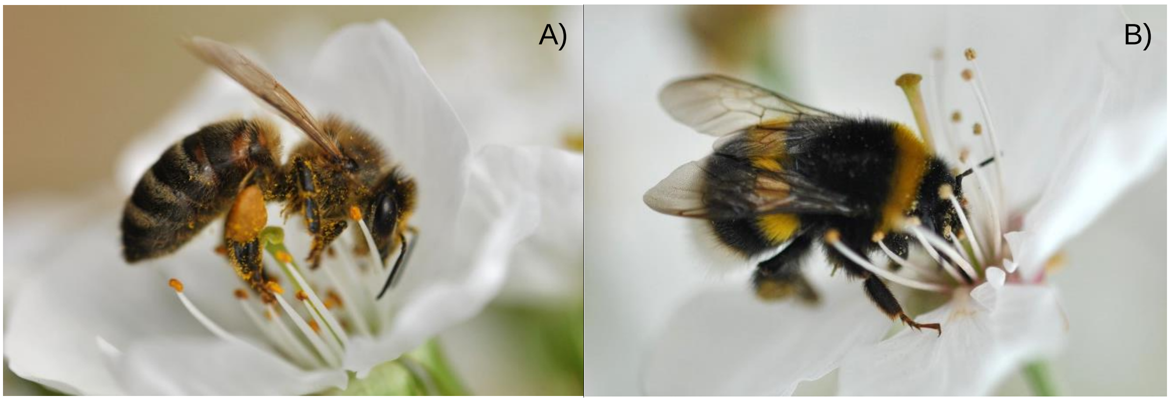

Figure 5.3. Managed pollinators visiting cherry blossoms: A) *Apis mellifera* (honeybee) and B) *Bombus terrestris* (buff-tailed bumblebee).

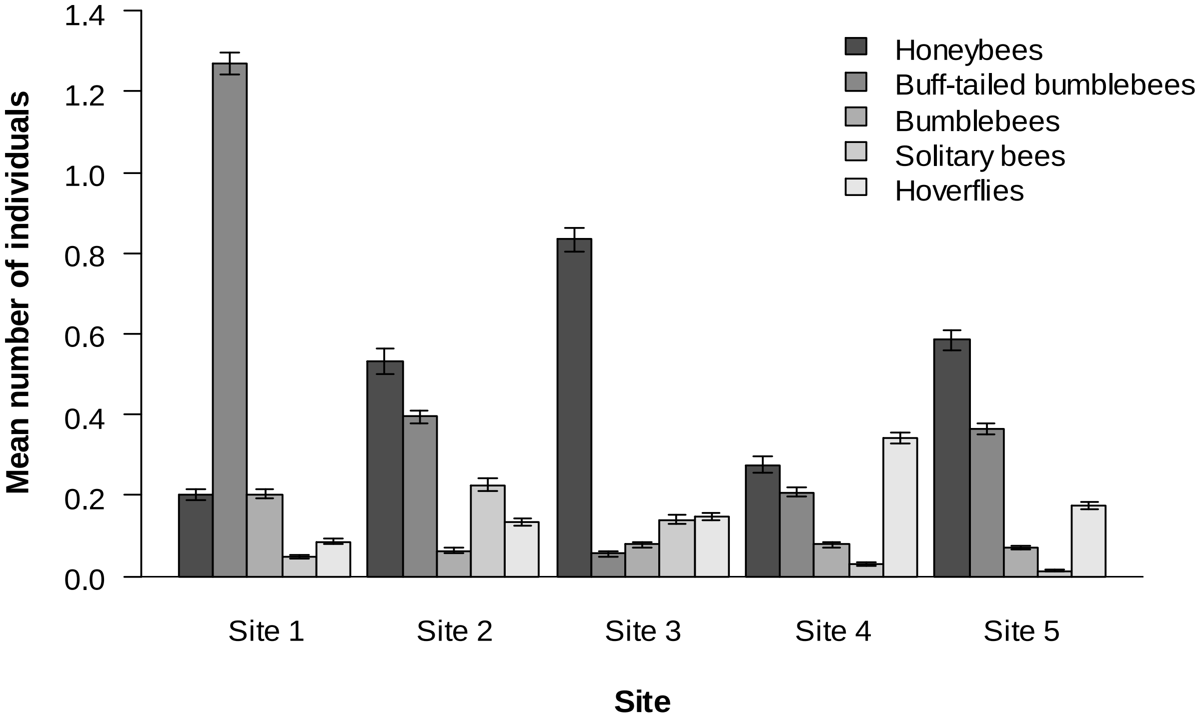

Figure 5.4. Mean number (± SE) of pollinator individuals per subsection according to site and pollinator guild recorded on transect surveys of cherry blossoms. Values are based on all three years of study. Butterflies were excluded due to scarce records.

Table 5.1 Mean number (± SE) of pollinator individuals and percentage within different pollinator guilds recorded per subsection on transect surveys of cherry blossoms throughout the three-year study according to activity. Percentages were calculated per activity, so that the sum of the four activities for each pollinator guild is 100%.

Pollinator guild	**Visiting cherry blossom**		**Visiting wildflower**		**Flying**		**Resting**	
	Mean	*%*	*Mean*	*%*	*Mean*	*%*	*Mean*	*%*
Honeybee	0.43 (± 0.01)	90.2	0.004 (± 0.001)	0.9	0.04 (± 0.002)	7.6	0.01 (± 0.001)	1.3
Buff-tailed bumblebee	0.26 (± 0.01)	54.6	0.004 (± 0.001)	0.7	0.19 (± 0.005)	40.9	0.02 (± 0.001)	3.8
Bumblebee	0.06 (± 0.002)	60.7	0.001 (± 0.0003)	1.0	0.03 (± 0.001)	30.3	0.01 (± 0.001)	8.0
Solitary bee	0.03 (± 0.002)	37.0	0.006 (± 0.001)	7.2	0.04 (± 0.002)	40.7	0.01 (± 0.001)	15.1
Hoverfly	0.04 (± 0.002)	24.3	0.006 (± 0.001)	3.3	0.06 (± 0.003)	32.5	0.07 (± 0.002)	39.9
Butterfly	0.0004 (± 0.0002)	10.4	0.00 (± 0.00)	0.0	0.003 (± 0.001)	77.1	0.0004 (± 0.0)	12.5

The abundance of individuals within the pollinator guilds differed according to activity (Table 5.1). Honeybees were the most recorded guild to visit cherry blossoms, followed by buff-tailed bumblebees, although these were more frequently recorded flying (40.9%). Of the wild pollinators, bumblebees visited the most cherry blossoms.

During the cherry blossom period very few wildflowers bloomed, and only four species were visited by pollinating insects. The most visited wildflower for all pollinator guilds combined was the unsown *Taraxacum officinale*, which accounted for 94.4% of the total visits to wildflowers (Appendix 5.1). The sown species *Silene dioica* started to bloom at the end of the cherry blossom period and was visited 2.8%; whilst 2.3% of pollinating insects were recorded visiting *Ranunculus repens* (unsown). *Brassica* spp. (unsown) received 0.5% of the total wildflower visits. Solitary bees used wildflowers the most, accounting for 7.2% of their total activity (Table 5.1).

5.4.2.2 Post-cherry blossom period

A total of 5,014 pollinators were recorded on the pollinator transect surveys of orchard alleyways conducted after the sweet cherry blossom period (from mid-May to late September) across the three-year study. The most abundant pollinator guild recorded was hoverflies (Figure 5.5A) with 2,749 individuals, followed by honeybees (1,220 individuals), bumblebees (Figure 5.5B) (760), butterflies (185), and solitary bees (100). The activities of pollinator guilds were highly variable (Table 5.2).

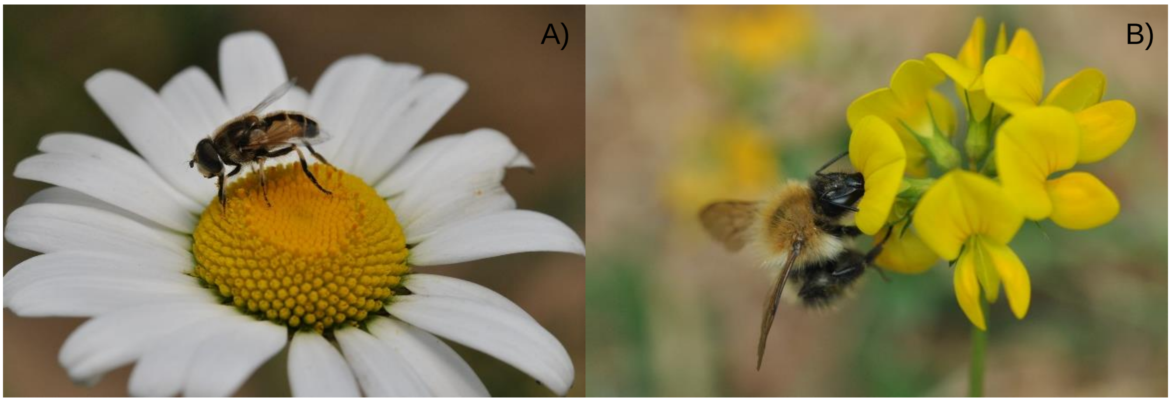

Figure 5.5. A) Hoverfly (*Eristalis arbustorum*) visiting the sown wildflower *Leucanthemum vulgare*. B) Bumblebee (*Bombus pascuorum*) visiting the sown wildflower *Lotus corniculatus*.

Of the total 5,014 pollinators, 1,310 were recorded visiting sown and unsown wildflowers (Appendix 5.2). This included 60 pollinator species, the honeybee, nine bumblebees, 15 solitary bees, 29 hoverflies, and six butterflies (Appendix 5.3). A total of 25 plant species were recorded being visited by pollinating insects (Appendix 5.2). The most visited wildflower was the sown *Leucanthemum vulgare* with 231 visits, followed by the unsown species *Trifolium repens* at 208 visits. Sown wildflowers were visited 612 times; whilst unsown wildflowers were visited 698 (Appendix 5.3). However, in year one, only unsown wildflowers were visited including *Brassica* spp., *Epilobium adenocaulon*, *Matricaria* spp., *Ranunculus repens*, *Senecio vulgaris*, *T. officinale*, and *T. repens*. Due to the large number of hoverflies recorded, these were the main visitors of wildflowers; their visits accounted for 28.2% of the observations (Table 5.2), being the pollinator guild that visited more sown wildflowers with 537 records compared to 286 visits to unsown wildflowers (Figure 5.6). Two hoverfly species (*Epysyrphus balteatus* and *Syritta pipiens*) were recorded visiting 16 wildflower species. Despite being scarcely recorded, solitary bees visited wildflowers on 44.0% of their total counts. Extrafloral nectaries were mostly visited by honeybees. Bumblebees and butterflies were recorded mainly flying.

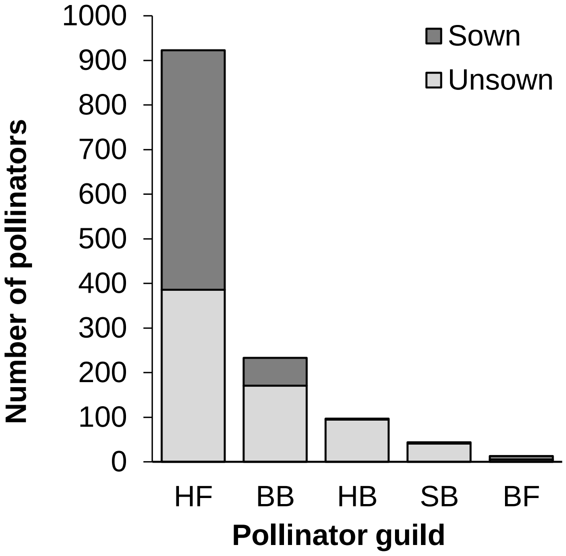

Figure 5.6. Total number of pollinating insects according to pollinator guild recorded visiting sown and unsown wildflower species on transect surveys of orchard alleyways throughout the three-year study. HF (Hoverfly), BB (Bumblebee), HB (Honeybee), SB (Solitary bee), BF (Butterfly).

Table 5.2. Mean number (± SE) of pollinator individuals and percentage within different pollinator guilds recorded per subsection on transect surveys of orchard alleyways throughout the three-year study according to activity. Percentages were calculated per activity, so that the sum of the four activities for each pollinator guild is 100%.

Pollinator guild	**Visiting wildflower**		**Visiting nectaries**		**Flying**		**Resting**	
	Mean	*%*	*Mean*	*%*	*Mean*	*%*	*Mean*	*%*
Honeybee	0.02 (± 0.002)	7.4	0.20 (± 0.01)	67.4	0.06 (± 0.004)	21.4	0.01 (± 0.004)	3.8
Bumblebee	0.05 (± 0.004)	28.7	0.03 (± 0.003)	18.9	0.09 (± 0.01)	50.3	0.004 (± 0.001)	2.1
Solitary bee	0.01 (± 0.002)	44.0	0.01 (± 0.001)	23.0	0.01 (± 0.001)	20.0	0.003 (± 0.001)	13.0
Hoverfly	0.19 (± 0.01)	28.2	0.003 (± 0.001)	0.5	0.30 (± 0.01)	44.8	0.18 (± 0.01)	26.5
Butterfly	0.002 (± 0.001)	5.4	0.00 (± 0.00)	0.0	0.03 (± 0.003)	77.3	0.01 (± 0.002)	17.3

5.4.3 Pollinating insect richness and diversity

In total, 104 different pollinator species were recorded throughout the growing season (April – September) over the three-year study (Appendix 5.4). 73 species were recorded during the blossom period, of those 18 were not recorded after. Whilst on transect surveys of orchard alleyways, 86 species were recorded, including 31 species which were only recorded from mid-May to September. Consequently, 55 species were found both during and after the blossom period. The 104 total species included one species of honeybee (*Apis mellifera*), ten bumblebee species, 33 species of solitary bee, 48 species of hoverfly, and 12 butterfly species. Pollinators in sweet cherry orchards were dominated by two single species, *A. mellifera* and *B. terrestris*, with ~30% of total records each. Of solitary bees, *Andrena haemorrhoa* was the most frequently recorded at 1.6%. *Episyrphus balteatus* was the most abundant hoverfly, being recorded on 3.5% of the total records, followed by *Eristalis pertinax* at 3.1%. Butterflies were the less frequent guild.

Only two species were recorded between the ranges of 3.0% and 4.0%, three species ranged the percentages of 2.0% and 3.0%, five species were recorded between 1.0% and 2.0%, whilst 92 species were rare and only recorded less than 1.0%, including 20 species that were solely recorded on a single occasion.

5.4.4 The influence of alleyway treatment on pollinating insects

5.4.4.1 During the cherry blossom period

The response of total pollinating insect abundance to alleyway treatment was affected by year (the most parsimonious model to analyse total pollinator abundance included the interaction between alleyway treatment and year) (Appendix 5.5). Accordingly, the most parsimonious model for all pollinator guilds except for bumblebees was also defined by the interaction between alleyway treatment and year (Appendix 5.5).

Overall, significantly more pollinators were recorded in association with Actively Managed Wildflower Strips (AMWS) than Control Strips (CS) or Standard Wildflower Strips (SWS); there was no difference between SWS and CS (Figure 5.7A; Appendix 5.6). Furthermore, in year three, a greater abundance was also recorded in AMWS compared to CS (Tukey test: $Z = 6.35$, $P < 0.001$) and SWS (Tukey test: $Z = -3.13$, $P < 0.01$), but more pollinators were associated with SWS than CS (Tukey test: $Z = 3.24$, $P < 0.01$) (Figure 5.7B). The greatest abundance of pollinators was recorded in year three with 2.0 (± 0.04) individuals per subsection sampled compared to 1.1 (± 0.02) and 1.2 (± 0.04) for years one and two respectively (Figure 5.7B; Appendix 5.6). Pollinator abundance in year two was also significantly greater compared to year one. The greater abundance in year three was due to an increased abundance of managed pollinators (honeybee and buff-tailed bumblebee) (Figure 5.8).

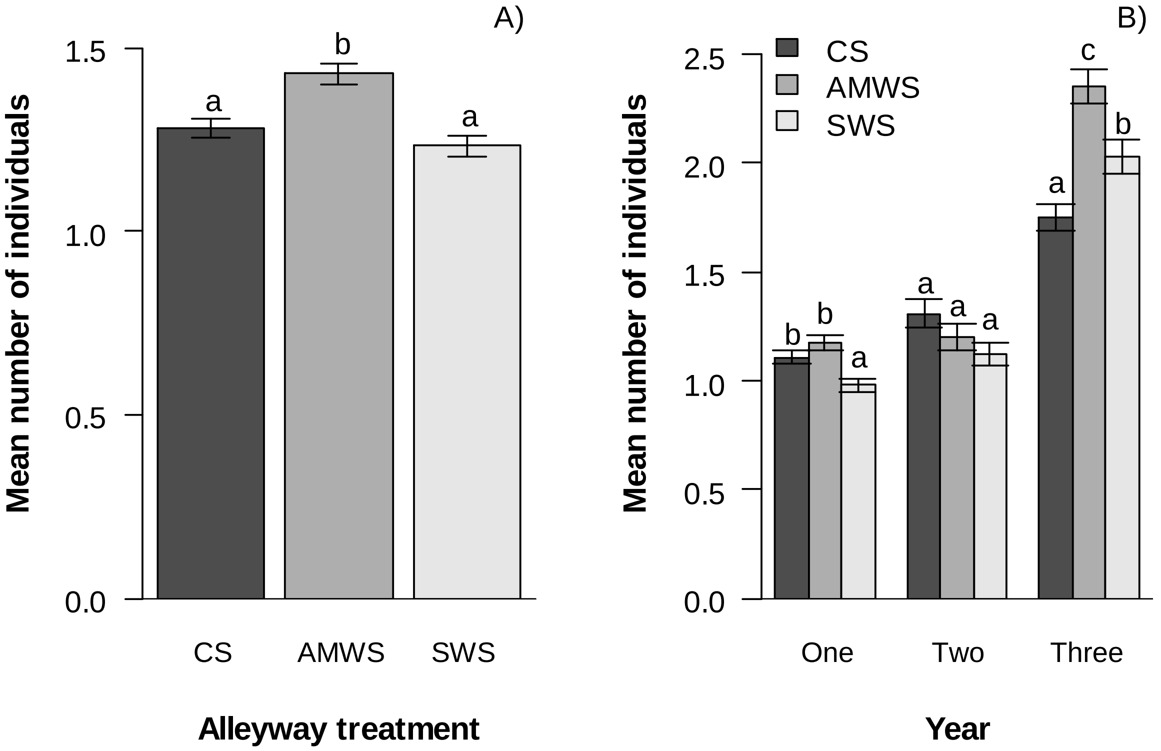

Figure 5.7. Mean number (± SE) of pollinator individuals recorded per subsection on the transect surveys of cherry blossoms across the three-year study A) according to alleyway treatment, and B) according to alleyway treatment and year. The same superscript letters indicate no significant differences according to the Tukey test (*P* > 0.05); for each category (year) in B). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

The abundances of buff-tailed bumblebees and solitary bees were affected by alleyway treatment and year (Figure 5.8; Appendix 5.5). A greater abundance of buff-tailed bumblebees was recorded in alleyways with AMWS compared to those containing SWS (Appendix 5.6). Whilst, solitary bee abundance was greater in association with CS than SWS (Appendix 5.6). No influence of alleyway treatment was found for the remaining pollinator guilds. Managed pollinators (honeybees and buff-tailed bumblebee) and wild solitary bees were more abundant in year three than in years one and two. Hoverflies were more frequently recorded in year two (Figure 5.8). Whilst butterfly abundance remained constantly low in all years.

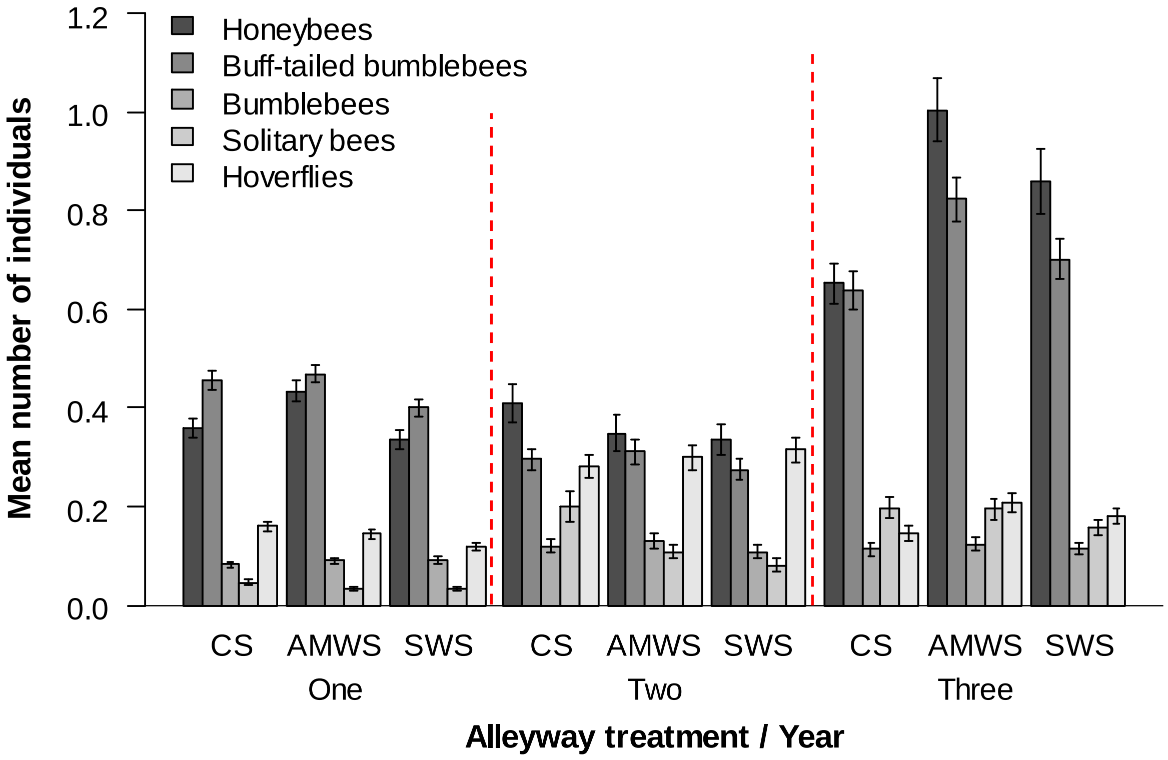

Figure 5.8. Mean number (± SE) of individuals for each pollinator guild recorded per subsection on the transect surveys of cherry blossoms according to alleyway treatment and year. Butterflies were excluded due to the low individuals recorded. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

The response of the abundance of total pollinating insects that were recorded visiting cherry blossoms to alleyway treatment strongly differed with time (Appendix 5.5). More pollinators were recorded visiting cherry blossoms on trees adjacent to AMWS (0.9 (± 0.02) pollinators per subsection) compared to CS and SWS, which recorded 0.8 (± 0.02), and 0.8 (± 0.02) pollinator visits, respectively. Visits to cherry blossoms were greater in year three compared to years one and two (Appendix 5.6). In year three, 1.3 (± 0.04) pollinators per subsection were recorded visiting cherry blossoms compared to 0.7 (± 0.02) in year one and 0.7 (± 0.03) in year two.

In contrast, the response of species richness and Shannon diversity to the three alleyway treatments was consistent between years, indicated by the most parsimonious model, which did not include the interaction (Appendix 5.5). AMWS and CS were associated with greater values of mean pollinator species richness and Shannon diversity than SWS (Table 5.3), but there was no significant difference between them (Appendix 5.6). Specifically for year three alone, a greater species richness was also recorded in AMWS compared to CS (Tukey test: $Z = 2.96$, $P < 0.01$) and SWS (Tukey test: $Z = -2.67$, $P < 0.05$), but there was no difference between CS and SWS (Appendix 5.6). There was also no difference between alleyway treatments for Shannon diversity in year three (Appendix 5.6). However, overall, year was an important factor influencing species richness and Shannon diversity (Appendix 5.6). Both the species richness and Shannon diversity values were greater in year three compared to years one and two; whilst diversity was greater in year two compared to year one (Table 5.3; Appendix 5.6).

Table 5.3. Values (± SE) of total species richness and Shannon diversity according to alleyway treatment and year. Values are based on transect surveys data of cherry blossoms. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Alleyway treatment	**Year one**		**Year two**		**Year three**	
	Species richness	Shannon diversity	Species richness	Shannon diversity	Species richness	Shannon diversity
CS	0.71 (± 0.08)	0.18 (± 0.01)	0.84 (± 0.12)	0.18 (± 0.01)	1.05 (± 0.12)	0.18 (± 0.01)
AMWS	0.74 (± 0.07)	0.18 (± 0.01)	0.82 (± 0.06)	0.18 (± 0.01)	1.21 (± 0.08)	0.19 (± 0.01)
SWS	0.64 (± 0.08)	0.19 (± 0.01)	0.77 (± 0.06)	0.19 (± 0.01)	1.06 (± 0.08)	0.18 (± 0.01)
Mean	0.70 (± 0.01)	0.10 (±0.003)	0.81 (± 0.02)	0.15 (±0.001)	1.11 (± 0.02)	0.21 (±0.001)

5.4.4.2 Post cherry blossom period

Total pollinator abundance in the orchard alleyways was inconsistent between years according to alleyway treatment, determined by the interaction between alleyway treatment and year (Appendix 5.7). A significant interaction was also found between alleyway treatment and year for three of the pollinator guilds (bumblebees, hoverflies, and butterflies). In contrast, the model for honeybees and solitary bees was more parsimonious with no interaction (Appendix 5.7). The total abundance of pollinators post cherry blossom was strongly influenced by the alleyway treatment (Figure 5.9A; Appendix 5.8). Overall, the total number of pollinators recorded in AMWS and SWS was greater than in CS. However, the number of pollinating insects recorded differed with time

(Appendix 5.8), and no differences were recorded in year one (baseline year) (Figure 5.9B). In addition, more pollinators were recorded in SWS compared to AMWS, mainly due to hoverflies (Figure 5.10).

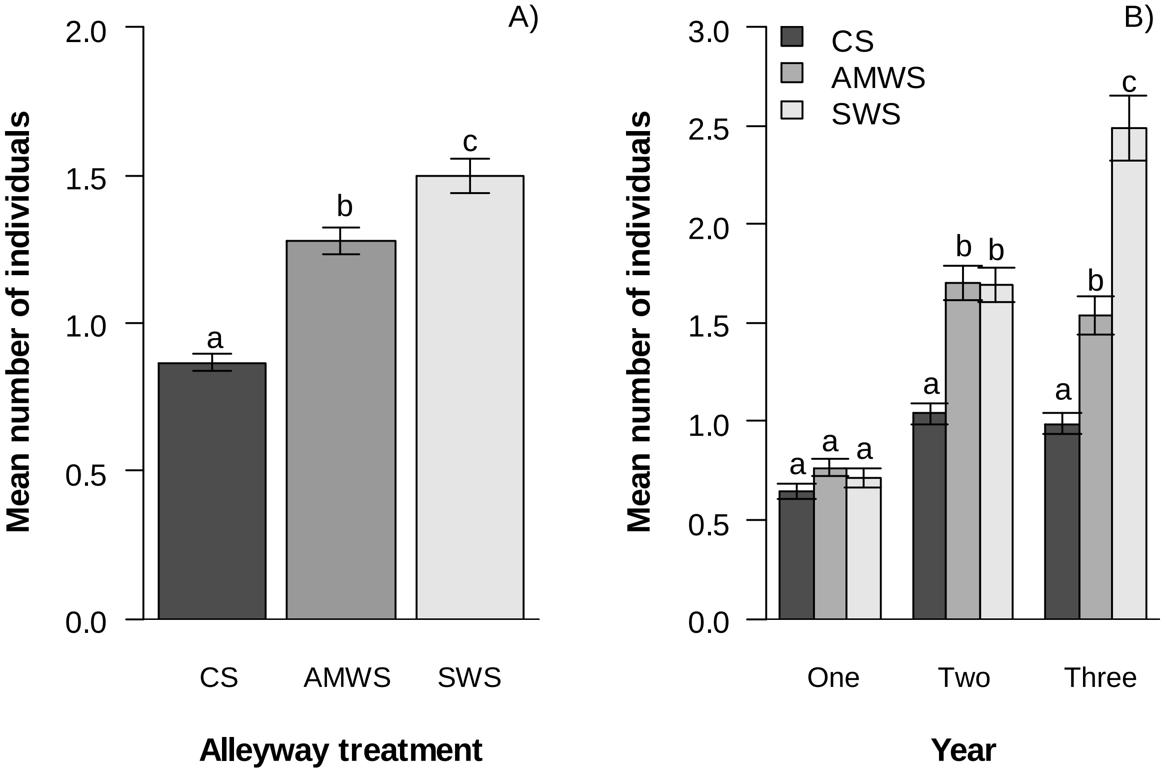

Figure 5.9. Mean number (± SE) of pollinator individuals recorded per subsection on the transect surveys of orchard alleyways across the three-year study A) according to alleyway treatment, and B) according to alleyway treatment and year. The same superscript letters indicate no significant differences according to the Tukey test (*P* > 0.05); for each category (year) in B). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Hoverflies were strongly influenced by alleyway treatment (Figure 5.10; Appendix 5.7). The greatest abundance was recorded in SWS with 0.9 (± 0.05) hoverflies per subsection compared to 0.7 (± 0.03) in AMWS and 0.4 (± 0.02) in CS. Hoverfly abundance in AMWS was also significantly higher than in CS. Honeybees were also affected by alleyway treatment (Appendix 5.7) and 0.1 individuals per subsection more were recorded in AMWS and SWS compared to CS (Appendix 5.8). Bumblebee abundance was only significantly higher in SWS with 0.21 (± 0.01) individuals per subsection compared to 0.16 (± 0.01) recorded in CS (Appendix 5.8). Their abundance in AMWS, which was 0.18 (±

0.01) individuals per subsection, was slightly higher compared to CS, but this was not significant. The greatest abundance of solitary bees was recorded in AMWS and the lowest in SWS by 0.02 (± 0.002) solitary bees per subsection, but there was no difference between AMWS and CS, nor SWS and CS (Appendix 5.8). Butterfly abundance was not influenced by alleyway treatment. Bumblebees (0.3 (± 0.02) individuals per subsection), and hoverflies (1.0 (± 0.1)) were more abundant in year three than in previous years (Figure 5.10). The abundances of honeybees, solitary bees, and butterflies were the greatest in year two with 0.4 (± 0.02), 0.05 (± 0.01), and 0.1 (± 0.01), respectively, compared to years one and three.

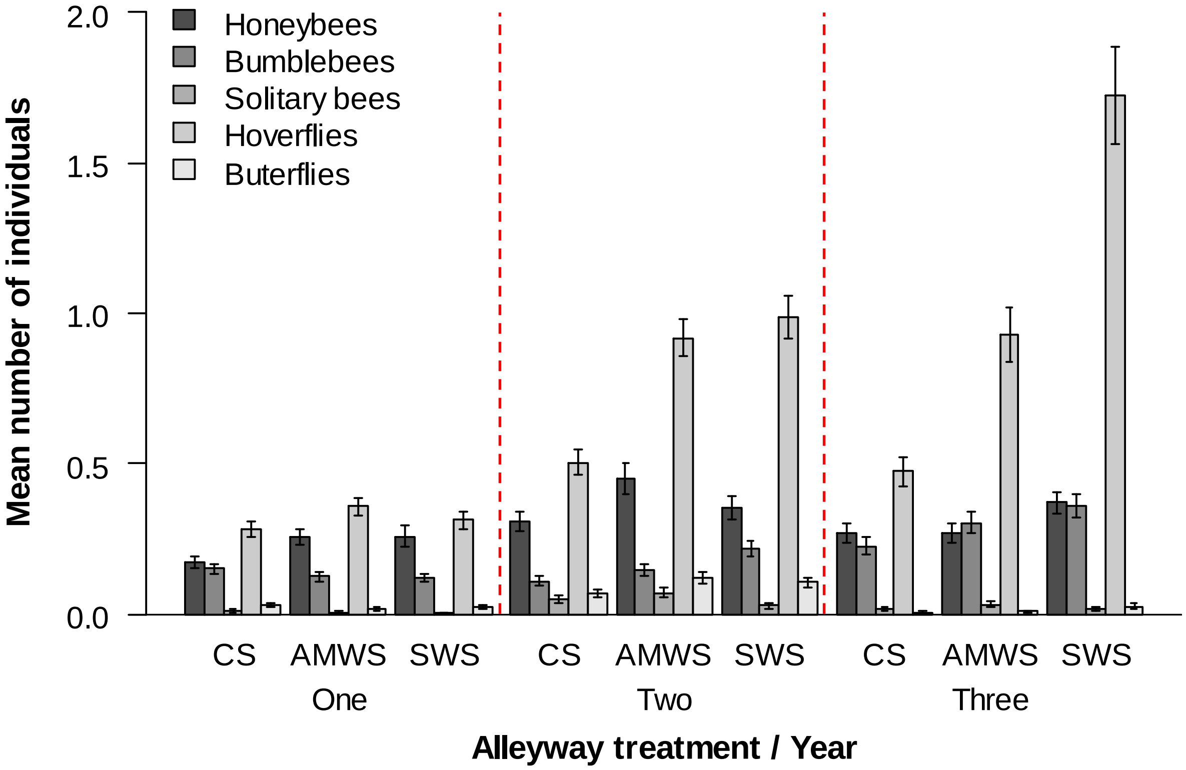

Figure 5.10. Mean number (± SE) of pollinator individuals per guild recorded per subsection on the transect surveys of orchard alleyways according to alleyway treatment and year. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

The total number of pollinators recorded as visiting wildflowers differed between alleyway treatments and also varied with time (interaction between alleyway treatment and year) (Appendix 5.7). Overall, a greater abundance was recorded in SWS, with 0.5 (± 0.04) pollinators per subsection compared to 0.3 (± 0.03) in AMWS, and 0.1 (± 0.01) in CS

(Appendix 5.8). The number of pollinators visiting wildflowers was greater in years two (0.3 (± 0.02)) and three (0.6 (± 0.05)) compared to year one (0.1 (± 0.01)).

The models for species richness and Shannon diversity were more parsimonious with the interaction between alleyway treatment and year, indicating that responses were not consistent between years according to alleyway treatment (Appendix 5.7). Both species richness and values of Shannon diversity were affected by alleyway treatment (Appendix 5.7). In AMWS and SWS a greater number of species were recorded compared to CS, and significantly more were recorded in SWS compared to AMWS (Appendix 5.8). Similarly, values of Shannon diversity were also higher in wildflower strips (AMWS and SWS) compared to CS, but no difference between wildflower strip treatments was found. The number of pollinator species recorded increased significantly each year (Table 5.4). Accordingly, Shannon diversity increased between years (Table 5.4), but there was no significant difference between years two and three (Appendix 5.8).

Table 5.4. Values (± SE) of total species richness and Shannon diversity per year according to alleyway treatment recorded on transect surveys of orchard alleyways. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Alleyway treatment	**Year one**		**Year two**		**Year three**	
	Species richness	Shannon diversity	Species richness	Shannon diversity	Species richness	Shannon diversity
CS	0.6 (± 0.1)	0.1 (± 0.02)	0.8 (± 0.1)	0.1 (± 0.03)	0.8 (± 0.1)	0.1 (± 0.02)
AMWS	0.6 (± 0.1)	0.1 (± 0.03)	1.1 (± 0.1)	0.2 (± 0.03)	1.1 (± 0.2)	0.2 (± 0.05)
SWS	0.6 (± 0.1)	0.1 (± 0.01)	1.1 (± 0.1)	0.3 (± 0.04)	1.7 (± 0.3)	0.4 (± 0.09)
Mean	0.6 (± 0.1)	0.1 (± 0.02)	1.0 (± 0.02)	0.2 (± 0.03)	1.2 (± 0.3)	0.2 (± 0.05)

5.4.5 The influence of edge effect on pollinating insects

5.4.5.1 During the cherry blossom period

Total pollinator abundance was affected by the distance from the orchard block edge (Appendix 5.5). The models showed that pollinators were recorded more frequently at the edge of orchard blocks, decreasing towards orchard centres (Figure 5.11; Appendix 5.9). The abundances of honeybees, bumblebees, solitary bees, and hoverflies were also influenced by the edge (Appendix 5.5), all being higher at the edge of the orchard block closest to the orchard edge and decreased towards the centre of orchards.

Distance from the orchard block edge was also a significant factor influencing pollinator visits to cherry blossoms (Appendix 5.5), with more visiting cherry blossoms near the edge (Figure 5.11; Appendix 5.9). A greater species richness and Shannon diversity of pollinating insects was also recorded at the edge of the orchard blocks (Figure 5.11; Appendix 5.9).

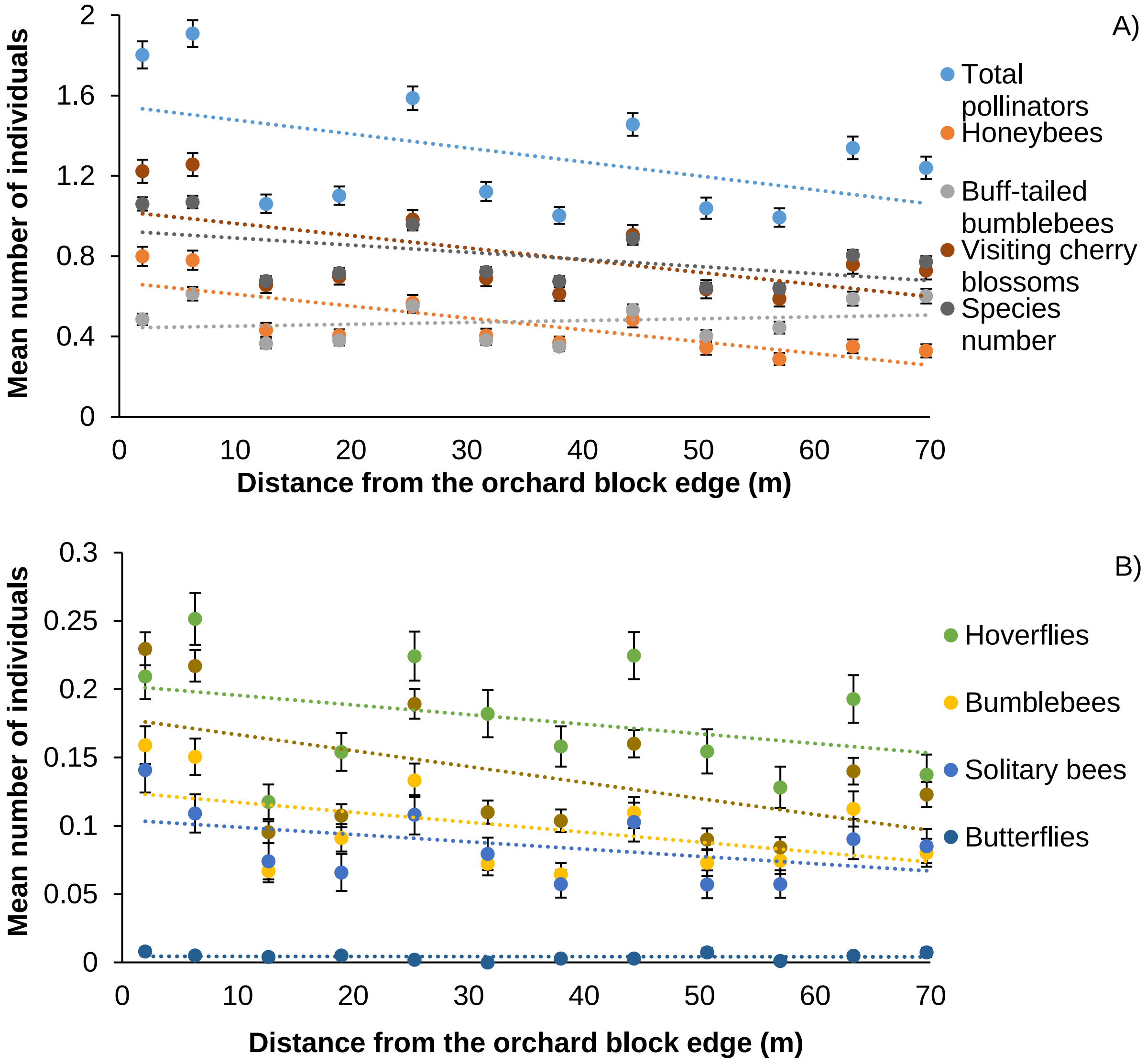

Figure 5.11. A) and B) Response (± SE) of the total pollinators, pollinator guilds, pollinators visiting cherry blossoms, species richness, and Shannon diversity to distance from the orchard block edge recorded on transect surveys of cherry blossoms in the three-year study. Buff-tailed bumblebees and butterflies are included but they were not significantly affected by the edge.

5.4.5.2 Post cherry blossom period

Total pollinator abundance was affected by proximity to the orchard block edge (Appendix 5.7). A greater number of pollinating insects was recorded at the edge of the orchard blocks, which decreased towards the centre (Figure 5.12; Appendix 5.9). This was consistent with honeybees, solitary bees, hoverflies, and butterflies. Pollinators visiting wildflowers were also more abundant near the edge. Similarly, distance from the orchard block edge also affected both species richness and Shannon diversity (Appendix 5.7) and greater values were registered close to the orchard block edge (Figure 5.12; Appendix 5.9).

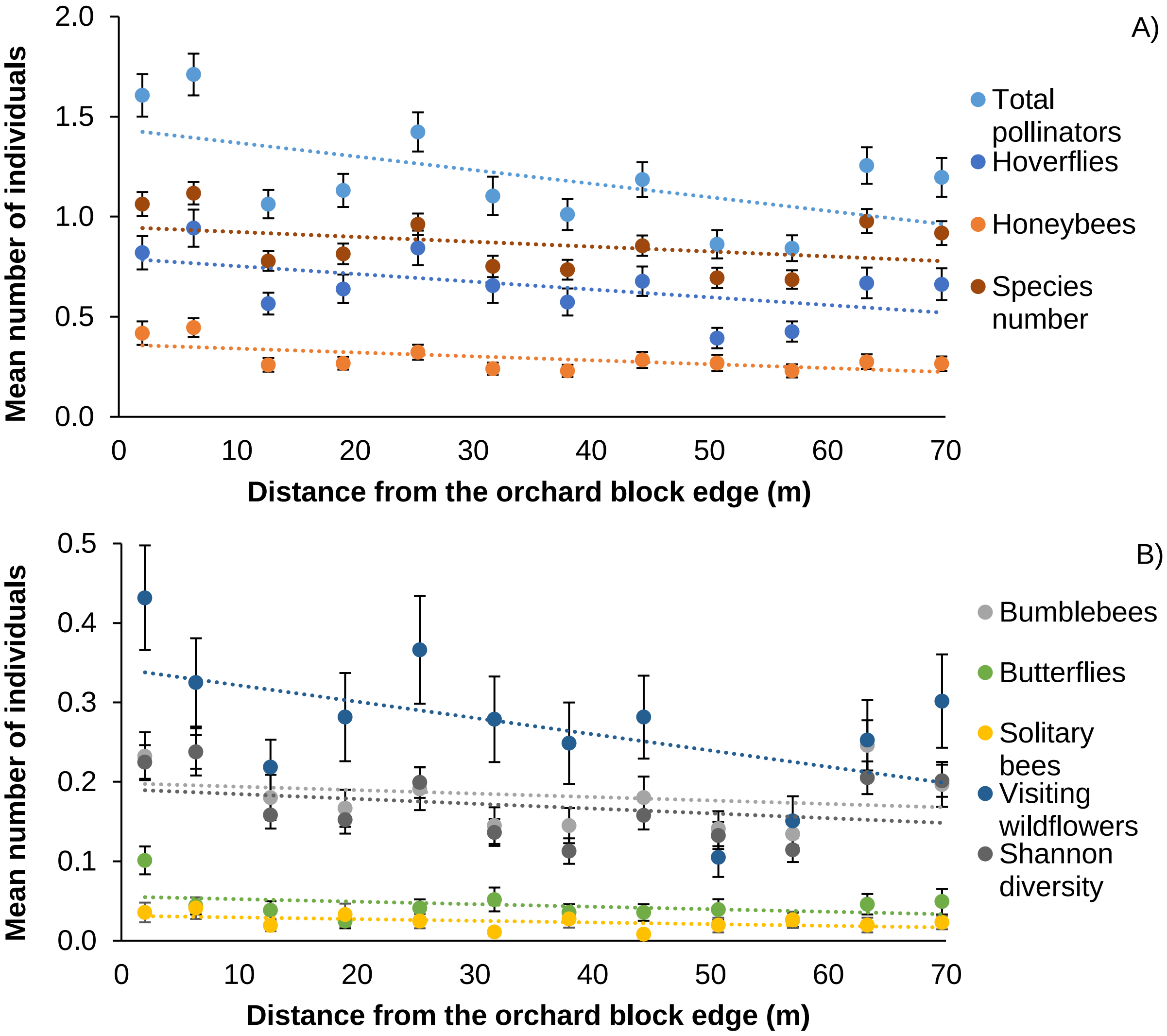

Figure 5.12. A) and B) Response (± SE) of the total pollinators, pollinator guilds, pollinators visiting wildflowers, species richness, and Shannon diversity to distance from the orchard block edge recorded on transect surveys of orchard alleyways in the three-year study. Bumblebees are included but they were not significantly affected by the edge.

5.4.6 The influence of survey time on pollinating insects

5.4.6.1 During cherry blossom period

The time of day pollinator surveys were done was also an important factor influencing pollinator abundance (Appendix 5.5). The number of pollinators recorded decreased through the day (Figure 5.13; Appendix 5.10). However, during year one, the trend was the opposite and more pollinators were recorded in the surveys conducted in the afternoon. Only honeybee and hoverfly abundances were affected by the survey time (Appendix 5.5). For both guilds, more individuals were recorded during the first surveys of the day and numbers were lower in the afternoon surveys (Figure 5.13).

Pollinators visiting cherry blossoms were also affected by the time of day (Appendix 5.5), and cherry blossoms were more visited in the morning (Figure 5.13; Appendix 5.10). Species richness and Shannon diversity were not affected by the time of surveys (Appendix 5.5).

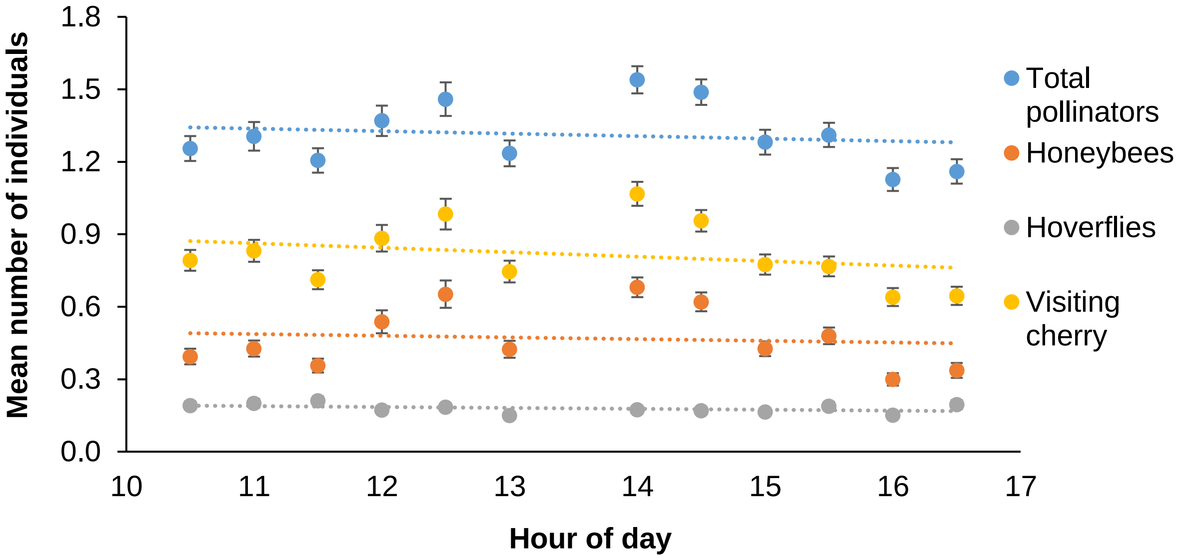

Figure 5.13. Response (± SE) of the total pollinators, honeybees, hoverflies, and pollinators visiting cherry blossoms to survey time (hour of day the survey took place) recorded on transect surveys of cherry blossoms throughout the three-year study.

5.4.6.2 Post cherry blossom period

The total number of pollinators recorded was not influenced by the time of day the survey took place (Appendix 5.7). However, most of the pollinator guilds were affected individually by survey time (Appendix 5.7). The three bee guilds (honeybees, bumblebees, and solitary bees) were more abundant in the afternoon surveys, in contrast to hoverflies, which were more often recorded in the morning (Figure 5.14; Appendix 5.10).

The abundance of pollinators visiting wildflowers was also influenced by the time of day surveyed (Appendix 5.7); more pollinators were recorded visiting wildflowers during the morning surveys (Figure 5.14; Appendix 5.10). Species richness and Shannon diversity did not vary throughout the day (Appendix 5.7).

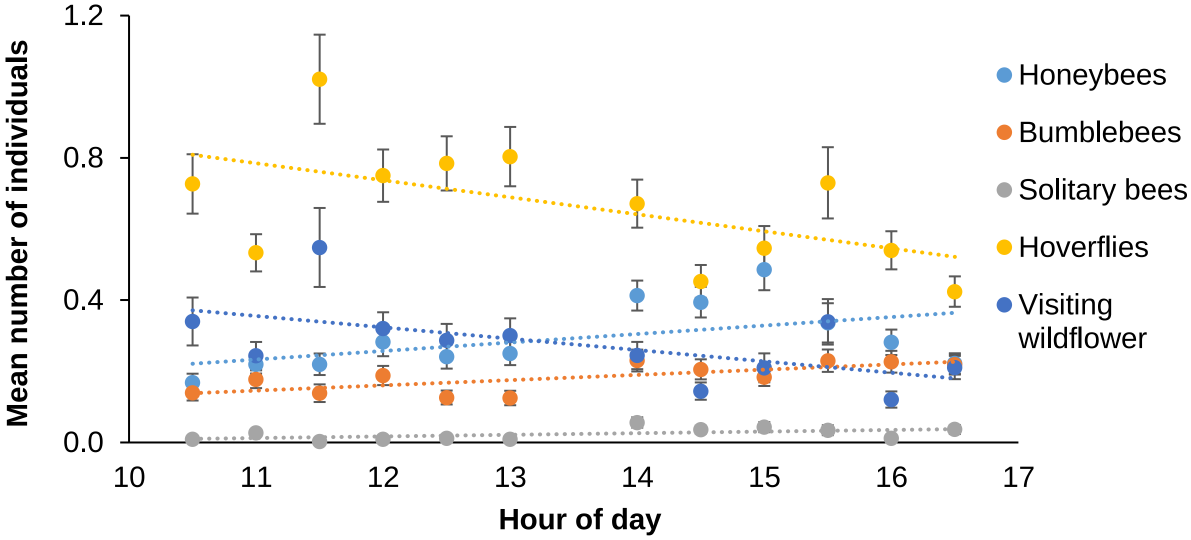

Figure 5.14. Response (± SE) of honeybees, bumblebees, solitary bees, hoverflies, and pollinators visiting wildflowers to survey time (hour of day the survey took place) recorded on transect surveys of orchard alleyways throughout the three-year study.

5.4.7 Pollinating insect efficacy to pollinate cherry blossoms

Across years two and three and all alleyway treatments, a total of 534 honeybees, 298 buff-tailed bumblebees, 116 bumblebees (Figure 5.15A), 134 solitary bees (Figure 5.15B), and 92 hoverflies (Figure 5.15C) were recorded during the stationary timed visitation surveys.

Figure 5.15. Wild pollinators visiting cherry blossoms: A) bumblebee (queen) (*Bombus terrestris*), B) solitary bee (*Andrena haemorrhoa*), and C) hoverfly (*Platycheirus albimanus*).

5.4.7.1 Visitation time (duration of pollinator visit per flower)

The time that pollinators spent on a single flower differed between pollinator guilds (Appendix 5.11). Visit duration, however, was not affected by alleyway treatment, but values were inconsistent between years (defined by the interaction term between visitation time and year). Solitary bees and hoverflies spent more time on average per flower than other pollinator guilds at 20.7 (± 2.0) and 14.8 (± 3.3) seconds per flower, respectively (Figure 5.16). In contrast, bumblebees spent the shortest time at 4.8 s (± 0.3) per blossom.

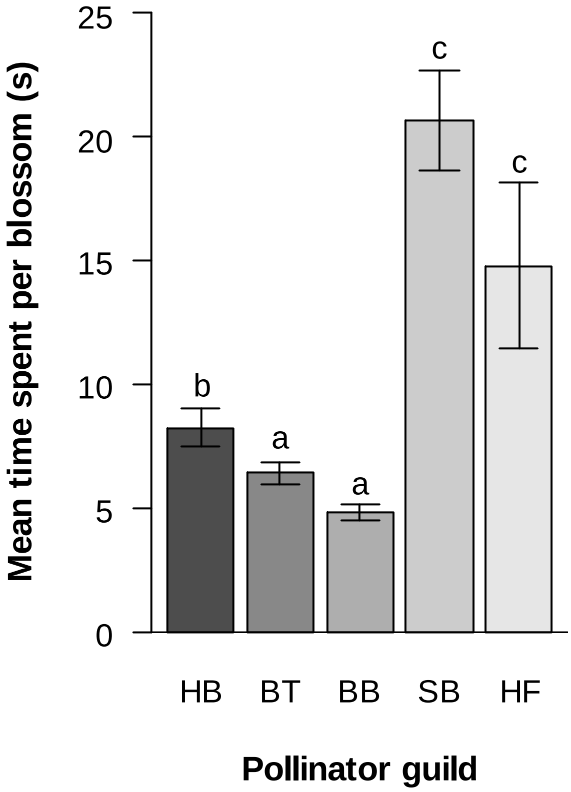

Figure 5.16. Mean time (± SE) spent (s) per individual cherry blossom on the stationary timed visitation surveys in years two and three combined. The same superscript letters indicate no significant differences according to the Tukey test (*P* > 0.05). HB (Honeybee), BT (Buff-tailed bumblebee), BB (Bumblebee), SB (Solitary bee), HF (Hoverfly).

5.4.7.2 Flowers visited per tree (number of flowers visited during the visit)

The number of individual blossoms visited by pollinators per cherry tree differed between guilds but was not affected by alleyway treatment (Appendix 5.11). The response was consistent between years (no interaction between alleyway treatment and year). Bumblebees visited more flowers during each survey than any other pollinator guild at 7.4 (± 0.7) (Figure 5.17). In contrast, hoverflies were only recorded visiting an average of 1.8 (± 0.1) blossoms on each tree surveyed.

The mean number of blossoms visited on each tree was also influenced by the time of day the survey took place (GLMER.NB: 0.02 ± 0.01, $Z = 2.02$, $P < 0.05$). Approximately 1.2 more blossoms were visited during the afternoon surveys.

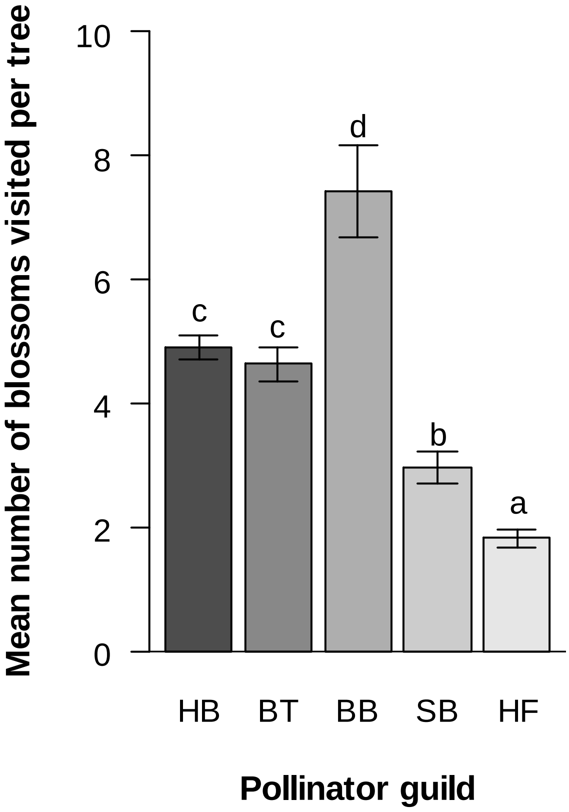

Figure 5.17. A) Mean number (± SE) of blossoms visited per cherry tree on the stationary timed visitation surveys in years two and three combined. The same superscript letters indicate no significant differences according to the Tukey test ($P > 0.05$). HB (Honeybee), BT (Buff-tailed bumblebee), BB (Bumblebee), SB (Solitary bee), HF (Hoverfly).

5.4.7.3 Visitation rate (flowers visited per minute)

Visitation rates differed according to pollinator guild but were similar between alleyway treatments (Appendix 5.11). Visitation rates according to alleyway treatment were consistent between years two and three (most parsimonious model did not include interaction between alleyway treatment and year). Bumblebees visited significantly more cherry blossoms per minute, recording a mean of 19.0 (± 1.3) (Figure 5.18). In contrast, solitary bees visited an average of 6.9 (± 0.7) blossoms per minute.

The number of blossoms visited per minute also differed between years (GLMER.NB: $Z = -11.96$, $P < 0.001$) (Appendix 5.11). In year three, fewer blossoms were visited per minute with a mean of 11.0 (± 0.4) compared to 19.4 (± 0.7) in year two.

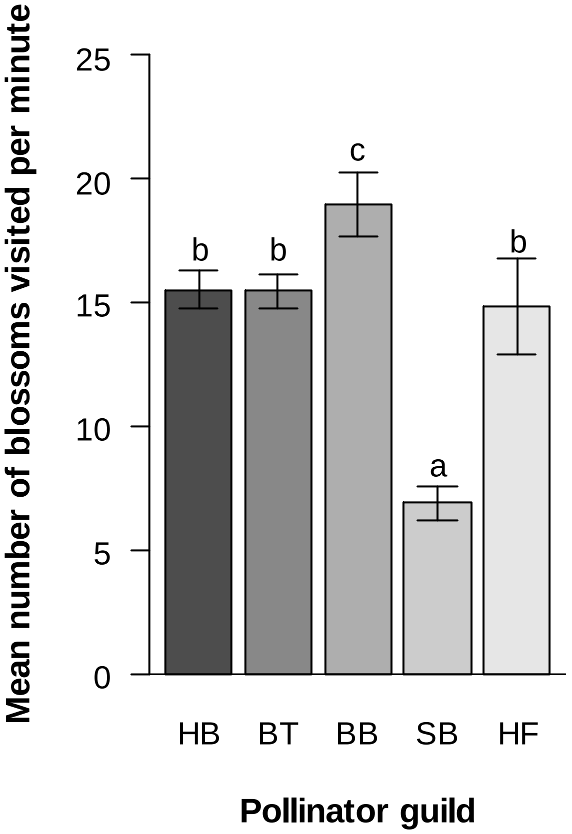

Figure 5.18. Mean number (± SE) of cherry blossoms visited per minute (visitation rate) on the stationary timed visitation surveys in years two and three combined. The same superscript letters indicate no significant differences according to the Tukey test ($P > 0.05$). HB (Honeybee), BT (Buff-tailed bumblebee), BB (Bumblebee), SB (Solitary bee), HF (Hoverfly).

5.4.7.4 Visit duration (time spent during the visit)

The duration of visits to cherry blossoms according to alleyway treatment was inconsistent between years (most parsimonious model with interaction). Visit duration was affected by pollinator guild, but not by alleyway treatment (Appendix 5.11). Solitary bees spent most time on cherry trees visiting blossoms averaging 56.6 seconds (± 5.5), followed by honeybees (44.7 (± 2.4) s) (Figure 5.19). Visit duration was strongly influenced by year (GLMER.NB: $Z = 10.92$, $P < 0.001$). Pollinators spent more time on trees in year three (51.6 s (± 2.3)) compared to year two (25.4 s (± 1.6)).

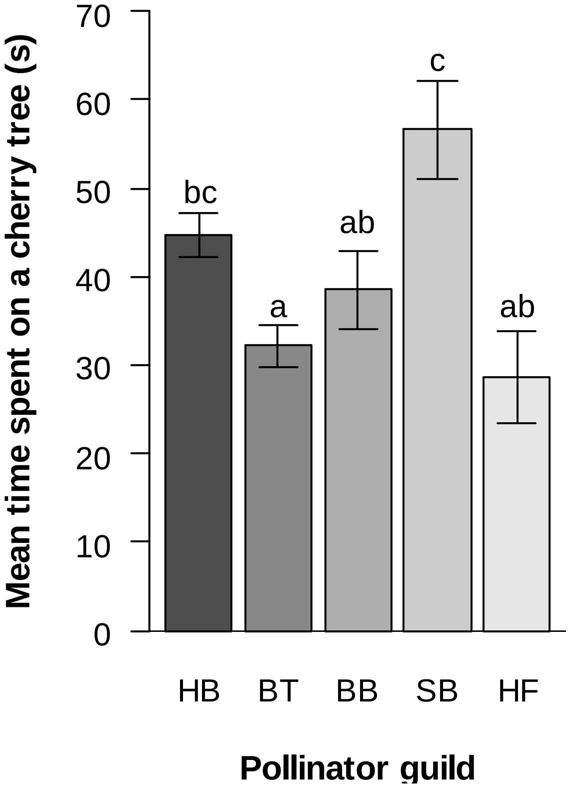

Figure 5.19. Mean time (± SE) spent (s) on each cherry tree (duration of the visit) on the stationary timed visitation surveys in years two and three combined. The same superscript letters indicate no significant differences according to the Tukey test ($P > 0.05$). HB (Honeybee), BT (Buff-tailed bumblebee), BB (Bumblebee), SB (Solitary bee), HF (Hoverfly).

5.4.7.5 Stigma contact

The number of times pollinators contacted the stigma varied between pollinator guilds but remained constant between alleyway treatments (Appendix 5.11). The stigma contact of cherry blossoms according to alleyway treatment was consistent between years. Bumblebees and solitary bees made contact with the stigma most of the times they visited cherry blossoms (Table 5.5). The percentage of visits that led to stigma contact also varied between years (Appendix 5.11). On average, pollinators made more contact with the stigma in year three (78.8% (± 1.7)) than in year two (67.7% (± 2.3)) (GLMER: $Z = 4.21$, $P < 0.001$).

Table 5.5. Percentage (± SE) of pollinators contacting the stigma of cherry blossoms across years two and three combined. The same superscript letters indicate no significant differences according to the Tukey test ($P > 0.05$).

Pollinator guild	**Honeybee**	**Buff-tailed bumblebee**	**Bumblebee**	**Solitary bee**	**Hoverfly**
Percentage of stigma contact	69.9% (± 2.2)^{a}	70.9% (± 2.8)^{a}	84.7% (± 3.4)^{b}	88.0% (± 2.9)^{b}	68.9% (± 5.0)^{ab}

Solitary bees contacted cherry stigmas with more body parts than the other pollinator guilds, and particularly with the head (Table 5.6). Hoverflies contacted the stigma on almost every visit, primarily with the legs. Whilst managed pollinators contacted the stigma a similar number of times, predominantly with the legs and sternum.

Table 5.6. Percentage (± SE) of three different pollinator body parts that contacted the stigma across years two and three. Due to the potential for pollinators to contact the stigma with more than one body part during the same visit, total values can exceed 100%.

Pollinator guild	**Head contact %**	**Legs contact %**	**Sternum contact %**
Honeybee	29.8 (± 1.5)	71.7 (± 1.6)	71.8 (± 1.6)
Buff-tailed bumblebee	35.1 (± 1.5)	69.6 (± 1.6)	74.9 (± 1.6)
Bumblebee	51.7 (± 1.5)	72.2 (± 1.6)	79.0 (± 1.6)
Solitary bee	67.1 (± 1.5)	91.4 (± 1.7)	80.5 (± 1.7)
Hoverfly	17.5 (± 1.5)	94.4 (± 1.6)	15.3 (± 1.6)

5.4.7.6 *Pollinator feeding of pollen and/or nectar*

The feeding behaviour of pollinators differed between guilds, but it was not influenced by alleyway treatment (Appendix 5.11). The most parsimonious model to analyse pollinator feeding behaviour did not include the interaction between alleyway treatment and year, indicating that the response on pollinator feeding to alleyway treatment was consistent between years. Buff-tailed bumblebees and solitary bees had similar feeding behaviours by spending about 30% of their time in nectar collection, and so did bumblebees and honeybees, which spent ~50% (Figure 5.20). In contrast, hoverflies behaved markedly different from the bee guilds, spending most of their time feeding exclusively on pollen (89.0% (± 3.5) of the visits).

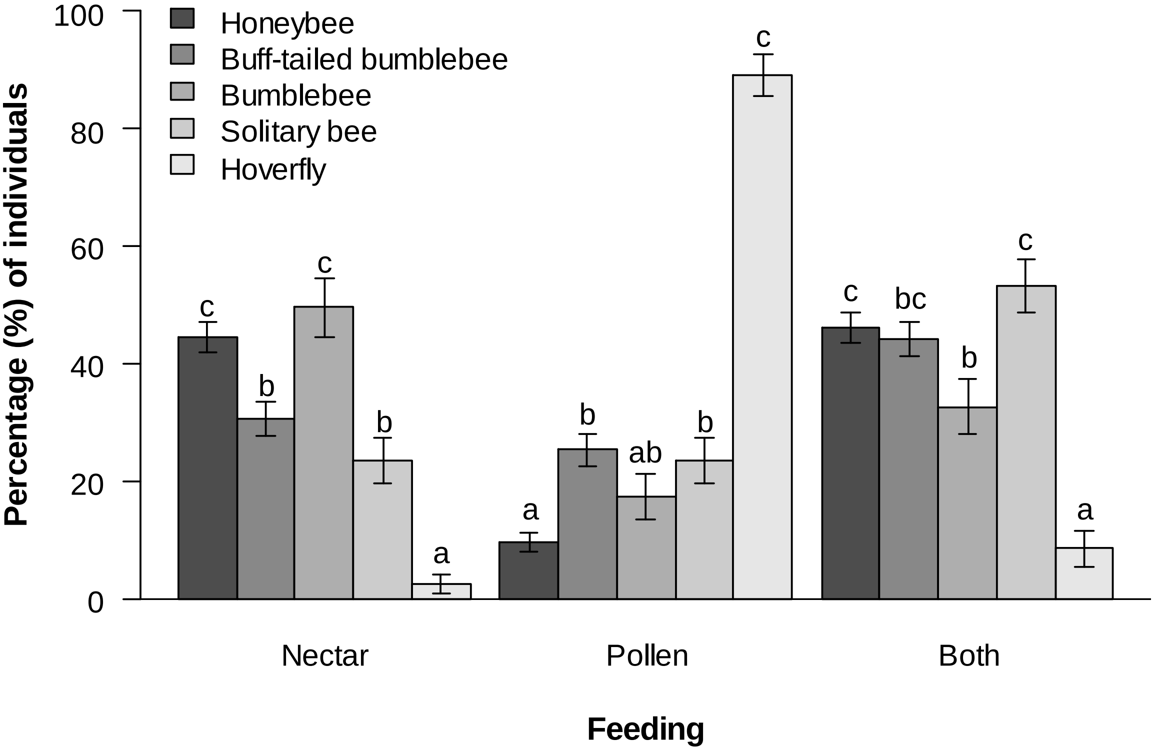

Figure 5.20. Percentage (± SE) of pollinator feeding of nectar, pollen or both (nectar and pollen) on cherry blossom recorded during the stationary timed visitation surveys in years two and three combined. The same letters above bars for each category (nectar, pollen, and both) indicate no significant differences according to the Tukey test (*P* > 0.05).

Pollen feeding was variable between years, and consequently, both (pollen and nectar) also varied. In year two, more pollen was collected than in year three, but in the latter year, pollinators also collected nectar during the same visit. Pollinator feeding behaviour was also affected by the time of day surveyed. Overall, nectar was collected more frequently in the morning whilst both pollen and nectar were more likely to be collected in the afternoon (nectar: GLMER: -0.04 ± 0.02, $Z = -2.15$, $P < 0.05$; pollen and nectar: GLMER: 0.04 ± 0.02, $Z = 2.03$, $P < 0.05$).

5.4.7.7 Flying behaviour for cross-pollination

The location of the tree subsequently visited after the surveyed tree significantly differed between pollinator guilds, but not between alleyway treatments (Appendix 5.11). Two of the flying behaviours, ‘stayed on tree’ and ‘same tree row’, were defined by the interaction between alleyway treatment and year, which indicated that their response to alleyway treatment varied between years. In contrast, the response of flying behaviours ‘adjacent tree row’ and ‘flew away’ was consistent between years. Buff-tailed bumblebees and bumblebees had similar flying behaviour, as did solitary bees and hoverflies (Figure 5.21).

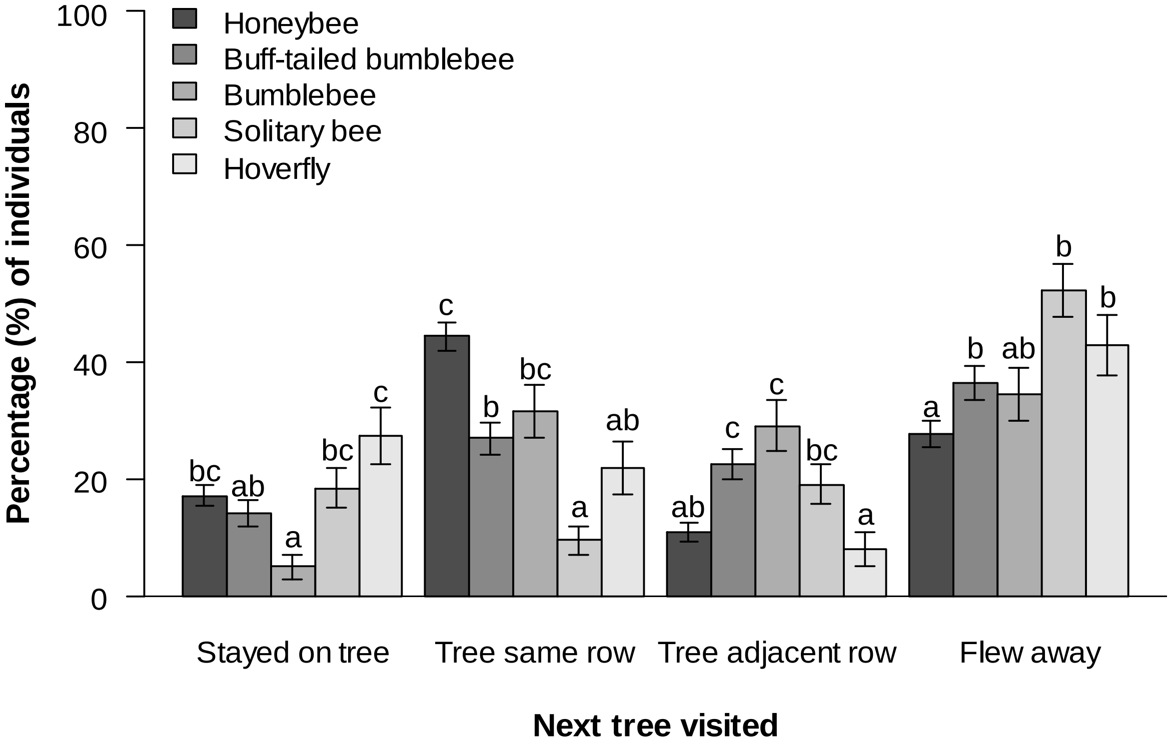

Figure 5.21. Percentage (± SE) of individuals according to location of the cherry tree subsequently visited recorded on the stationary timed visitation surveys in year two and three combined. The same letters above bars for each category (stayed on tree, same tree row, adjacent tree row, and flew away) indicate no significant differences according to the Tukey test ($P > 0.05$).

All bumblebees tended to forage in the same tree row, whilst solitary bees and hoverflies tended to fly away from the row within or outside the orchard blocks. Yet, buff-tailed bumblebees, bumblebees, and solitary bees were the pollinators which visited trees in the adjacent row more frequently. Honeybees had the most different flying behaviour and were more likely to visit trees in the same tree row than the other pollinator guilds, which accounted for 44.4% (± 2.4) of their flying behaviour. Honeybees and hoverflies were also the most infrequent pollinator guilds to visit a tree in the adjacent row after leaving the surveyed tree.

The time of day the survey took place affected whether pollinators stayed on the same cherry tree (GLMER: -0.05 ± 0.02, $Z = -2.87$, $P < 0.01$) or flew away (GLMER: 0.05 ± 0.02, $Z = 2.64$, $P < 0.01$) (Appendix 5.11). During the last surveys of the day, pollinators were more likely to be recorded flying away than during morning surveys. Furthermore, pollinators tended to remain on the same tree during surveys at the beginning of the day (until about 14 hrs).

5.4.8 Environmental factors

5.4.8.1 The influence of alleyway treatment on environmental factors

The responses of temperature, humidity, and wind speed to alleyway treatment were consistent between years, determined by the most parsimonious models, which did not include the interaction between alleyway treatment and year. Temperature and humidity recorded under protective covers were similar between alleyway treatments (Appendix 5.12). However, wind speed was influenced by alleyway treatment and significantly greater values were recorded in CS compared to AMWS (Tukey test: $Z = -3.71$, $P < 0.001$), but there was not significant difference compared to SWS.

Values of temperature, humidity, and wind speed varied between years (Appendix 5.12). Temperatures were generally lower in year one compared to years two and three. A lower average temperature was also recorded in year three compared to year two (Figure 5.22A; Table 5.7). In year three, humidity was lower compared to years one and two (Figure 5.22B; Table 5.7), whilst wind speed was greater in year one compared to year two (Figure 5.22C; Table 5.7).

Table 5.7. Pairwise comparisons (*Z* and *P* values) according to the *post-hoc* Tukey test between years for temperature, humidity, and wind speed recorded with a Kestrel weather meter on transect surveys during and post-blossom and stationary timed visitation surveys. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant.

Environmental factor	**Year one – Year two**	**Year one – Year three**	**Year two – Year three**
Temperature	Z = 23.02, **P < 0.001**	Z = 13.46, **P < 0.001**	Z = -7.80, **P < 0.001**
Humidity	Z = 1.23, P = 0.43	Z = -2.43, **P < 0.05**	Z = -3.10, **P < 0.01**
Wind speed	Z = -2.56, **P < 0.05**	Z = -1.71, P = 0.20	Z = 0.72, P = 0.75

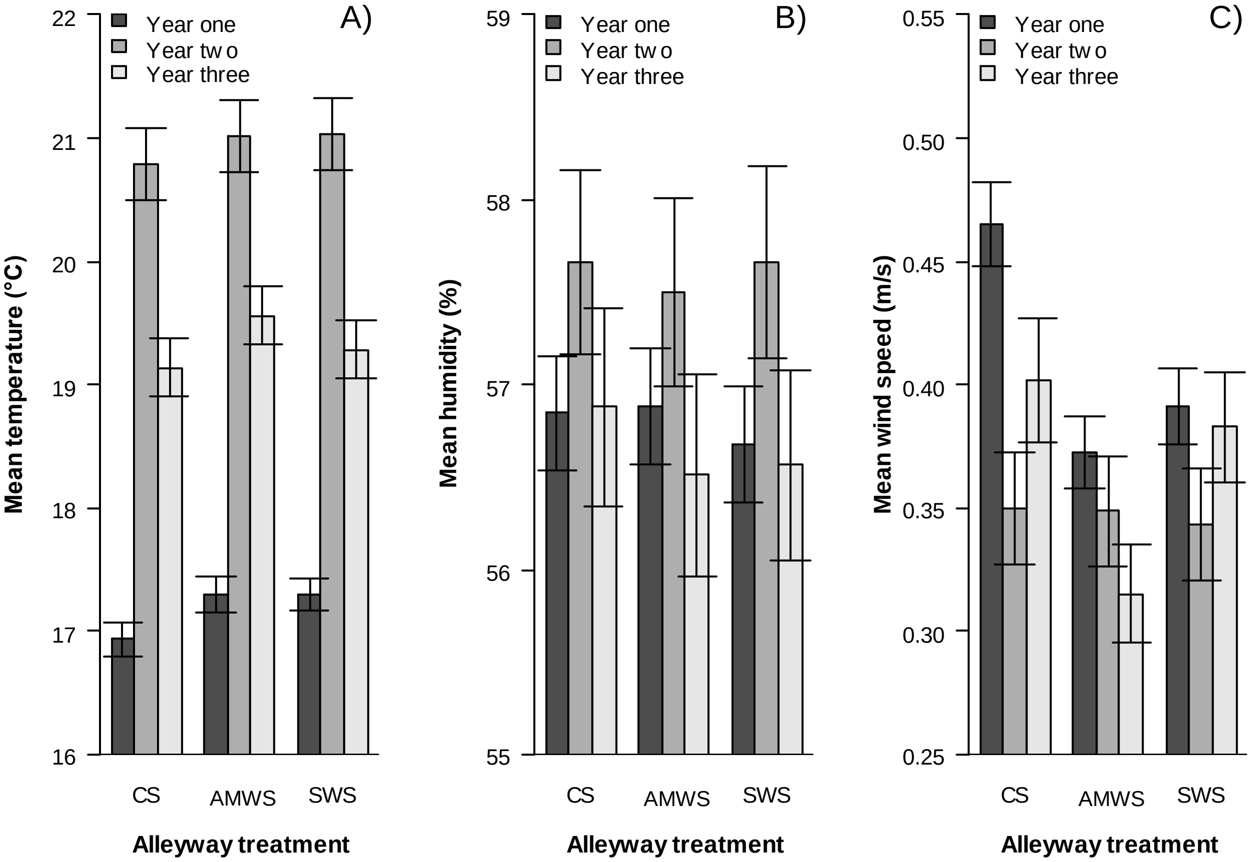

Figure 5.22. Mean (± SE) A) temperature, B) humidity, and C) wind speed recorded on transect surveys during and post-blossom and stationary timed visitation surveys according to alleyway treatment and year. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

5.4.8.2 The influence of edge effect on environmental factors

Environmental factors changed significantly with distance from the orchard block edge and varied between years (Appendix 5.12). However, the trend of each environmental factor remained constant for the three years. Overall, temperature increased towards the centre of the orchard block by around 0.8ºC, contrary to humidity and wind speed, factors which were higher at the edge of the orchard blocks by ~1.5% and 0.5 m/s, respectively (Figure 5.23; Table 5.8).

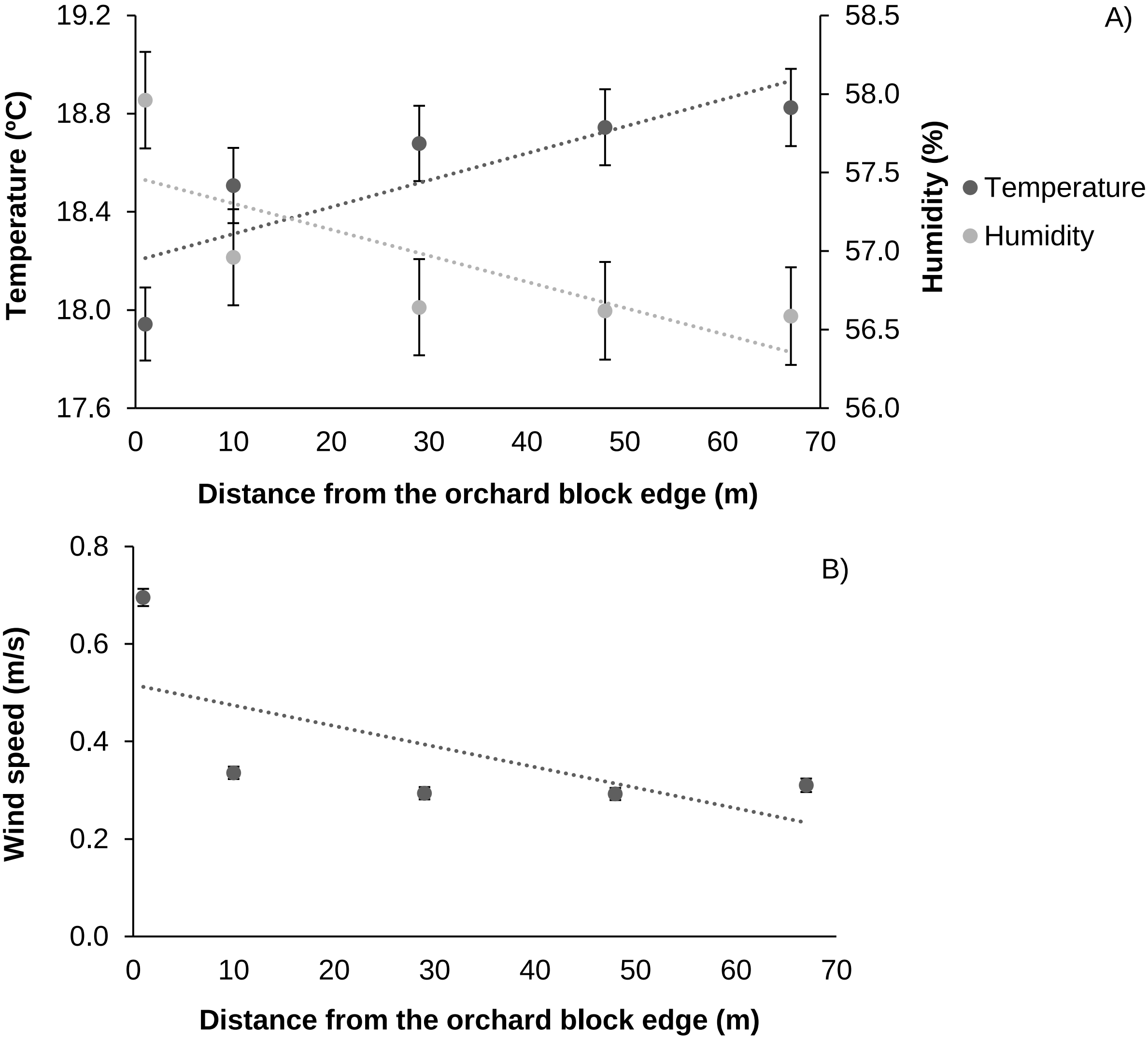

Figure 5.23. Response (± SE) of environmental factors A) temperature (ºC), humidity (%rh), and B) wind speed (m/s) to distance from the orchard block edge recorded with a Kestrel weather meter on transect surveys during and post-blossom and stationary timed visitation surveys throughout the three-year study.

Table 5.8. Response (estimated value ± SE, and *Z* and *P* values) of the orchard block edge and survey time according to the models used on temperature, humidity, and wind speed recorded with a Kestrel weather meter on transect surveys during and post-blossom and stationary timed visitation surveys. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant.

Environmental factor	**Generalized linear mixed model**
Distance from the orchard block edge	
Temperature	0.001 ± 0.0001, Z = 4.11, **P < 0.001**
Humidity	-0.0003 ± 0.0001, Z = -3.17, **P < 0.01**
Wind speed	-0.01 ± 0.001, Z = -13.56, **P < 0.001**
Survey time	
Temperature	0.01 ± 0.002, Z = 5.00, **P < 0.001**
Humidity	-00.1 ± 0.001, Z = -9.76, **P < 0.001**
Wind speed	-0.01 ± 0.01 = -0.56, P = 0.58

5.4.8.3 The influence of survey time on environmental factors

Temperature and humidity were significantly affected by the time of day surveyed during the blossom period, but wind speed was not (Appendix 5.12).

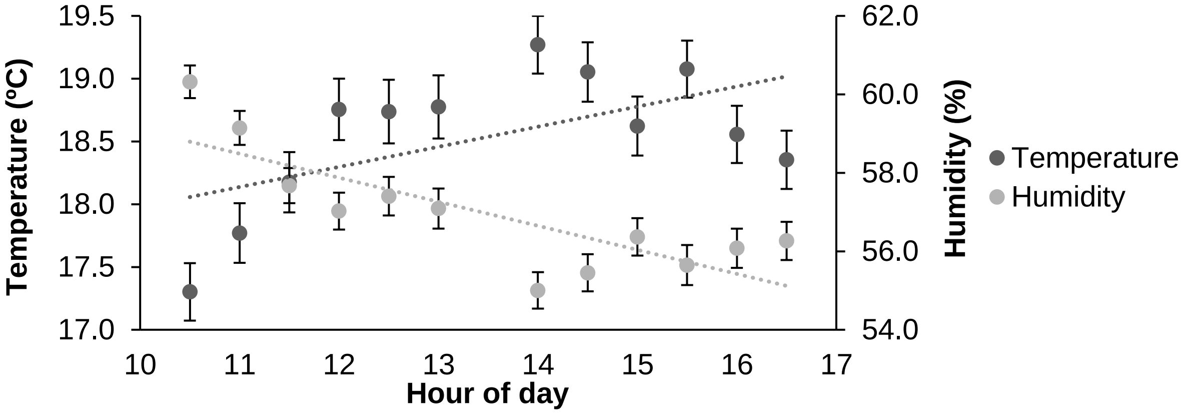

Figure 5.24. Response (± SE) of environmental factors temperature, and humidity to survey time (hour of day the survey took place) recorded with a Kestrel weather meter on transect surveys during and post-blossom and stationary timed visitation surveys throughout the three-year study.

Temperature increased with time during the surveys, whilst humidity decreased (Figure 5.24; Table 5.8). All three environmental factors remained constant between years.

5.5 Discussion

The key aim of this study was to investigate the range of insect pollinators that visited sweet cherry orchards and how they were influenced by the different alleyway treatments during and after the cherry blossom period. In addition, this study explored pollinating insect behaviour to investigate the efficacy of the different pollinator guilds at pollinating cherry blossoms as a proxy for the delivery of pollination services. The influence of edge effect and time of the day surveyed on pollinator behaviour, and environmental factors were also investigated.

5.5.1 Pollinating insects and pollination services in sweet cherry orchards

Managed pollinators dominated pollination services in sweet cherry orchards. *Apis mellifera* contributed to 33.0% of total records and *Bombus terrestris* (buff-tailed bumblebee) 29.0%, of which 89.7% were for managed *B. terrestris* installed in the orchards by growers during the cherry blossom period. The remaining 10.3% composed were wild queen and male bumblebees. This shows the high reliance of growers on the use of managed pollinators to pollinate sweet cherry. However, relying on a single or few species presents a risk to fruit production (Goulson, 2003). In addition, *A. mellifera* hives and *B. terrestris* colony boxes involve an on-going cost to growers for pollination services. Within wild pollinators, *Andrena* species were the most abundant solitary bees in this study, as found in pear (Fountain *et al.*, 2019) and apple orchards (McKerchar *et al*., 2020) in Kent, UK. Rosaceous fruit tree species have the same actinomorphic type of flower (Hummer & Janick, 2009) and similar blooming time early in spring (Wilkie *et al.*, 2008), which indicates that similar pollinators can visit rosaceous tree crop blossoms (Sedivy & Dorn, 2014). *Osmia* species, despite being considered an important pollinator to fruit orchard crops, including sweet cherry (Bosch *et al.*, 2006; Ryder *et al.*, 2019), was only recorded twice in the three years of study. The most abundant bumblebee species recorded in this study were also consistent with Fountain *et al.* (2019), suppoting that orchards are mostly visited by few common species (Kleijn *et al.*, 2015). Wildflowers may not meet the special requirements of rarer species, whose resource requirements are normally met outside of agricultural systems (Kleijn *et al.*, 2015).

Overall, pollinating insects responded positively to the wildflower habitats established in alleyways between rows of cherry trees. However, this response was only observed from year two, once the alleyway treatments were instigated and the sown wildflower strips had established successfully providing a greater abundance of floral resources (see Chapter 3). Due to continued cutting in year one of all alleyway treatments, preventing the flowering of the sown species, differences in pollinator abundance and richness between treatments during this baseline year was not expected (McKerchar *et al*., 2020).

5.5.2 The influence of wildflower interventions on pollinating insects

This is the first time the effects of sown wildflowers under protective covers on pollinating insects has been investigated. Wildflower strips were an effective approach to enhance wild pollinators in protected sweet cherry orchards, and this now provides a range of opportunities for other protected crops. The research has also demonstrated that wildflower interventions have potential to underpin a more sustainable approach towards the delivery of pollination services in sweet cherry, as found in other studies (Blaauw & Isaacs, 2014). The abundance and species richness of pollinating insects associated with the wildflower treatments increased over the three years. During the cherry blossom period, the 13.5% increase in abundance, 9.1% in species richness, and 17.2% in diversity recorded in AMWS and CS compared to SWS, could be a result of more nesting sites being available for bees. Greater vegetation in SWS (see Chapter 3) could have reduced access to the soil by ground nesting bees (Steffan-Dewenter & Tscharntke, 2000), whilst fewer resources in CS detracted bees to nest in those alleyways. Therefore, the novel approach of maintaining a height of 20 cm throughout the growing season is a promising management regime that can be implemented in orchard alleyways to minimise inconvenience to workers operating in the orchards and reduce the efficacy of spray applications, whilst enhancing the pollinator community.

Differences in pollinator abundance, richness and diversity, including more rare species, between wildflower strips and CS were more apparent after the blossom period. Due to the increase of 61.6% in abundance, 39.7% in species richness, and 81.8% in diversity recorded in AMWS and SWS compared to CS, pollinator abundance, richness and diversity could increase in subsequent years during blossom period if the wildflower strips were maintained (Blaauw & Isaacs, 2014). However, in order to meet pollen and nectar requirements for a greater range of pollinator species, more resources (e.g. more wildflower strips) may be needed (Dicks *et al*., 2015). Wildflower habitats could be used to improve pollination, by increasing abundance and diversity, which can lead to an increase the resilience of pollination services (Blaauw & Isaacs, 2014; Pywell *et al.*, 2015) and more efficient pollination of cherry blossoms, and consequently, better yields (Holzschuh *et al.*, 2012). Wild pollinating insects enhanced by wildflower habitats are therefore a promising alternative to managed pollinators, but benefits will take time to appear (Buhk *et al.*, 2018). The cost of wildflower establishment also has to be considered (Blaauw & Isaacs, 2014) (see Chapter 7).

5.5.3 Efficacy of pollinating insects to pollinate sweet cherry blossoms

This study also showed that pollinator behaviour in sweet cherry orchards differed between pollinator guilds. Solitary bees spent more time on individual cherry blossoms than other observed pollinator guilds. Solitary bees made a greater number of stigma contacts with more of their body parts, consequently increasing the likelihood of pollen deposition (Willmer *et al.,* 2017). Pollen grains are more often incidentally attached on solitary bees when collecting pollen or nectar due to the greater time spent on a flower (Woodcock *et al.*, 2013). These grains placed for instance, on the head or sternum, are dry and loose, and as a result, more accessible for the pistil (Woodcock *et al.*, 2013). Moreover, pollen collected by solitary bees on scopae is also fully available for pollination, rather than being collected and placed in the meta-tibial corbiculae (pollen baskets) (Parker *et al.*, 2015; Garratt *et al.*, 2016), which is moist and compact and consequently not normally available for pollination (Parker *et al.*, 2015). Importantly, solitary bees were the pollinator guild that visited fewer trees in the same row, which is important for cross-pollination of self-incompatible cultivars (Brittain *et al.*, 2013b). Bumblebees (queens) were also efficient visitors of cherry blossom since they visited more flowers than other pollinator guilds and, along with solitary bees, made more contact with the stigma. Due to the large size of queen bumblebees, these also made stigma contact with several body parts on each visit, increasing the likelihood of depositing available pollen on the stigma. In addition, bumblebees often visited cherry trees in adjacent rows. Consequently, solitary bees and bumblebees are probably more efficient pollinating insects of cherry blossoms than managed pollinators (Holzschuh *et al.*, 2012; Eeraerts *et al.*, 2020). *Bombus terrestris* workers are not efficient and in the longer time growers might be better advised to invest in wildflower interventions than managed bumblebees. In order to target and encourage wild pollinators, growers could implement orchard management strategies, including incorporation of wildflower strips in the alleyways of orchards.

5.5.4 Edge effects on pollinating insects

Pollinating insects were affected by the proximity of the floral resources to the edge of the cherry orchards even though wildflower resources were provided consistently along the alleyways. This is particularly important during the blossom period. More pollinators and greater species richness were recorded near the orchard edge, which might affect fruit set and, consequently, production along the tree rows (Nguyen & Nansen, 2018) (see Chapter 6). However, this trend may be as a result of the protective covers, since pollinator visits are affected by these (Hall *et al.*, 2019). In contrast, the even distribution of nest boxes of managed buff-tailed bumblebee along the alleyways meant this pollinator was recorded uniformly in the orchard blocks.

Reducing orchard size could be an alternative to ensure an even distribution of pollinators, but trade-offs between costs of orchard design and production should be considered. However, wild pollinator abundance and diversity in orchards can also be influenced by the surrounding landscape, particularly in conventional orchards (Kennedy *et al.*, 2013).

5.5.5 The influence of environmental factors and time of day surveyed on pollinating insects

The changes in the environmental factors throughout the day had an impact in pollinator abundance recorded during different times of day, particularly important during the sweet cherry blossom period. Differences in pollinator abundance due to time of day can indicate differences in foraging behaviour which can directly affect fruit set (Pisanty *et al.*, 2016). For example, the decrease in abundance of one of the key pollinators of sweet cherry, the honeybee, was affected by the time the survey took place, which could have been related to changes in environmental factors. Although temperature increased and humidity decreased with time during the surveys, this trend was observed approximately until 15 hrs. After that time, the decrease in temperature and increase in humidity coincided with the beginning of honeybee abundance decrease. Temperature can affect directly honeybee flying behaviour (Vicens & Bosch, 2000b), whilst nectar concentration in blossoms decreases with low humidity (Corbet *et al.*, 1979). Honeybees primary collected nectar and could have been therefore discouraged to visit cherry blossoms in late afternoon. Consequently, under colder and more humid conditions, honeybee abundance, and visits, decrease. This is especially important in sweet cherry as weather conditions during blossom in the West Midlands can be unfavourable for this pollinator. Particularly, since the number of honeybee visits can determine the success of the fruit set (Vicens & Bosch, 2000a; Garratt *et al.*, 2016). The overall greater pollinator visits to cherry blossoms, which directly impact fruit set, during the first surveys can be partly explained by nectar concentration but also it could have been biased by honeybee abundance. This greater number of pollinators recorded visiting cherry blossoms in the morning surveys is consistent with visitation times reported for the pollination of watermelon flowers (Pisanty *et al.*, 2016), which was associated with a lower fruit set in the afternoon. The even abundance of bees, which increased pollinator diversity, throughout the day suggests that collectively these pollinators can provide a resilient pollination service under daily changes in climate, since environmental factor effects can be minimised by enhancing pollinator diversity (Brittain *et al.*, 2013a). This could ensure adequate pollination, although more research is needed to confirm this in sweet cherry orchards. In addition, honeybee efficacy should be measured in the morning to determine whether this pollinator can pollinate sufficient cherry blossoms compensating the lower activity during the late afternoon.

5.5.6 Conclusions

This study has demonstrated that UK sweet cherry production is highly dependent on managed pollinators but relying on a small number of pollinating insect species could put production at risk. Solitary bees and wild bumblebees are efficient pollinators and their presence will increase the resilience of pollination services, in part because they are more efficient at pollinating cherry blossoms than managed pollinators. However, their influence on cherry production and quality still needs to be quantified. Wildflower strips in the alleyways of protected cherry orchards are an effective approach to enhance wild pollinator abundance and richness and improve pollination and resilience to environmental change.

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Chapter 6. Sweet cherry production: the role of pollination

6.1 Abstract

Sweet cherry is an economically important crop worldwide and production has increased in recent decades due to novel growing approaches and increasing demand. In order to underpin commercial yields, sweet cherry needs pollinating insects, which provide essential cross-pollination. Managed pollinators are often used for pollination, but wild pollinators often provide better pollination services. However, pollinators continue to decline and this could lead to pollination deficits. Wildflower habitats could be used to enhance wild pollinators in protected sweet cherry orchards to maximize production and avoid these deficits. Pollinator exclusion experiments were done to investigate the dependence of sweet cherry production on pollinating insects and detect potential pollination deficits. Fruit quality was also evaluated to investigate to what extent pollinating insects affected cherry quality over a three-year period. To investigate effects of wildflower habitat on pollination services, two wildflower strips in alleyways, one managed with a single cut in September (SWS) and another actively managed with regular cutting to a height of 20 cm (AMWS) were compared to untreated control alleyways (CS) under protective covers. In addition, to develop a protocol to help farmers assess pollination deficits in cherry, the frequency of hand pollination needed to maximise pollination was established.

Pollinating insects were key to achieve commercial yields and satisfactory fruit quality. Fruit set was approximately 30% on blossoms exposed to insect visits whilst only ~1% was achieved when insects were excluded. Furthermore, hand pollination resulted in ~50% fruit set, indicating pollination deficits in the study orchards. However, the presence of AMWS was shown to influence pollination services with more harvestable fruits being associated with this treatment compared to CS and SWS, although fruit quality was similar. Fruit set and quality responded to edge effects and greater yields were produced near the orchard edges, but with smaller fruits. This study showed sweet cherry is highly dependent on pollinating insects to achieve commercial yields, but production could be increased by up to 20% if different orchard pollinator management strategies were applied, such as the wildflower interventions deployed in this study.

6.2 Introduction

Sweet cherry is a commercially important fruit crop with an annual worldwide production of 2.56 million tonnes (FAO, 2020). Its production has increased in recent decades due to the adoption of novel approaches to improve cultivation, including high tunnels and dwarfing rootstocks (Bujdoso & Hrotko, 2017). Numerous studies have been conducted in sweet cherry to maximize yield and improve fruit quality over the years, including the development of new cultivars (Blažková, 2004; Sitarek & Grzyb, 2010; Ampatzidis & Whiting, 2013; Lang, 2014; Demirsoy *et al.*, 2017; Lillrose *et al.*, 2017; Quero-García *et al.*, 2017a; Vercammen *et al.*, 2019). Cultivars can be genetically modified to resist pests, diseases and viruses, spring frosts, fruit cracking, and reduce tree structure, but cultivars are also developed to meet marketable demands, such as flavour (Blažková, 2004; Lang *et al.*, 2011; Quero-García *et al.*, 2017b). There are hundreds of sweet cherry cultivars, and some are considered of global importance (e.g. c.v. Kordia) (Quero-García *et al.*, 2017b). Pollinating insects are key, providing successful cross-pollination to sweet cherry blossoms (Koumanov & Long, 2017; Radunic *et al.*, 2017), since most sweet cherry cultivars are self-incompatible (Quero-García *et al.*, 2017b), and compatible pollen is needed to set fruit which subsequently underpins commercial yields (Herrero *et al.*, 2017). Self-compatible sweet cherry cultivars also need insect pollination to achieve maximum yields (Lane & Schmid, 1984). The probability of compatible pollen being transferred onto the stigma of cherry blossom may be dependent on the number of insect visits to receptive flowers (Jacquemart *et al.*, 2006; Garratt *et al.*, 2016), but also the behaviour of pollinators when foraging (e.g. flying pattern to enhance cross-pollination and visit duration) (see Chapter 5). However, in addition to the effective transfer of pollen from a compatible cultivar donor to the flower stigma, other factors influence pollination success. These include the quality of pollen reaching the stigma (Wilcock & Neiland, 2002), the delivery of insufficient or excessive amounts of pollen (Wilcock & Neiland, 2002), pistil receptivity (which determines the effective pollination period) (Sanzol & Herrero, 2001), insect visits occurring at the incorrect stage of anthesis (Wilcock & Neiland, 2002), the condition of flowers (e.g. flowers not damaged by frost) (Szpadzik *et al.*, 2008), and environmental factors (e.g. temperature) (Radunic *et al.*, 2017).

Managed pollinators such as honeybees (*Apis mellifera*) and buff-tailed bumblebees (*Bombus terrestris*) are used in commercial sweet cherry orchards during the blossom period to help ensure successful pollination, and subsequently, production (Koumanov & Long, 2017). However, some wild pollinating insects can provide a better pollination service to sweet cherry (Eeraerts *et al.*, 2020), but they continue to decline globally potentially leading to insufficient pollination services resulting in limited yields (pollination deficits) (Potts *et al*., 2016). This could directly impact sweet cherry production (Holzschuh *et al.*, 2012). To mitigate pollination deficits in regions associated with low pollinator abundance and richness, pollen dusting is sometimes used, although this is not always a suitable replacement for insect pollination (Allsopp *et al.*, 2008). Pollen collected from compatible cultivars is mechanically applied using aircraft or pollen blowers (Allsopp *et al.*, 2008). Another approach to overcome pollination deficits is hand pollination (Garibaldi *et al.*, 2009). For example, in Sichuan Provence, China, apple blossom, and sometimes cherry blossom, is hand pollinated (Partap & Ya, 2012; Forbes & Northfield, 2017). However, due to labour costs, this approach is not common (Partap & Ya, 2012). An alternative approach is to enhance wild pollinators in orchards; a greater abundance and richness of wild pollinating insects compared to managed pollinators can lead to greater yields in sweet cherry orchards (Eeraerts *et al.*, 2020). Wild pollinators are highly dependent on semi-natural habitats (Cole *et al*., 2017) and their abundance and richness can be enhanced in orchards when wildflower habitat is provided (Campbell *et al.*, 2017). However, how the management of wildflower habitats affect pollination services, particularly in protected sweet cherry orchards is not known: a single cut at the end of the growing season a common practice (Haaland *et al.*, 2011). In addition, pollinating insects may be influenced by edge effects with pollinator abundance and species richness decreasing towards the centre of orchards, which can impact production (Nguyen & Nansen, 2018).

Insufficient pollination can lead to negative impacts in commercial production, so that detecting whether a crop is suffering from pollination deficits is important (Garratt *et al.*, 2014b). Hand pollination can be used to detect pollination deficits in crops through insect exclusion treatments (Button & Elle, 2014). The difference in fruit set, and subsequently yield between blossoms exposed to pollinating insects (insect pollination) versus those receiving hand pollination can indicate a pollination deficit (Garratt *et al.*, 2014b). Insect exclusion treatments are also utilized to determine the dependence of crops on insect pollination (Garratt *et al.*, 2016).

Hand pollination is also commonly used in cultivar compatibility programmes (Quero-García *et al.*, 2017a), where there is a need to identify the best cultivars as pollinizers to maximize yields and quality (Mehmet, 2011; Radičević *et al.*, 2011). This is of particular importance for crops that are predominantly self-incompatible, such as sweet cherry (Radičević *et al.*, 2011). Fruit set is determined by the number of blossoms that develop fruit (Holzschuh *et al.*, 2012), whilst fruit quality is normally determined by measuring parameters such as fruit mass and size (height, width, and length), firmness, brix, and dry matter (Koumanov *et al.*, 2016; Overbeck *et al.*, 2017; Toivonen *et al.*, 2017). Determination of the optimal frequency of hand pollination for sweet cherry is therefore important to detect pollination deficits and the development of new cultivars as sub-optimal hand pollination will either over- or under-estimate pollination. Furthermore, knowing how to efficiently maximise pollination by hand will help develop protocols for farmers enabling them to determine whether they have a pollination deficit (Garratt *et al.*, 2019). The optimum number of hand pollination events to maximise sweet cherry fruit set and its influence on fruit quality is not currently known. Blossoms are therefore hand pollinated numerous times when developing new cultivar compatible programmes (Beyhan & Karakaş, 2009), or when investigating abiotic effects on fruit set (Zhang *et al.*, 2018). There is clearly a need to determine the optimal frequency of hand pollination events to achieve optimal pollination in sweet cherry.

This study aimed to investigate the contribution of insect pollinators to sweet cherry production and the extent of any deficits using insect exclusion and supplementary pollination experiments. This study also aimed to investigate the influence of wildflower habitat established in alleyways, and their management (20 cm height throughout the growing season vs. a single cut at the end of the growing season) on pollinating insect foraging preferences and sweet cherry production (quantity and quality), and to what extent this was influenced by orchard edge effects. A further aim was to investigate the optimal frequency of hand pollination events to maximize fruit set.

6.3 Materials and methods

6.3.1 Study site and experimental design

The study was carried out in the West Midlands, UK, at three sites in Herefordshire and two in Staffordshire. At each site, two orchard blocks (defined as a separate parcel of land) were investigated. In each orchard block, three alleyways adjacent to rows of the cherry cultivar Kordia were selected to be studied. Two alleyways in each orchard block were randomly selected to receive one of two wildflower treatments, whilst the third was an untreated control, which consisted of the original alleyway vegetation (see Chapter 3). The three alleyway treatments were therefore:

i) **Control Strips (CS)**. Conventionally managed alleyways not sown with wildflowers that were cut regularly to a height of 10 cm from May to September, and then to a height of 8 cm in late September.

ii) **Standard Wildflower Strips (SWS)**. Cut annually in late September to a height of 8 cm.

iii) **Actively Managed Wildflower Strips (AMWS)**. Cut regularly (twice/three times per month) to a height of 20 cm from May to September, and then to a height of 8 cm in late September.

The length of strips was 95 m, starting from the orchard block edge (first cherry tree) towards the centre of the orchard. This allowed standardization among orchard blocks due to the length of five out of the 30 alleyways studied was 95 m, and the other edge was therefore at that distance. However, the second half of the five alleyways which total length was 95 m was not included in the analysis because the centre of the orchard block was at 47.5 m, and from that point, sections would become closer to the other block edge (as in Chapter 4). The distance between alleyway treatments varied from 26 to 48 m depending on the availability of Kordia in the orchard blocks. The distance between orchard blocks also varied. At four sites, the distance was between 250 and 975 m. At Site 2, the distance between blocks was 30 m (see Chapter 2). Consequently, due to the close proximity between alleyway treatments and the high mobility of pollinators (Zurbuchen *et al.*, 2010), the study was designed to assess the pollination services delivered by pollinating insects according to foraging preferences rather than impacts at the orchard scale on production.

The wildflower strips were sown in autumn 2016, but establishment was poor (see Chapter 3). Consequently, alleyways were re-sown in year one (2017) at Sites 1, 2, and 3 in April and at Sites 4, and 5 in September. To promote the establishment of wildflowers, all alleyway treatments were cut regularly to a height of 10 cm throughout year one (baseline year) (see Chapter 3). During the establishment year, baseline data were collected to gain an understanding of the dependence of sweet cherry on insect pollination. The different alleyway treatments were applied from May in year two (2018). All cuttings from mowing activities remained *in situ*.

6.3.2 Contribution of pollinating insects to sweet cherry production

6.3.2.1 Dependence of sweet cherry production on insect pollination

In years one and two, eight Kordia cherry trees in each alleyway treatment were selected to investigate the dependence of sweet cherry on insect pollination. To understand the extent to which insect pollination is affected by edge effects, trees were located at different distances from the orchard edge (Table 6.1).

Table 6.1. Distances (m) from the orchard block edge at which study Kordia cherry trees were located.

Tree number	Distance from the orchard block edge (m)
1	4.75
2	14.25
3	23.75
4	33.25
5	42.75
6	52.25
7	61.75
8	71.25

On each tree an insect exclusion experiment was set up (Garratt *et al.*, 2016) to compare pollination in the absence and presence of pollinating insects, ‘insect excluded pollination’ vs. ‘open pollination’. Prior to the blossom period, a branch with at least 20 buds was randomly selected on each tree for the insect exclusion treatment. A PVC mesh bag (mesh gauge 1.2 mm^2) was used. The mesh size permitted pollen to move through the mesh, whilst excluding potential pollinating insects. On the same tree, a second branch with at least 20 buds was also randomly chosen at approximately the same height. This ‘open pollination’ treatment allowed blossoms to be exposed to pollinating insects. Mesh bags were removed after the flowering period. In year two, all the blossoms were counted and the number of fruits set (initial fruit set) was determined two weeks after bag removal (Radunic *et al.*, 2017). Fruit retained to maturity (final fruit set) was determined by conducting a further survey in July, just prior to harvest (Holzschuh *et al.*, 2012). Initial fruit set was calculated by subtracting the number of fruits that set minus the number of flowers on each spur, whilst final fruit set was calculated by subtracting the fruit that matured minus the fruit that set initially. Blossoms were not counted in year one, and therefore, year one was excluded from fruit set but not for fruit quality analysis.

6.3.2.2 Determination of pollination deficits

In year three, to investigate potential pollination deficits in sweet cherry orchards, an additional 'hand pollination' treatment (optimum pollination) treatment was set up. At the five sites, four trees per alleyway treatment were used, at distances of 4.75, 23.75, 42.75, and 61.75 m from the orchard block edge (Table 6.1). On each tree, three spurs were randomly selected (see 6.3.2.1). As in years one and two, one spur had blossoms exposed to pollinating insects, whilst the second spur was bagged to exclude pollinating insect visits. The third spur was bagged to exclude insect visits but it also received the hand pollination treatment (Figure 6.1).

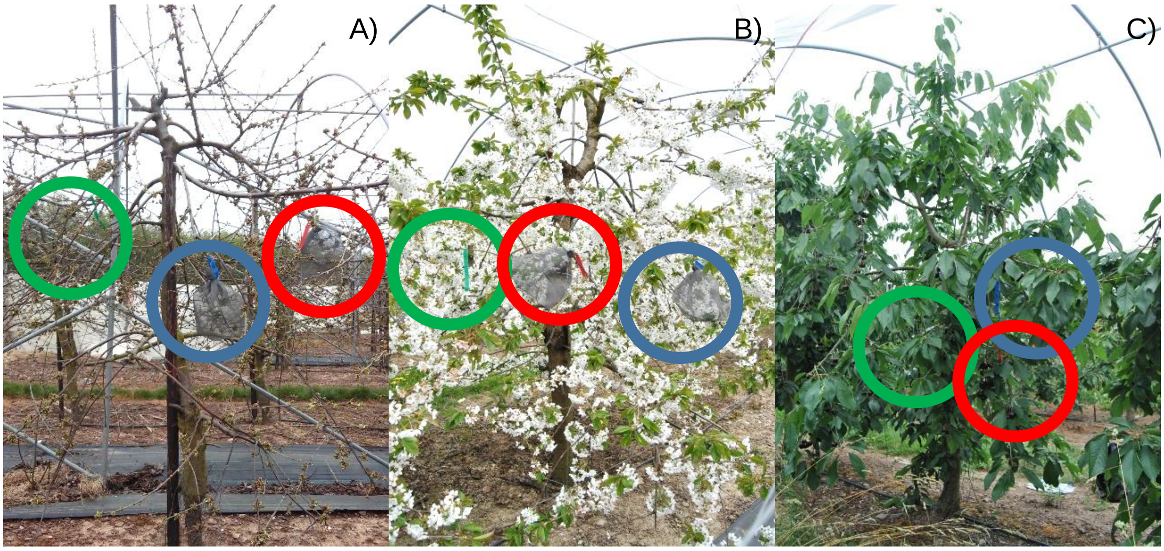

Figure 6.1. Spurs randomly selected to determine pollination deficits, A) before, B) during, and C) after the cherry blossom period. Open pollination treatment (green), insect excluded pollination treatment (blue), and hand pollination treatment (red).

Two different approaches can be used to compare the efficacy of hand pollination with insect pollination. Blossoms pollinated by insects can either be compared with those that are also hand pollinated after already being exposed to pollinating insects (Holzschuh *et al.*, 2012), or blossoms that are insect pollinated, can be compared with blossoms from which insect pollinators are excluded prior to, and during anthesis, and then hand pollinated (Garratt *et al.*, 2014a). The second approach was adopted, and mesh bags (as described above) were set up and removed before and after anthesis. Blossoms at the balloon flowering stage (stage 59 (Fadón *et al.*, 2015)) were hand pollinated twice with an interval of 24 hours, during which the mesh bags were temporarily removed. Unopened blossoms were removed to ensure all blossoms within the bags were hand pollinated; blossoms were not emasculated due to the potential negative impact on fruit set (Hedhly *et al.*, 2009).

Due to the self-incompatibility of the cultivar Kordia (Lech *et al.*, 2008), to ensure cross-pollination for the hand pollination treatment, pollen from Regina trees was used as this cultivar is a compatible donor for Kordia (Sagredo *et al.*, 2017) and was present at all five sites (see Chapter 2). Spurs with unopen blossoms were excised from Regina trees and placed with their end immersed in water at room temperature in a laboratory for 48 h. Once anthers were dehisced, blossoms were gently tapped over a petri dish, where the pollen was collected (Shivana & Rangaswamy, 1992; Nikolić & Milatović, 2016). A paintbrush (Professional Water Colour Sable Round, Winsor & Newton; number 1) was used to transfer pollen onto the stigma of opened flowers. Initial and final fruit set were determined as above (section 6.3.2.1).

6.3.3 The influence of wildflower interventions on insect pollination

In order to investigate whether the wildflower habitats and their management affected sweet cherry production, data from the open pollination treatment collected in years two and three was used. This included data from the dependence of sweet cherry production on insect pollination and pollination deficits. It was assumed that only blossoms available to pollinating insects (open pollination) could be affected by the wildflower interventions, and they would not affect the bagged insect excluded and hand pollination treatments.

6.3.4 Optimal frequency of hand pollination

In year two, the optimal frequency of hand pollination on cherry flowers was investigated in one orchard block at one of the sites (Site 1). Prior to the blossom period, ten trees were randomly selected from a different alleyway to those with the three alleyway treatments. On each tree, three branches were chosen. All 30 branches were bagged with at least twenty cherry buds at the beginning of April (as in sections 6.3.2.1 and 6.3.2.2). The pollen used for hand pollination was collected as in section 6.3.2.2.

All the flowers of each selected branch were hand pollinated either once, twice or three times on all ten trees. Bags were temporally removed each time to hand pollinate the flowers during the peak bloom stage (Choi & Andersen, 2001) (stage 62 (Fadón *et al.*, 2015)) with a paintbrush (Professional Water Colour Sable Round, Winsor & Newton; number 1) (Garratt *et al.*, 2014a). Second and third pollinations were undertaken 24 h after the previous pollination.

At the end of the blossom period (early May), the bags were removed; initial fruit set was determined two weeks after bag removal, whilst the final fruit set was recorded in mid-July.

6.3.5 Fruit quality evaluation

Two days before commercial harvest (July), cherries were picked. In year one, up to ten fruits per spur were harvested, whilst in years two and three following power analysis (MacCallum *et al.*, 1996) (data not presented), the number harvested was reduced to a maximum of three fruits per spur. The fruit was placed in sealable polythene bags and stored in a cold room (6ºC) prior to evaluation, which occurred within 48 hours.

Fruit quality was determined by measuring fresh mass, height, width, firmness, and dry matter in all years (Overbeck *et al.*, 2017; Toivonen *et al.*, 2017). In addition, in years one and three fruit length was also measured (Koumanov *et al.*, 2016) (Figure 6.2). Seed mass, height, width, and length (the latter only in year one and three) (Figure 6.2) were also evaluated each year to examine the possible relationship between pollination and fruit quality.

Values of mass were measured with an electronic scale (Precision Balances Entris®, model 822 -1SUS) and dimensions were measured with an electronic digital calliper. A firmness tester (Agrosta® 100USB) was used to determine fruit firmness and values are reported as Durofel units (Zoffoli *et al.*, 2017), which indicate resistance (1 to 100) (Belge *et al.*, 2017). A mean firmness value was calculated for each cherry by measuring two perpendicular sides. Prior to these assessments, all cherry stalks (Figure 6.2) were removed. Seeds were extracted with a stainless-steel cherry pitter. Seeds were then placed in a 1% pectinase solution for 24 h so that the remaining flesh could be removed. The cherry flesh was dried in an oven at 65ºC for 48 h. 100 cherry seeds were weighed before and after the adhered flesh was cleaned in order to estimate values of total fruit flesh.

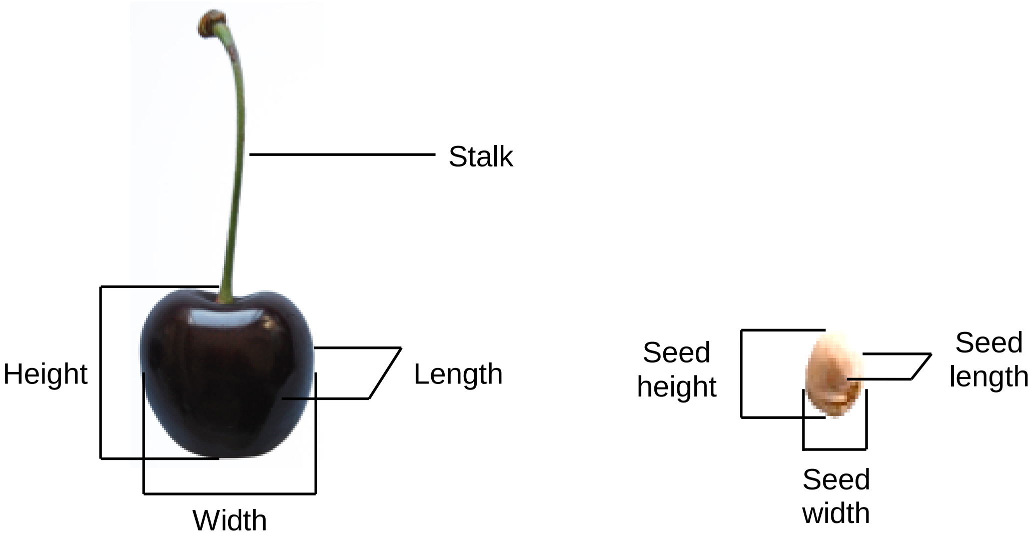

Figure 6.2. Dimensional parameters to determine size of sweet cherries and sweet cherry seeds.

6.3.6 Statistical analysis

All data were analysed using the software R (version R-3.6.1) (R Core Team, 2019).

6.3.6.1 Fruit set

6.3.6.1.1. Contribution of pollinating insects to sweet cherry production

To determine whether initial and final fruit set were affected by pollination treatment, two generalized linear mixed models with binomial error structures (lme4 package, function = GLMER, family = binomial (Bates *et al.*, 2014)) were used. The response variable for the initial fruit set was obtained from combining the number of blossoms that set fruit initially and blossoms that did not, whilst the response variable for final fruit set combined fruits that matured and fruits that did not. The response variables for each model were the initial fruit set and the final fruit set. Pollination treatment, year, and distance from the orchard block edge (continuous factor) were specified as fixed factors, while alleyway treatment nested within orchard blocks nested within sites were the random effects. All models were also run to test the importance of alleyway treatment as a fixed factor (with and without the interaction between alleyway treatment and year). The Akaike's Information Criterion (AIC) (Burnham & Anderson, 2002) was used to determine the most parsimonious model and hence, the model showing the lowest AIC was selected. Due to all models with alleyway treatment specified as a fixed effect had higher AIC values compared to models with alleyway treatment specified as a random effect, the latter models were used.

The models were also run to investigate the relative importance of the interaction between pollination treatment and year, and between pollination treatment and distance from the orchard block edge. For each model, the relative significance of the model terms was calculated by taking an information theoretic approach using the AIC. Each of the fixed effects were individually removed from the global model and the difference in AIC values was calculated for the reduced model (ΔAIC). The AIC was used to select the most parsimonious model in each case; AIC > 2 was considered to have a substantial level of empirical support (Burnham & Anderson, 2002). The Tukey's *post-hoc* test (multcomp package (Hothorn *et al.*, 2008)) was further used to analyse pairwise comparisons between factors with more than two levels. Significant differences were accepted when $P < 0.05$.

6.3.6.1.2. The influence of wildflower interventions on insect pollination

To investigate whether sweet cherry fruit set responded to alleyway treatment, two generalized linear mixed models with binomial error structures (lme4 package, function = GLMER, family = binomial (Bates *et al.*, 2014)) were used. One model included the initial fruit set as response variable, whilst the response variable in the second model was the final fruit set as above (section 6.3.6.1.1). However, alleyway treatment, year, and distance from the orchard block edge were specified as fixed factors for these two models, while orchard blocks nested within sites were set as random effects.

The two models were re-run to investigate the relative importance of the interaction between alleyway treatment and year, and between alleyway treatment and distance from the orchard block edge. The AIC was used to determine the most parsimonious models and calculate the relative significance of the model terms, whilst Tukey tests were used to investigate pairwise comparisons, as above (section 6.3.6.1.1).

6.3.6.1.3. Optimal frequency of hand pollination

For the hand pollination frequency experiment conducted in year two, two generalized linear mixed models with binomial error structures (lme4 package, function = GLMER, family = binomial (Bates *et al.*, 2014)) were also used to analyse the initial and final fruit set (as in section 6.3.6.1.1). However, these response variables were tested with hand pollination frequency (once, twice or three times) as a fixed effect and tree as a random effect. Tree was previously specified as a fixed effect to ensure there were no differences between trees.

For both models, empirical scale parameters (residual scaled deviance divided by the degrees of freedom) were calculated to determine over- or under-dispersion and plots of residuals were created to check homoscedasticity, normality of errors and negligible influence of outliers in the data. The Tukey’s *post-hoc* test (multcomp package (Hothorn *et al.*, 2008)) was used to assess differences between treatments.

6.3.6.2 Fruit quality evaluation

6.3.6.2.1 Contribution of pollinating insects to sweet cherry production

To investigate whether pollination treatment (hand, open, and insect excluded) affected fruit quality, the ten fruit quality parameters were first tested for normality with the Shapiro-Wilk test for each of the pollination treatments. All parameters (each as an independent response variable) were non-parametric and were analysed using individual generalized linear mixed effect models (lme4 package, function = LMER (Bates *et al.* 2014)). For each of the ten fruit quality parameters (ten global models), pollination treatment, year, and distance from the orchard block edge were specified as fixed factors. Whilst alleyway treatment nested within orchard blocks nested within sites were set as random effects. The relative importance of the interaction between pollination treatment and year, and pollination treatments and the distance from the orchard block edge were studied. The relative significance of the model terms was calculated, and Tukey tests were conducted as above.

To investigate whether fruit length and seed length can be estimated based on other parameters, Spearman's rank correlation tests were used. Correlation tests included fresh fruit mass and length, fruit height and length, fruit width and length, seed mass and seed length, seed height and seed length, and seed width and seed length.

6.3.6.2.2 The influence of wildflower interventions on insect pollination

The ten fruit quality parameters were tested for normal distribution with the Shapiro-Wilk test individually according to alleyway treatment. All parameters were non-parametric and were analysed using individual generalized linear mixed effect models (lme4 package, function = LMER (Bates *et al.*, 2014)). A global model for each parameter was therefore analysed as in section 6.3.6.2.1. However, alleyway treatment, year, and distance from the orchard block edge were specified as fixed factors. Whilst orchard blocks nested within sites were set as random effects. The relative importance of the interactions between alleyway treatment and year, and between alleyway treatment and the distance from the orchard block edge were studied. The relative significance of the model terms was calculated as above. Pairwise comparisons were also conducted between alleyway treatments and years with the Tukey’s *post-hoc* test tests (multcomp package (Hothorn *et al.*, 2008)).

6.3.6.2.3 Optimal frequency of hand pollination

To determine whether hand pollination frequency affected fruit quality, the eight fruit quality parameters (length and seed length were not measured in year two) were tested individually according to pollination frequency. Parameters meeting the assumptions of parametric tests were analysed using one-way ANOVA, whilst non-parametric data were analysed with generalized linear mixed effect models (lme4 package, function = LMER (Bates *et al.* 2014)). A total of eight global models were therefore analysed, one for each response variable. The number of hand pollination events was specified as a fixed effect, whilst the tree on which the experiment took place (ten trees) was specified as a random effect. The relative significance of the model terms on non-parametric data was calculated as above. Pairwise comparisons were also further investigated with the *post-hoc* Tukey test (multcomp package (Hothorn *et al.*, 2008)).

6.4 Results

6.4.1 Impacts of pollination on fruit set

6.4.1.1 Contribution of pollinating insects to sweet cherry production

6.4.1.1.1 Initial and final fruit set

Values of initial fruit set in response to hand, open and insect excluded pollination treatments were inconsistent between years (defined by the interaction between pollination treatment and year) (Table 6.2). The initial fruit set in open (Tukey test: $Z = -5.47$, $P < 0.001$) and insect excluded (Tukey test: $Z = -5.73$, $P < 0.001$) blossoms was greater compared to hand pollinated blossoms (Figure 6.3). Year was also found to be an important factor influencing initial fruit set (GLMER: $Z = -20.92$, $P < 0.001$); greater percentage values were recorded in year two (70.6% (± 0.7)) compared to year three (59.0% (± 1.1)).

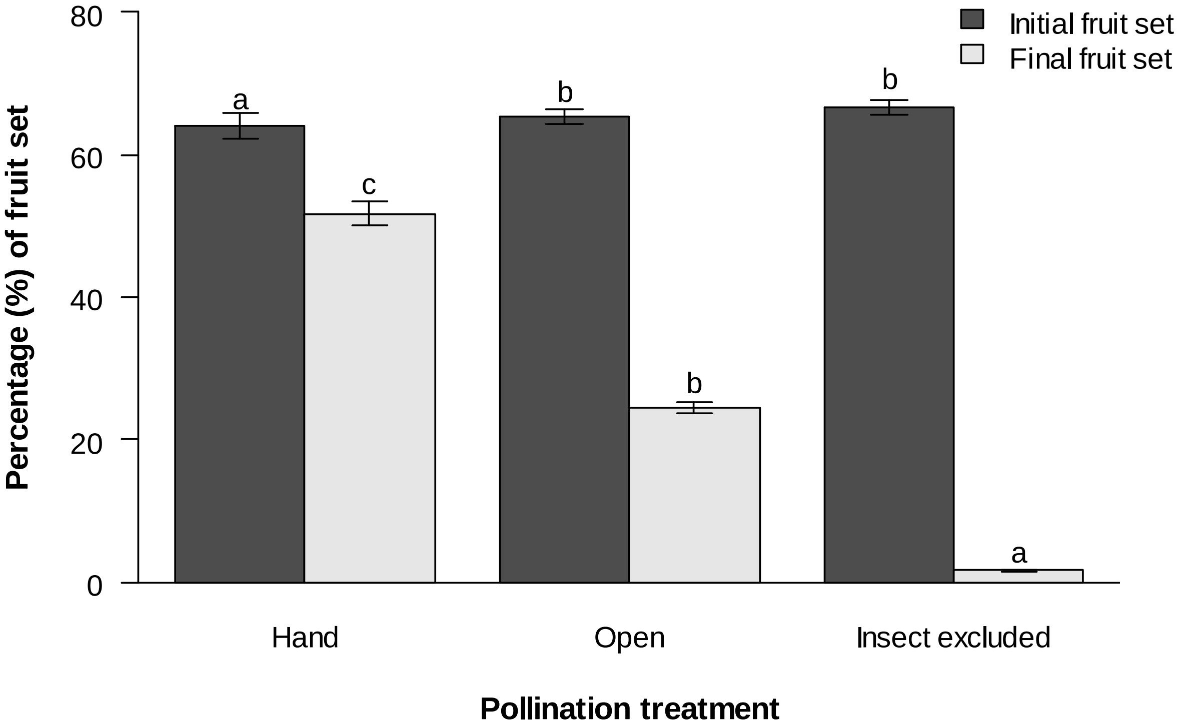

Figure 6.3. Mean percentage (± SE) of initial and final fruit set recorded in years two and three combined according to pollination treatment. The same superscript letters for each series (initial fruit set and final fruit set) indicate no significant differences according to the Tukey test ($P > 0.05$).

Table 6.2. Comparisons in the generalized linear mixed models for the contribution of pollinating insects to sweet cherry production using the Akaike Information Criteria (AIC). Interaction between pollination treatment and year represented by *Pollination treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Initial fruit set			
Initial fruit set ~ Pollination treatment: Year + Distance from the edge + (random: Site/Orchard/Alleyway treatment)			
Global model		9972.0	0.0
Pollination treatment: Year	1	9976.9	**4.9**
Distance from the edge	1	9998.8	**26.8**
Final fruit set			
Final fruit set ~ Pollination treatment: Year + Distance from the edge + (random: Site/Orchard/Alleyway treatment)			
Global model		4436.6	0.0
Pollination treatment: Year	1	4479.7	**43.1**
Distance from the edge	1	4506.2	**69.6**

The most parsimonious model for final fruit set also included an interaction between pollination treatment and year, indicating that the number of fruits that matured in hand, open, and insect excluded pollination treatments varied between years two and three (Table 6.2). Pollination treatment was an important factor determining the number of fruits that finally set. The greatest percentage was obtained from blossoms that were hand pollinated compared to insect pollinated (Tukey test: $Z = -11.57$, $P < 0.001$) and insect excluded blossoms (Tukey test: $Z = -32.09$, $P < 0.001$) (Figure 6.3). The insect pollination treatment was also associated with more fruit being retained to maturity compared to blossoms that had insect pollinators excluded (Tukey test: $Z = -36.70$, $P < 0.001$). The percentage of final fruit set varied between years, in year three more fruit matured at 27.8% (± 1.3) compared to 11.6% (± 0.6) in year two (GLMER: Z = -3.53, P < 0.001).

6.4.1.1.2 Edge effect

The number of fruits that set initially varied with distance from the orchard block edge (tree location along the alleyway) (Table 6.2) (GLMER: -0.002 ± 0.0004, Z = -5.36, P < 0.001), being lower towards the centre of the orchard block (Figure 6.4). An edge effect was also found (GLMER: -0.01 ± 0.001, Z = -8.44, P < 0.001) for final fruit set (Table 6.2). A negative correlation showed that trees closer to the orchard block edge retained more final fruit when blossoms were open and hand pollinated, which decreased as the distance from the orchard block edge increased (Figure 6.4). However, insect excluded blossoms retained similar number of cherries along the tree rows.

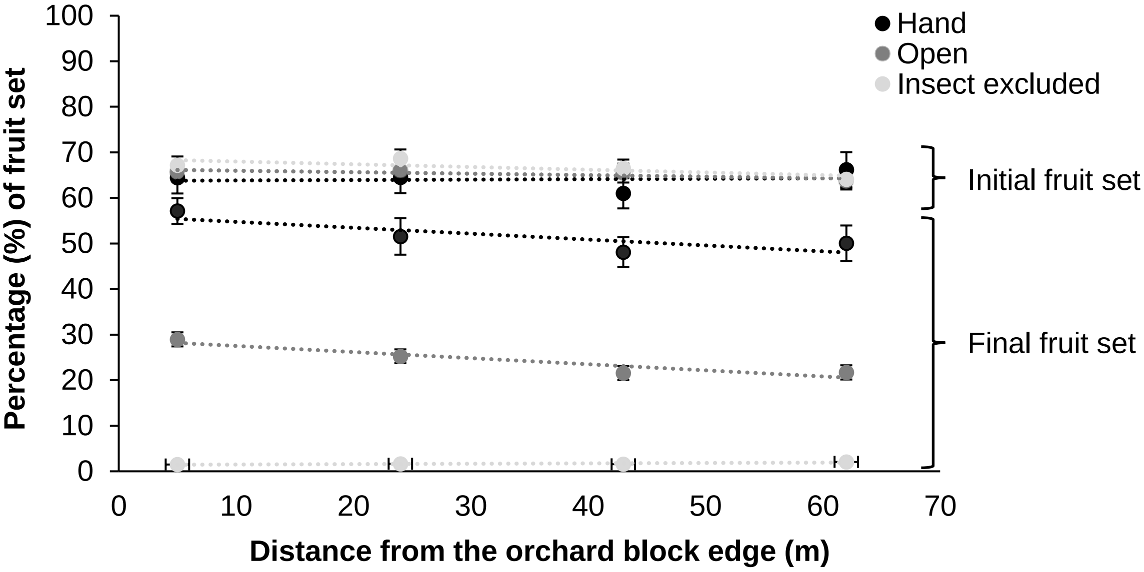

Figure 6.4. Mean percentage (± SE) of the initial and final fruit set recorded in years two and three combined according to distance from the orchard block edge (m) (tree location along the alleyways) and pollination treatment.

6.4.1.2 The influence of wildflower interventions on insect pollination

6.4.1.2.1 Initial and final fruit set

The influence of alleyway treatment on values of initial fruit set when blossoms were open pollinated varied between years (the most parsimonious model included an interaction between alleyway treatment and year) (Table 6.3). A significant effect of alleyway treatment was found for values of initial fruit set, and initial fruit set was greater on trees adjacent to CS (Tukey test: *Z* = -3.77, *P* < 0.001) and AMWS (Tukey test: *Z* = -2.38, *P* < 0.05) compared to trees next to SWS (Figure 6.5). The initial number of fruit that set varied between years (GLMER: *Z* = -12.87, *P* < 0.001) and was greater in year two (69.9% (± 1.0)) compared to year three (55.8% (± 2.0)).

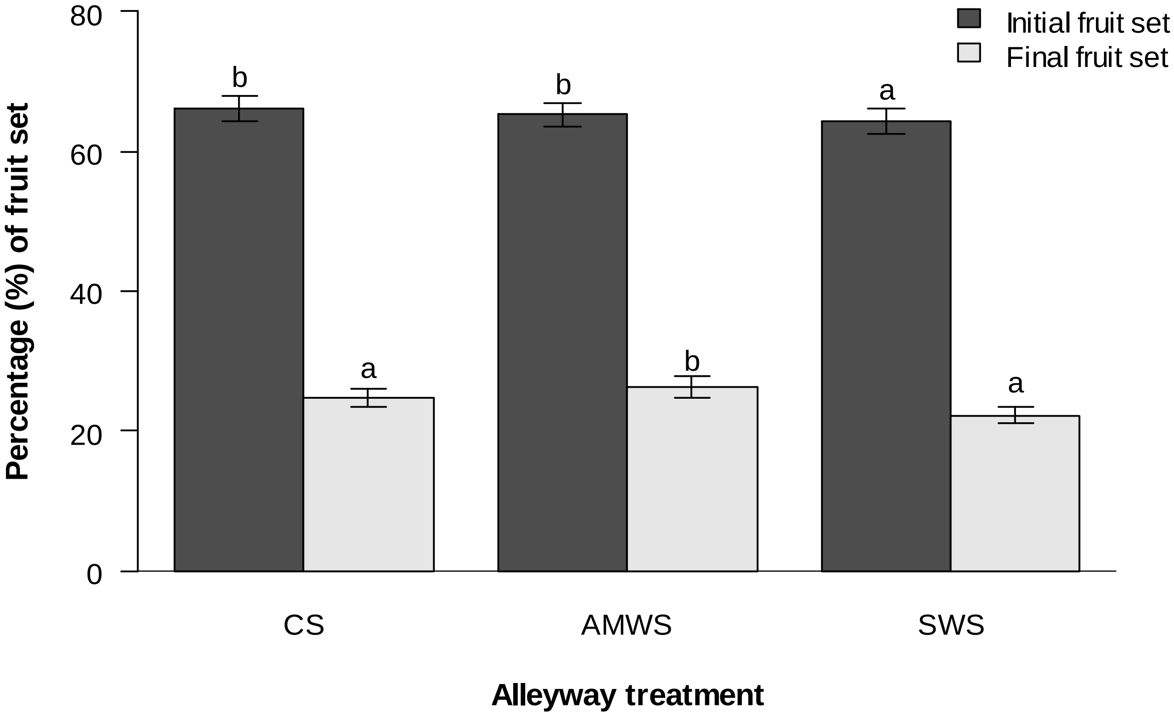

Figure 6.5. Mean percentage (± SE) of initial and final fruit set recorded on the blossoms open pollinated in years two and three combined according to alleyway treatment. The same superscript letters for each series (initial fruit set and final fruit set) indicate no significant differences according to the Tukey test (*P* > 0.05). CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

The most parsimonious model for the final fruit set was also defined by an interaction between alleyway treatment and year (Table 6.3). Alleyway treatment also affected the number of cherries that reached maturity. However, a greater percentage value was recorded on trees adjacent to AMWS compared to trees next to CS (Tukey test: *Z* = 2.93, *P* < 0.01) and SWS (Tukey test: *Z* = -4.55, *P* < 0.001) (Figure 6.5). Year also affected the final number of fruits that set (GLMER: *Z* = 2.28, *P* < 0.05), but contrary to the initial fruit set, a greater value was recorded in year three (30.2% (± 1.4)) compared to year two (21.4% (0.9)).

Table 6.3. Comparisons in the generalized linear mixed models for the influence of wildflower interventions on insect pollination using the Akaike Information Criteria (AIC). Interaction between alleyway treatment and year represented by *Alleyway treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	Degrees of freedom	AIC	ΔAIC
Initial fruit set			
Initial fruit set ~ Alleyway treatment: Year + Distance from the edge + (random: Site/Orchard)			
Global model		4475.6	0.0
Alleyway treatment: Year	1	4476.6	1.0
Distance from the edge	1	4479.7	**4.1**
Final fruit set			
Final fruit set ~ Alleyway treatment: Year + Distance from the edge + (random: Site/Orchard)			
Global model		2779.6	0.0
Alleyway treatment: Year	1	2802.6	**23.0**
Distance from the edge	1	2848.1	**68.5**

6.4.1.2.2 Edge effect

Distance from the orchard block edge was an important factor for initial fruit set (GLMER: -0.002 ± 0.001, $Z = -2.47$, $P < 0.05$) (Table 6.3), and a greater percentage of initial fruit set was recorded on trees closer to the orchard boundary (Figure 6.6). The final number of fruit that set was also affected by the distance from the orchard block edge (GLMER: -0.01 ± 0.001, $Z = -8.37$, $P < 0.001$); cherry production decreased towards the centre of the orchard blocks (Figure 6.6).

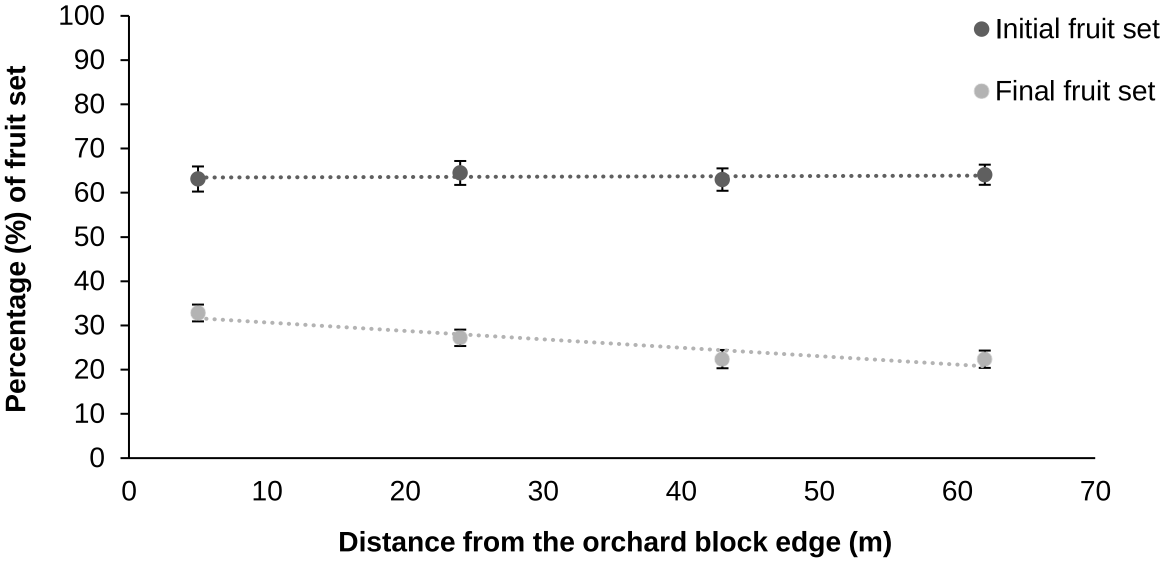

Figure 6.6. Mean percentage (± SE) of the initial and final fruit set recorded on the blossoms open pollinated in years two and three combined according to distance from the orchard block edge (m) (tree location along the alleyways).

6.4.1.3 Optimal frequency of hand pollination

6.4.1.3.1 Initial and final fruit set

The frequency of hand pollination had a significant effect on values of initial fruit set (Table 6.4). A greater percentage of blossoms set fruit in flowers that were hand pollinated three times (69.9% (± 4.69)), compared to blossoms hand pollinated only once (52.4% (± 6.24); Tukey test: $Z = 6.24$, $P < 0.001$) or twice (50.4% (± 4.25); Tukey test: $Z = 7.32$, $P < 0.01$) (Figure 6.7). There was no difference between blossoms pollinated either once or twice (Tukey test: $Z = -1.17$, $P = 0.47$).

The percentage of final fruit set was also affected by the number of times the blossoms were hand pollinated (Figure 6.7; Table 6.4). Blossoms hand pollinated twice retained 27.6% (± 5.1) of fruits compared to those pollinated once (11.1% (± 2.7); Tukey test: $Z = 5.09$, $P < 0.001$). In addition, a greater number of fruits developed to maturity in blossoms hand pollinated three times (19.86% (± 4.61)) than with a single hand pollination event (Tukey test: $Z = 3.18$, $P < 0.01$). However, there was no difference between blossoms hand pollinated twice or three times (Tukey test: $Z = -1.65$, $P = 0.17$).

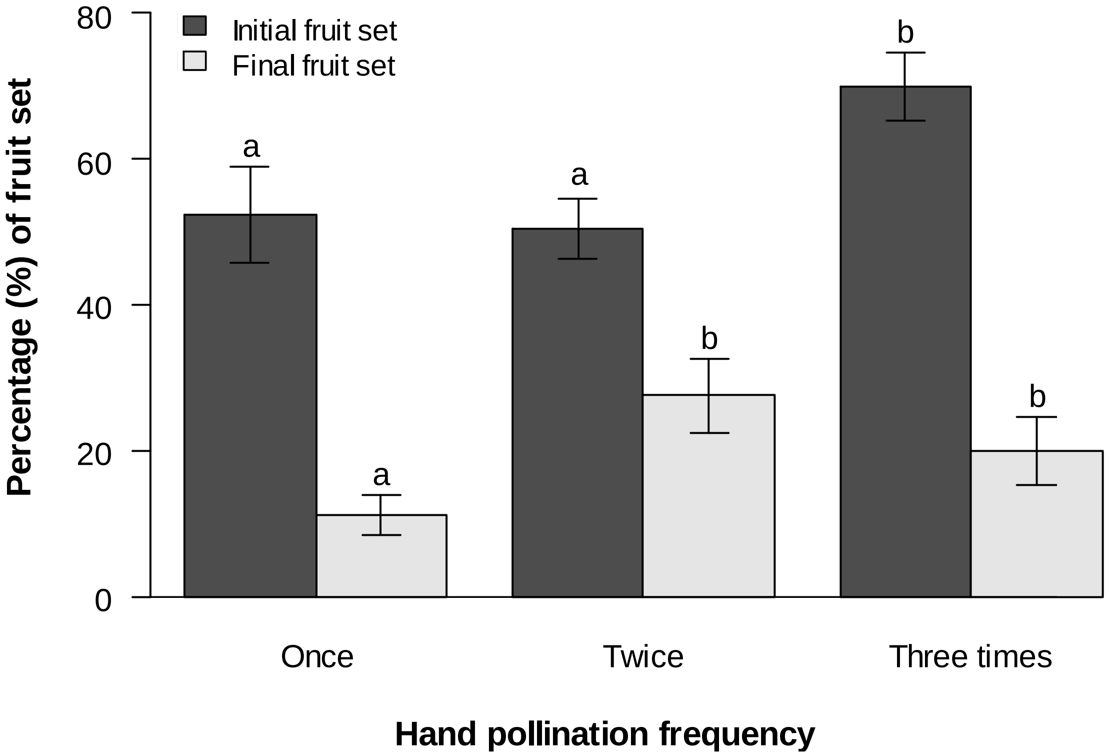

Figure 6.7. Mean percentage (± SE) of initial and final fruit set recorded in year two according to frequency of hand pollination of cherry blossoms. The same superscript letters for each series (initial fruit set and final fruit set) indicate no significant differences according to the Tukey test (*P* > 0.05).

Table 6.4. Comparisons in the generalized linear mixed models for the optimal frequency of hand pollination using the Akaike Information Criteria (AIC). Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Initial fruit set			
Initial fruit set ~ Hand pollination frequency + (random: Distance from the edge)			
Global model		262.1	0.0
Hand pollination frequency	2	318.3	**56.3**
Final fruit set			
Final fruit set ~ Hand pollination frequency + (random: Distance from the edge)			
Global model		172.9	0.0
Hand pollination frequency	2	195.0	**22.1**

6.4.2 Impacts of pollination on fruit quality

6.4.2.1 Contribution of pollinating insects to sweet cherry quality

The most parsimonious model for nine out of the ten fruit quality parameters was defined by an interaction between pollination treatment and year, indicating the response of fresh cherry mass, height, width, length, firmness, dry matter, seed mass, seed height, and seed length to pollination treatments was inconsistent between years (Appendix 6.1). Only the response of seed width was consistent between years.

Pollination treatment was an important factor influencing all fresh cherry quality parameters except for firmness (Appendix 6.2). Values of height and dry matter were greater when blossoms were open pollinated compared to hand pollinated or insect excluded (Table 6.5). Whilst, values with hand pollination were greater than for cherries produced under the insect excluded treatment. Other parameters did not differ significantly between hand and open pollination but these were greater than insect excluded pollinated blossoms. This included fruit mass, and width, and seed mass, seed width, and seed length. The length of cherries open pollinated was greater compared to hand or insect excluded blossoms. Seed height was greater in cherries from blossoms hand pollinated compared to insect excluded. There was a significant difference between all cherry fruit quality parameters between years, except for length (Appendix 6.1). Overall, in years one and three greater values were recorded compared to year two (Table 6.5; Appendix 6.2).

Distance from the orchard block edge affected some fruit quality parameters (Appendix 6.1). Fresh mass increased from the orchard block edge towards the centre by 0.3 (± 0.2) g, and so did height by 1.2 mm (± 0.3), width by 1.1 mm (± 1.2), length by 4.5 mm (± 0.7), dry matter by 0.1 g (± 0.1), and seed height by 1.4 mm (± 0.6) (Appendix 6.3). In contrast, values of seed width decreased towards the centre of the orchard blocks by 1.4 mm (± 0.01), seed length also decreased towards the centre by 1.2 mm (± 0.2).

The correlation between parameters to determine length and seed length with Spearman's rank correlation tests was positive in all cases (Table 6.6), indicating that cherry length and seed length values can be predicted based on other parameters.

Table 6.5. Means (± SE) of fresh mass, height, width, length, firmness, dry matter, seed mass, seed height, seed width, and seed length according to pollination treatment and year.

Fruit quality parameter	**Pollination treatment**								
	Hand			**Open**			**Insect excluded**		
	Year One	Year Two	Year Three	Year One	Year Two	Year Three	Year One	Year Two	Year Three
Fresh mass (g)	-	-	12.1 (± 0.1)	12.0 (± 0.04)	9.7 (± 0.1)	12.1 (± 0.1)	10.0 (± 0.2)	8.3 (± 0.1)	11.2 (± 0.4)
Height (mm)	-	-	28.5 (± 0.1)	28.9 (± 0.04)	25.3 (± 0.1)	28.5 (± 0.1)	27.4 (± 0.2)	24.3 (± 0.1)	28.2 (± 0.3)
Width (mm)	-	-	29.0 (± 0.1)	29.3 (± 0.04)	26.8 (± 0.1)	28.9 (± 0.1)	27.7 (± 0.2)	25.4 (± 0.2)	27.9 (± 0.4)
Length (mm)	-	-	25.4 (± 0.1)	26.3 (± 0.04)	-	25.4 (± 0.1)	24.1 (± 0.2)	-	24.7 (± 0.3)
Firmness (Durofel units)	-	-	69.4 (± 0.4)	66.2 (± 0.2)	63.2 (± 0.3)	69.7 (± 0.5)	61.4 (± 0.9)	66.3 (± 0.5)	69.3 (± 1.1)
Dry matter (g)	-	-	1.65 (± 0.02)	1.79 (± 0.01)	1.68 (± 0.02)	1.75 (±0.03)	1.18 (± 0.05)	1.41 (± 0.03)	1.53 (± 0.07)
Seed mass (g)	-	-	0.36 (± 0.003)	0.35 (± 0.001)	0.36 (± 0.002)	0.37 (± 0.004)	0.31 (± 0.005)	0.32 (± 0.003)	0.32 (± 0.01)
Seed height (mm)	-	-	13.1 (± 0.1)	12.6 (± 0.02)	12.4 (± 0.02)	13.2 (± 0.1)	12.8 (± 0.1)	12.2 (± 0.04)	13.3 (± 0.1)
Seed width (mm)	-	-	10.0 (± 0.03)	9.7 (± 0.01)	9.6 (± 0.02)	9.9 (± 0.03)	9.6 (± 0.04)	9.4 (± 0.03)	9.8 (± 0.1)
Seed length (mm)	-	-	8.3 (± 0.03)	7.9 (± 0.01)	-	8.3 (± 0.03)	7.7 (± 0.04)	-	8.0 (± 0.1)

Table 6.6. Spearman's rank correlation tests to determine the correlation between parameters and length and seed length. Values in bold are significant.

Parameters tested	S value	P value	R value
Fresh mass and length	1417118716	**P < 0.001**	0.71
Height and length	1954110870	**P < 0.001**	0.60
Width and length	1602662487	**P < 0.001**	0.67
Seed mass and seed length	1541351350	**P < 0.001**	0.69
Seed height and seed length	3399147865	**P < 0.001**	0.31
Seed width and seed length	835629738	**P < 0.001**	0.83

6.4.2.2 The influence of wildflower interventions on insect pollination

The most parsimonious model for fresh mass, height, width, firmness, dry matter, seed mass, and seed height included an interaction between alleyway treatment and year, but not for length, seed mass, and seed length (Appendix 6.4). This indicates that the effects of alleyway treatment on most fruit quality parameters were not consistent between years, contrary to the response of length, seed mass, and seed length, which were. However, the Tukey test only confirmed differences for firmness and seed mass (Appendix 6.5). Cherries on trees adjacent to the CS treatment were firmer (66.5 Durofel units (± 0.3)) compared to trees adjacent to AMWS (65.2 Durofel units (± 0.3)). Seeds developed in fruit from trees associated with SWS had a greater mass (0.36 g (± 1.7)) than seeds from cherries on trees adjacent to AMWS (0.35 g (± 1.5)). For most of the cherry fruit quality parameters, year was an important factor (Appendix 6.5), and percentage values of parameters tended to be similar between years one and three, and both greater compared to year two (Table 6.5).

Distance from the orchard block edge also influenced the majority of fruit quality parameters; only fruit firmness, seed mass, and seed height were not affected (Appendix 6.4). Fresh cherry mass was greater towards the centre of the orchard blocks with a difference of 1.9 g (± 0.1). Differences of 0.2 mm (± 0.1) for height, 1.5 mm (± 0.7) for width, 3.7 mm (± 0.4) for length, and 0.1 g (± 0.1) for dry matter were also found for these parameters, being greater towards the centre of the orchard block. In contrast, the values of seed width and seed length were 1.1 mm (± 0.1) and 1.0 mm (± 0.1), respectively, greater close to the orchard block edge (Appendix 6.5).

6.4.2.3 Optimal frequency of hand pollination

The frequency of hand pollination on cherry fruit quality was significant only for fruit firmness, seed mass and seed width (Appendix 6.6). Cherries were significantly firmer following two hand pollination events compared to those hand pollinated once (Tukey test: $Z = 2.59$, $P < 0.05$), and three times (Tukey test: $Z = -3.52$, $P < 0.01$) (Table 6.7). However, although cherries produced from blossoms hand pollinated twice had a lower seed mass than flowers hand pollinated once or three times, post-hoc analysis (Tukey test: $Z = 2.30$, $P = 0.06$) revealed no significant differences between treatments. The seed width of blossoms pollinated once were greater than those pollinated twice (Tukey test: $Z = -2.50$, $P < 0.05$).

Table 6.7. Mean (± SE) and statistical results of the fruit quality parameters with frequency of hand pollinations in cherry blossoms. Parameters significantly affected are in bold and values with the same superscript letters do not differ significantly ($P > 0.05$).

Fruit quality parameter	**Hand pollination frequency mean (± SE)**		
	Once	Twice	Three times
Fresh mass (g)	8.2 (± 0.3)	7.5 (± 0.2)	7.64 (± 0.2)
Height (mm)	24.2 (± 0.3)	23.5 (± 0.3)	23.6 (± 0.2)
Width (mm)	25.1 (± 0.3)	24.2 (± 0.3)	24.3 (± 0.3)
Firmness (Durofel units)	**62.6 (± 0.8)**^{a}	**64.9 (± 0.5)**^{b}	**61.30 (± 0.7)**^{a}
Dry matter (g)	1.08 (± 0.05)	1.04 (± 0.04)	1.09 (± 0.04)
Seed mass (g)	0.30 (± 0.01)	0.28 (± 0.01)	0.30 (± 0.01)
Seed height (mm)	12.1 (± 0.1)	11.9 (± 0.1)	11.8 (± 0.1)
Seed width (mm)	**9.3 (± 0.1)**^{b}	**9.0 (± 0.1)**^{a}	**9.2 (± 0.1)**^{ab}

6.5 Discussion

6.5.1 Contribution of pollinating insects to sweet cherry production

This study demonstrates that insect pollination is essential for achieving marketable yields in sweet cherry (Holzschuh *et al.*, 2012), with the exclusion of pollinators during flowering resulting in a lower fruit set (1.7% (± 0.1)) at harvest compared to insect-pollinated blossoms (24.4% (± 0.8)). Consequently, this highlights the importance of insect pollination in sweet cherry orchards for fruit production, as found in many other crops including apple (Garratt *et al.*, 2014b), blueberry (Button & Elle, 2014), and strawberry (Hodgkiss *et al.*, 2018). However, a greater percentage of final fruit set achieved in blossoms hand pollinated compared to the blossoms pollinated by insects suggests there was a pollination deficit in the study orchards of approximately 20%, indicating that profitability could be increased. Detecting shortfalls in pollination is therefore useful in pollinator-dependant crops as it helps target management and investment to increase production (Garratt *et al.*, 2014b). Better orchard management, such as the use of managed pollinator species or by boosting wild pollinators through newly created habitats, could enhance pollination and increase yields (Bosch & Kemp, 1999; Christmann *et al.*, 2017), the latter being more environmentally sustainable.

Cherry quality was also underpinned by insect pollination, although parameter values from hand pollinated blossoms were also high. Fruit size is one of the most important attributes of cherries, which is directly linked to commercial value (Whiting *et al.*, 2006). A minimum mass of 11-12 g and a diameter of 29-30 mm being a standard requirement for industry (Kappel *et al.*, 1996), although a width of 20 mm can be admissible into the first quality category according to the UNECE (United Nations Economic Commission for Europe) (UNECE standard, 2017). Blossoms which had insects excluded produced fruit of reduced mass, size and shape, which subsequently would negatively impact on the proportion of marketable fruit (Klatt *et al.*, 2013). Again, this highlights the importance of pollinating insects not only for yield in tonnes per hectare but also for maximum fruit quality.

Despite the importance of pollen being successfully transferred from a different compatible variety of cherry, the lower initial fruit set under the hand pollination treatment compared to open or insect excluded was not expected (Holzschuh *et al.*, 2012). However, the difference was small (~ 1.8% lower) to probably be commercially significant, additionally only the final fruit set is relevant for the industry (Holzschuh *et al.*, 2012). Lower initial fruit set in hand pollinated blossoms might have been a consequence of removing unopened blossom buds conducted as part of the study, which may induce selective abortion leading to the early abortion of neighbouring fruit (Sutherland, 1987). However, the greater percentage of final fruits set compared to open and insect excluded pollination treatments could have resulted following a better pollination. Hand pollination might increase the success of fertilization, leading to an increase of the likelihood of blossoms being selectively retained to maturity (Sutherland, 1987). In sweet cherry, the establishment of fruit set occurs 3-4 weeks after pollination, whilst, flower and fruit drop occurs within 2-4 weeks after pollination (Fadón *et al.*, 2015). This shows the need to understand additional effects that experimental treatments may have on pollination. The study has also highlighted the importance of using appropriate metrics to assess pollination deficits, particularly those that reflect final crop output such as final fruit set (Garratt *et al.*, 2018).

6.5.2 Effect of the wildflower interventions on insect pollination

The greater fruit set achieved on trees adjacent to AMWS compared to trees located in alleyways with CS and SWS was consistent with a greater abundance, species number and Shannon diversity of pollinating insects also recorded during the cherry blossom period in AMWS (see Chapter 5). This indicates that pollinating insect foraging preferences for wildflower habitats managed to a height of 20 cm can have a positive effect on sweet cherry production. Even though more pollinators were recorded in SWS compared to AMWS throughout the season (see Chapter 5), the novel cutting regime could have fostered more nesting sites for bees, which were unavailable with the greater vegetation in SWS (Steffan-Dewenter & Tscharntke, 2000). This could have resulted in more efficient insect pollinators being recorded the following spring during the cherry blossom period in this treatment. Otherwise, no differences were expected between wildflower strips during the blossom period due to vegetation height and the number of wildflower species were similar until mid-May (after the blossom period).

This study reveals, for the first time, that establishing wildflower strips in alleyways between rows of trees in protected sweet cherry orchards provides benefits due to the associated enhanced pollination services leading to better fruit yields. This result is encouraging and indicates the potential value of growers implementing the pioneering approach of actively maintaining wildflower alleyway strips at a height of 20 cm throughout the growing season in sweet cherry orchards.

Growers are highly reliant on managed pollinators (see Chapter 5), and this study demonstrates that wildflower habitats can be implemented in commercial sweet cherry orchards to promote more resilient pollination services as part of ecological intensification by increasing the abundance and diversity of wild pollinators (Williams *et al.*, 2019). The reliance of a single species (*Apis mellifera*) and sometimes *Bombus terrestris* for sweet cherry pollination (Koumanov & Long, 2017) needs to be carefully considered. Intensively produced commercial bees can be affected by diseases (Pirk *et al.*, 2017; Williams *et al.*, 2019), which puts production at risk. Enhancing wild pollinating insect diversity in crops is therefore an approach that can ensure more resilient pollination services to agriculture (Williams *et al.*, 2019). Moreover, since wild pollinators can provide better pollination services to sweet cherry than commercially produced and managed bees (Holzschuh *et al.*, 2012; Eeraerts *et al.*, 2020), fruit yield and quality could be improved. Wildflower habitats can be established to enhance wild pollinator diversity (Blaauw & Isaacs, 2014b), and seed mixes used to establish these habitats can be designed to target wild pollinators (Nichols *et al.*, 2019). As greater cherry yields and better fruit quality can be achieved through the enhancement of wild pollinators rather than using commercially produced and managed bees (Holzschuh *et al.*, 2012; Eeraerts *et al.*, 2020), the deployment of wildflower habitats could be used to complement pollination services (Garibaldi *et al.*, 2013; Mallinger & Gratton, 2015). In turn, expenditure on managed pollinators could be reduced (Feltham *et al.*, 2015), although costs of wildflower establishment and management should also be considered (Blaauw & Isaacs, 2014a) (see Chapter 7).

Competition for pollinators between crop floral resources and additional non-crop resources may be a concern for growers to implement wildflower habitats (Free, 1968; Foulis & Goulson, 2014). However, this study demonstrated that visits by pollinating insects to crop flowers are not negatively affected by changes to alleyway management, although this could also be influenced by the fact that only few sown (*Silene dioica*) and unsown (*Taraxacum officinale*, *Ranunculus repens*, and *Brassica* spp.) floral units were present in alleyways during the cherry period and were visited by pollinators (see Chapter 5). Wildflowers did not deter but enhanced fruit set in other studies including almond orchards (Lundin *et al.*, 2017), and strawberry crops (Feltham *et al.*, 2015). In addition, as farmers tend to maximize profits by cropping arable land, areas (patches) to establish wildflower may be not available (Christmann *et al.*, 2017; Williams *et al.*, 2019). However, wildflowers can be provided in the alleyways of perennial orchards allowing farmers to optimise ground cover and land use.

Findings of the study provide important evidence that could incentivise fruit growers to establish wildflower strips in their orchards to enhance production. Such an approach could also provide additional benefits, including the delivery of multiple ecosystem services (Balzan *et al.*, 2014; Sidhu & Joshi, 2016). For example, pest regulation services can be increased in sweet cherry orchards with wildflower interventions (see Chapter 4). The wider adoption of wildflower interventions as part of Integrated Pest Management programmes, which aim primarily to reduce PPP inputs, will further support pollinators in cropped habitats, as PPPs are a key driver of pollinator declines (Goulson *et al.*, 2015). Establishing wildflower habitats is also an effective approach to address habitat loss, reduce fragmentation, and foster arthropod and plant conservation (Freemark *et al.*, 2002; Haaland *et al.*, 2011; Bretzel *et al.*, 2016).

6.5.3 Edge effects on pollination services

The influence of an orchard edge effect on the behaviour of pollinating insects (see Chapter 5), is consistent with Hall *et al.* (2019), as both initial and final fruit set were greater near the orchard edges. In contrast, in open crops it has been shown that pollinator visits and subsequent yields are not affected (Button & Elle, 2014; Woodcock *et al.*, 2016; Fountain *et al.*, 2019). This may indicate that protective tunnels over crops may deter pollinator visits towards the centre despite wildflower resources being available throughout the orchards. Consequently, farmers could consider implementing protective covers after the cherry blossom, but this would risk blossom damage by rainfall or hail. This damage along with fungal incidence (e.g. *Monilinia laxa* and *Botrytis cinerea*) could be reduced if blossoms were covered on rainy days during the cherry blossom period (Børve & Stensvand, 2003). In this study, the greater fruit set near the edges of the orchards suggests a relationship between successful pollination and pollinator abundance and richness, which can be reduced in large cropped areas (Garibaldi *et al.*, 2011; Eeraerts *et al.*, 2017). It also suggests that wild pollinators are more efficient than managed pollinators, since despite the abundance of honeybees (Garibaldi *et al.*, 2011) and the consistent abundance of buff-tailed bumblebees throughout the crop (see Chapter 5), fruit set was greater near the edge, where wild pollinators were more abundant and diverse (Garibaldi *et al.*, 2011; Eeraerts *et al.*, 2017). Although lower temperature and greater humidity and wind speed were recorded near the edge of the orchard blocks (see Chapters 4 and 5), the use of protective covers may have detracted honeybees and wild pollinators to deliver pollination services towards the centre. Therefore, orchard design, size, length and width should be carefully considered.

This edge effect was also observed in the hand pollination treatment, but this is unlikely to be a response to pollinator behaviour. The greater percentage of final fruit set with hand pollination near the edge may be as a result of better stigma receptivity on flowers of those trees during the hand pollination. Stigma receptivity and therefore fruit set can be influenced by physiological and climatic factors (Blanusa *et al.*, 2006; Gratacós *et al.*, 2017). These were different at the start of the orchard blocks, and changed towards the centre (Hall *et al.*, 2019), as shown by values of temperature, humidity and wind speed (see Chapters 4, and 5). This could have led to a different stage of receptivity during the time of hand pollination and, consequently, different numbers of fruits set initially and finally. Moreover, warm temperatures during flower development can increase fruit abscission (Fadón *et al.* 2015), which could have also affected open pollinated blossoms. This may have not been realised for insect excluded pollinated blossoms due to the low number of cherries that reached maturity. Other edge conditions such as soil moisture, nutrition and microclimate cannot be ruled out as a cause.

The greater burden (number of fruits) on trees at the edge of orchards might also explain why values of fruit quality were greater towards the centre of orchard blocks, where larger cherries were produced (Whiting *et al.*, 2006). Similarly, seed size may have been affected, which decreased towards the centre, and producing therefore smaller seeds in larger fruits. This could be commercially important since the mesocarp (flesh)/endocarp (seed) rate increased (Olmstead *et al.*, 2007), meaning more flesh is produced. However, differences were small (<1 mm in cherries averaging 28.5 mm). Trade-offs between quantity/quality would not compensate (Spornberger *et al.*, 2014) since cherries with a width of 20 mm are admissible into the first quality category (UNECE standard, 2017) or other quality categories which threshold is 25 mm (Pérez-Sánchez *et al.*, 2010). Farmers could benefit from the higher quality cherries that were produced farther from the orchard block edges if higher quality standards for cherries are applied, such as a width of 29-30 mm (Kappel *et al.*, 1996). However, a more intense thinning could reduce fruit loads and trees would develop fewer but larger cherries (Spornberger *et al*., 2014).

6.5.4 Optimal frequency of hand pollination

The greater initial fruit set achieved in blossoms hand pollinated three times could be indicative of insufficient pollen being deposited during a single pollination event or that a greater number of visits increases the chance of pollen landing on flower stigmas when the flower is most receptive (Sanzol & Herrero, 2001). Visitation rates of insects to blossom is therefore an important factor determining the efficacy of pollinating insects (Vicens & Bosch, 2000), with low visitation rates being linked to pollen deficits. For example, crops such as watermelon and strawberry need to be visited several times to ensure adequate pollination (Kremen *et al.*, 2002; Hodgkiss *et al.*, 2018), whilst apple blossoms need only a single visit to underpin commercial fruit set when pollinated by solitary bees (Vicens & Bosch, 2000). Generally though, fruit set increases with the number of visits (Garratt *et al.*, 2016). However, higher fruit drop occurred in blossoms pollinated three times than blossoms hand pollinated twice, which resulted in fewer mature fruit. Competition between pollen tubes when pollen density is excessive can result in an adverse effect on fecundity and pollen tube growth in *Cichorium intybus* (d'Eeckenbrugge, 1990), but this has not been found in sweet cherry (Beyhan & Karakaş, 2009). Nonetheless, excessive fruit set can lead to a greater demand on the tree to support the development of each fruit, and is more likely to lead to self-thinning to a level that the tree can sustain under the prevailing conditions (Blanusa *et al.*, 2006; Racskó *et al.*, 2007). Also, a third hand pollination event could have damaged the stigmas reducing viable fertilization (Zhou *et al.*, 2002), as shown in raspberries where fruit set was reduced by style damage following excessive visits (Saez *et al.*, 2014). When hand pollination is used to underpin commercial yields, blossoms are hand pollinated a single time, but a second hand pollination is also often applied (Partap & Ya, 2012). Twice hand pollination event is the minimum recommended to pollinate crops in other studies (Allsopp *et al.*, 2008; Hodgkiss *et al.*, 2018). A second hand pollination increases the probability to pollinate blossoms at the correct stage (effective pollination period), but also increases labour cost (Partap & Ya, 2012).

A key finding from the study is that when investigating pollination deficits in sweet cherry, blossoms should be hand pollinated twice. The study has also highlighted the unreliability of using values of initial fruit set to predict yields at harvest. Consequently, studies investigating pollination deficits should always consider values of final fruit set. This approach can be employed by growers to help them affectively assess the extent of pollination deficits in their orchards helping target investment in pollinator management practices (Garratt *et al.*, 2019). It can also be applied in new cultivar development programmes, which would reduce the labour required compared to the daily hand pollination approach (Mehmet, 2011), or maximize fruit set compared to a single hand pollination event.

This study also revealed that hand pollination frequency can affect some key fruit quality parameters of relevance to industry. Fruit firmness is an important variable to measure due to its importance in post-harvest storage (Wani *et al.*, 2014) and quality evaluation (Campoy *et al.*, 2015). Consequently, the firmer cherries obtained from twice hand pollination can be of importance for marketable fruit. In addition, narrower cherries influence the relation mesocarp/endocarp (e.g. mesocarp (flesh) volume), increasing their profitable value (Olmstead *et al.*, 2007).

6.5.5 Conclusions

This study has demonstrated the importance of pollinating insects to achieve commercial yields of sweet cherry, although it was apparent that pollination deficits still existed in these orchards. To further reduce deficits and maximise cherry production, wildflower habitats created in alleyways between rows of trees have the potential to be an effective tool to increase wild insect pollinators and the pollination services they provide.

6.6 References

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Chapter 7. General discussion

7.1 Abstract

Over a three-year period (2017 to 2019), this study investigated the effect of sown native perennial wildflower strips in alleyways on natural enemies and pollinators and their influence on pest regulation and pollination services in ten British protected sweet cherry orchards. In addition, this research examined the most effective wildflower strip management cutting regime to maximize ecosystem services whilst minimising inconvenience for growers. The study compared a standard management approach (SWS) of a single cut at the end of the growing season and a novel approach of actively managing cutting to a height of 20 cm throughout the growing season (AMWS). Both wildflower treatments were compared to control (commercial standard practice) alleyways (CS). The establishment and development of the wildflower strips and their impact on abiotic and biotic factors was investigated including pests and natural enemies, pollinators and pollination, and crop yield and quality.

Wildflower establishment and development was successful, with a cover of 75.7% (± 6.1) in year three, with no differences between wildflower treatments. This enabled natural enemies to increase by 73.9% in alleyways and by 12.9% in adjacent cherry trees compared to CS. As a result, pest regulation services were also increased by 25.3% in cherry trees adjacent to wildflower strips compared to CS. Enhanced resources in wildflower strips also led to an increase in pollinating insects in summer (after the cherry blossom period) in SWS and AMWS compared to unsown alleyways, but more pollinators were also recorded in SWS than in AMWS. However, in spring during the cherry blossom period more pollinators were recorded in AMWS compared to SWS and CS, which was associated with an increase of 6.1% in fruit set. Pollination exclusion experiments revealed that pollinating insects are key to underpin yields. 30.2% of blossoms exposed to insect visits became marketable fruit but only 1.4% of blossoms set fruit when insects were excluded. However, hand pollinated blossom achieved 51.7%, indicating pollination deficits in the study orchards. The value of pollinating insects to sweet cherry in the UK was estimated to be £11.3 million (£14,731.8 (± 196.2) ha^{-1}) in 2018, which, based on the study findings, could be increased to £25,607.9 ha^{-1} if a different pollination management was applied. Farmers spend, on average, £896.5 ha^{-1} per year on pollination and insecticides and acaricides, compared to £328.2 ha^{-1} for wildflower establishment, which

can enhance beneficial arthropods to deliver pollination and pest regulation services for at least three years.

In conclusion, this study has shown that deploying wildflower interventions in protected orchards can be an effective approach to enhance the ecosystem services delivered by natural enemies and pollinators. In turn, this could enable growers to reduce Plant Protection Product inputs whilst increasing yields, subsequently increasing profits.

7.2 Wildflower strips in protected orchards

7.2.1 Vegetation development

Assessments of vegetation cover, percentage occurrence, and floral unit counts reported in Chapter 3 demonstrated that establishing and managing wildflower strips under protective covers in modern sweet cherry orchards is possible (Figure 7.1). The wildflower establishment can be deemed a success compared to other studies in open fields (Carvell *et al.*, 2004; Blackmore & Goulson, 2014). This is the first time wildflower habitats have been studied under protective covers to deliver a resource for natural enemies and pollinators. Irrespective of management, the wildflower habitat increased the availability and diversity of pollen and nectar, supporting beneficial arthropods (Feltham *et al.*, 2015) to a greater extent than alleyways managed conventionally (regularly mown standard alleyways with no sown wildflower species). The contribution of the sown wildflowers to alleyway composition improved each year in percentage cover and the associated floral resources. In contrast, the presence and cover of unsown forbs and grasses and bare ground cover decreased. The introduction of perennial species increased consistency in the swards and reduced variability among unsown species over time (Campbell *et al.*, 2017). The use of perennial species also negates the need to re-sow or create gaps in the sward on an annual basis (Pfiffner *et al.*, 2019). Reduced variability in perennial wildflowers support the development of beneficial arthropod populations better (Pfiffner *et al.*, 2019), which was demonstrated by the greater abundance of natural enemies and pollinators over time (see Chapters 4 and 5). Furthermore, the use of native species, which are adapted to British weather conditions, could have facilitated their establishment and development under protective covers (Isaacs *et al.,* 2009).

Figure 7.1. A) Standard Wildflower Strip and B) Actively Managed Wildflower Strip.

However, not all the sown species performed equally, for instance *Dactylis glomerata* was the most abundant, whilst, in contrast, *Leontodon hispidus* a species selected to provide floral resources later in the season was poorly represented throughout the three year study, coinciding with other studies (Pywell *et al.*, 2003; Blackmore & Goulson, 2014). The overall successful establishment was possible because alleyways were re-sown and seed rate used was high (200 seeds / m^2 for forbs, and 100 seeds / m^2 for grass). However, the use of Roundup^{TM} (Glyphosate) during the first sowing in order to remove existing vegetation may have limited seed development (Helander *et al.*, 2019). Roundup is normally used 2-4 weeks prior to sowing to promote wildflower establishment (Frances *et al.*, 2010). Yet, due to toxicity and issues to a range of animals (Gill *et al*., 2018) and human health (e.g. cancer) reported by the International Agency for Research on Cancer (IARC, 2015), this herbicide has been banned in Austria, for the first time in Europe (Peng *et al*., 2020), and it is likely more countries will withdraw it for all uses. However, the debate on the toxicity of glyphosate is still on-going and EU assessments have deemed this herbicide as non-hazardous to public health nor associated with cancer in humans and its use is allowed in the EU (Tarazona *et al*., 2017; Berry, 2020). In addition, the toxicity on glyphosate also depends on its excessive use (Singh *et al*., 2020).

7.2.2 Wildflower mix performance

When designing a seed mix to increase pollen and nectar availability for beneficial arthropods, it is important to increase the number and diversity of floral units (Carvell *et al.*, 2015). In the seed mix used, *Prunella vulgaris* and *Leucanthemum vulgare* were the sown species with more floral units recorded per 0.5 x 0.5 m quadrat, with 9.0 (± 1.5) and

7.0 (± 1.5), respectively. *Prunella vulgaris* was mainly visited by bumblebees, as found in other studies (Carvell *et al.*, 2004; Blackmore & Goulson, 2014), but it was also frequently visited by hoverflies. *Leucanthemum vulgare* supported other pollinators (e.g. hoverflies and solitary bees) and natural enemies (e.g. parasitic wasps and spiders), coinciding with previous works (Haaland *et al.*, 2011; Carrié *et al.*, 2012; Wood *et al.*, 2016; Hatt *et al.*, 2017). It is also important to include species in the mix that provide shelter for natural enemies, such as *D. glomerata* (Hajek, 2004; Pywell *et al.*, 2005). Moreover, the pollen of this species (an anemophilous grass species) is also collected by hoverflies, especially *Platycheirus* spp. and *Melanostoma* spp. (Syrphinae: Bracchini) (Wäckers & van Rijn, 2012; Inouye *et al.*, 2015), which were frequently recorded collecting pollen from this plant. The enhancement of hoverflies within these genera is important in crops because they provide multiple ecosystem services; larvae are voraciously aphidophagous (Solomon *et al.*, 2000), whilst adults are effective pollinators (Hodgkiss *et al.*, 2018). However, flowering stems of *D. glomera* can grow to 140 cm (Hubbard, 1992), and flower heads were frequently removed with cutting in the AMWS.

Soil disturbance prior to the sowing of the bespoke seed mix also stimulated the germination and establishment of unsown species. However, some of these, including *Trifolium repens*, *Ranunculus repens*, and *Taraxacum officinale*, have potential to enhance beneficial arthropods (Altieri *et al.*, 1977; Steinbach & Gottsberger, 1994), such as solitary bees (e.g. *Andrena* spp. and *Lasioglossum* spp.), which frequently visited these plants. As a consequence, the presence of sown and unsown species in alleyways provided a wider range of resources for a greater range of beneficial species (Blaauw & Isaacs, 2012, 2014b).

7.2.3 The influence of wildflower interventions on environmental factors

A key concern of growers with the novel approach of establishing wildflower habitat under protected crops was the potential influence on humidity and temperature. However, the fact that differences between treatments were small demonstrates that this should not be a barrier to their uptake. A difference of < 1.0% in humidity between CS and wildflower strips and a mean increase of ~ 0.2ºC in temperature in SWS when compared to AMWS and CS suggests that tall vegetation in alleyways is unlikely to have significant biological impacts or affect cherry fruit development or cherry quality. This was also supported by the finding that there were no effects of alleyway treatments on the incidence of fungal disease on harvested cherries. However, further study is needed to confirm whether this limited effect would remain if more alleyways in orchard blocks were established with wildflower habitats.

7.3 Natural enemies and pest regulation service

7.3.1 The influence of wildflower interventions on natural enemies and pest regulation services

In Chapter 4, the effects of wildflower strips on natural enemies and pest regulation were investigated in order to promote Integrated Pest Management (IPM) strategies through Conservation Biological Control (CBC). It was found that wildflower strips can not only enhance natural enemies in alleyways, but also boost their numbers in the adjacent cherry trees. In turn, this resulted in an increase in pest regulation services despite the continued use of Plant Protection Products (PPPs) throughout the season. The greater abundance of natural enemies in alleyways sown with wildflowers compared to unsown control alleyways could have resulted from the creation of a more complex habitat (Balzan *et al.,* 2014), which could have provided a greater range of opportunities for natural enemies. For example, through the provision of pollen, alternative prey, and shelter (Blaauw & Isaacs, 2012, 2014b; Campbell *et al.*, 2017). The benefits of providing additional wildflower resources was demonstrated after the baseline year (year one) through enhanced predator abundance and richness, and parasitoid wasp abundance compared to unsown alleyways. The increase of natural enemies in cherry trees adjacent to AMWS and SWS compared to CS probably resulted from a spill-over from the wildflower strips (natural habitats) to trees (cropped land) (Woodcock *et al.*, 2016) along the whole row of trees. This indicates that non-cropped areas are important reservoirs for beneficial arthropods (Egan *et al*., 2020). The greater abundance, richness, and diversity of natural enemies associated with wildflower habitats is likely to have underpinned the observed higher depletion rates in the baited cards (pest regulation services), as found in other studies (Marc & Canard, 1997; Blaauw & Isaacs, 2015; Campbell *et al.*, 2017; Dainese *et al.*, 2017). Consequently, the increase in depletion of 25.4% in wildflower strips compared to CS has clearly demonstrated that the deployment of wildflower strips in sweet cherry orchards to foster CBC as part of an IPM programme can be an effective approach to increase pest control. In turn, this can reduce the need for growers to use PPPs (Hatt *et al.,* 2017). A reduction in PPPs could lead to greater benefits being achieved with regards to pest regulation as some products are also toxic to natural enemies (Beers *et al.,* 2016). Other non-aphid cherry pests are also likely to be controlled by enhanced natural enemies in trees associated to wildflower treatments, since some natural enemies groups were generalist, and would prey on a range of pests. Moreover, alive pests are more likely to be attacked by non-scavenger predators (e.g. spiders) and parasitoid wasps.

The benefit of providing perennial wildflower habitat in the sweet cherry orchards is that the response of the enhanced natural enemies was consistent with the wildflower development, which increased throughout the three-year study. This indicates that more natural enemies, and subsequently, pest control, can be enhanced in sweet cherry orchards over time (Chaplin-Kramer *et al*., 2011). The similar results in natural enemy abundance, richness and diversity that were recorded between AMWS and SWS during the study suggests that wildflower strips can be actively managed without significant negative impacts on pest regulation services. This could bring benefits to growers and workers by allowing easier movement along the alleyways and to undertake management activities such as pruning. However, the greater height of wildflowers and greater number of floral units recorded in SWS compared to AMWS in the last year of study started to create a more structurally complex sward (Chaplin-Kramer *et al.,* 2011). This could result in a tendency for greater natural enemy enhancement and an associated pest regulation service in SWS compared to AMWS in future years.

7.3.2 Natural enemy diversity

It is apparent that Araneae (spiders) were the natural enemy taxonomic group which benefited most from the wildflower strips (Figure 7.2A), as they were more abundant and had greater species richness with these treatments. Abundance and richness of Araneae depend on landscape complexity and are enhanced when natural habitats are present (Schmidt & Tscharntke, 2005; Schüepp *et al.,* 2014). Spill-over from the sown alleyways also meant they were highly abundant on the adjacent cherry trees, second only to Anystidae (whirligig mites). Wildflower habitats also provided shelter and resources including alternative prey, pollen (protein), and nectar (sugar) to other taxonomic groups (Wäckers & van Rijn, 2012; Blaauw & Isaacs, 2015). This allowed Anystidae, Coleoptera (Figure 7.2B), Hemiptera, Neuroptera, Formicidae, Opiliones, and Parasitic wasps to increase in number in wildflower strips compared to CS. Resources are therefore essential to enhance natural enemies and maintain them in orchards when pests are scarce (Wäckers & van Rijn, 2012; Blaauw & Isaacs, 2015). This is likely to explain why generalist natural enemies (e.g. Anystidae, Araneae, Coleoptera, and Opiliones) were more abundant than more specialist groups (e.g. parasitoid wasps, and Syrphidae) (Balzan *et al.*, 2014), as they are able to survive feeding on alternative prey (Bogya, 1999; Solomon *et al.*, 2000; Harwood & Obrycki, 2005; Drummond *et al.*, 2010; Cuthbertson *et al.*, 2014). Yet, these natural enemies are important in orchards since they provide efficient pest regulation services (Chant, 1956; Wyss *et al.,* 1995; Marc & Canard, 1997; Markó *et al.,* 2009; Cichocka, 2007; Drummond *et al.,* 2010; Stutz & Entling, 2011; de Roincé *et al.,* 2013; Cuthbertson *et al.,* 2014; Schüepp *et al.,* 2014).

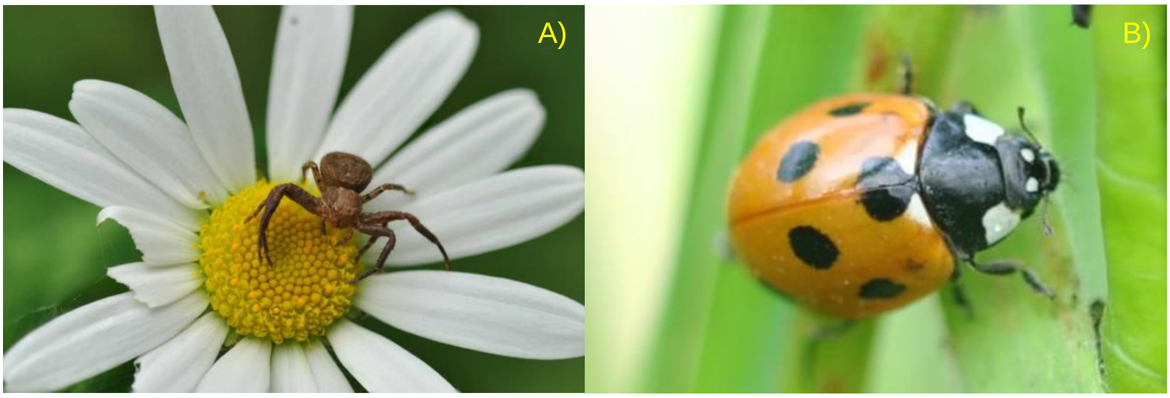

Figure 7.2. A) Crab spider (Thomisidae) on the sown wildflower *Leucanthemum vulgare*. B) Coleoptera (Coccinellidae: *Coccinella septempunctata*) in a Standard Wildflower Strip.

Due to the continued use of PPPs in the study orchards, pest populations in cherry trees were low, which is likely to have resulted in reduced prey availability for natural enemies. In turn, this can influence the abundance and species richness of natural enemies in cropped areas (Markó *et al.,* 2009). This is further compounded by the direct impacts of PPPs on natural enemies (Dib *et al.,* 2016). In contrast, PPPs were not used on wildflower strips allowing herbivores populations to develop, providing alternative prey for natural enemies (Wyss *et al.,* 1995). Moreover, swards can act as refuges for PPP applications (Pekár, 2012). This highlights the importance of introducing wildflower habitats to also provide resources for non-crop prey, supporting natural enemy populations when pest populations are low (Wäckers & van Rijn, 2012).

Despite adult hoverflies being more abundant in AMWS and SWS compared to CS (see Chapter 5), the abundance of hoverfly larvae was low and no detectable effects were observed with the implementation of alleyway treatment, which could have resulted from PPP exposure. For example, pirimicarb and spinosad had a toxicity of 2.0 (see Chapter 4), which is slightly toxic based on an average for natural enemies. However, these PPPs can cause 100% and 60% mortality on hoverfly larvae, respectively (Moens *et al.*, 2011), affecting hoverfly larvae abundance more than other natural enemy groups. In addition, about 40% of all British hoverfly species have aphidophagous larvae or feed on other soft-bodied insects, including species within Syrphini, such as *Epysirphus balteatus* (Ball & Morris, 2015). Species with zoophagous larvae were dominant in alleyways accounting for 86.7% of the total adult hoverflies recorded. Aphids are the main prey of these hoverflies (Tenhumberg, 1995), which were scarce in trees and probably in the alleyway vegetation (although prey assessments in alleyways were not conducted). This could have affected hoverfly laying behaviour and females laid eggs in the surrounding landscape where aphids were more abundant (Almohamad *et al.,* 2009). This would also support the greater adult hoverfly abundance and richness near the orchard block edges (see Chapter 5), but not for hoverfly larvae abundance, which was consistent throughout the alleyway (see Chapter 4). Adults are highly mobile and could have flown to the wildflower strips from boundaries solely to collect nectar and pollen (Almohamad *et al.,* 2009), remaining close to the edge.

7.3.3 Edge effect on natural enemies and pest regulation services

The lack of edge effect on natural enemy abundance, species richness, and pest regulation services suggests that wildflower strips can provide resources and shelter for natural enemies throughout the whole strip, although this could have been also influenced by protective covers. The increase of temperature and decrease of humidity under protective covers is likely to provide the environmental conditions to support some pests (e.g. *Tetranychus urticae* (two spotted spider mite)) (Lang, 2009; Leach & Isaacs, 2018), and greater pest abundance results in natural enemy enhancement (Leach & Isaacs, 2018). However, the scarce pests recorded throughout the rows of trees and the greater abundance of *M. cerasi* colonies near the orchard block edge in this study suggests that natural enemies were more likely to be enhanced evenly along the alleyways by the wildflowers rather than due to pest abundance. In addition, with other wildflower approaches such as field margins, hedgerow edges or wildflower patches near cropped areas in open fields, natural enemy enhancement decreases in the crop when the distance from natural habitats increases (Blaauw & Isaacs, 2015; Woodcock *et al.*, 2016). The wildflower strips used in this study provided resource for natural enemies and brought them into the orchard centres, mitigating the effects of the edge. Hence, the establishment of wildflower strips under protective covers can positively impact on the sweet cherry industry because protective covers do not deter natural enemy distribution in the orchards, and wildflowers strips can support even pest regulation services along the whole alleyway, as demonstrated by the bait cards.

7.4 Pollinating insects and pollination service

7.4.1 The influence of wildflower interventions on pollinating insects and pollination services

In Chapters 5 and 6, the responses of pollinators and the pollination services they provide to sweet cherry under protective covers were investigated. The study showed, for the first time, that wildflower strips were an effective approach to enhance wild pollinators (Figure 7.3) in protected sweet cherry orchards, although pollinating insects were only enhanced in AMWS during the cherry blossom period, which led to greater fruit set. This study reveals the benefits of sown wildflower strips established in alleyways between rows of trees in promoting pollinating insect enhancement and production. It is therefore evident that the deployment of wildflower habitats in sweet cherry orchards could be a sustainable approach underpinning pollination (Blaauw & Isaacs, 2014a).

Figure 7.3. A) Bumblebee worker (*Bombus pascuorum*) visiting the sown wildflower *Centaurea nigra*. B) Hoverfly (*Rhingia campestris*) visiting the sown wildflower *Silene dioica*.

The positive response of pollinating insects to the presence of wildflower habitats can be as a result of the greater floral resources recorded compared to unsown alleyways (see Chapter 3), although it is evident that the effects only appeared after the baseline year, once the cutting regimes were implemented. Interestingly, despite the similar abundance, richness, and diversity of pollinating insects associated to both wildflower strips after the blossom period in year two, the novel cutting regime could have fostered more nesting sites for bees, which were unavailable to reach with the greater biomass in SWS (Steffan-Dewenter & Tscharntke, 2000). Although percentages of bare ground were similar between alleyway treatments, and floral resources were not significantly different between wildflower strips, the higher vegetation in SWS and the reduced resources in CS could have deterred bees from nesting in these alleyways. Higher vegetation could have made it more difficult to reach the ground for bees to nest, more accessible with 20 cm height vegetation. As a consequence, a greater pollinator enhancement was recorded in AMWS during the blossom period in year three. The greater pollinator abundance and species richness in AMWS compared to SWS and CS resulted in a greater fruit set on trees located in alleyways with AMWS. This might indicate that pollinating insect foraging preferences for wildflower habitats managed to 20 cm height can have a positive effect on sweet cherry production. However, in year three, the greater abundance of pollinators, species richness, and diversity associated with SWS compared to AMWS and CS after the cherry blossom period might derive in an enhancement of pollinators in these alleyways during the blossom period in subsequent years (Blaauw & Isaacs, 2014a; Buhk *et al.*, 2018), which may lead to an increase in pollination services.

7.4.2 Sweet cherry pollination: insect pollinators and their efficacy

The high reliance of growers on managed pollinators for sweet cherry pollination was demonstrated by the dominance of pollination events by *Apis mellifera* (honeybee) and *Bombus terrestris* (buff-tailed bumblebee). However, relying on one or two species is a high risk strategy that can lead to yield losses (Goulson, 2003; Williams *et al.*, 2019). Honey bees and buff-tailed bumblebees can both be affected by parasites and pathogens (Pirk *et al.*, 2017; Williams *et al.*, 2019), which can put crop production at risk. For example, the mite *Varroa destructor* can dramatically reduce honeybee colonies (Vanbergen *et al.*, 2014). *Apis mellifera* hives and *B. terrestris* colony boxes also represent an on-going cost for growers. *Osmia* (mason bees) species are considered important managed solitary bees in some fruit orchard crops (Sedivy & Dorn, 2014), including sweet cherry (Bosch *et al.*, 2006; Ryder *et al.*, 2019), but this also entails a cost. In addition to reducing the risk of relying on one or two pollinator species, a greater abundance and species richness of wild pollinators can lead to a more resilient and efficient pollination of cherry blossoms (Blaauw & Isaacs, 2014a; Pywell *et al.*, 2015), and could underpin greater yields (Holzschuh *et al.*, 2012). Enhancing wild pollinating insect diversity in crops is therefore an approach that can ensure more resilient pollination (Williams *et al.*, 2019).

Wildflower habitats can be established to enhance wild pollinator diversity (Blaauw & Isaacs, 2014b) (see also Chapter 5), whilst wildflower mixes can be selected to target wild pollinators (Nichols *et al.*, 2019). For example, the mix used in this study was designed to enhance major wild pollinator guilds, such as *L. vulgare* and *Achillea millefolium*, which were mostly visited by solitary bees and hoverflies, as were *P. vulgaris* and *Trifolium pratense* by bumblebee species. Although butterflies were scarce throughout the study, they visited sown wildflowers (e.g. *Centaurea nigra*). Nonetheless, the two single records of butterflies visiting cherry blossoms indicate the low impact this pollinator guild has in sweet cherry pollination. If greater yields can be achieved with wild pollinator enhancement rather than using managed pollinators, these could be used to complement pollination services (Garibaldi *et al.*, 2013; Mallinger & Gratton, 2015), and costs for managed pollinators could be reduced (Feltham *et al.*, 2015). However, costs of wildflower establishment and management should also be taken into account (Blaauw & Isaacs, 2014a) (see Section 7.8).

In this study, the higher efficacy of wild bees to pollinate cherry blossoms was demonstrated. The dominance of *Andrena* species within solitary bees recorded visiting cherry blossoms (97.1% of the total records) determined the average solitary bee behaviour, since mason bees despite being considered good pollinators were only recorded twice throughout the three years. The greater time solitary bees (Figure 7.4A) spent on individual cherry blossoms and the greater number of blossoms visited by queen bumblebees (Figure 7.4B), along with both contacting the stigma more frequently than other observed pollinator guilds make wild bees very efficient pollinators of cherry blossoms. Moreover, both pollinator guilds were likely to subsequently visit trees in adjacent rows, which is essential for cross-pollination of self-incompatible cultivars (Brittain *et al.*, 2013b). In particular, the large size of queen bumblebees facilitated contacting the stigma often, whilst solitary bees made a greater number of stigma contacts with more of their body parts, consequently increasing the likelihood of pollen deposition (Willmer *et al.,* 2017). Pollen collected by solitary bees is dry and loose, and as a result, more accessible for the pistil (Woodcock *et al.*, 2013), the female part of the flower where the pollen lands to finalize the pollination and begin the fertilization (Woodcock, 2012). Greater numbers of visits to the pistil normally increases the chance of pollen contacting, which ensure fertilization (Garibaldi *et al.*, 2020). Consequently, solitary bees and wild bumblebees are believed to be more efficient pollinating insects of cherry blossoms than managed pollinators (Holzschuh *et al.*, 2012; Eeraerts *et al.*, 2020).

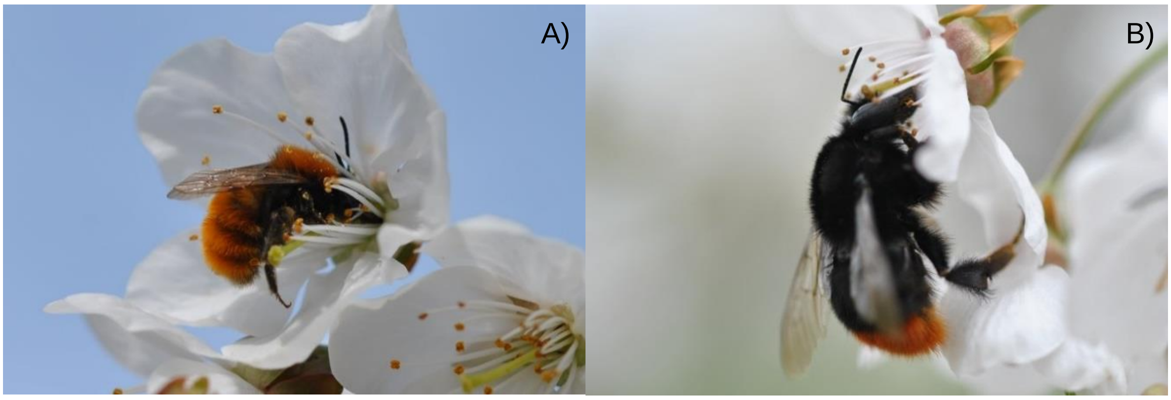

Figure 7.4. Wild bees, A) solitary bee (*Andrena fulva*) and B) queen bumblebee (*Bombus lapidarius*), visiting cherry blossoms.

Competition for pollinators between crop floral resources and additional non-crop resources may be a concern for growers to implement wildflower habitats (Free, 1968; Foulis & Goulson, 2014). However, this study demonstrated that visits by pollinating insects to crop flowers are not negatively affected by changes to alleyway management, although this could also be influenced by the fact that sown alleyways were not in bloom at the same time as cherry is flowering. Wildflowers did not detract but enhanced fruit set also in other studies including almond orchards (Lundin *et al.*, 2017), and strawberry crops (Feltham *et al.*, 2015). In addition, as farmers tend to maximize profits by arable cropping land, wildflower patches may be not available (Christmann *et al.*, 2017; Williams *et al.*, 2019). However, wildflower strips can be established in the alleyways of perennial orchards allowing farmers to optimise ground cover and land use.

7.4.3 Edge effect on pollinating insects and pollination services

In contrast to the responses of natural enemies (see Chapter 4), pollinating insects were influenced by the proximity of floral resources to the edge of cherry orchards under protective covers (see Chapter 5). The greater number of pollinators and species richness recorded near the orchard boundaries affected fruit set and quality, and consequently, production along the tree rows (see Chapter 6). The trees closest to the edge developed a greater number of cherries, but of smaller size, since greater fruit burdens on trees usually leads to the production of smaller fruit (Whiting *et al.*, 2006), indicating better cherries produced farther from the orchard block edges. The greater fruit set near the orchard boundaries suggests a relationship between successful pollination and abundance and diversity of pollinators. This indicates that wild visiting pollinators play a key role in sweet cherry pollination rather than resident managed pollinators, and if wild pollinators are detracted to visit blossoms in the centre of the orchards, lower yields could be expected in extensive cropped areas (Garibaldi *et al.*, 2011). Due to the limited pollination efficacy of managed pollinators, larger orchards would still suffer from pollination deficits regardless of honeybee (Garibaldi *et al.*, 2011; Eeraerts *et al.*, 2017) and managed buff-tailed bumblebee abundance (see Chapter 5).

The even distribution of nest boxes of managed bumblebees along the alleyways resulted in this pollinator not being affected by the proximity of the edge, and yet it did not result in an even fruit set along the tree rows. This suggests that buff-tailed bumblebees may not be an optimal pollinator for cherry blossoms. Buff-tailed bumblebees are known to forage for resources beyond the target crop (Trillo *et al.*, 2019), which could also impact on sweet cherry fruit set. A further study tracking managed bumblebees could help to confirm this. Honeybee hives are deployed near the orchards, so that honeybees have to fly into those to pollinate cherry blossoms. However, the use of protective covers could explain the limited visits from honeybees and wild pollinators towards the centre of the orchards since pollinator visits are affected by these (Hall *et al.*, 2019), even though higher temperature, and lower humidity and wind speed were recorded towards the centre (see Chapters 4 and 5). Environmental factors might affect pollination services, and subsequently fruit set, particularly delivered by honeybees (Brittain *et al.*, 2013a). For example, orchards situated in exposed locations (e.g. Orchard block 9) might suffer from greater wind speed, which limits honeybee flying behaviour (Brittain *et al.*, 2013a). Since pollinator services are not affected by edge effects in open crops (Button & Elle, 2014; Woodcock *et al.*, 2016; Fountain *et al.*, 2019), protective covers could be only installed after the cherry blossom period or during rainy days to protect blossoms from rain and fungal disease (e.g. *Monilinia laxa* and *Botrytis cinerea*) (Børve & Stensvand, 2003).

7.4.4 Pollination management

To maximise cherry production the design of orchards should be carefully considered since fruit production is affected by the distance from the orchard edge. Reducing orchard size could be an alternative to ensure an even distribution of pollinators. For example, rectangular orchard design with rows of trees of 50 m length might allow pollinators to reach the centre of the orchards. However, trade-offs between costs of orchard design and production should be considered. Although managed buff-tailed bumblebees can be evenly distributed under protective covers by deploying nesting boxes along the alleyways, and honeybees can visit orchards in open fields evenly (e.g. setting protective covers after the cherry blossoms period), pollination deficits would still be present. This is because managed pollinators cannot underpin maximum yields, and increasing their numbers in sweet cherry orchards would still probably lead to pollination deficits. Furthermore, this would pose an extra cost to growers. In contrast, wild pollinators are deemed to be more efficient pollinating cherry blossoms (see Chapter 5), so that increasing nesting sites for wild bees should be an essential objective. Particularly, since *Andrena* and ground-nesting *Bombus* species, are the key visitors of cherry blossoms. Nesting sites should be focused on soil characteristics, for example, most of solitary bees tend to nest in sandy soils (Cane, 1991). Sweet cherry blossoms provide resources from mid-April to early May, when *Andrena* spp. emerge and *Bombus* spp. queens forage to establish new colonies (Falk, 2015), whilst wildflowers provide resources throughout the rest of the growing season (until late September). *Andrena* spp. require normally bare ground in south-faced slopes to establish their nests, whilst ground-nesting *Bombus* spp. nesting sites are typically found in tussock forming grasses or old mammal burrows (Falk, 2015; Cole *et al.*, 2020). Such areas should be located near the orchards and not be disturbed. Alternatively, in larger orchards, nesting sites could potentially be located among the rows of trees, replacing one/two trees. However, the commercial feasibility of this management approach needs to be investigated since some inconveniences for growers may be encountered based on their established orchard designs.

Generally, wild pollinator abundance and diversity in orchards can also be influenced by the surrounding landscape, particularly in conventional orchards (Kennedy *et al.*, 2013). Wild pollinators differed between sites and orchard blocks due to landscape characteristics. For instance, at Site 1 more forest cover was present (see Chapter 2), which could support more pollinators in the landscape (Mallinger *et al.*, 2016). Next to Orchard block 6 at Site 3 and Orchard block 9 at Site 5, a patch of wildflower was established, which could have also resulted in higher pollinator diversity recorded compared to sites with less suitable landscape context (e.g. Site 4). However, at all sites, wild pollinating insects were enhanced by wildflower strips when the wildflowers established successfully, and these are therefore a promising alternative to managed pollinators.

7.5 Additional benefits of wildflower interventions in orchards

In addition to support ecosystem services and subsequently, crop production, the implementation of wildflower habitats can also contribute to climate change mitigation and improve air and water quality (Beard & Green, 1994; Aldrich, 2002). For example, wildflowers can uptake and retain pollutant particles, which is related to vegetation mass (Aldrich, 2002). Moreover, leguminous wildflowers such as *T. pratense* can fix nitrogen (Huss-Danell *et al.*, 2007), which could be taken up by the cherry trees, enabling N fertilizer inputs to be reduced (Huss-Danell *et al.*, 2007). Wildflower habitats can also improve water infiltration and prevent soil erosion (Burel, 1996), reduce the occurrence of undesirable weed species, and regulate tree development and productivity (Denys & Tscharntke, 2002; Granatstein & Sánchez, 2009). Furthermore, wildflower habitats can help to promote ecological intensification in sweet cherry orchards since wildflowers are an effective approach to restore habitat loss, and foster arthropod and plant biodiversity conservation (Freemark *et al.*, 2002; Haaland *et al.*, 2011; Blaauw & Isaacs, 2012; Bretzel *et al.*, 2016). However, in cropped areas only a few common species may benefit (Kleijn *et al.*, 2015), indicating that different approaches might also be necessary to conserve rarer species, especially those not associated with agricultural land.

7.6 The economics of wildflower strips in sweet cherry orchards

Insect pollination is essential for achieving marketable yields in sweet cherry (see Chapter 6). However, the greater percentage of final fruit set achieved in hand pollinated blossoms compared to the blossoms pollinated by insects suggests there was a pollination deficit in the study orchards of approximately 20% in fruit set. This is of considerable importance for commercial sweet cherry production. The value of cherries in the UK in 2018 was £11.7 million (£11,734,726.98) from a harvested area of 765 ha (DEFRA, 2019). Consequently, the value of cherries in the UK per hectare was £15,339.5 ha^{-1}, which is the sum of blossoms pollinated by insect pollinators and blossoms not receiving insect visits that retained fruit to maturity (final fruit set). The annual value of sweet cherries was calculated per each study site according to blossoms insect excluded, open, and hand pollinated (Table 7.1). The estimated annual value of pollinating insects for sweet cherries in the UK is therefore £14,731.8 (± 196.2) ha^{-1}. This represents a total value of £11.3 million in the UK. Whilst £607.7 (± 196.2) ha^{-1} can be produced without pollinators.

Hand pollination showed that fruit set could be increased by a further 51.7%, which results in an income of £25,607.9 (± 1,545.1) ha^{-1}. Assuming yields are consistent over the whole tree, hand pollination represents an increase of 66.9% (± 10.1). Consequently, current deficit across the study orchards represents £10,268.4 ha^{-1}. However, this value may change due to costs on wildflower strip establishment have been not included; further research would be needed to provide a more accurate estimation.

In addition, better cherry quality was underpinned in blossoms exposed to insect visits and hand pollinated. Fruit size is one of the most important attributes of cherries, which is directly linked to commercial value (Whiting *et al.*, 2006). Cherries with a width of 20 mm can be admissible into the first quality category according to the UNECE (United Nations Economic Commission for Europe) (UNECE standard, 2017). Consequently, cherries <20 mm width (diameter) would be discarded. This represents a 0.3% loss from hand pollinated blossoms, 0.1% for open pollinated, and 1.1% for insect excluded blossoms (Table 7.1). However, quality standards for first quality cherry in Spain consider a minimum of 25 mm width (Pérez-Sánchez *et al.*, 2010), whilst in Canada the sweet cherry standard requirement for industry ranges between 29-30 mm, although 25 mm is also considered a minimum width (Kappel *et al.*, 1996). In fact, Kordia cherries average 29.1 mm in diameter (Long *et al.*, 2008), which would imply a greater loss for not meeting marketable requirements (Table 7.1). In the UK, consumer preferences aim for large cherries (Wermund *et al.*, 2005).

Wildflower habitats are an alternative to enhance ecosystem services. Although Dicks *et al.* (2015) determined that 2% of wildflower habitat in cropped areas is sufficient to provide resources to wild bees, this may not be appropriate for cherry orchards, and an additional increase in floral resources could benefit beneficial arthropods. 1 x 50 m wildflower strips every four tunnels would be recommended (Figure 7.5) in a one ha-orchard block (200 x 50 m) with eight metre-tunnels (high tunnel system). This represents 6.25% of the area (6.25 strips; 200 ÷ 8 ÷ 4) to be sown per one ha-orchard block. Alternatively, 1 x 100 m wildflower strips every two tunnels in one ha-orchard block (100 x 100 m), which also represent 6.25% of the area.

Table 7.1. Percentage of final fruit set (%) and annual value (£ ha^{-1}) of insect excluded (IE), open (O), and hand (H) pollinated blossoms according to site.

Site	**Percentage of final fruit set (%)**			**Annual value (£ ha^{-1})**			**% increase with hand pollination**
	IE	**O**	**H**	**IE**	**O**	**H**	
1	2.5	31.1	56.5	1,137.9	14,201.6	25,788.7	68.1
2	0.0	20.5	41.4	0.0	15,339.5	31,025.8	102.3
3	2.0	33.6	58.8	879.8	14,459.7	25,313.5	65.0
4	0.9	34.3	49.3	394.8	14,944.7	21,505.8	40.2
5	1.3	31.7	52.5	626.0	14,713.5	24,405.8	59.1
Mean (± SD)	1.4 (0.4)	30.2 (± 2.5)	51.7 (± 3.0)	607.7 (± 196.2)	14,731.8 (± 196.2)	25,607.9 (± 1,545.1)	66.9 (± 10.1)

Table 7.2. Percentage of fruit set (%) and its value (£ ha^{-1}) of cherries from insect excluded, open, and hand pollinated blossom that retained fruit to maturity (final fruit set), which would be lost according to different width (diameter) thresholds that determine fruit quality.

Pollination treatment	**< 20 mm**		**< 25 mm**		**< 29 mm**	
	Fruit set (%)	Loss value (£ ha^{-1})	Fruit set (%)	Loss value (£ ha^{-1})	Fruit set (%)	Loss value (£ ha^{-1})
Insect excluded	1.1	168.6	27.7	4,256.3	83.8	12,853.2
Open	0.1	14.2	5.0	762.2	49.9	7,650.8
Hand	0.3	43.6	1.7	261.5	47.2	7,234.0

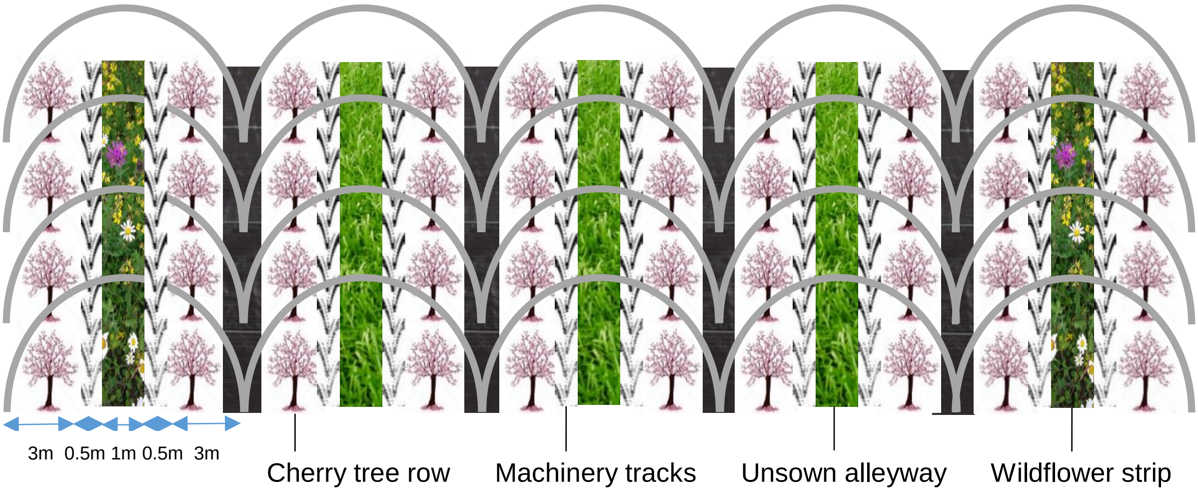

Figure 7.5. Diagram for wildflower strips established every four tunnels (eight alleyways) for a one ha orchard block (200 x 50 m) and eight metre-tunnels (two rows of trees per tunnel).

The cost for wildflower seeds (eight wildflower species) per one hectare-orchard using 2% for wildflower habitat (Dicks *et al.*, 2015) would be £44.5 ha^{-1}, compared to £139.0 ha^{-1} using 6.25% (Table 7.3). The total cost of wildflower strip establishment is £328.2 ha^{-1} (Table 7.3). However, the wildflowers selected in the mix are perennial which means that can survive for several years. Based on a lifespan of seven years (Beattie, 2019), the cost of establishment wildflower strips could be reduced to £46.9 ha^{-1} per year. In addition, if wildflowers are sown at the same time as the orchard plantation, investment can be substantially reduced, since there would be no need for the original sward to be removed or alleyways cultivated. This means that the total cost of wildflower strip establishment would be £247.1 ha^{-1}.

In addition, farmers can apply for Countryside Stewardships (GOV.UK, 2020), which provide financial support for adopting actions aimed to environmental habitat enhancement, such as Wildlife Offers (e.g. wildflower strips). For example, farmers can receive £511 per ha for sowing strips with a nectar-flower mix in bush orchards. The nectar-flower mix should include at least four nectar-rich plants and at least two perennials (e.g. *T. pratense*, *C. nigra* and *Lotus corniculatus*) (GOV.UK, 2020). This offer can compensate for the wildflower strips investment costs.

Table 7.3. Cost of establishing wildflower habitats of 6.25% area in a one-hectare sweet cherry orchard.

Operation	**Cost (£ ha^{-1})**	**Source**
PPP (fumigant / herbicide)	18.1	Wilson *et al.*, 2011; Beattie, 2019
Cultivation	63.0	Beattie, 2019
Seed mix	139.0	John Chambers Wildflowers (www.johnchamberswildflowers.co.uk) and Yellow Flag Wildflowers (www.wildflowersuk.com)
Sand	11.1	B&Q (www.diy.com)
Sowing	31.0	Beattie, 2019
Rolling	19.0	Beattie, 2019
Fuel	47.0	Beattie, 2019
Total	328.2	

The implementation of 6.25% wildflower habitat could enhance wild pollinators to replace managed pollinators, and reduce PPP inputs associated with pest control delivered by enhanced natural enemies. Farmers deployed an average of 6.5 buff-tailed bumblebee nest boxes and 2.4 honeybee hives per ha (see Chapter 2). Each single buff-tailed bumblebee box (Figure 7.6A) costs around £29-30, but multi hive boxes, which contain up to three colonies (Figure 7.6B), range £73-81 (personal communication). As both types of buff-tailed bumblebee boxes were used in the study orchards, the average for a box is therefore £53. Honeybees were rented from beekeepers, being the rate for a honeybee hive (Figure 7.6C) around £68 (personal communication). Consequently, growers spend annually in pollination services a total of £507.7 ha^{-1} (£344.5 for bumblebees and £163.2 per honeybees).

The average cost for an insecticide application is £54 ha^{-1}, whilst the cost for the spray application is approximately £18 ha^{-1} (Cross *et al.*, 2015). In the study sweet cherry orchards, insecticide and acaricide applications averaged 5.4 per orchard block and year (see Chapter 4). Consequently, the estimation of the cost for a cherry spray programme is £388.8 ha^{-1} per year.

Figure 7.6. A) Single and B) multi hive buff-tailed bumblebee (*Bombus terrestris*) nest boxes deployed within the orchard blocks along the alleyways. C) Honeybee (*Apis mellifera*) hives deployed next to the orchard blocks.

In total, farmers spend an estimate of £896.5 ha^{-1} per year in pollination and pest control, compared to £328.2 ha^{-1} for wildflower establishment, which can enhance beneficial arthropods to deliver pollination and pest regulation services for several years.

7.7 Experimental design limitations

For findings of the study to be of direct relevance to the sweet cherry industry, this study was conducted in commercial orchards. As a consequence, this presented a number of challenges when implementing the trial. Due to the project being focused on a particular sweet cherry cultivar of commercial importance (Kordia), distances between alleyway treatments adjacent to Kordia tree rows may not have been sufficient nor consistent between orchard blocks. Sweet cherry orchards include self-incompatible cultivars and therefore are not planted as solid blocks, different cultivars are included to enhance cross-pollination (Koumanov & Long, 2017), which varied from two to five (see Chapter 2). As a result, it was not possible to establish numerous alleyways in orchard blocks. Also, due to the high mobility of pollinators and the lack of independence between alleyway treatments, it was not possible to demonstrate the whole influence of alleyway treatment on pollinator and pollination (Zurbuchen *et al.*, 2010).

Although the contribution of wildflowers to the composition of alleyway vegetation was good despite some management actions by growers being counterproductive (e.g. pruning left on top of the wildflower strips), wildflower development could have been better. In addition, probably as a result of a shallow scarification of the alleyways at Site 4, which creates a niche for the seeds to germinate (Blake *et al.*, 2011), wildflower establishment was still particularly poor even after re-sowing, and this site was dropped from studies in year three.

Natural enemy studies were influenced by the continued use of PPPs, which resulted in the complete elimination of cherry arthropod pests. As a consequence, populations of *Drosophila suzukii*, *Myzus cerasi* and *T. urticae* in cherry trees and fruit could not be adequately monitored. It was therefore also not possible to investigate natural enemy/prey interactions and consumption rates (pest regulation services). The study with baited cards was therefore conducted, but this is a proxy using dead aphids. Consequently, non-scavenger predators (e.g. spiders) may not have predated from these bait cards. In addition, pest control delivered by parasitoid wasps could not be investigated with the bait cards used. The lack of time during the project limited experimentation with alternative bait for the cards, such as Lepidoptera eggs of *D. suzukii* pupae, which could have possibly allowed examining predation and parasitoidism. PPPs can also be detrimental for pollinators (Goulson *et al.*, 2015), and consequently, abundance and species richness could have been reduced to some extent and behaviour altered.

7.8 Future work

Key findings of the study are likely to be of importance for growers, but to gain a greater understanding further research is required to investigate the following:

- Different management approaches to ensure first sowing, including alleyway preparation without using the glyphosate Roundup.
- Alternative seed mix testing a combination of different species and removing species which performed poorest (e.g. *L. hispidus*).
- Assessments on wildflower interventions to identify alternative prey for natural enemies.
- The impact of floral mixes on *T. urticae* and associated phytoseiid mites is critical, as this is one of the main pests of cherry.
- Long-term (> three years) influence of wildflower strips on responses from natural enemies and insect pollinators.
- The impact of implementing wildflower strips over a greater cropped area in each orchard block to determine whether the spatial benefits seen in this study can be scaled up.
- Whether in the absence of PPPs, natural enemies can control cherry pests below economic thresholds (e.g. organic orchards).
- Natural enemy identification to species level to detect the most important predator and parasitoid wasp species controlling cherry pests.
- Yield and fruit quality of blossoms pollinated by wild pollinating insects vs. managed pollinators when there is sufficient distance between treatments.
- Edge effects in protected orchards with smaller distance from the edge.
- Pollen analysis to determine the percentage of cherry pollen on insect pollinator loads.

7.9 Recommendations for growers

Based on the performance (establishment and development) of the sown perennial native wildflowers, the species and sowing rate in Table 7.4 would be recommended. This mix aims to enhance beneficial arthropods to deliver multiple ecosystem services in the UK, and includes all the species used in this study except for *L. hispidus*. Mixes should include plants that support similar beneficial arthropods to increase probability of any of these species being established (e.g. *A. millefolium* and *L. vulgare*, which both support solitary bees and hoverflies). The rate followed in this study at 1.74 g seed m^{-2} would be recommended, which is also similarly used in other studies (Feltham *et al.*, 2015).

To maximize the probability of establishment, a correct sowing process should be carried out. This consists of a disturbance of the soil through cultivation followed by an herbicide application. Due to the possible withdrawal of the glyphosate Roundup, other herbicide such as fusilade could be employed, although this herbicide is only effective against grass species and a second herbicide such as glufosinate may be needed if alleyways include non-grass (broad leaf) species. Alternatively, a fumigation could also be applied, including products such as metam sodium and dazomet (Aldrich, 2002), which are approved for use in the UK. Sowing should take place after two weeks (Frances *et al*., 2010). For perennial wildflowers, sowing in autumn would be recommended as they can develop leaves to overwinter and will bloom sooner in spring (Aldrich, 2002), as followed in this study. In addition, this study has shown that crop blossoms do not compete with wildflower resources pollinating insect visits. Cultivation prior to sowing of the alleyways is essential to create spaces for seedlings to develop, whilst, rolling after-sowing is essential to firm contact of the seed with the soil. The seeds should be mixed with sand to ensure an even distribution by hand. It is also key to enable a baseline year of regular 10 cm-cutting to promote establishment.

The management cutting regime recommended would be to a height of 20 cm from May to late September and then to a height of 8 cm, as followed in this project. Mowing could be carried out at the same time of spraying saving cost. The immediate removal of branches left after thinning (during blossom) and pruning (after harvest) is key to avoid the bare patches. Accordingly, the removal of leaves in November / December would be advantageous. Yet, cuttings may be left *in situ*. The disposition of bare ground and tussock grass areas for wild bees to nest should also be encouraged.

This recommendation aims to maximize benefits from ecosystem services compared to the standard wildflower management with only cutting in the autumn. It is a promising cutting regime that can be implemented in protected orchard alleyways to minimise inconvenience to vehicle movement and workers operating in the orchards, whilst both natural enemy and pollinator communities are enhanced. This study has shown that such an approach would be cost effective.

Table 7.4. Seed mix composition, sowing rate recommended, and price (£) to establish wildflower strips in sweet cherry orchards under protective covers. Wildflower seeds from John Chambers Wildflowers (www.johnchamberswildflowers.co.uk) and Yellow Flag Wildflowers (www.wildflowersuk.com).

Scientific name	**Common name**	**Sowing rate (seeds m^2)**	**Sowing rate (g/m^2)**	**% by weight**	**Price (£) per ha**	**Price (£) per 2% of orchard**	**Price (£) per 6.25% of orchard**
Achillea millefolium	Yarrow	200	0.03	1.91	36.6	0.73	2.29
Centaurea nigra	Knapweed	200	0.44	25.48	286.0	5.72	17.88
Dactylis glomerata	Cock's-foot	100	0.10	5.70	44.9	0.90	2.81
Leucanthemum vulgare	Ox-eye daisy	200	0.10	5.73	64.4	1.29	4.03
Lotus corniculatus	Bird’s-foot trefoil	200	0.40	22.93	866.7	17.33	54.17
Prunella vulgaris	Selfheal	200	0.20	11.46	285.4	5.71	17.84
Silene dioica	Red campion	200	0.20	11.46	237.3	4.75	14.83
Trifolium pratense	Red clover	200	0.27	15.29	403.0	8.06	25.19
Total		**1,500**	**1.74**	**100**	**2,224.10**	**44.49**	**139.02**

7.10 Conclusions

Wildflower strips can be successfully established in protected commercial sweet cherry orchards to enhance pest regulation and pollination services to ultimately improve yields. A further important finding is that contrary to the expectations of growers, the presence of wildflower strips did not increase values of humidity, which might have otherwise resulted in the greater incidence of fungal diseases. The successful establishment and development of the wildflower strips led to a diverse range of sown and unsown floral resources providing nectar, pollen and structure for natural enemies and pollinators. More pollinators were associated with wildflower strips, although better pollination services were only recorded in AMWS, probably due to more accessible nesting sites for wild bees. This suggests that both nest sites and wildflowers are highly important resources for pollination services delivered by wild pollinators. Pollinating insects are essential to achieve sweet cherry commercial yields, which is mainly achieved by managed pollinators (*A. mellifera* and *B. terrestris*). However, relying on a small number of pollinating insect species is a risk, and wild pollinators (solitary bees and queen bumblebees) are more efficient visiting cherry blossoms than managed pollinators. Moreover, a diverse community of wild pollinators can provide resilient pollination. The pollination deficits recorded also support the lower pollination efficacy of managed pollinators, and greater yields, and subsequently profitability, can be achieved if different orchard pollinator management approaches were applied. This could include newly created wildflower habitats that can enhance wild pollinators and subsequently, pollination services in sweet cherry orchards.

Greater yields and better pest control obtained on trees associated with the novel approach of wildflower strips to a height of 20 cm (AMWS) compared to unsown alleyways (CS) is encouraging for growers to establish wildflower strips across the protected orchards. In addition, this approach can bring benefits to growers and workers through the improvement of movement along the alleyways and facilitation of management activities. This offers benefit in terms of production and the potential reduction in the number of PPP applications to control pests. In addition, the response of natural enemy abundance and richness and pest regulation services were not associated to edge effects, indicating that pest control can be evenly distributed within the orchards. However, less pollinators were recorded towards the centre of the orchard, which had an impact in fruit production. Consequently, consideration must be taken in large orchards. Although benefits will take time to appear (at least two years), bespoke wildflower strips have the potential to support sustainable production of sweet cherry through enhanced pollination and pest regulation services.

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Appendices

Appendix 3.1. Mean number (± SE) of floral units in both wildflower strips (Actively Managed Wildflower Strips and Standard Wildflower Strips) and Control Strips of the sown and unsown species per quadrat (0.5 x 0.5 m) across all surveys and years.

Plant species	**Sown / Unsown**	**Number of floral units**	
		Wildflower Strips	**Control Strips**
Trifolium repens	Unsown	14.0 (± 4.7)	4.7 (± 1.7)
Prunella vulgaris	Sown	9.0 (± 1.5)	-
Leucanthemum vulgare	Sown	7.0 (± 1.5)	-
Achillea millefolium	Sown	3.0 (± 0.7)	-
Dactylis glomerata	Sown	2.6 (± 0.3)	-
Lotus corniculatus	Sown	2.1 (± 0.7)	-
Ranunculus repens	Unsown	2.1 (± 0.6)	3.5 (± 1.5)
Silene dioica	Sown	1.5 (± 0.4)	-
Matricaria discoidea	Unsown	1.5 (± 0.7)	0.4 (± 0.2)
Centaurea nigra	Sown	1.4 (± 0.4)	-
Trifolium pratense	Sown	1.4 (± 0.4)	-
Epilobium adenocaulon	Unsown	1.2 (± 0.4)	1.4 (± 0.5)
Plantago lanceolata	Unsown	0.9 (± 0.3)	-
Senecio jacobaea	Unsown	0.4 (± 0.2)	-
Leontodon hispidus	Sown	0.3 (± 0.1)	-
Brassica sp.	Unsown	0.3 (± 0.2)	-
Capsella bursa-pastoris	Unsown	0.2 (± 0.2)	-
Veronica chamaedrys	Unsown	0.1 (± 0.1)	-
Plantago major	Unsown	0.1 (± 0.1)	0.1 (± 0.1)
Taraxacum officinale	Unsown	0.1 (± 0.03)	0.1 (± 0.1)
Cardamine sp.	Unsown	0.1 (± 0.1)	-
Geranium dissectum	Unsown	0.03 (± 0.03)	0.2 (± 0.1)
Bellis perennis	Unsown	-	0.3 (± 0.2)
Epilobium angustifolium	Unsown	-	0.03 (± 0.03)

Appendix 4.1. Comparisons in the generalized linear mixed models with negative binomial error structures for the natural enemy studies in alleyway vegetation using the Akaike Information Criteria (AIC). Interaction between alleyway treatment and year represented by *Alleyway treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Total natural enemies			
Total natural enemies ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		7966.2	0.0
Alleyway treatment: Year	4	8005.8	**39.6**
Distance from the edge	1	7964.9	-1.3
Time of day surveyed	1	7965.3	-0.9
Araneae			
Araneae ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		4757.1	0.0
Alleyway treatment: Year	4	4803.2	**46.1**
Distance from the edge	1	4755.5	-1.6
Time of day surveyed	1	4756.5	-0.6
Coleoptera			
Coleoptera ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		4078.1	0.0
Alleyway treatment: Year	4	4078.3	0.2
Distance from the edge	1	4078.7	0.6
Time of day surveyed	1	4081.6	**3.5**
Formicidae			
Formicidae ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		3500.9	0.0

Alleyway treatment: Year	4	3510.5	**9.6**
Distance from the edge	1	3499.0	-1.9
Time of day surveyed	1	3499.2	-1.7
Hemiptera			
Hemiptera ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1409.0	0.0
Alleyway treatment: Year	4	1409.9	0.9
Distance from the edge	1	1407.0	-2.0
Time of day surveyed	1	1408.0	-1.0
Lithobiidae			
Lithobiidae ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1031.5	0.0
Alleyway treatment: Year	4	1033.8	**2.3**
Distance from the edge	1	1030.3	-1.2
Time of day surveyed	1	1037.0	**5.5**
Neuroptera			
Neuroptera ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		934.3	0.0
Alleyway treatment: Year	4	939.6	**5.4**
Distance from the edge	1	932.5	-1.8
Time of day surveyed	1	932.7	-1.6
Opiliones			
Opiliones ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1604.9	0.0
Alleyway treatment: Year	4	1607.8	**2.9**
Distance from the edge	1	1605.7	0.8
Time of day surveyed	1	1603.2	-1.7

Parasitoid wasps			
Parasitoid wasps ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		4037.8	0.0
Treatment: Year	4	4064.2	**26.4**
Distance from the edge	1	4037.6	-0.2
Time of day surveyed	1	4035.9	-1.9
Syrphidae			
Syrphidae ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		542.2	0.0
Alleyway treatment	2	538.7	-3.5
Year	2	556.5	**14.2**
Distance from the edge	1	540.6	-1.6
Time of day surveyed	1	540.3	-2.0
Family richness			
Family richness ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		5163.5	0.0
Alleyway treatment: Year	4	5181.2	**17.7**
Distance from the edge	1	5161.9	-1.6
Time of day surveyed	1	5168.4	4.9
Shannon diversity			
Shannon diversity ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1390.2	0.0
Alleyway treatment: Year	4	1401.7	**11.5**
Distance from the edge	1	1389.1	-1.1
Time of day surveyed	1	1391.5	1.3

Appendix 4.2. Comparisons in the generalized linear mixed models with negative binomial error structures for the natural enemy studies on cherry trees using the Akaike Information Criteria (AIC). Interaction between alleyway treatment and year represented by *Alleyway treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Total natural enemies			
Total natural enemies ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		11156.3	0.0
Alleyway treatment: Year	4	11175.0	**18.7**
Distance from the edge	1	11155.7	-0.6
Time of day surveyed	1	11156.1	-0.2
Anystidae			
Anystidae ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		7848.3	0.0
Alleyway treatment	2	7845.5	-2.8
Year	2	7918.1	**69.8**
Distance from the edge	1	7856.6	**8.3**
Time of day surveyed	1	7850.1	1.8
Araneae			
Araneae ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		5683.4	0.0
Alleyway treatment	2	5685.2	1.8
Year	2	5798.5	**115.1**
Distance from the edge	1	5686.4	**3.0**
Time of day surveyed	1	5685.6	**2.2**
Coleoptera			
Coleoptera ~ Alleyway treatment + Year + Distance from the edge + Time period +			

(random: Site/Orchard)			
Global model		986.1	0.0
Alleyway treatment	2	983.6	-2.6
Year	2	1012.8	**26.7**
Distance from the edge	1	984.1	-2.0
Time of day surveyed	1	984.8	-1.3
Forficulidae			
Forficulidae ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		142.6	0.0
Alleyway treatment	2	139.6	-2.9
Year	2	139.1	-3.5
Distance from the edge	1	143.1	0.6
Time of day surveyed	1	141.4	-1.2
Formicidae			
Formicidae ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1114.2	0.0
Alleyway treatment	2	1112.5	-1.7
Year	2	1248.3	**134.1**
Distance from the edge	1	1112.3	-1.9
Time of day surveyed	1	1112.4	-1.8
Hemiptera			
Hemiptera ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		2337.3	0.0
Alleyway treatment	2	2334.3	-3.0
Year	2	2340.9	**3.6**
Distance from the edge	1	2337.6	0.3
Time of day surveyed	1	2336.6	-0.7
Neuroptera			
Neuroptera ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1071.6	0.0

Alleyway treatment: Year	4	1072.1	0.5
Distance from the edge	1	1069.6	-2.0
Time of day surveyed	1	1074.3	**2.7**

Opiliones

Opiliones ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)

Global model		2955.1	0.0
Alleyway treatment: Year	4	2964.7	**9.6**
Distance from the edge	1	2955.0	-0.1
Time of day surveyed	1	2953.6	-1.5

Parasitoid wasps

Parasitoid wasps ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)

Global model		2157.2	0.0
Alleyway treatment: Year	4	2159.0	1.8
Distance from the edge	1	2155.2	-2.0
Time of day surveyed	1	2161.2	**4.0**

Syrphidae

Syrphidae ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)

Global model		1019.5	0.0
Alleyway treatment	2	1020.9	1.4
Year	2	1024.3	**4.8**
Distance from the edge	1	1017.8	-1.7
Time of day surveyed	1	1022.0	**2.5**

Family richness

Family richness ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)

Global model		7365.0	0.0
Alleyway treatment	2	7365.5	0.5
Year	2	7466.6	**101.6**
Distance from the edge	1	7363.6	-1.4
Time of day surveyed	1	7366.0	1.0

Shannon diversity			
Shannon diversity ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		3247.7	0.0
Alleyway treatment	2	3248.1	0.4
Year	2	3350.4	**102.7**
Distance from the edge	1	3246.6	-1.1
Time of day surveyed	1	3248.0	0.3

Appendix 4.3. Comparisons in the generalized linear mixed models for the abiotic factors (temperature, humidity, and wind speed) using Akaike Information Criteria (AIC). Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	Degrees of freedom	AIC	ΔAIC
Temperature			
Temperature ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		27584	0
Alleyway treatment	2	27582	-2
Year	2	27763	**179**
Distance from the edge	1	27603	**19**
Time of day surveyed	1	27613	**29**
Humidity			
Humidity ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		35975	0
Alleyway treatment	2	35973	-2
Year	2	36114	**139**
Distance from the edge	1	35975	0
Time of day surveyed		36079	**104**
Wind speed			
Wind speed ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		4242	0
Alleyway treatment	2	4242	0
Year	2	4319	**77**
Distance from the edge	1	4564	**322**
Time of day surveyed	1	4241	-1

Appendix 5.1. Number of visits to wildflowers species by pollinating insects and the total of visits to wildflower species according to pollinator guild recorded on transect surveys of cherry blossoms throughout the three-year study. *Taraxacum officinale*, *Ranunculus repens*, and *Brassica* spp. are unsown species. *Silene dioica* is a sown species.

Pollinator guild	**Pollinator species**	**Visits to *Taraxacum officinale***	**Visits to *Silene dioica***	**Visits to *Ranunculus repens***	**Visits to *Brassica* spp.**	**Total visits by pollinator guild**
Honeybee	*Apis mellifera*	45	0	2	0	47
Bumblebee	*Bombus lapidarius*	2	0	0	0	47
	Bombus lucorum	1	0	0	0	
	Bombus pascuorum	2	0	0	0	
	Bombus terrestris (wild)	2	1	0	0	
	Bombus terrestris (managed)	39	0	0	0	
Solitary bee	*Andrena* spp.	4	0	1	0	69
	Andrena angustior	1	0	0	0	
	Andrena chrysosceles	2	0	0	0	
	Andrena cineraria	4	0	0	0	
	Andrena dorsata	1	0	0	0	
	Andrena flavipes	1	0	0	0	
	Andrena haemorrhoa	24	0	0	0	
	Andrena nigroaena	6	0	0	0	
	Andrena nitida	17	0	0	0	
	Halictus rubicundus	1	0	0	0	
	Lasioglossum albipes	5	0	1	0	
	Nomada fabriciana	1	0	0	0	
Hoverfly	*Cheilosia pagana*	1	0	0	0	52
	Episyrphus balteatus	3	0	1	0	
	Eristalis spp.	3	0	0	0	
	Eristalis pertinax	6	0	0	0	
	Eristalis tenax	2	0	0	0	
	Helophilus pandulus	1	0	0	0	
	Leucozona lucorum	1	0	0	0	
	Melanostoma scalare	4	1	0	0	
	Platycherius spp.	3	0	0	0	

	Platycherius albimanus	9	0	0	0	
	Platycherius peltatus	3	0	0	0	
	Platycherius scutatus	4	1	0	0	
	Rhingia campestris	1	3	0	0	
	Sphaerophoria scripta	1	0	0	1	
	Syrphus ribesii	3	0	0	0	
Total		203	6	5	1	215
Percentage		94.4	2.8	2.3	0.5	100

Appendix 5.2. Number and percentage of visits to wildflower species according to pollinator guilds recorded on transect surveys of orchard alleyways throughout the three-year study. HB (honeybee), BB (bumblebee), SB (solitary bee), HF (hoverfly), BT (butterfly).

Plant species	**Pollinator guild**						
	HB	**BB**	**SB**	**HF**	**BF**	**Total visits**	**% visits**
Leucanthemum vulgare	1	0	1	229	0	231	17.6
Trifolium repens	74	117	2	19	0	212	16.2
Achillea millefolium	0	0	0	129	1	130	9.9
Matricaria spp.	0	0	1	128	0	129	9.8
Prunella vulgaris	0	16	1	102	4	123	9.4
Ranunculus repens	3	7	24	68	1	103	7.9
Epilobium adenocaulon	13	23	4	44	1	85	6.5
Taraxacum officinale	1	10	9	35	2	57	4.4
Centaurea nigra	0	22	0	23	1	46	3.5
Trifolium pratense	1	18	1	17	0	37	2.8
Brassica spp.	0	9	1	26	0	36	2.7
Dactylis glomerata	0	0	0	21	0	21	1.6
Plantago lanceolata	0	0	0	20	0	20	1.5
Cirsium vulgare	4	2	0	8	1	15	1.1
Sonchus arvensis	0	2	0	11	0	13	1.0
Leontodon hispidus	0	0	0	8	2	10	0.8
Senecio vulgaris	0	0	0	10	0	10	0.8
Silene dioica	0	1	0	7	0	8	0.6
Lotus corniculatus	0	5	0	1	0	6	0.5
Persicaria maculosa	0	1	0	4	0	5	0.4
Capsella bursa-pastoris	0	0	0	4	0	4	0.3
Rumex obtusifolius	0	0	0	3	0	3	0.2
Lolium perenne	0	0	0	3	0	3	0.2
Polygonum aviculare	0	0	0	2	0	2	0.2
Plantago major	0	0	0	1	0	1	0.1
Total visits	97	233	44	923	13	1310	-
% visits	7.4	17.8	3.4	70.5	1.0	-	100
Total wildflower species visited	7	13	9	25	8	-	-

Appendix 5.3. Number of the plant species visited by pollinating insects, number of the visits to sown and unsown wildflower species by pollinating insects and the total of visits to wildflower species (sown plus unsown) according to pollinator guild recorded on transect surveys of orchard alleyways throughout the three-year study.

Pollinator guild	**Pollinator species**	**Wildflower species visited**	**Visits to sown wildflowers**	**Visits to unsown wildflowers**	**Total visits by pollinator guild**
Honeybee	*Apis mellifera*	7	2	95	97
Bumblebee	*Bombus hortorum*	5	7	2	233
	Bombus hypnorum	2	1	1	
	Bombus jonellus	1	0	1	
	Bombus lapidarius	9	10	53	
	Bombus lucorum	1	1	0	
	Bombus pascuorum	10	35	50	
	Bombus pratorum	5	2	9	
	Bombus sylvestris	1	0	1	
	Bombus terrestris	1	0	1	
	Bombus terrestris/lucorum	10	6	53	
Solitary bee	*Andrena bicolor*	1	0	1	44
	Andrena chrysosceles	3	0	3	
	Andrena cineraria	1	0	1	
	Andrena flavipes	1	0	1	
	Andrena haemorrhoa	2	0	12	
	Andrena minutula	3	0	3	
	Andrena nigroaena	3	0	5	
	Andrena nitida	1	0	4	
	Chelostoma florisomne	1	0	1	
	Lasioglossum spp.	1	0	3	
	Lasioglossum albipes	4	2	2	
	Lasioglossum lativentre	1	0	1	
	Lasioglossum leucozonium	1	0	2	
	Lasioglossum malachurum	1	0	1	
	Lasioglossum morio	1	1	0	
	Lasioglossum punctatisimum	1	0	1	
Hoverfly	*Cheilosia albitarsis*	1	0	2	923
	Cheilosia pagana	6	2	5	

	Chrysotoxum bicinctum	1	0	1	
	Dasysyrphus albostriatus	3	1	2	
	Episyrphus balteatus	16	136	47	
	Eristalis arbustorum	5	15	20	
	Eristalis intricaria	2	1	1	
	Eristalis nemorum	2	3	6	
	Eristalis pertinax	5	2	5	
	Eristalis tenax	13	84	64	
	Eupeodes corollae	12	20	16	
	Eupeodes luniger	15	21	10	
	Helophilus pendulus	4	2	3	
	Helophilus trivittatus	1	1	0	
	Melanostoma mellinum	5	7	5	
	Melanostoma scalare	7	12	9	
	Myathropa florea	2	0	2	
	Neoascia podagrica	1	3	0	
	Platycherius albimanus	15	56	45	
	Platycherius granditarsus	1	1	0	
	Platycherius peltatus	6	5	4	
	Platycherius rosarum	1	0	1	
	Platycherius scutatus	4	1	6	
	Rhingia campestris	4	3	4	
	Scaeva pyrastri	1	0	1	
	Sphaerophoria scrita	15	30	26	
	Syritta pipiens	16	83	85	
	Syrphus ribesii	11	46	14	
	Syrphus vitripennis	4	2	2	
Butterfly	*Aglais io*	2	2	0	13
	Aglais urticae	1	0	1	
	Anthocharis cardamines	1	1	0	
	Pararge aegeria	1	0	1	
	Pieris napi	4	1	3	
	Pieris rapae	2	4	0	
Total		-	612	698	1310

Appendix 5.4. Pollinator species recorded on the transect surveys of cherry blossoms (CB) and transect surveys of orchard alleyways (OA) throughout the three-year study. X represents pollinator species being recorded. *Bombus terrestris* / *lurorum* and pollinators identified to genus are also included.

Genus	**Species**	**Species number**	**Year one**		**Year two**		**Year three**		**Records**	**%**
			CB	OA	CB	OA	CB	OA		
Andrena	*angustior*	1	-	-	X	-	-	-	1	0.005
	bicolor	2	X	-	X	X	X	X	10	0.05
	chrysosceles	3	X	-	X	-	X	X	10	0.05
	cineraria	4	X	X	X	X	X	X	104	0.5
	dorsata	5	X	-	-	X	X	-	3	0.01
	flavipes	6	-	-	X	-	-	X	2	0.01
	fucata	7	X	-	-	X	-	-	3	0.01
	fulva	8	X	-	X	-	X	-	51	0.25
	haemorrhoa	9	X	X	X	X	X	X	339	1.63
	helvola	10	X	-	X	X	X	-	5	0.02
	minutula	11	-	-	-	X	-	X	3	0.01
	nigroaena	12	X	-	X	X	X	X	74	0.36
	nitida	13	X	X	X	X	X	X	82	0.39
	scotica	14	X	-	X	X	X	-	167	0.80
	synadelpha	15	-	-	-	X	-	-	1	0.005
	spp.	-	X	X	X	X	X	X	172	0.83
Anthophora	*plumipes*	16	X	-	-	-	X	-	9	0.04
Apis	*mellifera*	17	X	X	X	X	X	X	6861	32.95
Bombus	*hortorum*	18	X	-	X	X	X	X	24	0.12
	hypnorum	19	X	X	X	X	X	X	64	0.31
	jonellus	20	-	-	-	X	-	-	1	0.005
	lapidarius	21	X	X	X	X	X	X	415	1.99
	lucorum	22	X	-	X	X	X	X	101	0.49
	pascuorum	23	X	X	X	X	X	X	169	0.81
	pratorum	24	X	X	X	X	X	X	42	0.20
	sylvestris	25	-	X	-	X	-	-	2	0.01
	terrestris	26	X	X	X	X	X	X	6032	28.97
	vestalis	27	-	-	X	-	X		4	0.02
	spp.	-	X	X	X	X	X	X	20	0.10
	terrestris /	-	-	X	-	X	-	X	511	2.45

	lucorum									
Chelostoma	*florisomne*	28	-	-	-	-	-	X	1	0.005
Halictus	*rubicundus*	29	-	-	X	-	-	-	1	0.005
Lasioglossum	*albipes*	30	-	X	X	X	X	X	15	0.07
	lativentre	31	-	X	-	-	-	-	1	0.005
	leucozonium	32	-	-	-	X	-	-	2	0.01
	morio	33	-	-	-	X	-	-	1	0.005
	punctatisimum	34	-	-	-	-	-	X	2	0.01
	zonulum	35	-	X	-	-	-	-	1	0.005
	spp.	-	-	X	-	X	-	X	3	0.01
Osmia	*bicornis*	36	X	-	-	-	-	-	2	0.01
Nomada	*fabriciana*	37	X	-	X	X	X	-	5	0.02
	flava	38	X	X	X	-	X	-	9	0.04
	goodeniana	39	X	-	-	-	X	-	2	0.01
	lathburiana	40	-	-	X	-	-	-	1	0.005
	marshamella	41	X	-	-	-	X	-	12	0.06
	panzeri	42	X	-	X	-	-	-	3	0.01
	ruficornis	43	-	-	X	-	-	-	1	0.005
	spp.	-	X	X	X	-	X	-	16	0.08
Sphecodes	*monillicornis*	44	-	-	X	-	X	-	4	0.02
Baccha	*elongata*	45	-	X	-	-	-	X	2	0.01
Cheilosia	*albipila*	46	-	-	X	-	-	-	1	0.005
	albitarsis	47	-	-	-	-	-	X	2	0.01
	ilustrata	48	-	-	-	-	-	X	1	0.005
	pagana	49	X	X	X	X	X	-	15	0.07
	proxima	50	-	-	-	X	-	-	1	0.005
Chrysotoxum	*bicinctum*	51	-	-	-	-	-	X	1	0.005
Dasysyrphus	*albostriatus*	52	X	-	X	-	-	X	11	0.05
Epistrophe	*eligans*	53	X	-	X	-	X	X	22	0.11
Episyrphus	*balteatus*	54	X	X	X	X	X	X	728	3.50
Eristalis	*arbustorum*	55	-	X	-	X	-	X	37	0.19
	intricaria	56	X	-	-	X	X	X	7	0.03
	nemorum	57	X	-	X	X	-	X	20	0.10
	pertinax	58	X	X	X	X	X	X	634	3.05
	tenax	59	X	X	X	X	X	X	277	1.33
	spp.	-	X	X	X	X	X	X	231	1.11
Eupeodes	*corollae*	60	X	X	X	X	X	X	264	1.27

	latifasciatus	61	-	-	-	X	-	-	3	0.01
	luniger	62	X	X	X	X	X	X	501	2.41
	spp.	-	X	X	X	X	X	X	87	0.42
Helophilus	*pendulus*	63	X	X	X	X	X	X	30	0.14
	trivittatus	64	-	-	-	-	-	X	1	0.005
Leucozona	*lucorum*	65	X	-	X	-	X	-	9	0.04
Melanostoma	*mellinum*	66	X	X	-	X	-	X	60	0.29
	scalare	67	X	X	X	X	X	X	168	0.81
Meliscaeva	*auricollis*	68	-	X	X	-	-	-	4	0.02
Myathropa	*florea*	69	-	X	-	X	-	-	3	0.01
Neoascia	*podagrica*	70	-	-	-	-	-	X	4	0.02
Parasyrphus	*nigritarsis*	71	-	-	X	-	-	-	1	0.005
Pipiza	*noctiluca*	72	X	X	X	X	-	-	4	0.02
Pipizella	*viduata*	73	-	-	-	X	-	-	1	0.005
	virens	74	-	-	-	-	-	X	2	0.01
Platycheirus	*albimanus*	75	X	X	X	X	X	X	570	2.74
	ambiguus	76	-	-	X	-	X	-	4	0.02
	clypeatus	77	-	X	X	X	-	X	6	0.03
	europeaus	78	-	X	-	-	-	X	7	0.03
	granditarsus	79	-	X	-	X	-	-	9	0.04
	peltatus	80	X	X	X	X	X	X	38	0.18
	rosarum	81	-	-	-	X	-	X	4	0.02
	scutatus	82	X	X	X	X	X	X	51	0.25
	tarsalis	83	X	-	-	-	-	-	1	0.005
	spp.	-	X	X	X	X	X	X	154	0.74
Rhingia	*campestris*	84	X	X	X	X	X	X	98	0.47
Scaeva	*pyrastri*	85	-	X	-	X	-	X	6	0.03
Sphaerophoria	*scripta*	86	X	X	X	X	X	X	124	0.60
Siritta	*pipiens*	87	X	X	-	X	X	X	499	2.40
Syrphus	*ribessi*	88	X	X	X	X	X	X	288	1.38
	torvus	89	X	X	-	-	-	-	4	0.02
	vitripennis	90	X	X	X	X	X	X	46	0.22
	spp.	-	X	X	X	X	X	X	164	0.79
Xanthogramma	*pedissequum*	91	-	-	-	X	-	-	2	0.01
Xylota	*segnis*	92	X	-	-	-	-	-	2	0.01
Aglais	*io*	93	X	-	X	X	X	-	13	0.06
	urticae	94	X	-	X	X	X	X	5	0.02

Anthocharis	*cardamines*	95	X	X	X	X	X	-	15	0.07
Maniola	*jurtina*	96	-	X	-	X	-	X	3	0.01
Noctua	*pronuba*	97	-	X	-	-	-	-	1	0.005
Pararge	*aegeria*	98	-	X	-	X	X	X	19	0.09
Pieris	*brassicae*	99	X	-	X	-	-	X	8	0.04
	napi	100	X	X	X	X	-	X	28	0.13
	rapae	101	-	X	-	X	-	X	12	0.06
	spp.	-	X	X	X	X	-	X	107	0.5
Polygonia	*c-album*	102	X	X	-	X	-	-	4	0.02
Vanessa	*atalanta*	103	X	X	X	X	X	X	29	0.14
Zygaena	*filipendulae*	104	-	-	-	-	-	X	1	0.005

Appendix 5.5. Comparisons in the generalized linear mixed models with negative binomial error structures for the pollinators during the cherry blossom period using the Akaike Information Criteria (AIC). Interaction between alleyway treatment and year represented by *Alleyway treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Total pollinators			
Total number of pollinators ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		34359.6	0.0
Alleyway treatment: Year	4	34384.3	**24.7**
Distance from the edge	1	34483.7	**124.1**
Time of day surveyed	1	34364.1	**4.5**
Honeybees			
Honeybees ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		18365.8	0.0
Alleyway treatment: Year	4	18378.3	**12.5**
Distance from the edge	1	18543.7	**177.9**
Time of day surveyed	1	18371.6	**5.8**
Buff-tailed bumblebees			
Buff-tailed bumblebees ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		17611.0	0.0
Alleyway treatment: Year	4	17613.1	**2.1**
Distance from the edge	1	17609.4	-1.6
Time of day surveyed	1	17611.3	0.3
Bumblebees			
Bumblebees ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		7228.2	0.0
Alleyway treatment	2	7225.2	-3.0

Year	2	7244.4	**16.2**
Distance from the edge	1	7257.3	**29.1**
Time of day surveyed	1	7228.4	0.2
Solitary bees			
Solitary bees ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		5581.6	0.0
Alleyway treatment: Year	4	5583.2	1.6
Distance from the edge	1	5595.5	**13.9**
Time of day surveyed	1	5579.6	-2.0
Hoverflies			
Hoverflies ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		10689.4	0.0
Alleyway treatment: Year	4	10698.2	**8.8**
Distance from the edge	1	10694.7	**5.3**
Time of day surveyed	1	10692.8	**3.4**
Butterflies			
Butterflies ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		593.0	0.0
Alleyway treatment: Year	4	601.9	**8.9**
Distance from the edge	1	591.3	-1.7
Time of day surveyed	1	592.1	-0.9
Visiting cherry blossoms			
Pollinators visiting cherry blossoms ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		27044.5	0.0
Alleyway treatment: Year	4	27061.7	**17.2**
Distance from the edge	1	27168.3	**123.8**
Time of day surveyed	1	27060.3	**15.8**

Species richness			
Species richness ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		26895.5	0.0
Alleyway treatment	2	26915.8	**20.3**
Year	2	27244.2	**348.7**
Distance from the edge	1	26983.9	**88.4**
Time of day surveyed	1	26893.6	-1.9
Shannon diversity			
Shannon diversity ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1194.5	0.0
Alleyway treatment	2	1210.3	**15.8**
Year	2	1522.8	**328.3**
Distance from the edge	1	1262.2	**67.7**
Time of day surveyed	1	1192.3	-2.2

Appendix 5.6. Pairwise comparisons (*Z* and *P* values) according to the *post-hoc* Tukey test between alleyway treatments and between years for total pollinator abundance, pollinator guild abundance, pollinators visiting cherry blossoms, species richness, and Shannon diversity recorded on transect surveys of cherry blossoms. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Factor	**Pairwise comparisons**		
Between alleyway treatments			
	CS – AMWS	*CS – SWS*	*AMWS – SWS*
Total pollinators	Z = 2.97, **P < 0.01**	Z = -0.88, P = 0.65	Z = -3.80, **P < 0.001**
Honeybees	Z = 1.71, P = 0.20	Z = -0.11, P = 0.99	Z = -1.81, P = 0.17
Buff-tailed bumblebees	Z = 2.13, P = 0.08	Z = -0.92, P = 0.63	Z = -3.02, **P < 0.01**
Bumblebees	Z = 1.01, P = 0.57	Z = 0.30, P = 0.95	Z = -0.71, P = 0.76
Solitary bees	Z = -2.20, P = 0.07	Z = -3.80, **P < 0.001**	Z = -1.59, P = 0.25
Hoverflies	Z = 1.88, P = 0.14	Z = -0.09, P = 0.99	Z = -1.99, P = 0.12
Butterflies	Z = -0.22, P = 0.97	Z = 1.22, P = 0.40	Z = 0.25, P = 0.96
Visiting cherry blossom	Z = 2.55, **P < 0.05**	Z = -0.32, P = 0.95	Z = -2.84, **P < 0.05**
Species richness	Z = 2.25, P = 0.06	Z = -2.58, **P < 0.05**	Z = -4.83, **P <0.001**
Shannon diversity	Z = 0.23, P = 0.97	Z = -2.38, **P < 0.05**	Z = -2.61, **P < 0.05**
Between years			
	Year one – Year two	*Year one – Year three*	*Year two – Year three*
Total pollinators	Z = 3.71, **P < 0.001**	Z = 23.45, **P < 0.001**	Z = 15.28, **P < 0.001**
Honeybees	Z = 1.75, P = 0.18	Z = 19.11, **P < 0.001**	Z = 13.75, **P < 0.001**
Buff-tailed bumblebees	Z = -9.37, **P < 0.001**	Z = 13.03, **P < 0.001**	Z = 17.57, **P < 0.001**
Bumblebees	Z = 3.50, **P < 0.01**	Z = 3.79, **P < 0.001**	Z = 0.16, P = 0.98
Solitary bees	Z = 9.43, **P < 0.001**	Z = 16.26, **P < 0.001**	Z = 5.43, **P < 0.001**
Hoverflies	Z = 13.71, **P < 0.001**	Z = 4.40, **P < 0.001**	Z = -7.25, **P < 0.001**
Butterflies	Z = 2.05, P = 0.08	Z = -0.19, P = 0.98	Z = -0.23, P = 0.97
Visiting cherry blossom	Z = 0.90, P = 0.64	Z = 18.4, **P < 0.001**	Z = 13.7, **P < 0.001**
Species richness	Z = 5.24, **P < 0.001**	Z = 19.25, **P < 0.001**	Z = 10.54, **P < 0.001**
Shannon diversity	Z = 6.01, **P < 0.001**	Z = 12.67, **P < 0.001**	Z = 4.84, **P < 0.001**

Appendix 5.7. Comparisons in the generalized linear mixed models with negative binomial error structures for the pollinators during post cherry blossom period using the Akaike Information Criteria (AIC). Interaction between alleyway treatment and year represented by *Alleyway treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	Degrees of freedom	AIC	ΔAIC
Total pollinators			
Total number of pollinators ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		11798.5	0.0
Alleyway treatment: Year	4	11846.8	**48.3**
Distance from the edge	1	11829.2	**30.7**
Time of day surveyed	1	11796.4	-2.1
Honeybees			
Honeybees ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		5396.1	0.0
Alleyway treatment	2	5402.7	**6.6**
Year	2	5432.2	**36.1**
Distance from the edge	1	5414.6	**18.5**
Time of day surveyed	1	5420.7	**24.6**
Bumblebees			
Bumblebees ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		4124.0	0.0
Alleyway treatment: Year	4	4130.7	**6.7**
Distance from the edge	1	4124.4	0.4
Time of day surveyed	1	4134.0	**10.0**
Solitary bees			
Solitary bees ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		816.7	0.0

Alleyway treatment	2	822.3	**5.6**
Year	2	852.0	**35.3**
Distance from the edge	1	818.9	**2.2**
Time of day surveyed	1	819.6	**2.9**
Hoverflies			
Hoverflies ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		8416.5	0.0
Alleyway treatment: Year	4	8454.8	**38.3**
Distance from the edge	1	8420.5	**4.0**
Time of day surveyed	1	8441.0	**24.5**
Butterflies			
Butterflies ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1409.8	0.0
Alleyway treatment: Year	4	1412.4	**2.6**
Distance from the edge	1	1412.6	**2.8**
Time of day surveyed	1	1408.6	-1.2
Visiting wildflowers			
Pollinators visiting wildflowers ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		4329.0	0.0
Alleyway treatment: Year	4	4391.7	**62.7**
Distance from the edge	1	4334.1	**5.1**
Time of day surveyed	1	4340.6	**11.6**
Species richness			
Species richness ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		10385.9	0.0
Alleyway treatment: Year	4	10428.3	**42.4**
Distance from the edge	1	10398.4	**12.5**
Time of day surveyed	1	10386.6	0.7

Shannon diversity			
Shannon diversity ~ Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		3752.9	0.0
Alleyway treatment: Year	4	3765.2	**12.3**
Distance from the edge	1	3756.0	**3.1**
Time of day surveyed	1	3751.9	-1.0

Appendix 5.8. Pairwise comparisons (*Z* and *P* values) according to the *post-hoc* Tukey test between alleyway treatments and between years for total pollinator abundance, pollinator guild abundance, pollinators visiting wildflowers, species richness, and Shannon diversity recorded on transect surveys of orchards alleyways. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant. CS (Control Strips), AMWS (Actively Managed Wildflower Strips), SWS (Standard Wildflower Strips).

Factor	**Pairwise comparisons**		
Between alleyway treatments			
	CS – AMWS	*CS – SWS*	*AMWS – SWS*
Total pollinators	Z = 7.40, **P < 0.001**	Z = 10.70, **P < 0.001**	Z = 3.40, **P < 0.01**
Honeybees	Z = 2.97, **P < 0.01**	Z = 2.69, **P < 0.05**	Z = -0.27, P = 0.96
Bumblebees	Z = 1.29, P = 0.40	Z = 2.96, **P < 0.01**	Z = 1.68, P = 0.21
Solitary bees	Z = 1.25, P = 0.42	Z = -1.87, P = 0.15	Z = -3.00, **P < 0.01**
Hoverflies	Z = 6.40, **P < 0.001**	Z = 9.54, **P < 0.001**	Z = 3.33, **P < 0.01**
Butterflies	Z = 0.467, P = 0.89	Z = 1.85, P = 0.15	Z = 1.44, P = 0.32
Visiting wildflowers	Z = 5.21, **P < 0.001**	Z = 4.72, **P < 0.001**	Z = 0.13, P = 0.99
Species richness	Z = 5.19, **P < 0.001**	Z = 8.54, **P < 0.001**	Z = 3.44, **P < 0.01**
Shannon diversity	Z = 4.36, **P < 0.001**	Z = 5.86, **P < 0.001**	Z = 1.63, P = 0.23
Between years			
	Year one – Year two	*Year one – Year three*	*Year two – Year three*
Total pollinators	Z = 16.13, **P < 0.001**	Z = 14.01, **P < 0.001**	Z = -1.29, P = 0.40
Honeybees	Z = 5.87, **P < 0.001**	Z = 0.69, P = 0.77	Z = -4.78, **P < 0.001**
Bumblebees	Z = 1.50, P = 0.29	Z = 7.37, **P < 0.001**	Z = 5.59, **P < 0.001**
Solitary bees	Z = 5.43, **P < 0.001**	Z = 2.74, **P < 0.05**	Z = -2.97, **P < 0.01**
Hoverflies	Z = 13.32, **P < 0.001**	Z = 12.39, **P < 0.001**	Z = -0.20, P = 0.98
Butterflies	Z = 7.38, **P < 0.001**	Z = -2.26, P = 0.06	Z = -6.59, **P < 0.001**
Visiting wildflowers	Z = 12.65, **P < 0.001**	Z = 13.15, **P < 0.001**	Z = 1.14, P = 0.48
Species richness	Z = 10.56, **P < 0.001**	Z = 13.99, **P < 0.001**	Z = 3.58, **P < 0.01**
Shannon diversity	Z = 7.78, **P < 0.001**	Z = 8.67, **P < 0.001**	Z = 1.03, P = 0.56

Appendix 5.9. Response (estimated value ± SE, and *Z* and *P* values) of the orchard block edge according to the generalized linear mixed models with negative binomial error structures on total pollinator abundance, pollinator guild abundances, pollinators visiting cherry blossom, pollinators visiting wildflowers, species richness, and Shannon diversity on transect surveys of cherry blossoms and orchard alleyways. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant.

Factor	**Generalized linear mixed model**
During blossom	
Total pollinators	-0.006 ± 0.001, Z = -11.21, **P < 0.001**
Honeybees	-0.01 ± 0.001, Z = -13.26, **P < 0.001**
Buff-tailed bumblebees	-0.0002 ± 0.001, Z = -0.30, P = 0.77
Bumblebees	-0.01 ± 0.001, Z = -5.55, **P < 0.001**
Solitary bees	-0.01 ± 0.002, Z = -3.97, **P < 0.001**
Hoverflies	-0.003 ± 0.001, Z = -2.55, **P < 0.05**
Butterflies	-0.004 ± 0.01, Z = -0.54, P = 0.59
Visiting cherry blossoms	-0.01 ± 0.001, Z = -11.21, **P < 0.001**
Species richness	-0.004 ± 0.0005, Z = -9.45, **P < 0.001**
Shannon diversity	-0.01 ± 0.001, Z = -7.53, **P < 0.001**
Post blossom	
Total pollinators	-0.005 ± 0.001, Z = -5.73, **P < 0.001**
Honeybees	-0.01 ± 0.02, Z = -4.52, **P < 0.001**
Bumblebees	-0.003 ± 0.002, Z = -1.55, P = 0.12
Solitary bees	-0.01 ± 0.01, Z = -2.03, **P < 0.05**
Hoverflies	-0.003 ± 0.001, Z = -2.44, **P < 0.05**
Butterflies	-0.01 ± 0.004, Z = -2.18, **P < 0.05**
Visiting wildflowers	-0.01 ± 0.002, Z = -2.66, **P < 0.01**
Species richness	-0.003 ± 0.001, Z = -3.91, **P < 0.001**
Shannon diversity	-0.004 ± 0.002, Z = -2.25, **P < 0.05**

Appendix 5.10. Response (estimated value ± SE, and *Z* and *P* values) of the survey time according to the generalized linear mixed models with negative binomial error structures on total pollinator abundance, pollinator guild abundances, pollinators visiting cherry blossoms, pollinators visiting wildflowers, species richness, and Shannon diversity on transect surveys of cherry blossoms and orchard alleyways. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant.

Factor	**Generalized linear mixed model**
During blossom	
Total pollinators	-0.01 ± 0.003, Z = -2.64, **P < 0.01**
Honeybees	-0.02 ± 0.01, Z = -2.86, **P < 0.01**
Buff-tailed bumblebees	-0.005 ± 0.004, Z = -1.09, P = 0.28
Bumblebees	-0.01 ± 0.01, Z = -1.49., P = 0.14
Solitary bees	0.003 ± 0.01, Z = 0.22, P = 0.83
Hoverflies	-0.02 ± 0.01, Z = -2.19, **P < 0.05**
Butterflies	-0.05 ± 0.05, Z = -1.05, P = 0.30
Visiting cherry blossoms	-0.02 ± 0.004, Z = -4.33, **P < 0.001**
Species richness	-0.0004 ± 0.003, Z = -0.13, P = 0.90
Shannon diversity	-0.01 ± 0.01, Z = -1.00, P = 0.32
Post blossom	
Total pollinators	-0.0002 ± 0.009, Z = -0.02, P = 0.98
Honeybees	0.10 ± 0.02, Z = 5.13, **P < 0.001**
Bumblebees	0.07 ± 0.02, Z = 3.45, **P < 0.001**
Solitary bees	0.15 ± 0.07, Z = 2.18, **P < 0.05**
Hoverflies	-0.07 ± 0.01, Z = -5.14, **P < 0.001**
Butterflies	-0.04 ± 0.04, Z = -0.88, P = 0.38
Visiting wildflowers	-0.09 ± 0.02, Z = -3.69, **P < 0.001**
Species richness	-0.01 ± 0.005, Z = -1.66, P = 0.10
Shannon diversity	-0.01 ± 0.01, Z = -1.01, P = 0.31

Appendix 5.11. Comparisons in the generalized linear mixed models for the stationary timed visitation surveys using Akaike Information Criteria (AIC). Visitation time, flowers visited per tree, visitation rate, and visit duration models were analysed GLMER with negative binomial error structures. Stigma contact, flying behaviour for cross-pollination, and pollinator feeding models were analysed using GLMER with binomial error structures. Interaction between alleyway treatment and year represented by *Alleyway treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	Degrees of freedom	AIC	ΔAIC
Visitation time			
Visitation time ~ Guild + Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		5435.8	0.0
Guild	4	5699.2	**263.4**
Alleyway treatment: Year	2	5437.6	1.8
Distance from the edge	1	5434.0	-1.8
Time of day surveyed	1	5433.8	-2.0
Flowers visited per tree			
Flowers visited per tree ~ Guild + Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		4328.0	0.0
Guild	4	4458.8	**130.8**
Alleyway treatment	2	4325.4	-2.6
Year	1	4328.3	0.3
Distance from the edge	1	4326.2	-1.8
Time of day surveyed	1	4330.1	**2.1**
Visitation rate			
Visitation rate ~ Guild + Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		6163.1	0.0
Guild	4	6290.9	**127.8**
Alleyway treatment	2	6160.3	-2.8
Year	1	6308.2	**145.1**

Distance from the edge	1	6161.3	-1.8
Time of day surveyed	1	6161.9	-1.2
Visit duration			
Visit duration ~ Guild + Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		8146.6	0.0
Guild	4	8183.0	**36.4**
Alleyway treatment: Year	2	8147.4	0.8
Distance from the edge	1	8144.7	-1.9
Time of day surveyed	1	8145.7	-0.9
Stigma contact			
Stigma contact ~ Guild + Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1144.3	0.0
Guild	4	1164.1	**19.8**
Alleyway treatment	2	1141.4	-2.9
Year	1	1159.8	**15.5**
Distance from the edge	1	1142.6	-1.7
Time of day surveyed	1	1143.0	-1.3
Feeding on nectar			
Feeding on nectar ~ Guild + Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1275.2	0.0
Guild	4	1367.2	**92**
Alleyway treatment	2	1271.8	-3.4
Year	1	1273.4	-1.8
Distance from the edge	1	1273.6	-1.6
Time of day surveyed	1	1277.8	**2.6**
Feeding on pollen			
Feeding on pollen ~ Guild + Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		874.6	0.0
Guild	4	1124.1	**249.5**

Alleyway treatment	2	872.6	-2
Year	1	896.5	**21.9**
Distance from the edge	1	872.6	-2
Time of day surveyed	1	872.6	-2
Feeding on both			
Feeding on both ~ Guild + Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1364.7	0.0
Guild	4	1418.8	**54.1**
Alleyway treatment	2	1361.9	-2.8
Year	1	1380.1	**15.4**
Distance from the edge	1	1362.9	-1.8
Time of day surveyed	1	1366.8	**2.1**
Stayed on tree			
Stayed on tree ~ Guild + Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		893.8	0.0
Guild	4	914.1	**20.3**
Alleyway treatment: Year	2	896.8	**3.0**
Distance from the edge	1	891.8	-2.0
Time of day surveyed	1	891.9	-1.9
Same tree row			
Same tree row ~ Guild + Alleyway treatment: Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1194.5	0.0
Guild	4	1264.8	**70.3**
Alleyway treatment: Year	2	1191.8	-3.0
Distance from the edge	1	1192.5	-2.0
Time of day surveyed	1	1200.6	**6.1**
Different tree row			
Different tree row ~ Guild + Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		923.8	0.0

Guild	4	956.2	**32.4**
Alleyway treatment	2	920.7	-3.1
Year	1	934.0	**10.2**
Distance from the edge	1	922.2	-1.6
Time of day surveyed	1	921.8	-2
Flew away			
Flew away ~ Guild + Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		1265.9	0.0
Guild	4	1299.2	**33.3**
Alleyway treatment	2	1261.9	-4.0
Year	1	1281.1	**15.2**
Distance from the edge	1	1264.0	-1.9
Time of day surveyed	1	1143.0	-1.3

Appendix 5.12. Comparisons in the generalized linear mixed models with negative binomial error structures for the environmental factors (temperature, humidity, and wind speed) using Akaike Information Criteria (AIC). Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	Degrees of freedom	AIC	ΔAIC
Temperature			
Temperature ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		41244	0
Alleyway treatment	2	41245	1
Year	2	41810	**566**
Distance from the edge	1	41259	**15**
Time of day surveyed	1	41267	**23**
Humidity			
Humidity ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		50796	0
Alleyway treatment	2	50792	-4
Year	2	50802	**6**
Distance from the edge	1	50804	**8**
Time of day surveyed	1	50889	**93**
Wind speed			
Wind speed ~ Alleyway treatment + Year + Distance from the edge + Time period + (random: Site/Orchard)			
Global model		9620	0
Alleyway treatment	2	9630	**10**
Year	2	9624	**4**
Distance from the edge	1	9810	**190**
Time of day surveyed	1	9618	-2

Appendix 6.1. Comparisons in the generalized linear mixed models for the fruit quality parameters on the contribution of pollinating insects to sweet cherry production using Akaike Information Criteria (AIC). Interaction between pollination treatment and year represented by *Pollination treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Fresh mass			
Fresh mass ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)			
Global model		16072	0
Pollination treatment: Year	2	16079	**7**
Distance from the edge	1	16097	**25**
Height			
Height ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)			
Global model		33176	0
Pollination treatment: Year	2	33189	**13**
Distance from the edge	1	33195	**19**
Width			
Width ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)			
Global model		34160	0
Pollination treatment: Year	2	34158	-2
Distance from the edge	1	34169	**9**
Length			
Length ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)			
Global model		26088	0
Pollination treatment: Year	1	26115	**27**
Distance from the edge	1	26095	**7**

Firmness

Firmness ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)

Global model		28979	0
Pollination treatment: Year	2	29009	**30**
Distance from the edge	1	28978	-1

Dry matter

Dry matter ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)

Global model		59513	0
Pollination treatment: Year	2	59555	**42**
Distance from the edge	1	59550	**37**

Seed mass

Seed mass ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)

Global model		42355	0
Pollination treatment: Year	2	42352	-3
Distance from the edge	1	42355	0

Seed height

Seed height ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)

Global model		26123	0
Pollination treatment: Year	2	26153	**30**
Distance from the edge	1	26127	**4**

Seed width

Seed width ~ Pollination treatment + Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)

Global model		23450	0
Pollination treatment	2	23479	**29**
Year	2	23626	**176**
Distance from the edge	1	23458	**8**

Seed length			
Seed length ~ Pollination treatment: Year + Distance from edge + (random: Site/Orchard/Alleyway treatment)			
Global model		18405	0
Pollination treatment: Year	1	18406	1
Distance from the edge	1	18411	**6**

Appendix 6.2. Pairwise comparisons (*Z* and *P* values) according to the *post-hoc* Tukey test between pollination treatments and between years for the fruit quality parameters on the contribution of pollinating insects to sweet cherry production. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant.

Fruit quality parameter	**Pairwise comparisons**		
Between pollination treatments			
	Open – Hand	*Insect excluded – Hand*	*Insect excluded – Open*
Fresh mass	Z = 2.17, P = 0.07	Z = -7.34, **P < 0.001**	Z = -8.62, **P < 0.001**
Height	Z = 3.82, **P < 0.001**	Z = -5.24, **P < 0.001**	Z = -9.00, **P < 0.001**
Width	Z = 0.95, P =0.60	Z = -7.20, **P < 0.001**	Z = -6.98, **P < 0.001**
Length	Z = 5.66, **P < 0.001**	Z = -0.07, P = 0.99	Z = -10.37, **P < 0.001**
Firmness	Z = 1.16, P = 0.47	Z = -0-.91, P = 0.62	Z = -2.18, P = 0.07
Dry matter	Z = 4.47, **P < 0.001**	Z = -5.89, **P < 0.001**	Z = -10.34, **P < 0.001**
Seed mass	Z = 0.36, P = 0.93	Z = -5.20, **P < 0.001**	Z = -4.63, **P < 0.001**
Seed height	Z = 1.95, P = 0.12	Z = 3.98, **P < 0.001**	Z = 0.76, P = 0.72
Seed width	Z = -0.45, P = 0.89	Z = -4.03, **P < 0.001**	Z = -5.64, **P < 0.001**
Seed length	Z = -2.12, P = 0.08	Z = -4.54, **P < 0.001**	Z = -3.06, **P < 0.01**
Between years			
	Year one – Year two	*Year one – Year three*	*Year two – Year three*
Fresh mass	Z = -17.55, **P < 0.001**	Z = 3.55, **P < 0.01**	Z = 16.78, **P < 0.001**
Height	Z = -33.34, **P < 0.001**	Z = 1.37, P = 0.35	Z = 26.23, **P < 0.001**
Width	Z = -21.18, **P < 0.001**	Z = -0.66, P = 0.79	Z = 15.05, **P < 0.001**
Firmness	Z = 0.70, P = 0.76	Z = 7.05, **P < 0.001**	Z = 6.91, **P < 0.001**
Dry matter	Z = 2.32, P = 0.05	Z = 4.29, **P < 0.001**	Z = 2.80, **P < 0.05**
Seed mass	Z = 2.15, P = 0.08	Z = 2.74, **P < 0.05**	Z = 1.29, P = 0.40
Seed height	Z = -10.80, **P < 0.001**	Z = 10.19, **P < 0.001**	Z = 18.77, **P < 0.001**
Seed width	Z = -5.37, **P < 0.001**	Z = 11.04, **P < 0.001**	Z = 13.49, **P < 0.001**

Appendix 6.3. Response of the fruit quality parameters according to distance from the orchard block edge for the generalized linear mixed models on the contribution of pollinating insects to sweet cherry production. Models included the estimated value, its standard error and T value. Values in bold are significant.

Fruit quality parameter	**Generalized linear mixed model**
Fresh mass	**0.01 ± 0.001, T = 5.26**
Height	**0.1 ± 0.01, T = 4.54**
Width	**0.04 ± 0.01, T = 3.33**
Length	**0.04 ± 0.01, T = 3.02**
Firmness	**0.004 ± 0.01, T = 0.62**
Dry matter	**2.01 ± 0.32, T = 6.21**
Seed mass	0.1 ± 0.04, T = 1.43
Seed height	**0.01 ± 0.005, T = 2.42**
Seed width	**-0.01 ± 0.003, T = -3.15**
Seed length	**-0.01 ± 0.004, T = -2.96**

Appendix 6.4. Comparisons in the generalized linear mixed models for the fruit quality parameters on the influence of wildflower interventions on insect pollination using Akaike Information Criteria (AIC). Interaction between alleyway treatment and year represented by *Alleyway treatment: Year*. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Values in bold are significant.

Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Fresh mass			
Fresh mass ~ Alleyway treatment: Year + Distance from edge + (random: Site/Orchard)			
Global model		13057	0
Alleyway treatment: Year	4	13060	**3**
Distance from the edge	1	13073	**16**
Height			
Height ~ Alleyway treatment: Year + Distance from edge + (random: Site/Orchard)			
Global model		27152	0
Alleyway treatment: Year	4	27162	**10**
Distance from the edge	1	27162	**10**
Width			
Width ~ Alleyway treatment: Year + Distance from edge + (random: Site/Orchard)			
Global model		27796	0
Alleyway treatment: Year	4	27796	0
Distance from the edge	1	27798	**2**
Length			
Length ~ Alleyway treatment + Year + Distance from edge + (random: Site/Orchard)			
Global model		21516	0
Alleyway treatment	2	21515	-1
Year	1	21599	**83**
Distance from the edge	1	21522	**6**
Firmness			
Firmness ~ Alleyway treatment: Year + Distance from edge + (random: Site/Orchard)			

Global model		24017	0
Alleyway treatment: Year	4	24010	-7
Distance from the edge	1	24015	-2
Dry matter			
Dry matter ~ Alleyway treatment: Year + Distance from edge + (random: Site/Orchard)			
Global model		48738	0
Alleyway treatment: Year	4	48736	-2
Distance from the edge	1	48760	**22**
Seed mass			
Seed mass ~ Alleyway treatment: Year + Distance from edge + (random: Site/Orchard)			
Global model		34555	0
Alleyway treatment: Year	4	34551	-4
Distance from the edge	1	34553	-2
Seed height			
Seed height ~ Alleyway treatment: Year + Distance from edge + (random: Site/Orchard)			
Global model		21374	0
Alleyway treatment: Year	4	21376	2
Distance from the edge	1	21374	0
Seed width			
Seed width ~ Alleyway treatment + Year + Distance from edge + (random: Site/Orchard)			
Global model		19594	0
Alleyway treatment	2	19592	-2
Year	1	19731	**137**
Distance from the edge	1	19602	**8**
Seed length			
Seed length ~ Alleyway treatment + Year + Distance from edge + (random: Site/Orchard)			
Global model		15660	0
Alleyway treatment	2	15656	-4
Year	1	15817	**157**
Distance from the edge	1	15669	**9**

Appendix 6.5. Pairwise comparisons (*Z* and *P* values) according to the *post-hoc* Tukey test between alleyway treatments and between years for the fruit quality parameters on the influence of wildflower interventions on insect pollination. *P* value < 0.05 was accepted to be significantly different. Values in bold are significant. CS (Control Strips), AMWS (Actively Managed Wildflower), Strips SWS (Standard Wildflower Strips).

Fruit quality parameter	**Pairwise comparisons**		
Between alleyway treatments			
	CS – AMWS	*CS – SWS*	*AMWS – SWS*
Fresh mass	Z = 0.50, P = 0.87	Z = 0.35, P = 0.93	Z = -0.15, P = 0.99
Height	Z = -0.78, P = 0.99	Z = -1.04, P = 0.55	Z = -0.96, P = 0.60
Width	Z = 0.86, P = 0.67	Z = -0.86, P = 0.66	Z = -1.72, P = 0.20
Length	Z = 0.69, P = 0.77	Z = -0.75, P = 0.73	Z = -1.43, P = 0.32
Firmness	Z = -2.82, **P < 0.05**	Z = -0.66, P = 0.79	Z = 2.16, P = 0.08
Dry matter	Z = 0.20, P = 0.98	Z = 1.52, P = 0.28	Z = 1.31, P = 0.39
Seed mass	Z = -1.93, P = 0.13	Z = 0.56, P = 0.84	Z = 2.49, **P < 0.05**
Seed height	Z = -0.63, P = 0.80	Z = 0.15, P = 0.99	Z = 0.79, P = 0.71
Seed width	Z = -0.90, P = 0.64	Z = 0.63, P = 0.81	Z = 1.52, P = 0.28
Seed length	Z = 0.25, P = 0.97	Z = 0.77, P =0.72	Z = 0.52, P = 0.86
Between years			
	Year one – Year two	*Year one – Year three*	*Year two – Year three*
Fresh mass	Z = -29.74, **P < 0.001**	Z = 1.05, P = 0.54	Z = 20.62, **P < 0.01**
Height	Z = -50.59, **P < 0.001**	Z = -4.30, **P < 0.001**	Z = 29.77, **P < 0.001**
Width	Z = -32.93, **P < 0.001**	Z = -3.42, **P < 0.01**	Z = 18.83, **P < 0.001**
Firmness	Z = -7.06, **P < 0.001**	Z = 6.21, **P < 0.001**	Z = 10.10, **P < 0.001**
Dry matter	Z = -5.73, **P < 0.001**	Z = -0.96, P = 0.59	Z = 2.96, **P < 0.01**
Seed mass	Z = 2.95, **P < 0.01**	Z = 5.94, **P < 0.001**	Z = 3.23, **P < 0.01**
Seed height	Z = -7.56, **P < 0.001**	Z = 16.91, **P < 0.001**	Z = 19.77, **P < 0.001**
Seed width	Z = -4.84, **P < 0.001**	Z = 9.99, **P < 0.001**	Z = 11.92, **P < 0.001**

Appendix 6.6. Response of the fruit quality parameters according to distance from the orchard block edge for the generalized linear mixed models on the influence of wildflower interventions on insect pollination. Models included the estimated value, its standard error and T value. Values in bold are significant.

Fruit quality parameter	**Generalized linear mixed model**
Fresh mass	**0.01 ± 0.001, T = 4.27**
Height	**0.04 ± 0.01, T = 3.51**
Width	**0.03 ± 0.01, T = 2.07**
Length	**0.04 ± 0.01, T = 2.68**
Firmness	0.03 ± 0.01, T = 0.34
Dry matter	**1.76 ± 0.36, T = 4.93**
Seed mass	0.04 ± 0.04, T = 0.88
Seed height	0.01 ± 0.01, T = 1.41
Seed width	**-0.01 ± 0.004, T = -3.28**
Seed length	**-0.02 ± 0.004, T = -3.39**

Appendix 6.7. Comparisons in the parametric fruit quality parameters for the optimal frequency of hand pollination with One-Way ANOVA and non-parametric parameters with generalized linear mixed models. One-Way ANOVA include degrees of freedom, residuals, and *F* and *P* values. *P* < 0.05 was accepted to be significantly different. For the generalized linear mixed models the Akaike Information Criteria (AIC) was used. Fixed factors are removed in each reduced model to determine significant differences. Models include degrees of freedom, and the difference between models (ΔAIC). ΔAIC > 2 was accepted to be significantly different. Value in bold are significant.

Fruit quality parameter	**Parametric data: One-way ANOVA**			
	Degrees of freedom	**Residuals**	**F value**	**P value**
Seed mass				
Seed mass ~ Hand pollination frequency + (random: Distance from the edge)				
	2	215.6	3.66	**< 0.05**
Seed height				
Seed height ~ Hand pollination frequency + (random: Distance from the edge)				
	2	210.5	2.10	0.12
	Non-parametric data: LMER			
	Omitted terms in Model	**Degrees of freedom**	**AIC**	**ΔAIC**
Fresh mass				
Fresh mass ~ Hand pollination frequency + (random: Distance from the edge)				
	Global model		824.2	0
	Hand pollination frequency	2	823.9	-0.3
Height				
Height ~ Hand pollination frequency + (random: Distance from the edge)				
	Global model		1841.2	0
	Hand pollination frequency	2	1840.3	-00.9
Width				
Width ~ Hand pollination frequency + (random: Distance from the edge)				
	Global model		1988.9	

	Hand pollination frequency	2	1987.3	-1.6
Firmness				
Firmness ~ Hand pollination frequency + (random: Distance from the edge)				
	Global model		1355.7	0
	Hand pollination frequency	2	1366.3	**10.6**
Dry matter				
Dry matter ~ Hand pollination frequency + (random: Distance from the edge)				
	Global model		3145.2	0
	Hand pollination frequency	2	3146.9	1.7
Seed width				
Seed width ~ Hand pollination frequency + (random: Distance from the edge)				
	Global model		1300.1	0
	Hand pollination frequency	2	1304.5	**4.4**