Influential factors of cereal crop landscapes on aphidophagous syrphid communities

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Declaration

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Summary

Aphidophagous syrphids are a common group of insects in agriculture, attributed with benefiting farmers with pollination and pest control services. Farmed landscapes are also believed to have potential in supporting and conserving syrphid populations. However, there is still much to be understood in terms of how agricultural factors can influence aphidophagous syrphids, both positively and negatively. The more that is understood about influential factors, the more farmers and conservationists can do to optimize their ecosystem service provision and protect syrphid species. In this body of work, I aimed to investigate various agricultural factors for their influence on aphidophagous syrphids, with a focus on Irish cereal grain crops, and *Avena sativa* in particular.

First, I investigated the potential for the presence of harmful factors in agri-systems both organic and conventional. residues of pyrethroid (i.e. deltamethrin, esfenvalerate, permethrin) and neonicotinoid (i.e. clothianidin, imidacloprid, thiamethoxam) insecticides in nectar, pollen-laden stamens, and aphid food resources. While pyrethroids were not detected in any of the samples, neonicotinoid residues were detected in every sample media type (maximum residues for nectar < 2.40 ng/mL, stamens < 4.19 ng/g, aphids < 11.33 ng/g). Thiacloprid was detected seven times, imidacloprid once, clothianidin not at all, and no sample contained multiple pesticides. Furthermore, neonicotinoid residues were found in organic and conventional oat crops, suggesting mobility of pesticide residues into areas where they are not applied. These results suggest that aphidophagous syrphids can be exposed to dietary pesticide residues in adult and larval stages, even in fields that did not receive direct applications of pesticides.

I then conducted lab-based bioassays to investigate the lethal and sublethal effects on the adult of a common Irish syrphid (*Episyrphus balteatus*) from acute oral exposure to ten different concentrations of the neonicotinoid thiamethoxam in nectar food resources, non-control concentrations ranging from 0.01 to 24 ng/µL. While we determined that there were differences between some of the survival rates of the different concentrations ($P = 0.016$), they were not significant enough to be detected after pair-wise test and corrections for multiple testing. Mortality was not high enough to determine a 72-hour LD50, though an acute LD50 was estimated to be about 100-120 ng per fly 12 days after exposure. Temporary sublethal effects were also observed, with neonicotinoid exposure linked with increased time spent resting, decreased time spent flying and grooming, and a reduced ability to grip smooth surfaces. These sublethal effects were not seen beyond three days after exposure. From these results, it is possible that thiamethoxam dietary exposure in the field poses a low risk to aphidophagous syrphid health, particularly when compared to the detected residue levels from the first study.

Finally, I investigated what natural factors (i.e. hedge habitat structure, food resource availability, food resource competitor abundance) may influence their populations in cereal grain crops. Floral abundances were measured by counting floral units along transects in the crops and along the hedge, and timed walks of these same transects were done for observing adult aphidophagous syrphids and other visitors landing on flowers and oats. Aphids, juvenile aphidophagous syrphids and other invertebrate abundances on the oats were also measured along these transects in the crop from timed hand-searches. Both investigated food resources were determined to be influential to aphidophagous syrphid abundances in organic oat crops, where floral abundance had a positive effect, but crop aphid abundance had a negative effect. The pattern of aphid and aphidophagous syrphid abundances throughout the summer indicated a predator-prey dynamic, in which syrphid predatory activity helped control crop aphid abundance. For food resource competitors, flower-visiting bees and parasitized aphid abundances had positive relationships with aphidophagous syrphid abundance, indicating that any competition that occurs is inconsequential on syrphid abundances. Hedge habitat structure did not correlate to aphidophagous syrphid abundance, though this could be due to my methodology.

This work establishes a starting point of knowledge on dietary pesticide residue hazards faced by aphidophagous syrphids in Irish agriculture, which puts this anthropogenic factor into context of various natural factors. Future research can draw upon the findings of this work to further our understanding of pesticide residues in the agricultural landscapes and how syrphids and other beneficial insects are affected by them, which can inform ecologically friendly practices and policies on pesticide use. Farmers and conservationists can also refer to this work for insight into how the agricultural landscape can impact aphidophagous syrphids.

To my family

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List of main contributors to each chapter

Chapter	Name	Contributions
1	Sarah Gabel	Writing
	Jane Stout	Structure and comments on draft chapter
	Blánaid White	Comments on draft chapter
2	Sarah Gabel	Concept/experimental design
		Field site selection
		Data and sample collection
		Sample sorting and processing
		Pesticide extractions
		GC-MS analysis
		Chromatogram analysis and interpretation
		Statistical analysis and interpretation
		Writing
	Jane Stout	Concept/experimental design
		Structure and comments on draft chapter
	Blánaid White	Concept/experimental design
		Guidance on data analysis
		Structure and comments on draft chapter
	Michael Gaffney	Guidance in aphid sampling design
	Críostóir Mac Cuirc	Provided transportation for sampling
		Sampling of aphids, nectar, and stamens
	Gillian Collins	Provided transportation for sampling
		Sampling of nectar and stamens
	Alison Hall	Sampling of aphids, nectar, and stamens
	Jacqueline Stone	Provided transportation for sampling
		Sampled nectar and stamens
	Nan Hu	Sampling of aphids
	Tom Murphy	Sampling of aphids
	Aoife Delaney	Transportation and assistance with finding field sites
2 continued	Mary Ross	GC-MS guidance and assistance

	Imogen Hands	Assistance with lab work
		Assistance with GC-MS analysis
	Saorla Kavanagh	Transportation and assistance with finding field sites
		Provided training in extraction protocols
		Assistance with lab work
		Ran samples on UHPLC-MS/MS
		Guidance on data analysis
3	Sarah Gabel	Concept/experimental design
		Conducted bioassays
		Data collection
		Statistical analysis and interpretation
		Writing
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	Blánaid White	Comments on draft chapter
	Simon Hodge	Guidance and assistance with statistical analysis
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4	Sarah Gabel	Concept/experimental design
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		Field work and data collection
		Insect and plant identifications
		Statistical analysis planning and interpretation
		Writing
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	Felipe Guapo	Field work and data collection
	Tom Gittings	Identification verification for syrphid specimens
4 continued	Tomás Murray	Identification verification for bee specimens
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	Aoife Delaney	Identification verification for plant specimens
	Hannah Hamilton	Assistance with finding field sites
	Jackie Stone	Assistance with finding field sites
	Ben Malone	Assistance with finding field sites
	Laura Russo	Assistance with finding field sites Guidance on reviewing dataset
	Ruth Kelly	Guidance on reviewing dataset
	Simon Hodge	Guidance on statistical analysis and ran analysis on software
5	Sarah Gabel	Writing
	Jane Stout	Comments on draft chapter
	Blánaid White	Comments on draft chapter

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Chapter 1

General Introduction

1. Introduction

1.1 Syrphids in Irish Agriculture

There are about 6,000 known species of hover flies (Family Syrphidae, Order Diptera) globally (Sommaggio, 1999), and 183 species can be found in Ireland (Speight, 2008). On the British Isles, hover flies (or syrphids) are grouped into three sub-families: the largest sub-family Eristalinae (or Milesiinae), which are the species with larvae that mainly feed on decomposing matter or living plant material (and occasionally within living vertebrates or on invertebrates, Pérez-Bañón et al., 2020); Syrphinae, which are comprised of species with predatory larvae; and the very small sub-family Microdontinae, a difficult-to-find group of species with larvae that prey on the juveniles of ant colonies (Ball and Morris, 2015; Stubbs and Falk, 2002), which some authors consider could be assigned their own, separate family (Speight, 2008). Based on larval feeding type, in Ireland there are 65 species with saproxylic larvae (four of which also feed on plants, and one is also predatory), 29 species that feed on living plants (two of which are also predatory), and 96 species that are predatory (Speight, 2008). The most common species in Ireland are listed in Table 1.1. The general life cycle of syrphids occurs as winged adults feeding on floral food resources of nectar and pollen to mature and fuel their bodies for foraging and mating (or, given the time of year, overwintering or migrating), the females selectively oviposit their eggs in habitats and locations that optimize food-resource availability and suitability for their species' offspring, usually within a week larvae hatch from the eggs and feed to develop themselves through three instars and prepare them for pupation (or, given the time of year, overwintering as larvae or pupae), and finally adults eclose from the pupae and the cycle repeats (Ball and Morris, 2015; Rodríguez-Gasol et al., 2020). Most of a syrphid's life is spent as larvae, ranging from weeks to years depending on the species, whereas the adult stage typically lasts a few days or weeks (Ball and Morris, 2015). Because of this, the number of generations that can occur of a syrphid species can be one to four per year (Hassall et al., 2017). The habitats and landscapes that syrphid species are associated with are linked with the unique dietary needs of their less mobile larval stage and the abundance of resources needed for the mobile adult stage to nourish themselves and reproduce. Given that over half of the land area of the Ireland is used for agriculture (4,524,400 hectares in 2019 of the total land area of 6,888,300 hectares, Central Statistics Office (CSO) of Ireland, 2020; World Atlas, 2020), and that the behaviours of syrphids can be beneficial for waste decomposition, pest control, and pollination, it benefits syrphids for agricultural lands to be managed in such a way as to support their life cycles. It can also be in farmers' interest to have syrphid populations in their fields.

Table 1.1 Common syrphid species in Ireland (Speight, 2008).

Phytophagous	Predatory (soft-bodied hemipterans)	Predatory (other prey)	Saprophagous
Cheilosia albitarsis	*Chrysotoxum bicinctum*	*Volucella bombylans*	*Eristalinus sepulchralis*
Cheilsoia illustrata	*Dasysyrphus albostriatus*	*Volucella pellucens*	*Eristalis nemorum*
Cheilosia pagana	*Epistrophe eligans*		*Eristalis pertinax*
Cheilosia variabilis	*Episyrphus balteatus*		*Eristalis tenax*
	Melanostoma mellinum		*Lejogaster metallina*
	Melanostoma scalare		*Myathropa florea*
	Meliscaeva cinctella		*Neoascia podagrica*
	Platycheirus albimanus		*Neoascia tenur*
	Platycheirus clypeatus		*Rhingia campestris*
	Platycheirus scutatus		*Syritta pipiens*
	Syrphus ribesii		*Xylota segnis*
	Syrphus torvus		*Xylota sylvarum*
	Syrphus vitripennis		

The percent land area of the Ireland's total area in 2014 of the various agricultural sectors, ranking from highest to lowest, were grassland (60.70%), forests (10.60%), crops (9.50%), wetlands (16%), and other (3.20%) (CSO of Ireland, 2016). Pesticides are used to control and reduce crop damage from weed competition, feeding and disease-transmitting animal pests, and fungal-caused diseases. While winter and spring oat crops (the focal cereal crop of my research) have similar recommended fungicide treatment and timing (three treatments from development of first node to emergence of the flag leaf in oat plants), they differ for herbicides and insecticides (Teagasc, 2017a). Suggested winter oat chemical treatments for weeds and insect pests is more strongly based on monitoring (e.g. conduct aphid counts and use chemicals as a last-resort for control), whereas some pre-emptive approaches are used for spring oat chemical treatments (e.g. at least one spray to control aphids and avoid BYDV-transmission, monitor aphids for a possible second treatment) (Teagasc, 2017b, c, d, e). Overall, all farmers are encouraged to use IPM (integrated pest management) to reduce reliance on pesticides as much as possible. Such IPM practices include crop rotation, early sowing, and ploughing (PCS, 2020).

Various factors can impact the wildlife that reside in an agricultural landscape. Power et al. (2016) studied the factors that influenced syrphid populations on pastures at the local and landscape scale. They found that the floral abundance and diversity in pastures were influential, but the proportion of surrounding habitat area composed of grasslands also improved syrphid abundances in pastures, particularly the species *Eristalis tenax*, *Helophilus pendulus*, and *Rhingia campestris*. From a conservation standpoint, it is encouraging that the dominant pasture habitats in Irish agriculture can support various syrphids, but the benefits pasture farmers get from those syrphids may not be highly significant, as there are no crops to pollinate, and the prey of predatory syrphids (mainly soft-bodied Hemipterans, Ball and Morris, 2015; Stubbs and Falk, 2002) do not influence livestock (with the exception of crops used for feed, such as oats). While there are syrphid species that feed on and recycle the nutrients in vertebrate dung and in stagnant puddles and ponds which are common in Irish pastures, and this recycling aids in the removal of wastes that can harbour bacteria and foul the foliage livestock feed on, a few studies suggest that syrphids may play only a small role in the recycling of livestock waste (Curry et al., 1979; Laurence, 1954).

Sixty-two percent of adult hover fly species commonly found in farmland have aphidophagous larvae (Speight, 2008), so entomophilous crops prone to aphid infestations could reap pest control benefits from the presence of syrphids in their larval stage, as well as pollination services of adults. In terms of land area, oilseed rape is one of the most abundant nectar-producing crops in Ireland, accounting for 9,200 hectares in 2019 (not including production for fodder, CSO of Ireland, 2020). Oilseed rape (*Brassica napus*) has bright yellow flowers with open petal arrangements allowing easy access to pollen and nectar (Kunin, 1993; Phillips et al., 2018; Stanley et al., 2013). The yellow colour of the flowers has been noted as attractive to several kinds of flies, including syrphids (Lunau, 2014), and the accessibility of the pollen and nectar is important due to the typically short lengths of syrphid proboscis' (though there is variability in proboscis length among syrphid species and this has been seen to correlate with their preferred diets, Gilbert, 1981). These attributes make oilseed rape crops attractive as a food source for many syrphid adults, but oilseed rape can also be host to aphid colonies, providing a food source for some syrphids' predatory larvae. In turn, oilseed rape crops receive two benefits from the syrphids of pollination and pest control (Doyle et al., 2020). Several syrphids are believed to be important pollinators of oilseed rape crops (Phillips et al., 2018), particularly the saprophagous (aquatic, detritous-feeding) *Eristalis* spp. and the aphidophagous *Episyrphus balteatus* (Jauker and Wolters, 2008; Jauker et al., 2012; Stanley et al., 2013).

More abundant in the Irish cropped landscape are cereal grain crops, mainly barley, wheat, and oats, which in 2019 accounted for 267,700 hectares (CSO of Ireland, 2020). Unlike oilseed rape crops, however, cereal grain crops do not produce flowers to attract and provide nutrition for adult syrphids, their most mobile life stage. Some hover flies do feed on the pollen of graminoids (Leereveld, 1982; Ssymank and Gilbert, 1993), and while they have been documented feeding on the pollen of cereal grain crops such as corn, rice, and sorghum (Saunders, 2018), there does not appear to be literature on their use of the pollen specifically from the common grain crops in Ireland. Multiple syrphid species have been observed actively preying on aphids in cereal crops, one of the most damaging pests of cereals, and syrphid larvae are considered important predators that significantly impact the growth of aphid populations in crops (Chambers and Adams, 1986; Raymond et al., 2014; Tenhumberg, 1995; Tenhumberg and Poehling, 1995; Wotton et al., 2019). Irish agricultural authorities note that the primary concerns of aphid damage to cereal crops is via feeding and transmission of YDVs (yellow dwarf viruses), which can cause losses of 0.8 (BYDV, for barley) and 2 tons/hectare (CYDV, for cereals) of barley sown in April (Teagasc, 2017b; Walsh, 2020b). These concerns are compounded by the detections of pyrethroid resistance in cereal aphids across Ireland, and with the banning of neonicotinoids, the number of available and effective chemicals for aphid control are dwindling (Walsh et al., 2020a and b). Speight (2008) noted that Irish cereal crops can commonly serve as habitats to 11 syrphid species, all of which are aphidophagous: *Episyrphus balteatus*; *Eupeodes corollae* and *luniger*; *Melanostoma mellinum* and *scalare*, *Platycheirus albimanus*, *clypeatus*, and *scutatus*; *Scaeva pyrastri*; *Sphaerophoria scripta*; and *Syrphus ribesii*. In order for these syrphid species to be effective pest controllers for Irish cereal grain crops, some of the most common aphid pests on these crops (*Metopolophium dirhodum*, *Rhopalosiphum padi*, and *Sitobion avenae*) would need to be suitable hosts for their larval stage (Giller et al., 1995; Kennedy and Connery, 2001 and 2005; Teagasc, 2017b; Walsh, 2020b). Some of these syrphid species have been documented feeding on all three of these cereal aphid species (e.g. *Episyrphus balteatus*, Mushtaq et al., 2014 and Tenhumberg, 1995). However, some syrphid species, while commonly observed flying in and nearby cereal crops in their adult stage, may prefer other aphids other than those found on cereal crops as larval prey. For example, Alhmedi et al. (2009) surveyed aphids and juvenile syrphids on wheat crops, and on nearby pea crops and stinging nettle in Belgium for two years. While *Sitobion avenae* and *Metopolophium dirhodum* were the two aphid species found on the wheat crop, and *Episyrphus balteatus* was the most common juvenile syrphid observed and was also found on the wheat crop both years sampled, *Melanostoma mellinum* was the only other juvenile syrphid also found on the wheat crop, with the syrphids *Platycheirus scutatus*, *Eupeodes luniger*, *Sphaerophoria scripta*, and *Scaeva pyrastri* among others being found on the pea crop and the stinging nettle but not the wheat crop (Alhmedi et al., 2009). Syrphids can contribute to pest control services alongside other predators and parasitoids, but what factors optimize syrphid predation in cereal crops is not fully understood.

1.2 Physical factors in agriculture that influence syrphids and other beneficial invertebrates

1.2.1 Surrounding landscape composition

Farmland habitats appear to suit many syrphid species well, compared to other habitats such as those available in urban areas (Baldock et al., 2015; Luder et al., 2018; Persson et al., 2020; Verboven et al., 2014). The composition of the landscape can have an impact on the communities of syrphids in a given location, with studies demonstrating presence and cover of woody plants and grasslands to influence syrphids on farmland (Alignier et al., 2014; Power et al., 2016; Sarthou et al., 2005), and Haenke et al. (2014) found that the presence of oilseed rape crops seemed more closely linked to high predatory syrphid abundance than wheat crops. However, it is not just the features, but the diversity and quality of habitats that are important for supporting a variety of taxa of different needs. Dainese et al. (2019), in a global meta-analysis study, found that landscapes with fewer diverse habitats supported fewer pollinators and pest controllers, which in turn correlated with lower crop yields. While Albrecht et al. (2020) found that this (greater proportion of arable crops) only translated to reduced pollination services in crops and not for pest control services, Redlich et al. (2018) found that a diversity of crops in the landscape promoted crop pest control. Regardless, a richness of habitats can support a richness of ecosystem service providers, which can in turn improve crop yield (Dainese et al., 2019; Woodcock et al., 2019). Land managers may hesitate to allow a diversity of habitats though, due to the possibility of attracting pests. While there may be similarities in how pests and their predators can respond to various landscape features, there are also differences that, once better understood, could be used to manage these features in such a way as to support more predators such that the benefits surpass the damages of attracting pests. For example, Alignier et al. (2014) found that syrphid juvenile abundance in wheat crops responded to prevalence of woody and grassland habitats more positively than aphid abundance. The scale at which syrphid communities can be impacted by the surrounding landscape is very large as well. For instance, while Garratt et al. (2017) did not find any correlation between syrphid abundance and landscape composition in the UK within 500 metres of the surveyed point, Power et al. (2016) looked at an expanded range of 4 kilometres and did find landscape effects in Ireland, noting that landscapes with high coverage of grassland supported more syrphids. Farmers have much more control over local features though, where they can create habitats that support predatory syrphids and encourage their pest control activities in their crops.

1.2.2 Local landscape features

Hatt et al. (2017) found that aphid pests in a pea crop did not respond differently between having adjacent wildflower strips versus a forest edge, but aphidophagous syrphids and parasitoids were more abundant in the crop beside wildflower strips than forest. A global meta-analysis of crop characteristics with and without adjacent hedges or wildflower strips found no significant differences in crop yields (Albrecht et al., 2020), hence any additional abundances of pests or transmitted crop diseases appears to have had little effect. There was too much variability in the findings of Albrecht et al. (2020) in order to draw strong conclusions in terms of how these habitats can influence pollination, and only flower strips were observed to increase pest control (on average, 16% increase), so more research will be needed in order to give farmers the tools to manage habitats in a way that optimizes these services and boosts crop production while not exacerbating pest problems. Albrecht et al. (2020) also found that the benefits garnered from wildflower strips were independent of landscape effects, leading the authors to comment that the condition of the surrounding landscape should not deter farmers from creating habitats for ecosystem service provision.

In terms of conservation, hedge and wildflower strip habitats can be beneficial for providing habitat for syrphids. Power et al. (2016) found that hedges influence syrphid abundance, where wide hedges and greater land coverage of hedges supported more syrphids (Power et al., 2016). Maudsley (2000) noted that hedges are one of the most important habitats for invertebrates on farmland, as they generally have more invertebrate species associated with them than anywhere else in agricultural landscapes, and Haenke et al. (2014) found that hedges improved syrphid abundance in crops more than adjacent forests. Montgomery et al. (2020) also acknowledge that hedges can provide multiple ecosystem services to farmland, which can include supporting pollinating and pest controlling insects like syrphids. Hedges can also be an appealing habitat to manage for wildlife, as they already are prolific in the Irish landscape and take up far less space than woodlands and forests, freeing up more land for production.

1.2.3 Local landscape feature management

For habitats to be able to support aphidophagous syrphids, along with other beneficial invertebrates, they must be managed in such a way as to facilitate this. Graham et al. (2018) noted that large hedges provide more habitat, structural variability, and food resources than small hedges, thus over-managing to keep hedges “neat” may reduce the value of hedges as a habitat, in addition to causing excessive disturbances that can kill or injure residing wildlife (Maudsley et al., 2000). Also, syrphids can utilize the same habitat differently depending on seasonal needs, and habitats need to be managed with this in mind. Sarthou et al. (2005) studied the relationship of forest structure with the abundance of the syrphid *Episyrphus balteatus* and found that they were more abundant in southern edges during the winter due to higher temperatures than northern edges; however, higher floral abundances in northern edges attracted more syrphids in the summer. This temporal change in needs can be translated to hedges and flower strips, where it is important that there are a diversity and even-spread of plant species such that the seasonal changes in these plants provide year-round resources for invertebrates in terms of food, shelter, etc. (Maudsley, 2000). However, even though many would agree with Gontijo (2019) on how hedges and several other kinds of shelters can be implemented on farmland to support and disperse pest controllers, he acknowledged that there was still more to be understood in how to optimize these shelters for the benefit of farmers.

For example, it is not clear how crop-adjacent habitats can be maintained to disperse beneficial insects across an entire crop, and not just into the crop margins closest to the habitats. Garratt et al. (2017) noted that syrphids and bees were more abundant on the hedges than in the crops far from the hedge, and Woodcock et al. (2016b) found that the improved effects of predation in oilseed rape crops from having wildflowers in the margins extended only 50 metres into the crop (in fields of about 12 hectares). In addition, Albrecht et al. (2020) noted that hedges and wildflower strips on the margins of crops did not improve pollination services overall but did cause higher crop pollination services close to the margins and lower pollination in the crop far from the margins. Conversely, this disproportionate pollinating with crop depth was not observed in crops with no habitats in the margins (Albrecht et al., 2020). Regardless of the state of crop margins, Albrecht et al. (2020) also found that pest control services tended to diminish with crop depth. This issue could potentially be addressed with either smaller field sizes or in-field habitats such as wildflower patches or strips (Kirchweger et al., 2020).

One thing that is commonly deduced from the current state of the literature is that management of habitats should be done carefully, cautiously, and strategically rather than done with a heavy-hand in an attempt to “tidy” habitats to make them symmetrical and homogenous, with planning as to the time of year for management and what methods to use. Albrecht et al. (2020) observed that floral species richness and 2-year-old flower strips improved pollination by 27% compared to temporary strips with few floral species. For hedges, Maudsley (2000) pointed out that there should be a diversity in ages of woody plants to provide different structural shelters for invertebrates. This would suggest that, in order to optimize the benefits of habitats near crops, diversity of food resources, permanence, and commitment to proper management are key. Albrecht et al. (2020) are in agreement with this, pointing out that such habitats could be so attractive as their constancy and reduced disturbance allows individuals to nest and overwinter, building up populations and a stock of ecosystem service providers throughout the year, ready to pollinate and prey on pests when farmers plant crops. However, the effectiveness of specifically hedges at improving ecosystem services is uncertain, particularly compared to other habitats. Albrecht et al. (2020) found evidence from a global meta-analysis comparing pest control and pollination services in crops with hedges, wildflower strips, or plain margins, and found wildflower strips to improve ecosystem services better than hedges, though the authors did acknowledge that this may be due to wildflower strip design and management specifically for ecosystem service provision. While hedges are acknowledged to be good for wildlife, they are complicated habitats with complicated relationships in terms of wildlife support and ecosystem service provision that have yet to be fully understood (Maudsley et al., 2000). Wildflower strips, being much easier to implement and manage habitats, focus on catering to one of the fundamental needs of syrphids and other invertebrates – providing food resources, particularly nectar and pollen.

1.3 Biological factors in agriculture that influence syrphids and other beneficial invertebrates

1.3.1 Impacts of floral food resources and competitors on syrphids and other beneficial invertebrates

Food resources that make up the diet of syrphids can be some of the most influential in terms of attracting and retaining aphidophagous syrphids. This is especially true of floral communities, which serve as a visually attractive source of nectar and pollen for the adults, the most mobile syrphid stage. Garratt et al. (2017) noted that in a hedge with flowers provided on woody plants and forbs, syrphids visited forb flowers more often, so allowing wildflower growth beside and within the hedge would create a more attractive and supportive hedge for syrphids. Wildflower strips are another small-scale habitat that focuses on providing pollen and nectar foods for invertebrates that provide pollination and pest control services. As noted previously, one explanation for why wildflower strips may be better than hedges at improving ecosystem services in a crop is due to the fact that wildflower strips are often created specifically to attract beneficial invertebrates via provision of food resources, whereas hedges have different main purposes such as to serve as boundaries, to provide wind and rain shelter to livestock, to reduce soil erosion, and control water runoff (Baudry et al., 2000). For example, Tschumi et al. (2016) found that wildflower strips designed for the attraction of predators of aphid pests in potato crops did increase adult and juvenile richness and abundance of predators in the crops, including syrphids. They also found that having wildflower strips reduced aphid populations to a quarter of what were found in crops with no adjacent wildflower strips

(Tschumi et al., 2016). Wildflower strips can both support beneficial insects, as well as attract them into the crop to implement their ecosystem services.

This flower-feeding behaviour is common among predatory insects at the adult stage, including parasitoid wasps, lady birds, and lace wings (Campbell et al., 2017; Pontin et al., 2006; Varennes et al., 2016a; Varennes et al., 2016b), however, insects have preferences and limitations as to what floral resources they can use. Many syrphids have short proboscis lengths (<5 mm), which is a limiting factor to which flowers they feed from, so these species tend to favour open flowers (Larson et al., 2001). The species of the syrphid and flower visited also determine how the fly feeds – sometimes it will consume both the pollen and nectar, and sometimes only one or the other, with it being believed that the importance of pollen in the diet is lower in syrphids with a longer proboscis, as they can include more nectar in their diets due to being able to feed from tubular flowers (Gilbert, 1981). Syrphids use the nectar of flowers as a source of sugars for energy, and their consumption of pollen is mainly to stimulate development of reproductive organs and gametes (Branquart et al., 2000; Haslett, 1989; Rodríguez-gasol et al., 2020). Pinheiro et al. (2015) observed that female syrphid (*Episyrphus balteatus*) survival is enhanced by inclusion of pollen in a diet of honey, but this effect was not seen in males. Although females consume more pollen throughout their adult life than males, mainly to enable continuous egg production, how pollen consumption could be linked to increased longevity in females is unknown (Pinheiro et al., 2015). Syrphids not only feed on floral resources of pollen and nectar but have been known to feed on honeydew as well to supplement diets when floral food resources are scarce (Pinheiro et al., 2015). van Rijn et al. (2006) noted that the addition of high quantities of honeydew to the floral diets of the syrphid *Episyrphus balteatus* improved their survival. Sutherland et al. (2001) found that wildflower clusters were more attractive to syrphids if they were in the margins than the centres of crops, which the authors believed was due to additional resources in the margins, such as a diversity of aphids on forbs providing honeydew food and oviposition sites for predatory larvae. This was in spite of there being greater floral abundance in the crop wildflower patches than the margin patches, so it is not just the abundance but the availability of various required resources to meet other needs as well, such as aphids for the predatory larval stage (Sutherland et al., 2001).

The presence of other flower-visiting insects can decrease available food supplies, and this can also influence syrphid abundances. Lindström et al. (2016) found that honey bee hives near oilseed rape fields decreased the abundance of other floral visitors in the fields, including syrphids. On the other hand when looking solely at the impact on wild bees, Mallinger et al. (2017) found inconsistency of effects from competition for floral food resources from honey bees, instead suggesting that negative effects are more likely to stem from transmission of pathogens, which based on the limited available literature on pathogen transmission from bees does not appear to be of much concern for syrphids (Bailes et al., 2018; Evison et al., 2012; Manley et al., 2015). Additionally, behavioural differences may reduce competition, such as different foraging times of day, and foraging adaptation to competitor presence (Brosi et al., 2017; Jeavons et al., 2020). Landscape complexity can play a part in competitive effects on communities too, where greater complexity can allow for greater insect diversity even in the face of dominant, forage-intensive insects like honey bees (Herbertsson et al., 2016). Thomson and Page (2020) noted that, while there is evidence of competition for floral food resources between insect groups, how and under what circumstances this translates to community changes is still unclear.

1.3.2 Impacts of aphid food resources and competitors on predatory syrphids and other beneficial invertebrates

As previously noted, it is not just attracting the mobile adult stage of syrphids by providing them food resources that is important, but also ensuring that the crop is a preferable site for female syrphids to oviposit their eggs in for the subsequent larvae to prey on crop aphid pests. Syrphid ovipositing behaviour can be influenced by the crop host of the aphid, the aphid species, and the size of the aphid colony (Dunn et al., 2020; Rodríguez-Gasol et al., 2020), and these preferences are specific to syrphid species. Just as having wildflowers may attract floral visitors to pollinate crops, so too can having a diversity of plant host-aphid communities possibly boost oviposition on crops. In a review of the literature on the interactions between plants, pests, and natural enemies, Evans (2008) suggested that diverse and abundant invertebrate communities could serve as prey to maintain and build up generalist predator communities prior to sowing of fields, thus a stock of predators are available when pest infestations on crops occur. This idea links up with what was discussed previously from Albrecht et al. (2020), in that permanent habitats could also be attractive and create a stock of predatory insects because they allow non-pest prey populations to build up, providing food for predators when crop pests are not available. This idea of having additional, non-crop-pest prey available to create and sustain a reservoir of predators has been practiced in greenhouses via banker plants. Pineda and Marcos-García, (2008) attempted this practice specifically for syrphids in greenhouses, and found that a banker plant system (*Rhopalosiphum maidisi* aphids on barley) could increase visits to the greenhouse from wild syrphid predators, but they were not able to link this to increased pest control of the crops, and also found that it did not increase the retainment time of manually introduced predatory syrphids. Laurenz and Meyhöfer (2021) also found that banker plants can increase wild syrphid predation, whereby there were on average a 61.5% increase in syrphid larvae and a 17.3% decrease in white fly (*Aleyrodes proletella*) abundances on outdoor Brussels sprout crops (using Hokkaido squash bearing *Trialeurodes vaporariorum* as a banker plant system). However, the banker plant system of white flies (*Aleyrodes lonicerae*) on European columbine (*Aquilegia vulgaris*) did not affect syrphid abundances, and no banker plants effected aphid abundances (Laurenz and Meyhöfer, 2021). Recently, the banker plant system of bird cherry-oat aphids (*R. padi*) on barley and introduced syrphids (*Eupeodes americanus*) has shown potential in controlling foxglove aphids (*A. solani*) on green pepper crops in greenhouses (Bellefeuille et al., 2021). Bellefeuille et al. (2021) found that syrphids significantly reduced aphid abundances after six weeks (crop plants exposed to syrphids had on average 11 aphids per plant, those not exposed to syrphids had 1,131 per plant), though there are things to consider with this system (e.g. controlling ants that could prey on juvenile syrphids; juvenile syrphids may need to develop on the banker plant system in order to be effective, as syrphids released as adults in the greenhouse did not appear to mate and oviposit young on crops).

Another factor that could influence egg-placement in syrphids is presence of competition. In terms of competition between other syrphid larvae, Sadeghi and Gilbert (2000a) noted the scarcity of documented cases of different syrphid larvae species preying within the same aphid colonies, and because of this we cannot deduce much regarding the influence intraguild predation among syrphids has on syrphid communities. Aphidophagous syrphids will avoid other aphid consumers though, such as parasitized aphids (Dunn et al., 2020; Rodríguez-Gasol et al., 2020). Regardless, it is not just the competition for aphid food resources that can influence oviposition and larval survival, but also intraguild predation. Hindayana et al. (2001) found that other aphid predators, namely a gall midge (*Aphidoletes aphidimyza*), a lacewing (*Chrysoperla carnea*), and a ladybird (*Coccinella septempunctata*) could all prey on at least the eggs and early larval stages of *Episyrphus balteaus*. The superior predator (i.e. the predator that can prey upon other predators present) between different species or insect groups can depend on the species and, if a juvenile, the instar. Putra et al. (2009) demonstrated this by doing paired comparisons of how two species of syrphid larvae interacted with three species of ladybird larvae, and found that the more developed larvae preyed on the newly emerged larvae in all paired species cases, however when larvae were of similar instars the superior predator depended on the paired species involved. Putra et al. (2009) also found that ovipositing females could be influenced by the presence of ladybird larvae, where *Episyrphus balteatus* females would lay significantly fewer eggs in aphid colonies if superior ladybird species larvae were present, but this effect was not seen for the syrphid *Metasyrphus corollae*.

While competition and intraguild predation can influence the syrphid communities, for the farmer, the main question of interest is how these factors influence pest control services, and the answer is variable due to several factors. Sometimes having multiple kinds of pest consumers can result in higher pest control. For example, de Pedro et al. (2019) found medfly parasitism was higher when two species of parasitoid were present. However, the competition and predation between multiple predators could sometimes result in no improvement or a reduction of pest control. Rosenheim et al. (1993) ran multiple experiments with different methodologies, all comparing aphid-control performance between treatments with only one aphid predator or multiple predators present. Some experiments showed the growth rate of the aphid populations were similar in both cases of one or multiple predators, suggesting that any intraguild predation was made up for by the dominant predators consuming the same number of aphids in the absence of their consumed predators (Rosenheim et al., 1993). Other experiments showed that having various predators reduced the aphid population growth rates (i.e. having more predator variety resulted in better pest control), and some showed having various predators resulted in higher densities of aphids (this could be linked to intraguild predation, where predators preyed too much on each other rather than the pests) (Rosenheim et al., 1993). The authors believed their methodology for each experiment influenced these variable results (Rosenheim et al., 1993), which exemplifies the difficulty in understanding predator-prey dynamics, much more so optimizing biological pest control in fields or greenhouses.

1.3.3 Other biological factors that can impact syrphids

Syrphids do not just have to face intraguild predation, but also predators that specifically target them or include them in their diets. Syrphid larvae face parasitism by various hymenopterans, particularly ichneumonid wasps, and the adults are preyed upon by various animals, including hymenopterans, spiders, and birds (Dunn et al., 2020; Rodríguez-Gasol et al., 2020). They face many other non-invertebrate dangers as well, such as entomopathogenic fungi (especially *Entomophthora muscae*, Stubbs and Falk, 2002) and viruses and bacteria (Gilbert, 2005).

1.4 Chemical factors in agriculture that influence syrphids and other beneficial invertebrates

1.4.1 Natural chemicals

Pollen and nectar were previously discussed as important parts of syrphid diets that aid in reproductive functioning due to protein content in pollen, and to provide energy for activities due to carbohydrate content in nectar (Haslett, 1989; Rodríguez-gasol et al., 2020). However, these food resources in some plants can sometimes contain defence chemicals believed to be intended for herbivores (Jacobsen and Raguso, 2018; Stevenson, 2020). These toxins have been demonstrated to have negative impacts on flower visiting insects, such as the findings from Tiedeken et al. (2016) that honey bee survival was reduced when fed the plant defence chemical grayanotoxin I, and a solitary bee (*Andrena carantonica*) avoided consuming food that contained the toxin. Other nectar chemicals that do not benefit or directly harm flower visitors are deceptive chemicals. These chemicals deceive flower visitors into perceiving the flower as something else, such as a potential mate or an attractive place for oviposition, and this deception can sometimes result in the insect becoming trapped in the flower (Raguso, 2020). This chemical deception has been observed to target aphidophagous syrphids too, such as in orchids that deceive syrphids into perceiving the presence of an aphid colony for oviposition (Jiang et al., 2020; Stökl et al., 2011). Stökl et al. (2011) were able to isolate and identify some of the same chemicals in an orchid (*Epipactis veratrifolia*) and in an aphid species (*Megoura viciae*) that stimulate oviposition in *Episyrphus balteatus*. Chemical signals from aphids can inform syrphids where prey is, but it appears some plants also mimic these chemicals to attract syrphids for pollination.

Just as floral food resources can contain harmful chemicals produced from the plant, so can aphid prey contain harmful defence chemicals they consumed from the plants they damage. For example, Vanhaelen et al. (2002) found decreased survival and development in *Episyrphus balteatus* larvae that fed on specialist aphids (*Brevicoryne brassicae*) feeding on plants (*Sinapis alba*) that produced large quantities of glucosinolate defence chemicals. However, they did not find a difference in survival or development when aphids fed on plants producing low quantities of glucosinolates or when the aphid prey were generalists (Vanhaelen et al., 2002). Notwithstanding, these are all naturally occurring chemical stressors that have coexisted and evolved in tandem with syrphids, such that syrphids could develop defences and resilience. There are also anthropogenic chemicals that societies either release purposefully (e.g. pesticides, fertilizers) or dispose of or emit that can spread into the environment (e.g. fuel exhaust, pharmaceuticals, etc.). Syrphids can be exposed to and impacted by these too.

1.4.2 Synthetic chemicals

Both pollinator communities (Kevan, 1975; Mallinger et al., 2015; Tuell and Isaacs, 2010) and predator communities (Khan, 2017; Mansour, 1987; Monzo et al., 2014; Wilson et al., 1999) can be negatively influenced by pesticide use. Kevan (1975) attributed the failure of blueberry crops to the deaths of bees from exposure to pesticides in a nearby commercial forestry plot, while Wilson et al. (1999) were able to link predator declines to bigger pest populations in sprayed crops. In addition, there are many reviews that have discussed the links between pesticide use, damage to beneficial insect communities, and crop performance (Allen-Wardell et al., 1998; Kevan and Phillips, 2001; Pimentel et al., 1992; Sponsler et al., 2019). For syrphids specifically, Power et al. (2016) found that organic land management supported more syrphids than what was expected after adjusting for landscape factors and floral abundance, which the authors believed could be linked to synthetic pesticide use in conventional farms. These community-level effects can be due to individuals' contact exposure, either from being sprayed directly or from residues accumulating on crop vegetation, soil, and nearby grasses, forbs and woody plants. Chemical residues can degrade over time via exposure to oxygen, sunlight, rainwater, metabolic activity of microorganisms in soil and on plants, etc. (Katagi, 2020). Furthermore, rainwater can wash residues off plants, where they can end up in waterways and soils. So even though there are still noteworthy effects on insects from contact exposure with pesticide residues (e.g. decreased host-finding and ovipositing activities in parasitoids; Desneux et al., 2004; Salerno et al., 2002), this route of exposure is considered short-lived due to chemical degradation and removal over time. Moreover, in order for syrphids to be directly sprayed or come into contact with fresh residues, they would have to be in the field at the time of spraying or shortly after, and given their adult mobility that time period could be rather short – it would be eggs, larvae, and pupae that are at highest risks of exposure. Another route of exposure is via dietary exposure – consuming foods contaminated with pesticides. While it is possible that sprayed chemicals may sometimes end up in the pollen and nectar of open flowers with these resources readily accessible (e.g. Daniele et al., 2018; Giroud et al., 2013; Tong et al., 2018, note that these sprayed pyrethroid residues were found in bee-collected pollen or bee bread), systemically active pesticides are of more concern for this exposure route due to their water solubility which allows them to be taken up by plants and transported throughout their tissues (Bonmatin et al., 2015).

Pesticides can be purposed for targeting a specific group of organisms – fungicides, herbicides, acaricides, molluscicides, insecticides, and rodenticides to name a few. Of the chemicals that are used to protect plant crops from stressors, over 1,400 active ingredients are registered in the EU pesticide database, although less than 500 are approved for use (European Commission, 2020b). Of the insecticidal active ingredients considered to be of high concern to beneficial insects, much attention has been given to neonicotinoids as environmental contaminants due to their water solubility allowing dispersion in fields to be taken up by non-crop plants, and their persistence which can last after harvest and still be detected in soils and succeeding plants long after application (Bonmatin et al., 2015; Li, 2020; Long and Krupke, 2016; Wintermantel et al., 2020). An unintentional consequence is that the chemicals are also distributed to pollen and nectar – food resources that predators, pollinators, and other beneficial invertebrates (e.g. decomposers like the syrphid *Eristalis tenax*) and insects of conservational interest rely on. Residues in pollen and nectar have been documented to reach extremely high levels, with a review by Zioga et al. (2020) listing median concentrations of 1,400 ng/g carbofuran in pollen and 1,595 ng/g dimethoate in nectar. Significant variability can occur though, which depends on several factors including crop type and application method (Gierer et al., 2019; Zioga et al., 2020). Another unintended consequence is the distribution of the chemicals from runoff. Because systemic pesticides are preferred because they can be absorbed by the plants without having to spray large areas whereby non-target plants and wildlife will receive direct application, a common alternative is to purchase the crop seeds coated with the pesticide. However, most of coated systemic chemicals leach into the soil (up to 98%, Goulson, 2013), where water can distribute it across the field and even into the margins, where the chemical can be taken up by non-target habitat vegetation (Botías et al., 2015; Mogren and Lundgren, 2016). Furthermore, due to the persistence of these chemicals in soils (e.g. clothianidin has a 90% deterioration time in field soil of 387 days, and imidacloprid of 717 days, Lewis et al., 2016), subsequent crops and non-target plants can still absorb residues several months after the pesticides were introduced. Because of this, landscapes could bear contaminated pollen and nectar food resources for lengthy periods, such as Wintermantel et al. (2020) demonstrated by finding imidacloprid in oilseed rape crop nectar each of five years after it had been banned from use on flowering crops.

Additionally, there has been a growing interest in how contaminated pests can also be detrimental to insect communities. Pests that consume systemic pesticides while feeding on treated plants may survive, such that predators will still prey on them and thereby consume systemic pesticides from the pests (Douglas et al., 2015; Thornham et al., 2007; Tian et al., 2015). It is not just carnivores preying on contaminated pests, though, but invertebrates that feed on the honeydew that sap-sucking pests deposit on plants. Recent research has demonstrated how the honeydew of pests feeding on neonicotinoid treated plants contains neonicotinoid residues, which can increase mortality in insects that feed on the honeydew (Calvo-Agudo et al., 2019, 2020).

A number of sub-lethal effects can also arise from feeding on contaminated nectar, pollen and pests, a few examples being decreased learning efficiency, behavioural changes such as increases in grooming, and changes to circadian rhythms (El Hassani et al., 2008; Phelps et al., 2020; Smith et al., 2020; Tackenberg et al., 2020; Williamson et al., 2013). Sub-lethal effects can make it more difficult for invertebrates to withstand field-realistic stressors, such as increased susceptibility to pathogens, parasites, and other pesticides encountered (Baron et al., 2014; James and Xu, 2012; Tsvetkov et al., 2017); decreases in efficiency at pollen collecting, which can exacerbate nutrition deficits during times of food scarcity (Feltham et al., 2014); and decreases in body temperature which can make them less resilient to drops in temperature (Azpiazu et al., 2019). Furthermore, sub-lethal effects can persist through multiple life stages, or be delayed such that they only become apparent in subsequent life stages (Tadei et al., 2019; Tavares et al., 2017; Tesovnik et al., 2020). Pesticide exposure can also influence reproductivity thereby influencing subsequent generations, such as reduced reproductivity, smaller offspring, and skewed sex ratio of offspring (Baron et al., 2014; Baron et al., 2017; Sandrock et al., 2014; Siviter et al., 2020). The value of habitat and food provision on farmland for the conservation and encouragement of insect ecosystem services could be diminished by the presence of toxic chemicals harming the wildlife and inhibiting service provision.

1.5 Research objectives

Optimizing Irish farmed landscapes and fields to support predatory invertebrates and encourage pest control services could benefit cereal farmers, but benefits could be diminished by the use of pesticides that harm beneficial invertebrates, either directly by killing them or indirectly by reducing their food supplies of floral foods and prey. Any benefits derived from natural pest controllers can also be masked by altering prey population cycles, either by killing prey at similar or higher levels than provided by predators, or by killing too many natural predators thereby allowing pests that survived pesticide applications to reproduce unchecked (Pimentel et al., 1992). From a conservation standpoint, providing attractive habitats on land harbouring toxic chemicals could be an ecological trap for beneficial invertebrates, encouraging their residence in harmful environments (Kovács-Hostyánszki et al., 2017) and exacerbating current problems with population declines (Eggleton, 2020; Hallmann et al., 2017; Wagner, 2020). Before we could address questions on how to make crop fields attractive to syrphids, first we had to ask what hazards potentially exist on farmland and how might they influence syrphids.

In Chapter 2, I asked whether there are insecticides in predatory syrphid food resources in cereal crops. I investigated food resources utilized throughout predatory syrphid life cycles – wildflower nectar and pollen for the adult stage, and crop aphids for the larval stage. To my knowledge, there had been no investigations yet of pesticide residues in field-collected floral nectar or pollen in Ireland, or of residues in field-collected aphid crop pests anywhere prior to this study. It was important to establish some degree of a baseline to serve as a reference point of frequency and quantity of residues in food resources in Ireland before moving on to the next question – how does dietary exposure to pesticides influence predatory syrphids?

This question was explored in Chapter 3, where I exposed the Irish native syrphid *Episyrphus balteatus* to residues of the neonicotinoid, thiamethoxam, in their nectar diets, and monitor their survival and behaviour over 14 days. Behaviour was observed in the form of activity budgets to see if there were differences in syrphid general behaviour between pesticide doses received, and by testing their ability to grip smooth surfaces. The hope for the grip test was that it would add a new dimension of meaning to the survival data, in hopes that I could monitor the health changes preceding death and over the 14 days of observation. Even though individuals may have survived a long time after exposure to a single stressor in captivity, it would not mean the condition of the individual had remained the same or that the individual was capable of surviving in field-realistic conditions where multiple stressors abound.

After exploring the insecticide hazards syrphids may face in Irish farmland and how it affects them, I moved on to ask what natural factors, without the use of harmful pesticides, may make cereal grain crops (specifically organic oat crops) attractive or unattractive to predatory syrphids in Chapter 4. Given the prevalence of hedges in rural areas in Ireland, features of this habitat were investigated in terms of shape and gaps present. The provision of food resources (wildflowers and aphids), and potential competitors for those resources (floral visitors and parasitized aphids) were also investigated. It was hoped that this study could aid hedge management and IPM (integrated pest management) methods to create habitats that better attracted predatory syrphids to crop fields. Furthermore, understanding how food and competitor abundances could be linked to predatory syrphid abundances could shed light for farmers and conservationists in terms of how syrphids interact with other wildlife – what foods they need, the links between predatory syrphid populations and crop aphid populations, and what competitors may make a habitat difficult for syrphids to thrive in.

The final chapter, Chapter 5, drew together the findings of chapters 2 through 4. In this chapter, I discussed how chemical hazards (insecticides), physical (hedge habitats) and biological (food resources) attractants, and biological deterrents (food competitors) can all be linked together to reflect syrphid populations and service provision in cereal crop landscapes, what farmers and land managers can do for wildlife, and what are the benefits and costs for farmers. I also considered other points not explored in this work.

Chapter 2

Neonicotinoid residues in aphids and wildflowers in oat cropping systems

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2. Neonicotinoid residues in aphids and wildflowers in oat cropping systems

2.1 Abstract

Pyrethroids and – until recently – neonicotinoids are commonly used to protect arable crops from aphid pests, but can be mobile and persistent in the environment, resulting in residues in floral nectar and pollen, and in surviving aphids that feed on treated crops. These residues can poison flower-visiting pollinators, and natural pest controllers that prey on aphids and supplement their diets with floral resources. We collected samples from three organic and three conventional spring oat crops in the Ireland. At each site, we took one sample of aphids from the crop, and one sample each of the nectar and stamens (pollen-laden) from *Rubus fruticosus agg.* in the margins of the crops. We analysed each sample for pyrethroids (deltamethrin, esfenvalerate, and permethrin) and for neonicotinoids (clothianidin, imidacloprid, and thiacloprid). No pyrethroids were detected, but neonicotinoids were detected in at least one sample each of nectar, stamens, and aphids. Thiacloprid was detected the most often, imidacloprid once, and clothianidin not at all. There were a fairly equal number of detections between organic and conventional sites. These results suggest that neonicotinoids likely pose a greater dietary hazard to flower-feeding and predatory insects than pyrethroids. Hover flies feed on flowers as adults and on aphids as larvae, exemplifying exposure to dietary residues from unique sources throughout some predators’ lifecycles. More research is needed to better understand how pesticides impact biological pest control in agriculture.

2.2 Introduction

Insecticides are used all over the world to manage insect pests in agriculture, private and public areas, and dwelling places, with > 2.7 billion kgs used globally (Atwood and Paisley-Jones, 2017). The EU pesticide database lists 306 insecticidal ingredients, with 102 approved for use in member states (European commission, 2020b); however, some of the most used are from the neurotoxic classes of pyrethroids and neonicotinoids (Jeschke et al, 2011). Concerns exist regarding exposure to non-target insects that humanity benefits from, particularly pollinating bees. Bees can be exposed via contact but can also be exposed through their diets of nectar and pollen. Much attention has been drawn recently to pesticide residues in flower pollen and nectar that can pose a risk to beneficial invertebrates that depend on these as food resources (EFSA, 2012; Pisa et al., 2015; Wood and

Goulson, 2017). Pesticide-laced floral foods have been shown to have several negative impacts on bee health (Alkassab and Kirchner, 2018; Decourtye et al., 2004; Laycock et al., 2012; Sandrock et al., 2014; Stanley et al., 2015) and are considered a contributing factor to global bee declines (Goulson et al., 2015; Mason et al., 2013; Woodcock et al., 2016a). However, other beneficial insects can also be at risk of dietary exposure.

While bees are considered beneficial for their pollination services, there are many predatory insects that are valued for their provision of pest control services. Insect predators often have multitrophic diets, whereby one or more life cycle stages feed on pollen and nectar exclusively or to supplement their carnivorous diets (Coll and Guershon, 2002; Lundgren, 2009; Wäckers et al., 2005), therefore these beneficial predators are also at risk of residues in floral food resources. Another source of dietary pesticide residues for predators can also be found in the target pests they prey upon (Bonmatin et al., 2015; Pisa et al., 2015). For example, it was found that when thiamethoxam was used as a seed-treatment for soy beans, slugs that fed on the resulting plants were unaffected, but over 60% of predatory beetles that fed on the slugs experienced mobile impairment, with about half of these impaired beetles eventually dying, despite the fact that overall residual concentrations of the neonicotinoid decreased as it moved through the food chain (Douglas et al., 2015). The authors did note that thiamethoxam is targeted to insects, likely explaining why the slugs did not experience ill effects (Douglas et al., 2015). However, insecticide residues have been detected in aphids that survived treatment, including metabolites with insecticidal properties (Bass et al., 2011).

There are many insecticides used that can be found in non-target insect diets, and the classes of pyrethroids and neonicotinoids are some of the most common and regarded to have the highest exposure risk to flower-visiting insects (Sanchez-Bayo and Goka, 2014). These classes are both neurotoxic to insects, though the specific mechanisms are different – pyrethroids act on the voltage-gated sodium channels (Soderlund et al., 2002), while neonicotinoids act on the nicotinic acetylcholine receptor (Taillebois et al., 2018). Field studies have also provided evidence that decreases in the abundance and diversity of beneficial insects can correlate with the use of pyrethroids (Croft and Whalon, 1982; Douglas and Tooker, 2016; Frampton and Brink, 2007; Huusela-Veistola et al., 1994; Shires, 1985; Wiles and Jepson, 1994) and neonicotinoids (Douglas and Tooker, 2016; Saeed et al., 2016; Seagraves and Lundgren, 2012).

A number of factors determine the likelihood that these pesticides can end up as residues in the food resources of non-target wildlife, the likelihood that they will be in concentrations that will have any effect on the wildlife, and how long they will persist. These factors include the chemicals’ stability when exposed to water, sunlight and oxygen, biodegradation by microbes and fungi, and rates of dissipation and elimination in plant food resources and animal prey (Table 2.1). Plants and animal prey can transform pesticides into metabolites as well, some of which can exhibit comparable toxicity to the original pesticide, or they can be effective pesticides themselves. For instance, thiamethoxam is transformed to clothianidin in soil, insects, and plants, and both chemicals are commercially available neonicotinoids (Simon-Delso et al., 2015). The method of degradation and ultimate fates of pesticides are determined by the chemistry and application methods of the pesticides. For example, while neonicotinoids are considered moderately soluble (between 50 and 500 mg/L) or better, pyrethroids are considered hydrophobic (Bonmatin et al., 2015; Cycoń and Piotrowska-Seget, 2016; Lewis et al., 2016). Neonicotinoids' solubility allows them to be systemically absorbed with water by plants and distributed throughout the tissues internally. As a result, while there is reduced contact exposure with insects when they are applied as seed treatments, there is also potential for exposure resulting from the transport of residues into flower pollen and nectar. On the other hand, non-systemic pyrethroids are typically used as foliar sprays, increasing the risk of contact exposure for insects, with any residues that end up in pollen and nectar mainly due to spraying of or drift landing on flowers. There have already been several detections in nectar, pollen and the insects that forage on these of both pyrethroids and neonicotinoids (Chauzat et al., 2011; Hakme et al., 2017; Niell et al., 2017; Roszko et al., 2016; Tong et al., 2018), including in non-target wildflowers found in the margins of treated crops (Botías et al., 2015; David et al., 2016; Krupke et al., 2012).

For aphids, however, we are not aware of any studies that have attempted to measure pesticide residues in field-collected samples, despite the lab-based evidence of negative impacts on predators fed pesticide-treated aphids (Thornham et al., 2007; Wanumen et al., 2016; Wumuerhan et al., 2020). This pest-related risk includes the honeydew they produce, (EFSA, 2013; van der Sluijs et al., 2013) which many beneficial insects consume when floral nectar resources are scarce (Konrad et al., 2009; Pinheiro et al., 2015; Wäckers et al., 2008). The risk of dietary residue exposure in honeydew has recently been demonstrated in a greenhouse study that not only detected neonicotinoid residues in the honeydew of psyllid pests, but also found increased mortality among pest consumers (the syrphid *Sphaerophoria rueppellii* and the parasitic wasp *Anagyrus pseudococci*) that fed on the contaminated honeydew (Calvo-Agudo et al., 2019). Target pests are a potentially significant yet understudied source of dietary insecticide residues to beneficial insects.

In conventional cropping systems, particularly cereals, several pyrethroids and neonicotinoids are used that can result as dietary residues for non-target organisms. Cereal fields are often near land used for other crops, sometimes only separated by a hedge or a fence and a margin of a few metres in width. This closeness increases the likelihood of pesticides crossing from one field to the next via drift or runoff. Furthermore, residues can end up in waterways, where they can be distributed across the landscape (Casado et al., 2019; Silva et al., 2019), including onto nearby organic farms (Barański et al., 2014; EFSA, 2018; Walorczyk et al., 2013; Zohair et al., 2006). However, it is expected that residues will be detected less often and at lower concentrations in organic than conventional fields (Barański et al., 2014; EFSA, 2018). Regardless, some animals are very mobile (e.g. birds, butterflies and migratory hover flies) such that individuals can be exposed to residues in food resources in various conventional and organic fields.

Some of the pyrethroids and neonicotinoids now have restricted and limited use. For instance, permethrin is no longer used in any crop sector in Europe, due to its discontinuation in 2000 (European Commission, 2000). In 2013, clothianidin, imidacloprid and thiamethoxam were banned from use as seed and soil treatments on all outdoor crops except winter cereals, and as sprays on all outdoor crops prior to flowering (European Commission, 2013). Regardless, until late 2018, these neonicotinoids were still available for various uses, including agricultural uses on winter cereals and applications after crop flowering, and even now are still used as topical medications for pets and livestock, and to control pests in human dwelling places (Tables 2.2-3). We focussed on six compounds in this study: three pyrethroids and three neonicotinoids, which have various uses in the Ireland (Figure 2.2, Tables 2.2-3). These six were selected because they have recently been detected in honey samples from Ireland (Hu, 2018; Kavanagh, 2021). Very little research has been carried out for pesticides residues in floral resources in Ireland, but since honey is made from flower-collected nectar with trace amounts of pollen, it is assumed that these residues can be traced back to floral origins. Furthermore, if residues can be found in non-target floral resources, then there is potential that the target pests of these insecticide applications (i.e. crop aphids) harbour residues as well.

Table 2.1 Properties that selected pyrethroids and neonicotinoids exhibit in the environment.

Class	Pesticide	[a]Water solubility (mg/L)	[b]Plant ½-life (hours)	[cdefg]Animal ½-life (hours)
Pyrethroid	Deltamethrin	0.0002	90.24	15.9
	Esfenvalerate	0.001	38.4	12-14.4
	Permethrin	0.2	140.16	9
Neonicotinoid	Clothianidin	340	198.48	2.4
	Imidacloprid	610	88.8	1.33
	Thiacloprid	184	90.24	.833

[a] Source: Lewis et al., 2016. Measured at 20 ^{o}C.

[b] Source: Fantke et al., 2014. Values are from a model that estimates average half-lives for an average plant.

[c] Source for deltamethrin ½-life in animals: Mortuza et al., 2018. Measured from rat plasma after a 0.5 mg/kg injected dose.

[d] Source for esfenvalerate ½-life in animals: Shah and McGregor, 2012. Measured from rat excrement after a 4.2 mg/kg gavage administered dose.

[e] Source for permethrin ½-life in animals: Chata et al., 2019. Measured from rat plasma after a 0.4 mg/kg gavage administered dose.

[f] Source for clothianidin ½-life in animals: Yokota et al., 2003. Measured from male rat plasma after a 5 mg/kg injected dose.

[g] Source for imidacloprid and thiacloprid ½-life in animals: Ford and Casida, 2006. Measured from rat plasma after a 10 mg/kg injected dose.

Table 2.2 Outdoor agricultural uses of target pesticides in the Ireland in 2018 (country of origin of all samples in this study).

Crop	Pyrethroids			Neonicotinoids		
	Deltamethrin	Esfenvalerate	Permethrin	Clothianidin	Imidacloprid	Thiacloprid
Spring Oats[a]				**X**		
Other arable crops[a]	X	X		X		
Fodder crops (beet, swede, and turnips)[b]					X	
Outdoor vegetables[c]	X	X				X
Non-protected and semi-protected soft fruits[d]						X

[a] Source: Delaney et al., 2019.

[b] Source: Quirke, 2013.

[c] Source: Quirke, 2015.

[d] Source: Quirke, 2014.

Table 2.3 Number of products available for purchase containing target pesticides in the Ireland in 2018 (country of origin of all samples in this study).

Use Category	Pyrethroids			Neonicotinoids		
	Deltamethrin	Esfenvalerate	Permethrin	Clothianidin	Imidacloprid	Thiacloprid
Plant protection products[a]	7	1		1	6	5
Veterinary medicine (cattle, donkeys, horses, sheep)[b]	7		1			
Veterinary medicine (household pets)[c]	6		18		37	
Other biocides[d]	28		49		23	9

[a] Source: PCS, 2018; Includes crops, ornamentals, forests, gardens, and turfs.

[b] Source: HPRA, 2014; applications include spot-on and pour-on.

[c] Source: HPRA, 2014; applications include spot-on, collar, soaps, and ear medication,

[d] Source: PCS, 2019; Applications can be done by amateurs, professionals, or industries and include indoor and outdoor uses.

The purpose of this study was to identify residues occurring in food resources of predacious insects in cereal crop landscapes, and we chose to compare detection frequency between organic and conventional sites. Less than 5% of agricultural land in Ireland is under organic management (Utvik et al., 2019), so we selected spring oats – the crop that is most commonly grown organically (DAFM, 2019) – as the focus crop for this study. We sampled floral nectar and pollen of *Rubus fruticosus agg.* (an abundant wildflower in agricultural hedges surrounding crops, with high visitation from insects), and the aphid prey in the oat crops, on organic and conventional farms. These resources were selected specifically for their availability to predacious insects and for their sufficient abundance to meet our sampling size needs for chemical analysis, as well as the increased likeliness of pesticide residues (i.e. aphids directly off the crop, and flowers surrounding the crop). *Rubus fruticosus* agg. is also a wildflower commonly investigated for pesticide residues, thereby our data can contribute to that research and allow for comparisons between studies and geographical locations (Zioga et al., 2020). Specifically, we tested the following hypotheses:

- There are detectable insecticide residues in floral food resources of wildflower pollen and nectar.
- There are detectable insecticide residues in the prey food resource of crop aphids.

- Insecticides will be detected more often in samples from conventional farms than from organic farms.

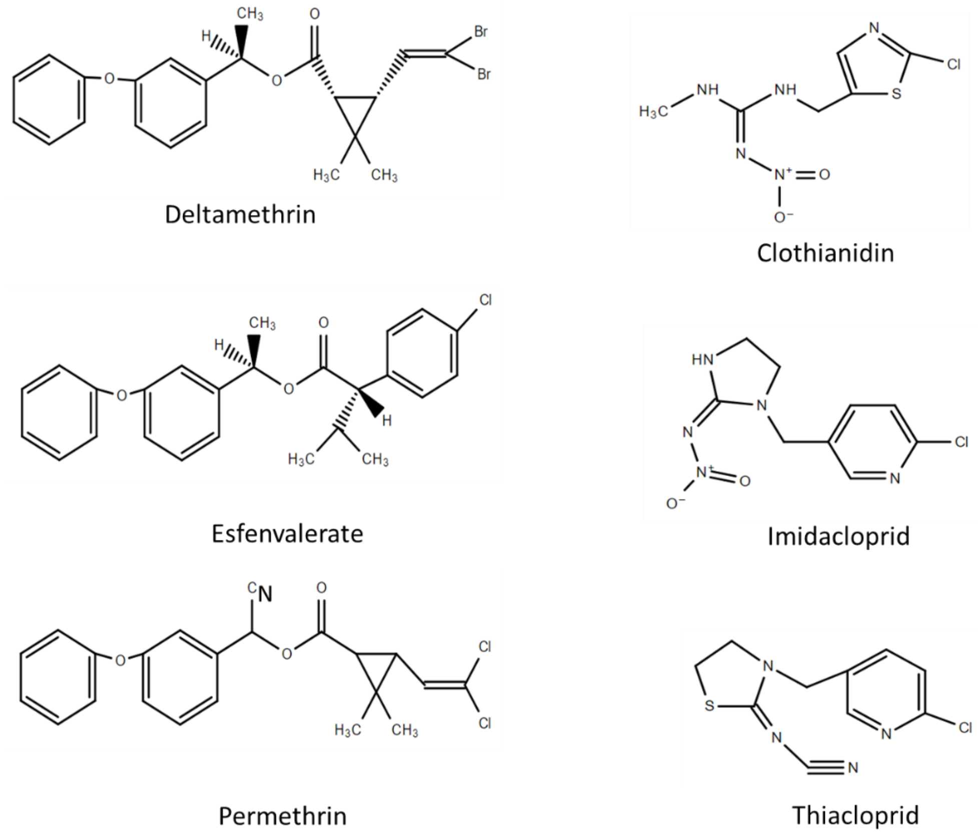

Figure 2.1 Chemical structure of target insecticides of this study (Structural references Iwasa et al., 2004 and Matsuo, 2019; Drawing Software ChemSpider, accessed 2020).

2.3 Methods

2.3.1 Field sampling and surveying

2.3.1.1 Sampling sites

Six spring oat crops in south-eastern Ireland were sampled during 2018: three conventionally managed crops, and three qualifying for organic status for at least nine years (Figure 2.2). Cropping history varied among all the sites, with rotations including cereals, other arable crops, and grassland (Table 2.4). At each site, aphids were sampled along transects within a single oat field, while floral samples were taken from the hedges surrounding the same oat field. The fields ranged in size from 2 to 13 hectares (Table 2.4).

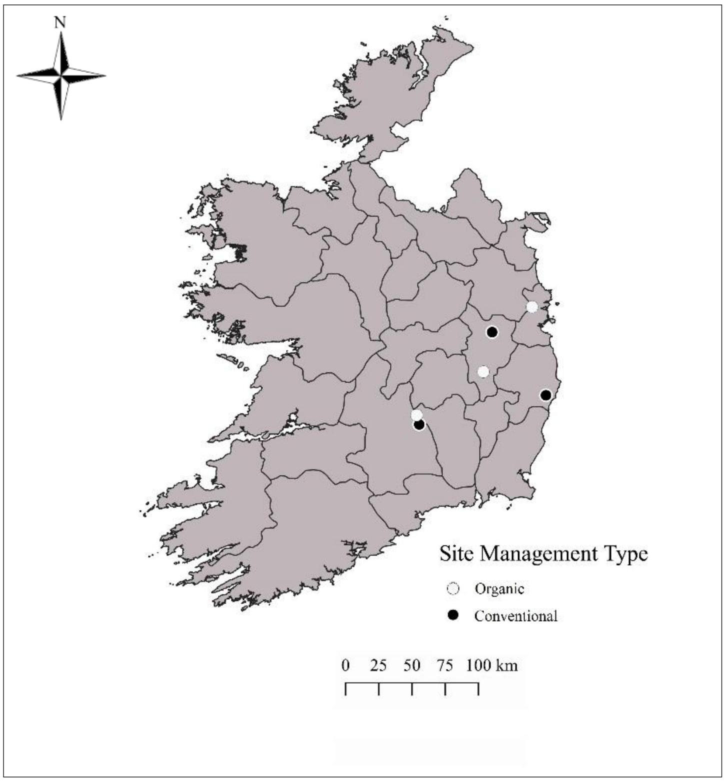

Figure 2.2 The six sampling sites in Ireland.

2.3.1.2 Aphid sampling

From June to the first week of July, aphids were collected along three 30-metre transects starting at roughly the centre of the eastern edge of the crop, running west, each separated by five metres. 30-metre length transects were chosen in order to remain within the limits of smaller fields, whilst still penetrating the crop in larger fields. At every metre along the transect, two people searched the oat tillers directly in front of them in an area of about 1 m^2 for at least two minutes. All live aphids not showing signs of parasitism or fungal infections were collected into Petri dishes before transferring to glass vials. The numbers of alate and apterous aphids collected were recorded, as some alate aphids may have only briefly migrated to the oat tillers at time of collection, and therefore would have had limited exposure to any pesticides in and on oat tillers. If vegetation was wet due to a previous rain, or it started to rain during collection, sampling would cease for that day. It was necessary that an aphid sample weigh at least 100 mg in order to have enough material for chemical analysis, therefore sampling continued until this much had been collected in total from each site, such that each site sample was pooled across transects and sampling days.

2.3.1.3 Stamen and nectar sampling

From the last week of June to mid-August, nectar and stamens from flowers of *Rubus fruticosus* agg. were collected from the hedges surrounding the spring oat crops. Periodically, before and during this time, flowers at various stages (i.e. from bud to fully open, but not senescing) were wrapped in mesh (bridal veil, about 1 mm diameter holes) to protect them from insects feeding on the nectar and pollen and returned to later for sampling. If vegetation was wet due to a previous rain, or it started to rain during collection, sampling ceased. Stamens were sampled from flowers using forceps cleaned with deionized water and acetone. Nectar was collected into 1 µL glass capillaries. It was necessary that a nectar sample be at least 50 µL and a stamen sample weigh at least four grams in order to have enough material for chemical analysis, therefore sampling continued until these amounts had been collected in total from each site, such that each site sample was pooled across transects and sampling days..

2.3.1.4 Sample storage

All samples were stored in glass vials wrapped in aluminium foil in the field. A few silica beads were added to vials with stamens and aphids to absorb moisture. Samples were kept on ice in the field and stored in a -20 °C freezer at the field lab for no more than 3 months, until they could be moved to a -80 °C freezer at the chemistry lab.

2.3.1.5 Syrphid surveying

Predatory syrphid juveniles and syrphid adults were surveyed to describe their abundance and the species present at sampled sites. On the first day of aphid sampling for chemical analysis, syrphid juveniles were surveyed on oat tillers along 2x30-metre transects in the crops starting at the East edge of the crop running West. These were placed 10 and 15 metres North of the transect used for sampling aphids for chemical analysis. Starting at zero metres and sampling every five metres after, one person would search the oat tillers for five minutes, collecting all juvenile syrphids (eggs, larvae, and pupae) into 15 mL tubes and they were reared to the adult stage in the lab for identification. Adult syrphids were surveyed in the oat crop and along the hedge. On a day when aphids were being sampled for chemical analysis, one of the transects used for sampling aphids for chemical analysis and one of the transects used for surveying juvenile syrphids were used to survey adult syrphids. For the adult surveying in the crop, transects were walked at a pace of one minute every five metres, and all adult syrphids within a one metre radius of the observer were recorded, resulting in a total observation time of 12 minutes in the crop per site. Observation time was short in the crop due to time limits, and because syrphid juvenile predators were of more relevance to the study than syrphid adults in the crop.

On days when flower materials were being sampled from the hedge, the West hedge was surveyed for adult syrphids. A 50 metre transect was laid parallel to and one metre from the hedge, with the 25-metre mark lined up roughly at the centre of the hedge. The transect was walked at a pace of three minutes every five metres, and all adult syrphids within a one metre radius of the observer were recorded. Surveys were done on two separate days at each site in the same location, resulting in an hour of total observation time of the hedge at each site.

2.3.2 Reagents, standards, and equipment

Acetonitrile, water, and hexane used for initial extraction and GC-MS analysis were HPLC grade of at least >95% purity and purchased from Sigma-Aldrich, Ireland. Pre-made QuEChERS extraction mix and PSA/C18/ENVI-Carb purification mix were purchased from Sigma-Aldrich, while bulk PSA (primary secondary amines) was purchased from Biotage, Sweden; all of which were analytical grade. All reagents for re-suspension and UHPLC –MS/MS analysis were LC-MS or analytical grade (acetonitrile, water, methanol, formic acid, and ammonium formate). Pyrethroid (deltamethrin, esfenvalerate, and permethrin) and neonicotinoid (clothianidin, imidacloprid, and thiacloprid) standards were analytical grade and acquired from Sigma-Aldrich. Glassware were purified to a resistance of at least 18 MOhm, rinsed with deionized water, and then rinsed with the reagent they were purposed for prior to each use.

2.3.3 Sample preparation for analysis and storage

Stamens and aphids were homogenized in liquid nitrogen and stored in centrifuge tubes for no more than 2 months until extraction. Nectar samples were emptied from capillaries into Eppendorf tubes a few days before extraction.

Table 2.4 Sampled site information from 2018. (Sp. = Spring; Wi. = Winter; “-“ means data not available).

Site	Area (hectares)	Field Rotations (previous 5 years)	Aphid sample dates	Nectar sample dates	Stamen sample dates
CON-1	2	Sp. Oat and mustard bird cover (2016-17) Sp. Barley (2013-15)	11 June	13, 24 July	31 July
CON-2	13	Wi. Wheat (2016-17) Other tillage (2013-15)	12 June	25, 26, 28 July 5, 9 August	25, 26, 28 July
CON-3	11	Sp. Oat (2017) Grassland (2013-16)	13, 15 June	28 June 3 July	28 June 3 July
ORG-1	5	Sp. Oat (2017) W. OSR (2016) Sp. Oat (2013-15)	2 July	9, 12 July	12, 19 July
ORG-2	10	-	25 June	4, 10 July	5 July
ORG-3	11	Beans (2017) Oat (2016) Wild, out of production) (2013-15)	26, 30 June	30 July 4 August	16 July

2.3.4 Extractions

2.3.4.1 Nectar and stamens

No study looking at field-collected floral or insect samples had occurred in Ireland yet at the time of this study – the closest matrix studied being Irish honey (Hu, 2018; Kavanagh et al., 2021). For ease, confidence, and comparison of results, the laboratories and equipment used in this study were the same used as the studies testing for pesticide residues in Irish honey, which tested for the same six pesticides as in this study. Extractions were carried out using a similar QuEChERS protocol as the one used by Mitchell et al. (2017), which was also used in the Irish honey studies, but with minor modifications for this study. Nectar stored in Eppendorf tubes were centrifuged at 4,000 g for 10 minutes to push debris into a pellet, and then 50 µL of nectar was taken off the top and added to a 15 mL polypropylene centrifuge tube. For stamens, 100 mg was added to a 15 mL tube. We aimed to use as much matrix as possible and within reason, given the difficulty of collection in the field, and these amounts are similar to those used in Botías et al. (2015, Supporting Information, Tables S2a-f, I and S3a), which allows for comparison between these studies. The following protocol was the same for both media types. For the first step, 9 mL of a 50:50 solution of HPLC acetonitrile and water was added to the tube and shaken for 10 minutes. QuEChERS extraction mix (4 grams magnesium sulphate, 1 gram sodium chloride, 0.5 grams sodium citrate dibasic sesquihydrate, and 1 gram sodium citrate tribasic dehydrate) was then added to the tube gradually and shaken for 5 minutes. Another 1 mL of the 50:50 solution of HPLC acetonitrile and water was added, and then shaken for 2 minutes. The tube was centrifuged at 4,000 g for 10 minutes, and 4 mL of the supernatant was added to a separate 15 mL centrifuge tube containing 0.15 grams $MgSO_4$ and 0.1 grams PSA (primary secondary amines). The tube was shaken for two minutes, centrifuged at 4,000 g for 10 minutes, and 3.6 mL of the supernatant was distributed evenly among 3x1.5 mL Eppendorf tubes. The Eppendorf tubes were placed in a centrifugal vacuum evaporator (MAXI Dry Plus, Medical Supply Co. Ltd., Ireland) at 40 ^{o}C for at least 3 hours until dry. Next, 100 µL of HPLC hexane was added to each of two of the Eppendorfs, sonicated for four minutes, the volumes were added to the third Eppendorf, and sonicated again for four minutes. The re-suspended extract was then filtered through a 13 mm diameter nylon filter with a 0.45 µm pore size (Aquilant Analytical Sciences) into an amber GC-MS vial with a glass insert and secured with a PTFE septa cap (Agilent, Ireland).

2.3.4.2 Aphids

Extractions followed the same protocol as for nectar and stamens but with minor modifications. For aphids, 100 mg was weighed out and added to a 15 mL centrifuge tube. The amount of 100 mg was selected also with the aim to use as much matrix as possible and within reason, as well as to keep it consistent with the amount used of the other dry matrix (stamens) in this study for comparison. Next, 9 mL of an acetonitrile and water solution was added to the tube and shaken for 10 minutes. QuEChERS extraction mix was then added gradually and shaken for five minutes. Another 1 mL of the acetonitrile and water solution was added, and then shaken for two minutes. The tube was centrifuged at 4,000 g for 10 minutes, and 4 mL of the supernatant was added to a separate 15 mL centrifuge tube containing 0.25 g of PSA/C18/ENVI-Carb mix (1.2 g magnesium sulphate, 0.4 g PSA, 0.4 g graphitized non-porous carbon, 0.4 g Discovery® DSC-18). The change in the mix for this step from the mix used for nectar and stamens was needed due to the high pigment content of the aphid matrix. The tube was shaken for two minutes, centrifuged at 4,000 g for 10 minutes, and 3.6 mL of the supernatant was distributed evenly among three Eppendorf tubes. The Eppendorf tubes were placed in a centrifugal evaporator until dry. 100 µL of HPLC hexane was added to each of two of the Eppendorfs, sonicated for four minutes, the volumes were added to the third Eppendorf, and sonicated again for four minutes. The re-suspended extract was then filtered through a 25 mm diameter nylon filter with a 0.45 µm pore size into an amber GC-MS vial with a glass insert and secured with a PTFE septa cap.

2.3.5 GC-MS analysis

2.3.5.1 Equipment

A Hewlett Packard 6890 Series GC System Plus + coupled to a Hewlett Packard 5973 Mass Selective Detector was used for the analysis, with an attached 7683 Series Auto-injector (Agilent). The column was an Agilent J&W GC Durabod DB-XLB Column of 15 metre length, 0.250 mm diameter, and 0.25 µm film.

2.3.5.2 Method

Sample injections were of a 2 µL volume. The samples were run in splitless mode, with the helium carrier gas flowing at a constant rate of 1.3 mL/min. The injection was made at 250 ^{o}C. The oven running method had an initial temperature of 60 ^{o}C for 1.40 minutes, ramped up by 20 ^{o}C/min to 140 ^{o}C and held for five minutes, and ramped up by 10 ^{o}C/min to 300 ^{o}C and held for five minutes. Signals were recorded after a delay time of 1.70 minutes and detected at a rate of 20 Hz with a minimum peak width of 0.01 retention minutes. The syringe was rinsed pre-injection with two rinses of hexane, one rinse of the sample, and six pumps of the sample. Post-injection rinse involved two rinses of hexane. Each sample batch included a blank hexane to ensure no contaminants were found in the hexane or were carried over from previous injections. All samples in the batch were in a randomized order that was run in triplicate.

2.3.5.3 Chromatogram analysis

The software used was the 2018 OpenChrom version 1.3.0 Dalton (OpenChrom, 2018). Chromatograms were reduced to extracted ion counts (EIC) for each pyrethroid analyte separately, selecting the two most abundant product ions (± 1 *m/z*). Peaks were identified by observing retention time and mass spectra, known retention time being determined by injections of hexane spiked with pyrethroid standards at 30 µg/mL, and known mass spectra pattern being determined by NIST reference spectra (Table 2.5). Peak identifications were confirmed by the probability-based matching algorithm. The reference chromatograms in Figure 2.3 show the EIC peak areas and retention times for each of the analytes.

Table 2.5 Chromatogram characteristics used for identifying pyrethroid analytes.

Pyrethroid	Retention Time (min)	EIC ions (*m/z*)
Deltamethrin	25.45	181, 253
Esfenvalerate	24.95	125, 167
Permethrin	22.96	183, 163

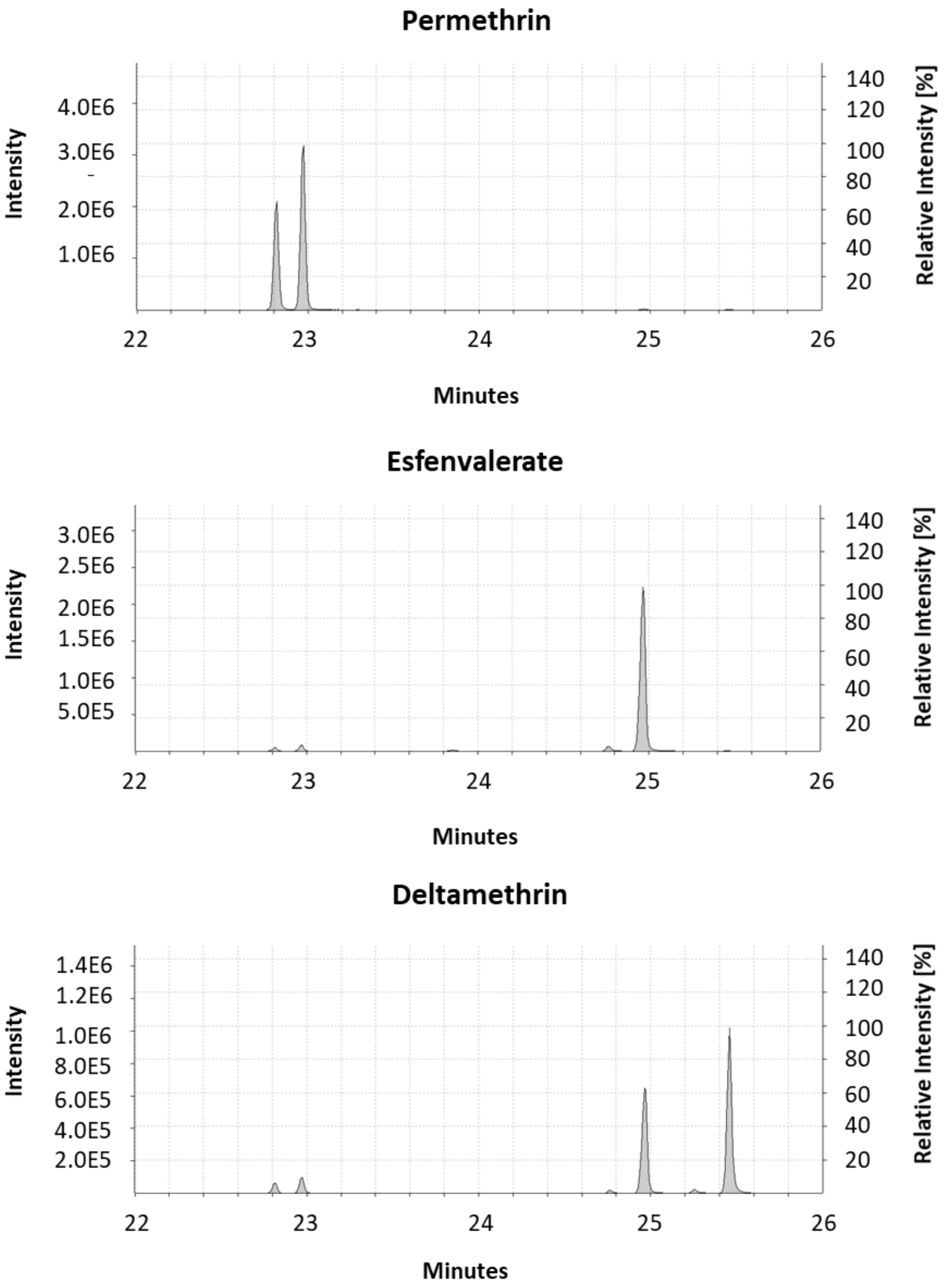

Figure 2.3 EIC reference chromatograms of GC-MS peaks corresponding to pyrethroid analytes, with x-axis showing minutes retention time, left y-axis showing intensity, and right y-axis showing relative intensity (OpenChrom, 2018).

2.3.6 UHPLC-MS/MS analysis

2.3.6.1 Equipment

A UHPLC Thermo Fisher Scientific UltiMate 3000 coupled to a tandem mass spectrometer LTQ Orbitrap XL was used for the analysis. The column was a Waters Xbridge UPLC BEH column of 3.5 µm particle size and an internal diameter of 4.6x100 mm.

2.3.6.2 Method

After GC-MS analysis, samples in vials were allowed to evaporate to dryness at room temperature before being sealed and stored at 5 °C until UHPLC-MS/MS analysis. When ready for analysis, samples were re-suspended in 25% LC-MS methanol and immediately run on the UHPLC-MS/MS. The mobile phases were (A) a 5% formic acid solution in water, containing 5 mM ammonium formate and (B) a 5% formic acid solution in acetonitrile. Both mobile phases were prepared by filtering using a vacuum filtration manifold with a 47 mm diameter nylon filter of 0.22 µL pore size. Sample injections were of a 5 µL volume. Valves were directed to waste for the first five minutes after injection, and then diverted to the ion source. The column was held at a constant temperature of 25 °C and flow rate of 0.3 mL/min. The gradient program of mobile phase B increased from 10% to 80% in 15 minutes, then 80% to 98% in 0.1 minutes, held for two minutes at 98%, then decreased to 10% in two minutes. The scans were programmed to delay detection for seven minutes, and then run in SIM mode following the specifications listed in Table 2.6. Normalized collision-induced dissociation energy was 35%, and signals were detected with an isolation width of 1 *m/z*. Each sample batch included a blank, and all samples were run in triplicate.

Table 2.6 Chromatogram characteristics used for identifying neonicotinoid analytes.

Neonicotinoid	Retention time range (min)	Selected Ion range (*m/z*)	Parent Ion (*m/z*)	Product Ions (*m/z*)
Clothianidin	10.9-11.2	249.5-250.5	250.0165	169, 132
Imidacloprid	11.2-11.5	255.6-256.6	256.0596	209, 175
Thiacloprid	12.5-12.9	252.5-254.5	253.0309	126, 226

2.3.6.3 Chromatogram analysis

The software used was the Thermo Xcalibur version 2.2 SP.48, released 12 August 2011. In the expected retention time range that the neonicotinoid signals were to appear, first the time range was checked for signals with a *m/z* value accurate to at least three decimal places. Next, the time range was checked for dissociation of the signals into expected product ions and general fragmentation pattern. The reference chromatogram in Figure 2.4 illustrates the signal generated for the neonicotinoid analytes.

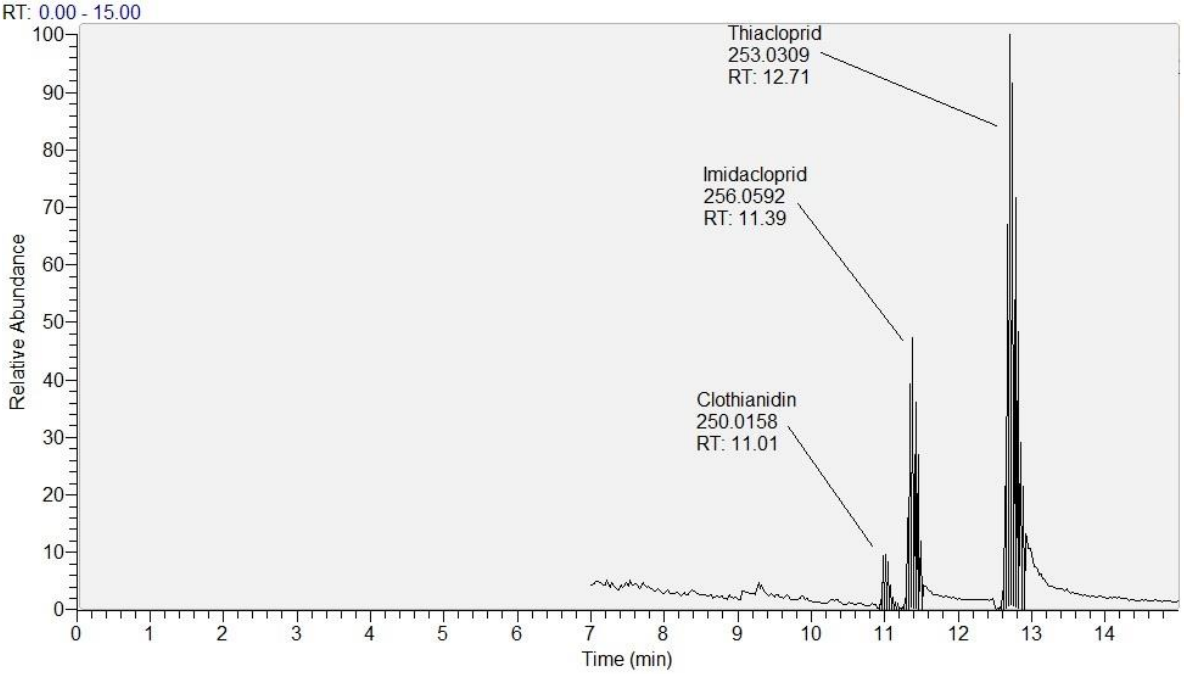

Figure 2.4 Reference chromatogram of UHPLC-MS/MS peaks corresponding to neonicotinoid analytes, with x-axis showing retention time in minutes, and y-axis showing relative abundance. All analysis parameters as described in Section 2.6.2.

2.3.7 Quantification and validation

2.3.7.1 GC-MS

Pyrethroids were quantified by a 5-point calibration curve of 1, 5, 10, 15, 20 µg/mL. Method detection and quantification limits (MDL and MQL) were determined by ten extractions of increasing quantities of spiked pyrethroid standards (0.01, 0.025, 0.0474, 0.095, 0.1874, 0.374, 0.75, 1.5, 3, and 6 µg), which was carried out separately for each purification extraction method (i.e. one for the method that used PSA and $MgSO_4$, and one for the method that used PSA/C18/ENVI-Carb mix). MDLs were defined as a signal intensity equivalent to three times the noise intensity, and MQLs were set at 10 times the noise intensity. The recovery rate was determined from a minimum of four extractions of each sampled media spiked with 6 µg of pyrethroid standards.

2.3.7.2 UHPLC-MS/MS

Neonicotinoids were quantified by a 10-point calibration curve of standards mixed at the following concentrations: 0.0015, 0.003, 0.0075, 0.015, 0.03, 0.15, 0.5, 1, 5, 10 µg/mL. Limits of detection and quantification (LOD and LOQ) were determined from the calibration curve, with LODs defined as a signal intensity equivalent to three times the noise intensity, and LOQs set at 10 times the noise intensity. Due to lack of sample to work with, the recovery rate was determined for only the stamens, with three samples spiked with 60 ng neonicotinoid standards, and three spiked with 0.6 ng.

2.4 Results

2.4.1 Syrphid surveying

For each growth stage, *Episyrphus balteatus* was the most abundant of the identified specimens, followed by *Platycheirus scutatus* for the juveniles and *Platycheirus granditarsus* for tying for second for the adults (Tables 2.7 and 2.8), all of which have aphidophagous larvae. *Syritta pipiens* was also the second-most common adult syrphid, which has saprophagous larvae. The other juvenile syrphids could not be identified due to death before reaching the adult stage, either due to eggs never hatching or parasitism. Very few juvenile syrphids were observed in the crop (n=21) compared to adults in the crop and along the hedge (n=450). While juvenile syrphids were more common at conventional sites in the crop, adults were more common at organic sites.

Table 2.7 Surveyed juvenile syrphids at sites sampled from for analysis of chemical residues.

	Number of crop observations		
Syrphid	Organic	Conventional	Total
Episyrphus balteatus	2	3	5
Platycheirus scutatus agg.	0	2	2
Emerged Parasite	2	1	3
Egg	0	11	11
TOTAL	4	17	21

Table 2.8 Surveyed adult syrphids at sites sampled from for analysis of chemical residues.

	Number of adult observations		
Syrphids	Organic	Conventional	Total
Episyrphus balteatus	192	80	272
Platycheirus granditarsus	18	2	20
Syritta pipiens	13	7	20
Melanostoma melinum	3	15	18
Eristalis spp.	15	0	15
Helophilus pendulus	6	0	6
Platycheirus angustatus	4	2	6
Eupeodes corollae	2	2	4
Eupeodes luniger	4	0	4
Eristalis arbustorum	1	2	3
Eristalis tenax	2	1	3
Platycheirus manicatus	2	1	3
Scaeva pyrastri	1	2	3
Eristalis nemorum (interruptus)	2	0	2
Neoascia podragica	2	0	2
Platycheirus or Melanostoma spp.	1	0	1
Rhingia campestris	1	1	2
Syrphus ribesii	2	0	2
Helophilus spp.	1	0	1
Platycheirus albimanus	1	0	1
Platycheirus clypeatus/occultus group	1	0	1
Sericomyia silentis	1	0	1
Syrphus vitripennis	1	0	1
Volucella pellucens	1	0	1
Other	50	8	58
TOTAL	327	123	450

2.4.2 GC-MS method validation, detection and quantification

No fragmentation patterns of the product ions characteristic of the pyrethroids were found in any of the samples. For determining MDLs using the Envi-Carb extraction method, the linear range was determined to be 0.01-6 µg analyte added for each pyrethroid. For the PSA method, the linear range was determined to be 0.01-1.5 µg analyte added for each pyrethroid. The detection parameters of extraction protocol and GC-MS method are shown in Table 2.9 and they could detect the pyrethroids at least as low as 2.69 µg/g for solid samples (aphids and stamens), and 5.38 µg/mL for liquid samples (nectar). The method was most sensitive for detecting permethrin in any sample media. Recovery rates were very low for esfenvalerate, as low as 9.29 % in nectar. For deltamethrin, however, the recovery rates were quite high, as high as 192.50 % in aphids. The permethrin recovery rate was also quite high in aphids, at 135.64%.

2.4.3 UHPLC-MS/MS method validation, detection and quantification

No analytes were detected in any solvent blanks, so the extract solvent was deemed clean of neonicotinoid analytes. Detections were based on three separate requirements. The first was fragmentation, whereby an analyte's pre-cursor and two product ions appeared together in the same injection. The second detection requirement was based on LOD, whereby an analyte's pre-cursor or one of the product ions appeared with an intensity greater than the LOD. None of the detected values were greater than the LOQ. The last detection requirement was based on repetition, whereby an analyte's pre-cursor or product ion appeared in all three injections of a sample at an intensity of $>10^{-2}$. In total, pesticides were detected in eight of the 18 samples, and each field site had at least one sample with a pesticide present (Table 2.10). Thiacloprid was detected seven times, imidacloprid was detected once, and clothianidin was not detected in any sample. No sample contained more than one pesticide. Detections were most commonly found by fragmentation (five hits) and repetition (five hits), and LOD resulted in two positive hits. Two detections met all three detection requirements, and one detection met two detection requirements. Pesticides were most commonly detected in stamen samples (four samples), and all detections that met multiple requirements were in stamens. Pesticides were detected in only one nectar sample. In three of the aphid samples pesticides were detected, one of which was imidacloprid. Solely looking at unique detections (regardless of the number of detection requirements met), more detections were found at organic sites (five samples) than conventional sites (three samples). Summing up the number of different requirements met, however, six detection requirements were met at organic sites, and seven at conventional sites. All the pesticide detections in aphid samples were from organic sites, the detection in a nectar sample was from a conventional site, and the detections in stamen samples were from organic and conventional sites.

Table 2.9 Validation parameters for GC-MS detection of pyrethroids. All calibration curves have a linearity of ≥ 0.98.

Media	Pyrethroid	Recovery rate (%) ± RSD	MDL[a]	MQL[a]
Aphids				
	Permethrin	135.64 ± 9	1.14	1.29
	Esfenvalerate	16.14 ± 11	2.62	3.49
	Deltamethrin	192.50 ± 10	2.00	2.26
Nectar				
	Permethrin	102.10 ± 17	0.99	1.21
	Esfenvalerate	9.29 ± 47	5.38	14.28
	Deltamethrin	102.56 ± 54	1.55	2.33
Stamens				
	Permethrin	102.57 ± 11	0.49	0.61
	Esfenvalerate	12.92 ± 11	2.69	7.14
	Deltamethrin	146.62 ± 10	0.77	1.17

[a]Aphids and stamens are measured in µg/g, and nectar is measured in µg/mL.

Table 2.10 Detections of neonicotinoids in sampled media in the six sites. Values in parentheses represent the detection requirements met (a = fragmentation, b = LOD, c = repetition). Neonicotinoid abbreviations are CLO = clothianidin, IMI = imidacloprid, and THL = thiacloprid.

[a]Site	Aphids	Nectar	Stamen
ORG-1	THL (a)		
ORG-2	THL (c)		THL (a, b)
ORG-3	IMI (c)		THL (a)
CON-1			THL (a, b, c)
CON-2			THL (a, b, c)
CON-3		THL (c)	

[a]Site labels starting with "ORG" are organic sites, and with "CON" are conventional sites.

For method validation, calibration curves were constructed with linear ranges of 0.003-1 µg/mL for clothianidin, 0.003-.5 µg/mL for imidacloprid and 0.003-0.03 µg/mL for thiacloprid, with linearities ≥ 0.97 (Table 2.11). LODs for UHPLC-MS/MS were lowest for thiacloprid (1.20 ng/g for solid media, 2.40 ng/mL for liquid media), and highest for clothianidin (27.49 ng/g in solid media, 54.97 ng/mL in liquid media). Recovery rates could not be determined from the samples spiked with 0.6 ng standards, so values are only reported from samples spiked with 60 ng. Recovery rates were different between the neonicotinoids, with imidacloprid having the highest recovery rate and the least variability (86.23% ± 7.15), clothianidin having the most variability (± 21.32), and thiacloprid having the lowest recovery rate at 34.44% (Table 2.11).

Table 2.11 Validation parameters for UHPLC-MS/MS detection of neonicotinoids. All calibration curves have a linearity of ≥ 0.97.

Media	Neonicotinoid	LOD[a]	LOQ[a]	Recovery rate (%) ± RSD
Aphids				
	Clothianidin	27.49	111.42	52.74 ± 21.32
	Imidacloprid	11.33	41.47	86.23 ± 7.15
	Thiacloprid	1.20	4.19	34.44 ± 9.65
Nectar				
	Clothianidin	54.97	222.84	52.74 ± 21.32
	Imidacloprid	22.66	82.94	86.23 ± 7.15
	Thiacloprid	2.40	8.37	34.44 ± 9.65
Stamens				
	Clothianidin	27.49	111.42	52.74 ± 21.32
	Imidacloprid	11.33	41.47	86.23 ± 7.15
	Thiacloprid	1.20	4.19	34.44 ± 9.65

[a] Aphids and stamens are measured in ng/g, and nectar is measured in ng/mL.

2.5 Discussion

Neonicotinoids were detected in every media type as hypothesized, mainly thiacloprid but there was one detection of imidacloprid. For the floral food source that supports pollinators and supplements predatory insect diets or comprises the diet of a stage of predatory insect, only one nectar sample had thiacloprid residues, while four of the six stamen samples had thiacloprid residues. Furthermore, three of the stamen samples met multiple requirements for detecting thiacloprid, improving confidence in the detections. This is in line with the findings of several other studies, where pesticide detections are more frequent and at higher concentrations in pollen than in nectar (Kyriakopoulou et al., 2017; Gierer et al., 2019; Zioga et al., 2020). This may be due to the sugars in nectar reacting with the pesticide residues to form conjugates, making it more difficult to extract and identify residues in nectar (Choudhary and Sharma, 2008; Gierer et al., 2019). For the prey food source, thiacloprid was detected in two aphid samples, and imidacloprid was detected in one aphid sample. However, like the nectar sample, these three aphid samples only met one detection requirement each. Interestingly, all three aphid detections were from organic sites, whereas the floral detections were a mix of organic and conventional. Looking at number of detections by requirements met, six were from organic samples and seven from conventional, which disagrees with our hypothesis that more detections would be found on conventional sites. However, this finding is not uncommon (Humann-Guilleminot et al., 2019; Mogren and Lundgren, 2016) and further adds to the evidence of neonicotinoid mobility in the landscape and the hazards wildlife face even on organically managed lands.

The thiacloprid detections in the floral food sources had concentrations between 1.20 and 4.19 ng/g for stamens, and 2.40 to 8.37 ng/mL or ppb for nectar. In 2013, Botías et al. (2015, Supporting information, Tables S2a-f, I and S3a) collected nectar and pollen from several wildflowers, including *Rubus fruticosus* agg., around conventional winter oilseed rape and winter wheat fields in England and tested them for the same three neonicotinoids. For the *Rubus fruticosus* agg. samples, they gathered three nectar samples at three separate oilseed rape sites, and clothianidin was detected once (0.17-0.50 ng/g or ppb) and thiacloprid (0.03-0.08 ppb) was detected twice (Botías et al., 2015, Supporting information, Tables S2a-f, I and S3a). Of eight pollen samples taken at five oilseed rape and two winter wheat sites, only imidacloprid was found once at a concentration of 4.19 ng/g (Botías et al., 2015, Supporting information, Tables S2a-f, I and S3a). The Botías et al. (2015) study was able to detect these neonicotinoids at lower concentrations than our study, yet overall, there was not much difference in total detection rates in *Rubus fruticosus* agg. between the two studies. This is despite all the sites in the Botías et al. (2015) study were conventional with recent or a history of neonicotinoid applications. Regardless, the levels of thiacloprid residues we found could have low impacts on invertebrates, as the acute oral LD50 for honey bees is 17,320 ng (Lewis et al., 2016), which could also suggest low impact to predators that use nectar and pollen to supplement aphid diets.

For aphids, the concentrations of detections were between 1.20 and 4.19 ng/g (ppb) for thiacloprid, and between 11.33 and 41.47 ng/g for imidacloprid. Calvo-Agudo et al. (2019) sampled honeydew across five days from sap-sucking pests (*Planococcus citri*) feeding on trees that had received soil-applications of thiamethoxam and imidacloprid and tested the honeydew for residues of the pesticides. Of these, 71.4% of the sampled trees had thiamethoxam in the aphid honeydew with an average concentration of 18.3 ng/mL (ppb), and 42.9% of the sampled trees had imidacloprid in the aphid honeydew with an average concentration of 15.6 ng/mL (Calvo-Agudo et al., 2019). These are higher detection rates and residue levels than we observed, though it should be noted that the Calvo-Agudo et al. (2019) study was conducted in a greenhouse, with sampling starting one day after pesticide treatment. While in our field study, three out of six samples of aphids feeding from crops had neonicotinoids, it was unexpected that all our residues were found in organic fields. Winged aphids can disperse widely in the landscape and travel between fields (Dixon, 1971), so it is possible that these individuals received sub-lethal pesticide exposure from a conventional field before travelling to one of our organic fields. It should be noted, however, that there were very few winged aphids in our site samples, where the number of winged individuals was less than 10% of all collected aphids at a site except at one organic site where almost a quarter of all collected aphids were winged. Regardless, contaminated food resources can have negative impacts on the health and pest-controlling performance of predators. Kumar and Santharam (1999) tested how imidacloprid exposure would influence lacewings (*Chrysoperla carnea*) when sprayed on them at the egg stage, when the larvae were fed sprayed prey, and when adults were fed treated honey water. Interestingly, they found that egg-hatching rate was not effected by imidacloprid, but larvae mortality before pupation was higher when they fed on treated prey (sprayed with 0.006% imidacloprid solution), and adult longevity was shorter and reproductive success was reduced when fed honey water with imidacloprid (0.006%) (Kumar and Santharam, 1999). It is clear that residues can be found in aphid prey and floral resources in the field, which could cause adverse effects to aphid predators.

From brief surveys of syrphids conducted at these sites during the sampling season, 450 adult syrphids were observed in the hedges and oat crops, and 21 juveniles (eggs, larvae, pupae) were observed on the oat crops. While more adults were observed on organic crops, more juveniles were observed on conventional crops. One might think that means adults are less at risk of dietary exposure to pesticides and juveniles are more at risk, but based on these findings of residues on organic and conventional sites this is not the case – as many organic as conventional sites had stamens with detected neonicotinoid residues, and all residues detected in aphids were sampled from organic sites. This was a very small study though, and more work will need to be done to understand the dietary pesticide exposure risks aphidophagous syrphids face in agricultural landscapes.

We only detected neonicotinoids in the samples, and no pyrethroids. This is likely due to the nature of these classes of pesticides, where neonicotinoids are systemic and therefore capable of being absorbed and moved throughout the plant and into sap-sucking aphids, whereas pyrethroids are non-systemic and samples would have to come into direct contact with sprays (Gierer et al., 2019). Sanchez-Bayo and Goka (2014) also noted that even though pyrethroids are extremely toxic to bees, they pose a low risk due to how uncommonly they are detected in floral nectar and pollen, which our study supports. However, this does not prove absence entirely, and given their high toxicity it would be worth including pyrethroids in future residue studies. Of the three neonicotinoids, thiacloprid was the most commonly detected, which was expected due to the fact that imidacloprid and clothianidin use were restricted since 2013 (European Commission, 2013), and our detection methods showed that thiacloprid had the lowest LOD (i.e. our methods were most sensitive to thiacloprid). Clothianidin was never detected, likely due to both restrictions in use and a high LOD (i.e. detection methods were least sensitive). Despite restrictions, we were able to detect imidacloprid in one of the samples, possibly because it had the highest recovery rate with least variability from our methods (i.e. little residue was lost during the extraction process). However, this does not agree with Wintermantel et al. (2020), who sampled nectar from oilseed rape crops in France after the 2013 restrictions were put in place, sampling from 2014 to 2018. They tested for the same neonicotinoids we tested for, and imidacloprid was detected the most often, even compared to unrestricted thiacloprid (Wintermantel et al., 2020). The findings of Pohorecka et al. (2012) did agree with our findings though. In their study, they applied the same three neonicotinoids we studied to oilseed rape crops, and found that thiacloprid was detected more frequently in the pollen and nectar than clothianidin and imidacloprid, so even when the three pesticides are applied at similar times thiacloprid was still detected more often (Pohorecka et al., 2012). There seems to be variability in terms of which neonicotinoids occur more commonly in floral resources, and more research will be needed in order to clarify this.

From this study, the neonicotinoids with legal restrictions in place were rarely detected in the samples, but the unrestricted thiacloprid was commonly detected and therefore insects that feed from flowers and on aphids, or combinations of these could be at risk of dietary exposure to thiacloprid at least. Future studies may benefit from searching for metabolites too, as pesticides degrade in the environment, sometimes producing metabolites that can also be toxic, and not analysing for these could lead to underestimating the significance of risks wildlife face from pesticide use. Future studies should also test pest predators for pesticide residues, to help determine how often and how much they are consuming or encountering residues. It should also be noted that, at the time of this study’s publication, thiacloprid was also banned in Europe starting in February of 2021 (when the grace period ended for using up any purchased product containing thiacloprid) (European Commission, 2020a), further reducing the risk of exposure to wildlife. While from this study it appears there is a low risk to the health of invertebrate predators that rely on these food resources, it is recommended that farmers use pesticides sparingly, as residues found on organic sites suggests mobility, and the detection of banned imidacloprid suggests that these chemicals can persist in the Irish landscape.

Chapter 3

Lethal and sub-lethal effects of thiamethoxam ingestion on the adult hover fly *Episyrphus balteatus*

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3. Lethal and sub-lethal effects of thiamethoxam ingestion on the adult hover fly *Episyrphus balteatus*

3.1 Abstract

Systemic pesticides (e.g. neonicotinoids) are designed for absorption by plants and transport throughout their tissues, thus controlling pests that feed on all parts of the plant. Systemic pesticide residues can be found in floral nectar, thereby posing a risk to nectar-feeding beneficial insects, such as pollinators (e.g. bees) and pest-controllers (e.g. parasitoid wasps). In extreme scenarios, acute poisoning can result in death of the beneficial insect, but even sub-lethal exposure can modify behaviour and reduce their ability to provide ecosystem services. We investigated the lethal and sub-lethal impacts of oral exposure to the systemic pesticide thiamethoxam on a common European hover fly, *Episyrphus balteatus*, which functions both as a pollinator and predator of aphid pests. Five days after eclosion from the pupal stage, adult flies were fed 5 µL of sugar-water spiked with thiamethoxam at one of ten concentrations ranging from 0 to 24 ng/µL (ppm). Mortality was insufficient to estimate LD50 values 72 hours after exposure. By 12 days after exposure, longevity of dosed flies was significantly reduced and the LD50 (50% lethal dose) was estimated at 5-6 ng thiamethoxam per mg bodyweight of flies. Sub-lethal effects were investigated by measuring the time spent doing various behaviours and the ability to grip smooth surfaces during the two weeks after ingestion. Resting, flying, and grooming behaviours all showed weak correlations with dose two days after treatment, with more resting as dose increased, but less flying and grooming, but these effects were not observed at later time points. The relationship between the ability to grip smooth surfaces and dose changed over two weeks in a non-linear fashion, with the strongest negative correlation occurring three days after thiamethoxam ingestion. These results indicate that *Episyrphus balteatus* appears relatively resilient to oral ingestion of this commonly used pesticide and suggest that the role of these hover flies as ecosystem service providers may be of increasing importance in agricultural systems still reliant on neonicotinoids for pest control.

3.2 Introduction

There is growing interest in the unintended accumulation of systemic pesticides, such as neonicotinoid insecticides, in the wider environment, and the subsequent consequences of this contamination on non-target insect species (Casado et al., 2019; Douglas et al., 2015; Mitchell et al., 2017). Because these insecticides can be administered using seed coatings and soil drenches as alternatives to foliar sprays, those who used them had the expectation that, as aerial drift was avoided, the pesticide would be found primarily within the treated plants and would be delivered only to pest insects feeding directly on crop foliage and sap. Recent research, however, has shown that systemic pesticides can also be detected in crop nectar and pollen, food sources used by several beneficial insects that provide ecosystem services such as pollination, pest control, and waste decomposition (Gierer et al., 2019; Wood and Goulson, 2017). This contamination of pollen and nectar is not confined to treated crops: neonicotinoids are generally water-soluble and persistent so aqueous drift can occur via runoff into field margins where the pesticide is then absorbed by wildflowers (Botías et al., 2015). This drift can be so substantial that neonicotinoids have been found in soil and plants on organic farms adjacent to conventionally managed farmland (Humann-Guilleminot et al., 2019).

Research on beneficial insects at risk of pesticide exposure has largely focused on bees as pollinators (Franklin and Raine, 2019; Uhl and Brühl, 2019; Wood and Goulson, 2017) along with a limited number of predatory insects, including beetles (Awasthi et al., 2013; Candolfi et al., 2000; Roubos et al., 2014; Wiles and Jepson, 1992) and parasitoid wasps (Desneux et al., 2007; Gnanadhas et al., 2010; Joseph et al., 2011; Prabhaker et al., 2007). While residual neonicotinoids in nectar may not always reach concentrations considered lethal to insect visitors (Botías et al., 2015; Humann-Guilleminot et al., 2019), they can potentially cause sublethal effects which could impact the efficiency of ecosystem service delivery. For example, nonlethal pesticide exposure can reduce the longevity of bees by weakening the immune system as has been observed in *Apis mellifera* (Di Prisco et al., 2013) and impair reproduction therefore reducing population size as has been observed in bumble bees (e.g. *Bombus terrestris*), solitary bees (e.g. *Osmia bicornis*), and honey bees (e.g. *A. mellifera*) (Laycock et al., 2012; Sgolastra et al., 2018; Siviter et al., 2020; Williams et al., 2015). Pesticides can also impact insect motor skills and basic behaviour patterns, such as pollen foraging in bees (e.g. *Bombus terrestris*) and host location in parasitoid wasps (e.g. *Trissolcus basalis*, a natural enemy of pentatomid bug crop pests), which would directly reduce their provision of ecosystem services (Feltham et al., 2014; Salerno et al., 2002).

A lesser-studied group of ecosystem service providers are hover flies (Syrphidae, Diptera). Hover flies are important pollinators in their adult stage, and play a range of beneficial roles as larvae, including decomposition, nutrient cycling, and pest regulation. About a third of all hover fly species prey on aphids in the larval stage (Doyle et al., 2020; Dunn et al., 2020; Wotton et al., 2019), yet there is little research into how pesticides directly or indirectly impact hover fly behaviour and performance. Most laboratory work has focussed on hover fly mortality resulting from contact exposure, although some studies have assessed sublethal effects such as development time of larvae, fertility in adults, and egg-laying responses to contaminated surfaces (Jansen et al., 2011; Moens et al., 2011; Pineda Gómez, 2008). In a recent study, Basley et al. (2018) investigated how contamination of lagoon water by thiamethoxam, a broad-spectrum neonicotinoid, impacted the aquatic larvae of the hover fly *Eristalis tenax*. They found that field-realistic doses of this pesticide had little impact on the development of the larvae and activity budgets of the eclosed adults, suggesting this species might be relatively tolerant of this compound.

Even less common are studies examining how dietary exposure of neonicotinoids impacts adult hover flies, which is surprising given the large number of studies investigating this exposure route in bees. One study demonstrated that newly-emerged adult hover flies that consumed honey dew from pests that fed on neonicotinoid-treated plants experienced acute lethal effects, the strongest response being 100% mortality after three days of consuming nothing but honey dew from pests treated with just 50% the recommended spray concentration of thiamethoxam for foliar spray (Calvo-Agudo et al., 2019). Interestingly, of the 14 honeydew samples collected from foliar treated trees across five days after treatment, they could only detect thiamethoxam in one sample, collected two days after treatment, albeit this was also the highest concentration detected in the entire study (290.32 ppb, Calvo-Agudo et al., 2019). From soil-treated trees, samples collected all on the same day had concentrations ranging from 1.86 to 43.11 ppb, and hover flies that fed from honeydew of these trees had 73% mortality by the end of three days (Calvo-Agudo et al., 2019). Overall, Calvo-Agudo et al. (2019) demonstrated that there can be much variability in pesticide residues in honeydew between days after treatment, and even on the same day, but this can still lead to high mortality of young adult hover flies. Another study compared the lethal effects of three different insecticides when consumed by *Episyrphus balteatus* with the effects observed in honey bees (Descher and Geusen-Pfister, 1991), and found large differences in the 24-hr LD50 values for these pesticides: the hover flies were less sensitive to acephate (4897 ng/insect) than bees (180 ng), more sensitive to dimethoate (12 ng) than bees (170 ng), with the sensitivity to methomyl being somewhat similar for flies (68 ng) and bees (80 ng) (Descher and Geusen-Pfister, 1991). It is therefore important to consider pesticide impacts on various beneficial insect groups in order to understand how and which ecosystem services may be affected.

This study aimed to identify the lethal and sublethal effects experienced by adults of the hover fly *E. balteatus* when fed a one-time dose of thiamethoxam in a nectar substitute (i.e. sugar water). *E. balteatus* was selected specifically for its importance as both a pollinator and a pest controller, which is in a geographically broad sense as an abundant migratory species within the palearctic region, and because it is commercially available as a supplement for crop pest control (Doyle et al., 2020; Pineda and Marcos-García, 2008). Thiamethoxam was chosen as a test compound because it is one of the most commonly used neonicotinoids globally (Simon-Delso et al., 2015). Although it has recently been banned from outdoor agricultural use in the European Union, it is considered moderately persistent with a DT90 (90% degradation time) in soils of 296.5 days (Lewis et al., 2016). Wintermantel et al. (2020) detected thiamethoxam in oilseed rape nectar in France one and four years after the 2013 moratorium that banned its use on flowering crops, suggesting that residues may have stayed in the soil years after the moratorium or persisted from previous non-flowering crops in the fields that were treated. Thiamethoxam is still allowed for greenhouse use (European Commission, 2018) and some greenhouses will periodically open walls or windows for temperature control and ventilation, which allows entry of wild invertebrates that could be exposed to applied pesticides (Pineda and Marcos-García, 2008). For farmers who may consider purchasing and introducing invertebrates into greenhouses for pollination and pest control, it is important to understand how pesticide residues can influence beneficial insects. This research has implications for syrphid health outside of Europe as well, as the latest regulatory statuses show that thiamethoxam is approved for outdoor agricultural use in Brazil, China, and the United States (Donley, 2019).

By using a multiple-concentration approach in a highly controlled laboratory setting, we assessed whether the dose of thiamethoxam consumed by adult flies affected (1) hover fly survival and longevity, (2) the time spent by surviving flies performing different activities, such as flying, feeding, grooming, and (3) grip strength of surviving flies, as measured by the ability of the fly to hold onto a smooth surface.

3.3 Methods

3.3.1 Study organisms

E. balteatus pupae were purchased in four separate batches over a period of six months (September 2019 through February 2020) from Katz Biotech AG (Germany). The pupae were placed into closed plastic Petri dishes (83 mm diameter; 13 mm depth; ≤ 100 pupae per dish), with bottled drinking water sprinkled on the inner surface of the lid to increase humidity and prevent desiccation of the pupae. The pupae were maintained in a controlled climate room at 50-60% relative humidity, 22 ± 2 ^{o}C, and a day: night cycle of 16:8 hours. Once flies started to emerge, the dishes were opened and placed into a mesh insect cage (40 cm x 40 cm x 90 cm). The cage was checked several times a day for newly emerged flies, which were moved to separate holding arenas consisting of 500 mL plastic boxes (17.15 cm x 11.75 cm x 3.25 cm) with 26 ventilation holes (about 1 mm diam.) in the lid. The climate conditions of each box were roughly the same temperature as the room, but relative humidity was higher at 60-70%.

Each holding arena was supplied with a cotton pad (6 cm diameter) soaked with a 500 g/L solution of caster sugar and sprinkled with a pinch of crushed pollen pellets that was rubbed into the cotton pad. Pollen used was purchased from the Natural Health Market (UK) as EU agricultural bee pollen certified organic by the Soil Association and meeting EU Organic Regulations. Sugar solution was added daily to the cotton pad (from here on, referred to as sugar pads), but no more pollen was added as this was never fully consumed. The sugar pads were replaced every 3-4 days to prevent spoiling. Holding arenas were randomly arranged on shelving illuminated at 190 μmol/m^2/second by plant grow lights (VÄXER LED cultivation light, model L1518, IKEA, Sweden).

Honey bees (30 returning foraging workers; *A. mellifera mellifera*) were collected in June 2020 from the entrances of three hives maintained on the Trinity College Dublin campus. The bees were collected into 60 mL tubes and maintained, without access to food or water, in the same controlled environment room in the dark described above until testing was performed 30 minutes later. Each tube held more than one bee, as preliminary tests indicated that bees were more likely to die if held in isolation.

3.3.2 Preparation of test solutions

Thiamethoxam standards were purchased from Sigma Aldrich (Ireland). On the day prior to exposure, the pesticide was dissolved in acetone at 10 concentrations in 2 mL clear glass vials with natural rubber/PTFE septa and stored at 5 °C. In the morning of the first day of exposure, the acetone dilutions were added to a 500 g/L sugar solution such that each resulting dilution contained 1% of the acetone solution by volume. The resulting concentrations of pesticide in sugar solution were: 0, 0.1, 0.5, 1, 2, 4, 8, 10, 12, and 24 ng/µL, with the 0 ng/µL dilution serving as the negative control (i.e. containing just acetone at 1% by volume). Unused sugar-water treatment solutions were stored at 5 °C and were not used 4 days or more after preparation. Acetone solutions were used to spike sugar-water solutions no longer than 14 days after being made.

3.3.3 Hover fly exposure

Adult flies were exposed to thiamethoxam five days after emergence. Flies were randomly assigned to treatments such that in each of the four batches of flies, each treatment had eight replicates, four males and four females. Thus, the entire experiment consisted of 320 flies (80 per batch) in total, and 32 in each thiamethoxam treatment. Additionally, 20 individuals, 10 males and 10 females, were treated with a solution of 200 ng/µL thiamethoxam to act as a positive control. Flies showing injuries or displaying unusual behaviours (e.g. twitching, weak movement, etc.) were not used in trials.

Before trials, flies were isolated in individual 60 mL tubes containing some damp paper towel to prevent dehydration but with no access to food. Prior to dosing, the flies were calmed by placing them in a Styrofoam box containing icepacks for 5-10 minutes. The flies were then gently placed in a feeding harness composed of a section of plastic drinking straw and a piece of clear adhesive tape that allowed only the head to emerge enough that the proboscis could reach the offered sugar solution (see Appendix Figure A.1). A cotton bud was used to gently push the fly through the straw. The fly was then presented with 5 µL of one of the sugar solution treatments as a drop on a folded piece of tape, and after consuming all the drop, it was released back into its holding arena.

3.3.4 Honey bee exposure

After the bees had been starved, they were separated into individual tubes and 20 µL of non-contaminated sugar-water added to the inside of the tube cap. The bees were then randomly assigned to one of three treatments which were also added to the inside of the tube cap: 5 µL of 4 ng/µL thiamethoxam in sugar solution containing 1% acetone, 5 µL of 0 ng/µL thiamethoxam in sugar solution containing 1% acetone, or 5 µL of 0 ng/µL thiamethoxam in sugar solution. Twenty bees received the caps with thiamethoxam in acetone, 10 bees received the control caps with acetone only, and five bees received the procedural control caps without acetone or thiamethoxam. The 4 ng/µL treatment, delivering 20 ng thiamethoxam, was expected to be a sufficient dose to kill all the test bees, as Laurino et al. (2010) reported that a thiamethoxam dose of about 17.5 ng was 100% lethal to honey bees within six hours of consumption.

After bees had consumed the sugar-water treatment from the cups, two cotton buds soaked in non-contaminated sugar-water and rubbed in pollen were added to the tube for food, with the sugar solutions and pollen being the same as used for the flies. These cotton buds were re-soaked in nectar in the evening and morning.

3.3.5 Survival and lethal effects

The hover flies were maintained and observed for 14 days after exposure. Every day, the numbers alive and dead were recorded, as well as any abnormal behaviour (e.g. twitching). Death was checked by prodding the insects with forceps four times to see if there was any movement. For the honey bee tests, bees were exposed in the afternoon and mortality was checked every hour for the first six hours after exposure, the following morning, and 24 hours after exposure using the same prodding technique as used for the flies.

3.3.6 Sub-lethal effects on hover fly behaviour

To assess the functioning of the hover flies, a grip test was conducted on the surviving flies each day for 14 days after exposure. Each fly was first positioned in its cage to be standing upright on a plastic surface (i.e. not the sugar pad). Then the cage was gently rotated such that the fly was upside down, held for a count of two seconds, then gently rotated to the upright position again. This was done four times for each fly daily, and the number of times the fly maintained its grip while flipped or upside down was recorded.

To assess how the general behaviour of the hover flies might be affected by thiamethoxam, activity time budgets were obtained two days after exposure and every three days after that for 12 days, resulting in five measurements per individual. Five treatment groups (0, 0.1, 1, 4, and 10 ng/µL thiamethoxam doses) were used for activity time budget observations. Flies were placed in individual clear plastic cages (28 x 34.25 x 24.75 cm) with a mesh cover, and two sugar pads with pollen on the base of the cage. These sugar pads in the activity budget cage were visually the same as the ones kept in their individual cages, so they had been conditioned to identify them as a food source for at least a week prior to their first activity budget measurement.

Each fly was given one minute to acclimate to the larger cage, and then its behaviour was recorded for 10 minutes following standard protocols (see Basley et al., 2018; Guest, 1984). Behaviours were categorized as walking, flying, grooming, feeding, probing (proboscis extension on anything other than sugar pads), resting (subtle or no movement), and righting itself (the time spent lying on back before standing on feet again). Captive *E. balteatus* have been observed showing highest activity levels between five and 22 days after emergence from the pupa (Guest, 1984). Since the flies were exposed to the pesticide at five days old, and the observation of their activities ended at 19 days old, our data were collected during the period that captive adult flies were expected to be most active.

3.3.7 Data analysis

3.3.7.1 Survival analysis and proportional hazards models

Analysis was performed using R 3.5.2 (R Core Team, 2018) with the packages *survival* (v3.1-12; Therneau, 2020), *survminer* (v0.4.6; Kassambara et al., 2019), and *coxme* (v2.2-16; Therneau, 2020). Kaplan-Meier survival estimates were calculated for each concentration over the 14-day period, and differences between the estimates were tested using log-rank tests. Cox proportional hazard mixed effects models were also run in order to determine relative hazards adjusting for covariates. The variable “sex” was determined not to significantly improve the models and so was not included in the final models, which contained the random factor “batch” and either one of two numerical explanatory variables: concentration of the test solution or dose per fly body weight. Dose per fly body weight was calculated by dividing the amount of pesticide consumed (all flies consumed a standard 5 µL of their assigned concentration treatment) by their individual body weight (measured upon emergence from pupae, before flies had access to food or water). The initial plan was to analyse data by concentration treatment so that there were an equal number of replicates per treatment. However, the flies used in this study showed a broad range of body weights, therefore a second analysis was done to identify any influence body weight had on the responses observed. The final selected models met the assumption that hazards were proportional to each other.

3.3.7.2 Mortality dose response relationships and estimation of LD50 and LC50 values

The relationship between fly mortality and thiamethoxam was assessed using the day 2 and day 12 data (Genstat v 19 software; VSN Ltd, UK). Initially, generalized linear models fitted mortality as a binary response variable, with a logit link function, to the categorical explanatory factors “batch” and “sex” and either thiamethoxam concentration or dose per mg body weight as numerical explanatory variables. These models found no relationship between day 2 mortality and thiamethoxam dose, so no further analyses were performed on these data.

For the day 12 data, the initial GLM models found that all two- and three-way interaction terms, and the main factor “sex”, had no significant effects on fly mortality. Subsequent logistic regression models were fitted that included only one of the numerical predictors, or with the numerical predictor and the factor “batch”, to obtain LC50 and LD50 estimates for the overall dataset and for each batch separately. To examine for consistency between LC50 and LD50 estimates, a REML meta-analysis approach was also used to find a weighted average estimate from the results obtained from the different batches of insects. To assess the effect of background mortality on LC50 estimates, Abbott’s correction (Abbott, 1987) was applied to the whole dataset and a corrected LC50 estimate obtained.

3.3.7.3 Activity budget analysis

The purpose of the activity budgets was to determine the relationship between time spent doing activities and the treatment concentrations flies received, and to see if this relationship changed on different days after exposure. A separate analysis was carried out on each day after exposure for the activity budgets (days 2, 5, 8, 11), and for three select activities (flying, grooming, resting), therefore 12 separate analyses. Analysis was not performed on Day 14 due to the low number of surviving flies. Flying, grooming, and resting were selected for further analysis because flies consistently spent the most time on these activities (see Appendix Figure A.3). On each day, the time spent doing an activity for all the surviving flies were averaged by batch and concentration, thus each analysis had 20 values (five treatments in each of four replicate batches). Rank correlations were used to examine relationships between concentrations and amount of time spent doing each activity, with P-values calculated to account for ties in the concentrations used (each concentration has four replicates, one from each batch).

3.3.7.4 Grip test analysis

The purpose of the grip test was to determine if there were differences in flies' abilities to grip onto smooth surfaces between the treatments, and how these differences persisted and changed over time after exposure. The first batch of flies (batch A) was not used in this analysis because several of these flies became trapped on the nectar pad, which was much more textured than the smooth plastic of the cage, therefore batch A flies could have appeared to have stronger grips than other batches solely due to the texture of the floor. Two generalized linear mixed models (GLMM) were formulated, using logit link functions, where the binomial response variable was the number of occasions each fly successfully held on to the plastic surface, batch was included as a random factor, and the explanatory variables were sex, dose, day, and the interaction of dose and day. While dose was always included as a numerical variable and sex always as categorical, we created separate models where day was included as a numerical variable or a categorical variable. These two separate models were created to test both whether the grip scores changed as a function of time since dosing or differed among daily assessments in a non-monotonic fashion. From the GLMM model where day was a categorical variable, it was found that the grip score changed with day since dosing in a non-monotonic fashion. To visualize any patterns in grip score with day and dose, the grip scores of all surviving flies for each dose treatment (Appendix Figure A.4) and across all dose treatments (Appendix Figure A.5) was averaged for each day and graphed against day.

3.4 Results

3.4.1 Survival analysis and proportional hazards models

In the positive control treatment (200 ng/µL thiamethoxam), 14 out of the 20 flies (70%) died within 24 hours, and 100% death was reached four days after exposure, indicating that the flies were ingesting the thiamethoxam-laced sugar solution. Of the 320 individual flies used in the actual dose-response trial, 226 (71%) survived the full 14 days of observation, 108 of which were male and 118 were female (see Appendix Table A.1).

From the 14-day Kaplan-Meier survival estimates, the 0 ng/µL control treatment had the highest survival probability (0.844), and the highest concentration of 24 ng/µL thiamethoxam had the lowest survival probability (0.500) (Table 3.1). When pooling all the data, there were significant differences in the survival of individuals based on treatment (log-rank test pooled data among all 10 treatments, P = 0.016; Figure 3.1), but we could not separate doses using pair-wise comparisons (log-rank tests with Bonferroni correction, P > 0.150). The log-rank tests performed on individual batches of flies only identified a significant difference in survival among doses for batch D (log-rank test pooled data among all 10 treatments within batch D, P = 0.030; see also Appendix Figure A.2).

The per-unit relative hazard for thiamethoxam calculated from the Cox proportional hazard models was highly significant (P < 0.001) but the actual effects were small, with values only slightly higher than 1 (Table 3.2). The effect of the random factor “batch” was also significant in the models, with batch C having the lowest relative hazard (0.486 and 0.491) and batch A having the highest (1.578 and 1.616) (Table 3.2).

Table 3.1 Survival probabilities 14 days after exposure of adult *Episyrphus balteatus* flies fed different concentrations of thiamethoxam in sugar water. Data are pooled for all batches, with 32 individuals per treatment.

Concentration (ng/µL)	Survival Probability
0	0.844
0.1	0.781
0.5	0.812
1	0.812
2	0.656
4	0.625
8	0.781
10	0.625
12	0.625
24	0.500

Table 3.2 Summary of Cox proportional hazard mixed effects models of adult *Episyrphus balteatus* at 14 days after oral exposure based on (a) thiamethoxam concentration in sugar solution and (b) thiamethoxam dose per the bodyweight (bwt) of individual flies.

Explanatory variable	Covariates	Relative hazard	p-value
(a) Concentration (ng/µL)		1.051	P < 0.001
	Batch factor		P < 0.001
	Batch A	1.578	
	Batch B	0.862	
	Batch C	0.486	
	Batch D	1.512	
(b) Dose (ng/mg bwt)		1.194	P < 0.001
	Batch factor		P < 0.001
	Batch A	1.616	
	Batch B	0.920	
	Batch C	0.491	
	Batch D	1.369	

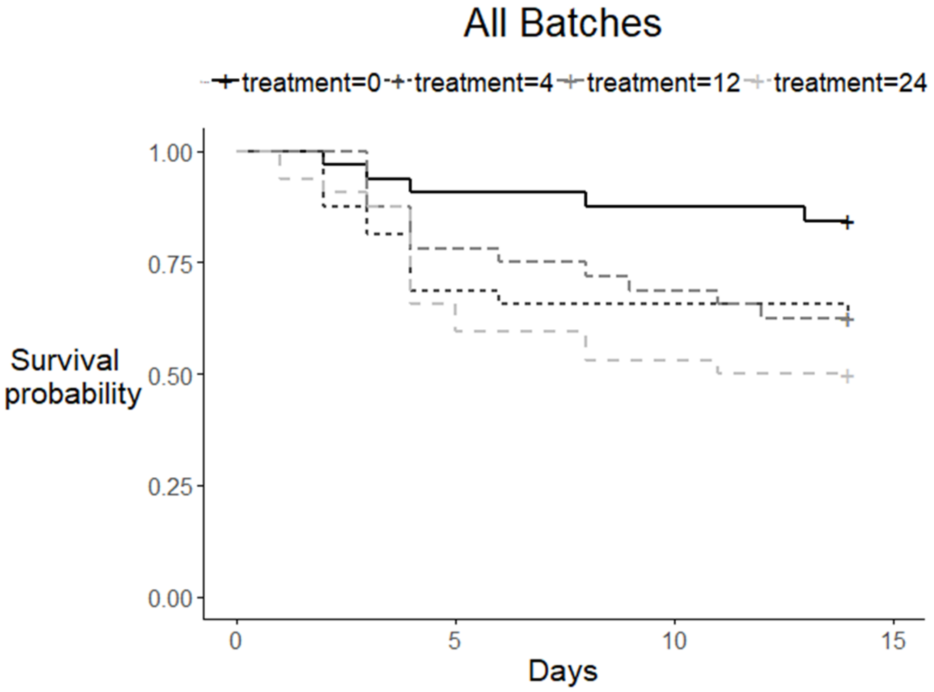

Figure 3.1 Kaplan-Meier survival curves over 14 days of select concentrations (0, 4, 12, and 24 ng/µL) for all the batches pooled (see Appendix Figure A.2 for each batch individually).

For the honey bee tests, 19 out of the 20 thiamethoxam treated bees died within six hours of treatment, and the last treated bee was found dead the next morning at 19 hours after exposure. All the standard and procedural control bees survived for over 40 hours.

3.4.2 Hoverfly mortality, and LD50 and LC50 estimates

At day 2 there was no significant relationship between mortality of the *E. balteatus* adults with either the concentration of thiamethoxam or the dose per mg body weight (Figure 3.2 a, b; Table 3.4 a). Although only 12 individuals from 320 (3.75%) had died by day 2, there were significant effects of the factors “sex” and “batch” on mortality, which arose because 10 of these dead individuals were males, and of these males six belonged to batch A, giving a relatively high mortality rate for this group of 15%.

At day 12 there were significant positive relationships between mortality and thiamethoxam concentration, and between mortality and dose of thiamethoxam (Figure 3.2 c, d; Table 3.4 b). There were also significant differences among batches of flies, with total mortality in batch C (12.5%) being considerably lower than that observed in batches A and D (> 35%). The interaction terms between batch and thiamethoxam were not significant (Table 3.4), and so a common coefficient was fitted for all batches in the regressions for mortality against concentration and dose (Figure 3.2 e, f; Table 3.3).

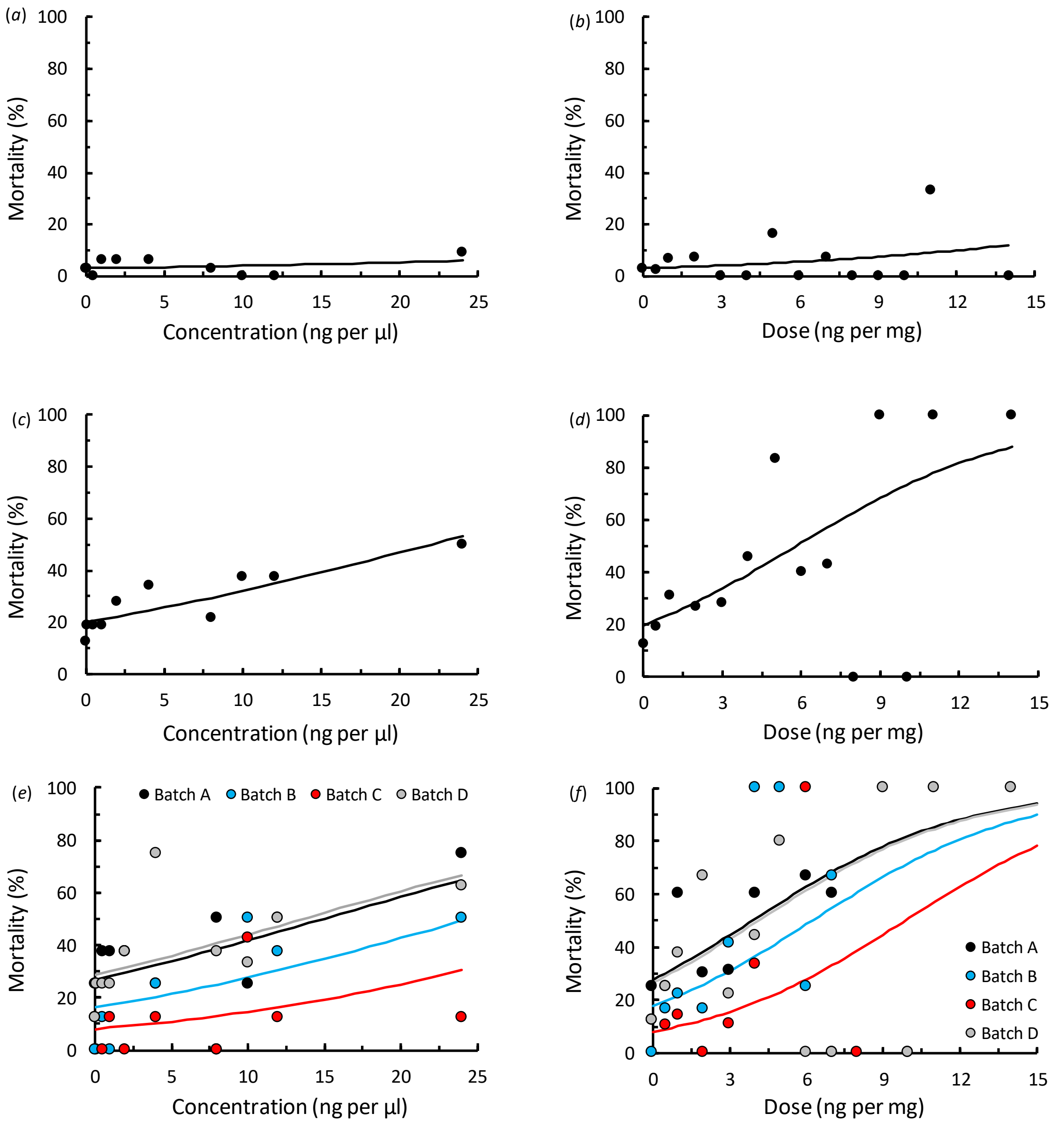

Figure 3.2 Relationships of *Episyrphus balteatus* mortality adults at (a-b) day 2 and (c-f) day 12 after oral treatment with thiamethoxam expressed as either concentration of solution (ng/µL) or dose per insect (ng per mg bodyweight). Graphs *a-d* show raw data and fitted curves for all data, whereas graphs *e* and *f* show separate trends for batches A-D. Observed mortality data for graphs *b, d, f* were calculated for intervals of 1 ng per mg.

Table 3.3 Summary of logistic regression analyses of mortality of adult *Episyrphus balteatus* after 12 days in response to oral application of thiamethoxam based on the concentrations of solutions fed to individual flies or dose in relation to bodyweight (bwt) of individual flies. Fitted models were of the form: logit(*p*)= *a* + *b*(thiamethoxam), where *p* is the probability of mortality. Abbott's correction was applied to adjust for control (0 ng/µL) mortality when estimating the corrected LC50. The meta-analysis estimates used a REML procedure that combined estimates from each batch, weighted inversely to standard errors.

		a (SE)	*b* (SE)	LC50 / LD50 (95% CI)
Concentration (ng/µl)	Batch A	-0.997 (0.265)	0.067 (0.017)	14.9 (7.9, 27.7)
	Batch B	-1.634 (0.300)	0.067 (0.017)	24.4 (15.6, 44.8)
	Batch C	-2.434 (0.377)	0.067 (0.017)	36.4 (23,4, 67.9)
	Batch D	-0.909 (0.260)	0.067 (0.017)	13.6 (6.7, 25.3)
	All data	-1.378 (0.063)	0.063 (0.016)	22.0 (16.0, 38.8)
	Corrected	-2.246 (0.308)	0.091 (0.025)	24.7 (17.2, 53.9)
	Meta-analysis			17.8 (11.3, 24.3)
Dose (ng/mg bwt)	Batch A	-0.967 (0.259)	0.248 (0.062)	3.9 (2.0, 7.2)
	Batch B	-1.544 (0.286)	0.248 (0.062)	6.2 (3.9, 11.4)
	Batch C	-2.440 (0.377)	0.248 (0.062)	9.9 (6.5, 17.8)
	Batch D	-1.022 (0.273)	0.248 (0.062)	4.1 (2.2, 7.3)
	All data	-1.401 (0.171)	0.241 (0.058)	5.8 (4.3, 9.5)
	Meta-analysis			4.9 (3.2, 6.6)

Table 3.4 Results of generalized linear models evaluating mortality of *Episyrphus balteatus* adults in response to thiamethoxam treatment at (a) 2 days and (b) 12 days after treatment. In each table, the top half is the model with thiamethoxam treatment as concentration (ng/ µL), and the bottom half is the model with thiamethoxam treatment as dose (ng per mg bodyweight).

(a)

Factor	df	Deviance	Mean deviance	Deviance ratio	P-value
Conc	**1**	**0.6152**	**0.6152**	**2.39**	**0.123**
Sex	1	6.0419	6.0419	23.50	<.001
Batch	3	4.6907	1.5636	6.08	<.001
Conc.Sex	1	1.2063	1.2063	4.69	0.031
Conc.Batch	3	4.3513	1.4504	5.64	<.001
Sex.Batch	3	7.2947	2.4316	9.46	<.001
Conc.Sex.Batch	3	0.0004	0.0001	0.00	1.000
Residual	304	78.1458	0.2571		
Total	319	102.3462	0.3208		
Dose	**1**	**0.7654**	**0.7654**	**2.97**	**0.086**
Sex	1	6.3336	6.3336	24.55	<.001
Batch	3	4.7031	1.5677	6.08	<.001
Dose.Sex	1	2.3752	2.3752	9.21	0.003
Dose.Batch	3	3.5218	1.1739	4.55	0.004
Sex.Batch	3	5.4485	1.8162	7.04	<.001
Dose.Sex.Batch	-	0.000	0.000	0.00	-
Residual	307	79.1986	0.2580		
Total	319	102.3462	0.3208		

(b)

Factor	df	Deviance	Mean deviance	Deviance ratio	p-value
Conc	**1**	**14.428**	**14.428**	**13.15**	**<.001**
Sex	1	1.983	1.983	1.81	0.180
Batch	3	18.897	6.299	5.74	<.001
Conc.Sex	1	0.044	0.044	0.04	0.841
Conc.Batch	3	1.858	0.619	0.56	0.639
Sex.Batch	3	6.653	2.218	2.02	0.111
Conc.Sex.Batch	3	0.830	0.277	0.25	0.860
Residual	304	333.657	1.098		
Total	319	378.351	1.186		
Dose	**1**	**18.550**	**18.550**	**17.10**	**<.001**
Sex	1	2.599	2.599	2.39	0.123
Batch	3	17.087	5.696	5.25	0.002
Dose.Sex	1	0.567	0.567	0.52	0.470
Dose.Batch	3	2.596	0.865	0.80	0.496
Sex.Batch	3	6.099	2.033	1.87	0.134
Dose.Sex.Batch	3	0.986	0.329	0.30	0.823
Residual	304	329.868	1.085		
Total	319	378.351	1.186		

The differences in the mortality curves resulted in substantial differences in the LC50 and LD50 estimates on day 12 obtained for the different batches of flies, and the scatter around the fitted values meant that these estimates were also associated with wide confidence intervals (Figure 3.2; Table 3.3). In terms of thiamethoxam concentration, 50% mortality was only just achieved at the highest concentrations, and was not attained in batch C, where a mortality of 12.5% was observed at the highest concentration of 24 ng/ µL. The response curves using dose in terms of bodyweight all surpassed 50% mortality, and thus the LD50 estimates, overall, had narrower confidence intervals. This analysis also indicated that dose-by-weights were not distributed evenly among batches, as the smaller flies that received high concentrations of thiamethoxam, and which tended to show high mortality, were all in batch D.

The overall and meta-analysis estimates of LC50 were in the region of 20 ng/µL, so at the upper end of the concentrations used. The adjusted LC50 value that accounted for background mortality was

24.3 ng/µL, so slightly above the highest concentration used. The LD50 estimates suggested that 50% of flies would be dead 12 days after ingesting approximately 5-6 ng of thiamethoxam per mg of bodyweight.

3.4.3 Activity budgets

On day 2, the time spent in flight (rho = -0.69, p < 0.001) and on grooming (rho = -0.43, p = 0.041) had negative correlations with thiamethoxam concentration, whereas time spent resting had a positive correlation with concentration (rho = 0.61, p = 0.005) (Figure 3.3). These relationships were no longer apparent 5, 8, and 11 days after exposure (Figure 3.3).

3.4.4 Grip test

In both GLMM models, the factor “sex” was found to have no significant effect on the ability of flies to grip the plastic surface (Table 3.5). In the model where day was included as a numerical variable, dose was found to have a small, but statistically significant, negative effect on grip (-0.040, P = 0.018). In the model where day was included as a categorical variable, dose again had a small negative effect on grip (-0.054, P = 0.025), and day and the interaction between day and dose were also found to be significant (P <0.001).

Table 3.5 Results of generalized linear models evaluating grip (measured as number of times flies were able to maintain grip when flipped four times) of *Episyrphus balteatus* adults in response to thiamethoxam treatment dose, analysed with the variable day as a (a) categorical variable and a (b) numerical variable.

Factor		(a) Day = Categorical			(b) Day = Numerical	
	df	f-stat	f-probability	df	f-stat	f-probability
Dose	1	5.13	0.025	1	5.65	0.018
Day	10	5.18	<0.001	1	0.02	0.898
Sex	1	0.75	0.386	1	0.68	0.412
Dose.Day	10	3.26	<0.001	1	0.12	0.730

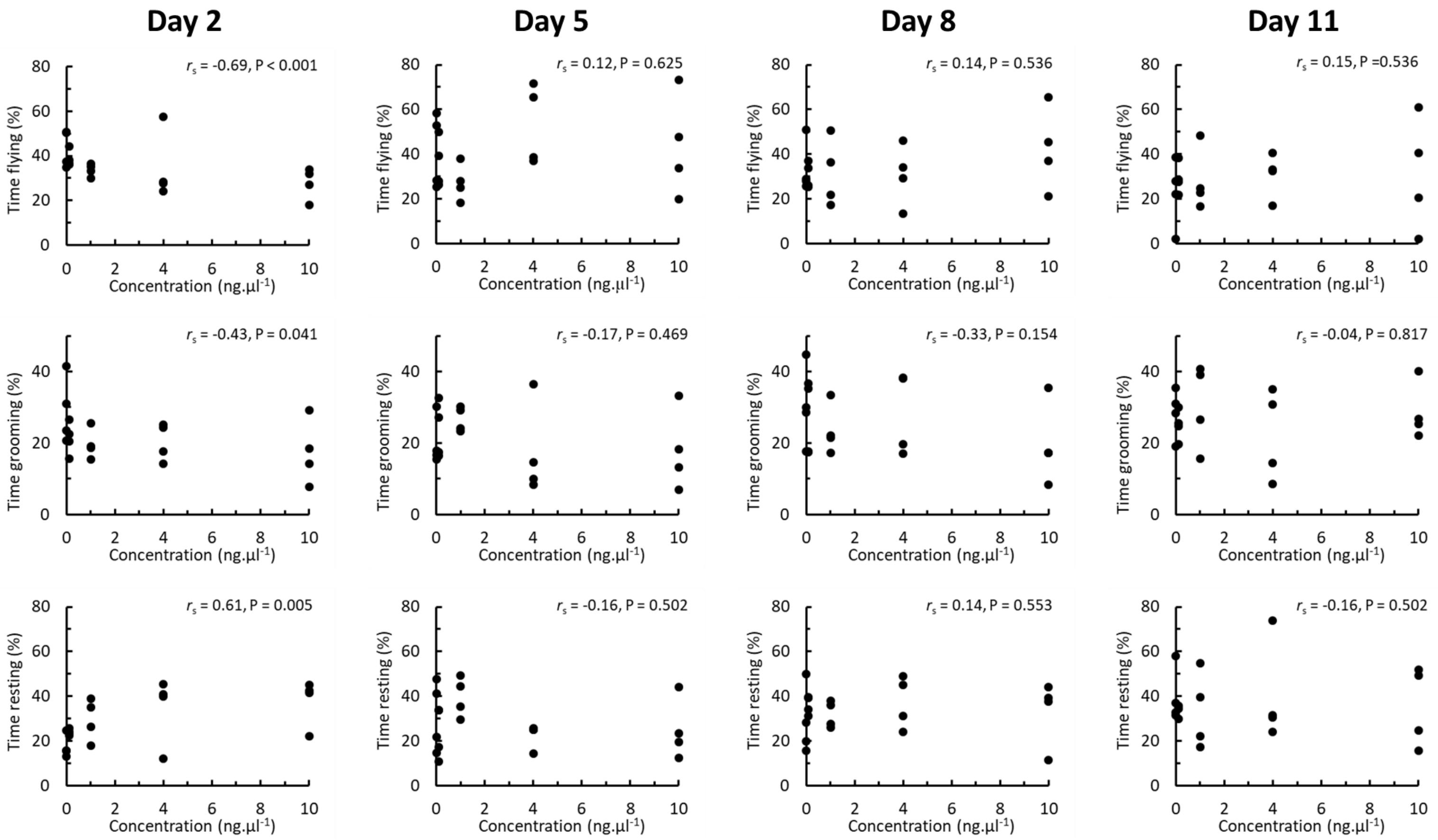

Figure 3.3 Percent time doing any activity graphed against concentration of thiamethoxam received orally by *Episyprhus balteatus* adult flies. Graphed for 2, 5, 8, and 11 days after exposure to pesticide. Rho and p-value given on each graph. Plotted points are the averaged percent times doing an activity of all the surviving flies for each treatment concentration group (5 groups) of each batch (4 batches), so that there are 20 data points on each graph.

3.5 Discussion

These results suggest that the hover fly *E. balteatus* appears to be resilient to acute oral exposure of the neonicotinoid thiamethoxam, especially in the context of field-realistic levels (Zioga et al., 2020). Botías et al. (2015) sampled nectar from thiamethoxam-treated oilseed rape crops in the U.K. and discovered a 54% detection rate of thiamethoxam in 13 samples, with the highest quantified amount being 13.30 ng/g nectar. Pohorecka et al. (2012) reported similar results for oilseed rape crops in Poland, with a 65% detection rate in 212 samples of pooled nectar and honey containing thiamethoxam as high as 12.9 ng/g. The density of the 500 g/L sugar-water solution used for this experiment is 1.14 g/mL, therefore even the smallest thiamethoxam concentration in sugar-water used in this experiment (0.1 ng/µL = 87.64 ng/g) was higher than what might typically be found in the field. Furthermore, we found that the 12-day LD50 of acute oral dose for flies was 5-6 ng per mg of fly body weight. With the average weight of flies used being 20 mg, an LD50 dose for the average fly would be 100-120 ng, meaning flies would have to consume 8-9 g of nectar with field-realistic residual thiamethoxam (13.30 ng/g, Botías et al., 2015), in a short period of time, in order to kill 50% of individuals within 12 days: this scenario is highly unrealistic.

It should be noted, however, that exposure through nectar in an agricultural field or a greenhouse is more likely to be chronic rather than a one-off dose, as both crop flowers and wildflowers within and near treated crops can contain residues (Botías et al*.*, 2015). Descher and Geusen-Pfister (1991) demonstrated that, even though *E. balteatus* would have to consume 4,897 ng of acephate in order to reach a 24-hour acute LD50, if provided a food source of sugar-water with 5 ppm acephate for 10 days, LD50 would be reached in 4.5 days with flies only having consumed 144 ng of acephate. Although it is beyond the scope of this present study of effects from acute exposure, it is possible that the flies in this study would have shown higher and quicker rates of mortality if they were chronically exposed to thiamethoxam.

Furthermore, this study occurred under constant optimal climate conditions, easily accessible food provided *ad libitum*, and protection against all other stressors (e.g. winds, pathogens, entomophagous fungi, predators, competitors, other pesticide residues, etc.), none of which reflect realistic field conditions. As inhibition of pollinators by pesticide toxicity is known to work synergistically with other abiotic and biotic stressors (e.g. Sánchez-Bayo et al., 2016) the mortality rates we observed are likely to be underestimates of what might be seen under natural conditions.

Within our highly controlled environment, even relatively high doses of thiamethoxam failed to cause total mortality of *E. balteatus*, indicating a high resilience of this hover fly species to dietary exposure of this compound. Basley et al. (2018) reached a similar conclusion for the hover fly *Eristalis tenax*, where no significant effect on survival was found after individuals had spent most of their aquatic larval stage in water with thiamethoxam concentrations up to 100 ppb. The high resilience of *E. balteatus* is further highlighted by comparison with the responses of honey bees under similar environmental conditions. We were able to confirm the LD100 result for honey bees in Laurino et al. (2010), where similar doses (approximately 17.5 ng in Laurino et al., 2010; 20 ng in this study) produced total mortality 24 hours after a single oral dose, with most bees determined dead at 6 hours after exposure. While Laurino et al. (2010) reported a 24-hour LD50 acute oral dose of thiamethoxam at 2.761-4.546 ng per *A. mellifera* forager, we estimated the required dose for an *E. balteatus* adult to be >220 ng per insect. The differences in body weights between these insects make the 24-hour lethal results all the more remarkable – the weights of the individual flies ranged between 9-30 mg with an average of 20 mg (measured within 24 hours after emergence from pupae, before offered food), while the weights of individual bees ranged between 76-167 mg with an average of 107 mg (measured after experiments with all collected bees euthanized and defrosted). Despite the much smaller size of *E. balteatus* compared with *A. mellifera*, the flies still showed much greater resilience than the bees. Similarly, Descher and Geusen-Pfister (1991) showed that the 24-hour acute oral LD50 dose of acephate was more than 27 times higher for *E. balteatus* than for *A. mellifera*. It should be noted that the 100% lethal concentration to bees reported by Laurino et al. (2010) was 0.5 ppm, which could mean ng/µL or ng/mg. However, our calculations suggest that the dose received by the bees in the study done by Laurino et al. (2010) was between 17.5 and 20 ng, and therefore our 20-ng dose was still a good confirmation of their results (see Section A.1).

One reason we found *E. balteatus* to be more resilient to thiamethoxam exposure than *A. mellifera* could be due to influences of their sociability. Bees' behaviour can range from high interdependence on each other (e.g. honey bee colonies with a caste system that nurtures the next generation to adulthood) to low interdependence (e.g. female solitary bees that prepare a nest stocked with food for their young) (Falk and Lewington, 2017). The activation of an individual honey bee's immunity can decrease their productivity, so it has been theorized that honey bees promote colony immunity through various social behaviours (e.g. grooming each other and identification and exclusion of unhealthy colony members) to compensate for a diminished individual immune response for the sake of optimizing colony productivity (Claudianos et al., 2006; Cremer et al., 2007; Evans and Pettis, 2005; Evans et al., 2006; Simone et al., 2009). Hover flies, however, do not occur in colonies or actively gather food or prepare a shelter for their young – at best, the females are selective on where they lay their eggs to optimize food availability for the larvae that emerge, which has been well-researched for aphidophagous species (Almohamad et al., 2009; Kan, 1989; Sadeghi and Gilbert, 2000b). The independent attribute in the life cycle and behaviour of hover flies may require a higher individual robustness against general stressors compared to bees in order to survive.

We also found that hover fly behaviour was not strongly affected by sub-lethal doses of thiamethoxam, although some small changes were detected. Correlations of the analysed activities (flight, grooming, and resting) as well as the ability to grip smooth surfaces were seen most strongly shortly after exposure, but any effects diminished or disappeared over time. We theorize that this is at least partly due to the death of individuals most influenced by the pesticide, thereby leaving only healthy individuals whose behaviour was not noticeably modified. This can be seen in the grip test when comparing the changes of the average held flips of all surviving flies to the average dose of all surviving flies over the course of 12 days (see Appendix Figure A.5). From this graph after day 3, the average dose of all surviving flies decreases across days as the average held flips increases, suggesting that unhealthy flies that received higher doses die off early leaving behind the healthy flies that either received lower doses or were resilient to the pesticide, thereby diminishing the effect of thiamethoxam on grip ability. Some changes, or converging, of behaviour could also occur due to the aging of the flies through the 14 days of the trial: the average lifespan of adult *E. balteatus* in captivity has been recorded as 30 days for females and 44 days for males, with highest activity levels being observed between 5 and 22 days after emergence from the pupa (Guest, 1984). Since flies in this experiment were exposed and observed between 5 and 19 days after emergence from the pupa though, we believe that age is likely to have a minimum effect on the behaviour. Future studies, however, would benefit from investigating how age of flies at the time of pesticide exposure affects both lethal and sub-lethal responses.

Although the strengths of the correlations between activities and thiamethoxam concentration were small to moderate and short-lived, these could still affect performance and survival under field conditions. Flight was negatively related to concentration, which might possibly impede foraging and reproduction, as mating occurs while the adults are in flight (Ball and Morris, 2015; Guest, 1984). Decreased mobility of females may mean ineffective egg-placement as well. For example, Guest (1984) found that older females laid eggs further from aphids and sometimes on the ground, which was attributed to lack of mobility from degraded wings. Grooming was the activity least correlated with concentration, but also had a negative relationship. Interestingly, grooming has been seen to increase in invertebrates when exposed to toxins through both contact and ingestion (Desneux et al., 2007; Hanna and Hanna, 2013; Hodge and Longley, 2000; Hurst et al., 2014; Neuman-Lee et al., 2013; Williamson et al., 2013) and some pesticide-exposed flies in this study were briefly observed grooming incessantly along with other agitated behaviours (e.g. rapid and constant walking, loud buzzing). One reason hover flies groom themselves is to collect and consume pollen from their bodies (Holloway, 1976), so both decreased grooming and decreased flight activity could mean overall decreased food intake. Grooming also aids in protection from parasites and pathogens, so a reduction may make the flies more vulnerable (James and Xu, 2012; Zhukovskaya et al., 2013). Resting was the one activity observed to increase with increasing concentration of thiamethoxam received. This can suggest lethargy which might be linked to food-intake. Ingestion of neonicotinoids has been documented to decrease food-intake in bees (Kessler et al., 2015; Laycock et al., 2012; Thompson et al., 2015), so if this also occurs in flies, it is possible that the flies rested more due to lower energy reserves. These behavioural changes have the potential to reduce longevity and productivity of individuals under field conditions, which may then translate to decreases in population size and ecosystem service delivery.

The grip ability test was a means by which to quantify declining health of exposed individuals before death, and our GLMM results (which incorporated grip scores of individual flies on each day) suggest that thiamethoxam dose did not affect grip ability in the same way on each day. One explanation for this might be that flies receiving higher doses were affected and died, leaving more healthy individuals of lower doses for later days. In Appendix Figure A.5,there seems to be a pattern when looking at the average grip ability of the flies: up to day three, the average daily dose received by flies changed little, while the average daily held flips steadily decreased, suggesting that even though treated flies were not killed immediately, their physical strength diminished. This would decrease their chances for survival in their natural habitat with more adverse conditions. On day four, the average daily dose decreased and the average number of held flips increased, suggesting death of flies most impacted by thiamethoxam. Beyond day four, the averages of daily dose and number of held flips changed little.

We found no other studies reporting the impacts of consumed chemicals on grip ability, though there are many studies that investigate grip *per se*. Flies have setose (i.e. bearing bristles) tarsi which excrete an adhesive fluid (Beutel and Gorb, 2001; Geiselhardt et al., 2020). This adhesive fluid has been found in multiple insect groups and can contain various biochemicals, such as fatty acids, amino acids, hydrocarbons, and saccharides (Dirks and Federle, 2011a, 2011b), and the biochemical composition can play a part in adhesive strength (Geiselhardt et al., 2020). Neonicotinoids have been observed to influence metabolic physiology (e.g. honey bees, Cook, 2019), which could influence the biochemicals in tarsal fluid secretion for grip. Another factor of grip success is the amount of fluid that is required to provide adhesion to a surface (Drechsler and Federle, 2006). Persson et al. (2005) briefly hypothesized that tarsal fluid secretion may be linked to the nervous system, which is targeted by neonicotinoids. Although Dirks and Federle (2011b) provided evidence that secretion of fluids stored in the tarsal pad occurs passively as a result of capillary action, they noted that when the fluids are depleted in the pads over time there must be something controlling the refill of the pads without overflowing, and that the nervous system may possibly play a part in this process. Regardless, in this experiment we observed that flies that lost their grip consistently (out of the four flips) on any given day were usually weak and less active, which might have impacted their ability to stand upright properly and make sufficient tarsal contact with the surface in order to create a good adhesive grip. On occasion we observed flies that moved around the cage well, but when held upside down their tarsi slipped across the plastic surface and they rearranged their legs in order to maintain a grip – this could be due to tarsal pads depleted of fluids, but whether this depletion is caused by poisoning or is simply due to a normal temporary depletion before recovery of fluids requires further study.

A final note should be made about the differences in responses to pesticide exposure observed between the batches, particularly for the mortality responses and 50% lethal doses and concentrations. Aside from knowing that the purchased *E. balteatus* came from a continuous culture grown under long-day conditions (exact hours unspecified) we do not know the specific rearing protocol the supplier company used, but their rearing conditions could change the susceptibility of *E. balteatus* to pesticide stressors between the batches, and in comparison to other captive-bred and especially wild *E. balteatus*. Captive breeding of insects can also cause behavioural, physiological, and genotypical divergences from natural populations, caused by events such as adapting to artificial conditions and inbreeding (Sørensen et al., 2012). We did observe differences in the average weights of syrphids between our purchased batches (see Appendix Table A.1), and different genotypes of the same species has been observed to cause different sensitivities to pesticides (e.g. *Apis mellifera* exposed to various neonicotinoids, Laurino et al., 2013). This should be taken into consideration when planning insect bioassays and contextualizing these results in a field setting where wild syrphids are exposed to pesticides.

3.6 Conclusions

The survival and behaviour of *E. balteatus* flies were remarkably resilient to acute oral exposure of thiamethoxam, especially in comparison to honey bees. Furthermore, a recent paper by Clem et al. (2020) demonstrated that some hover flies might be able to detect food resources contaminated with neonicotinoids at field-realistic levels and avoid those sources. Conversely, there is some evidence bees prefer neonicotinoid-laced food (Arce et al., 2018; Kessler et al., 2015), thereby making hover flies potentially even less susceptible to poisoning than bees. From these results, we propose that field-realistic one-off doses of thiamethoxam are unlikely to have major effects on *E.*

balteatus, but future work is important regarding repeated exposure, and the impacts on multiple ecosystem service provision. As some hover flies appear to be at lower risk from the harmful effects of some pesticides than bees in terms of both resilience and avoidance, it would benefit farmers to consider hover flies as well when managing fields for attraction and support of beneficial invertebrates.

Chapter 4

Factors driving aphidophagous Syrphidae (Diptera) abundance in cereal grain cropping systems

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4. Factors driving aphidophagous Syrphidae (Diptera) abundance in cereal grain cropping systems

4.1 Abstract

Non-cropped habitats can support a range of beneficial invertebrates on farmland, including syrphids (Syrphidae, Diptera), which can provide both pollinating and pest-controlling services to the adjacent crop. Adult syrphids feed on flowers in the hedges and margins of the crop, whilst the larvae of some species are aphidophagous. Syrphid abundance may be driven by a range of factors, including the physical and biological aspects of habitats, as well as the abundance of food resources and competitors for those food resources. This study investigated the relationships between hedge structure and composition, floral resource availability and the abundance of other floral visitors, and the abundance of aphids and other aphid consumers. Nine organic oat (*Avena sativa)* crop fields were surveyed in the Ireland in 2017. Hedge structure was quantified in terms of hedge dimensions and percent gaps, whilst composition was quantified as richness of woody genera. Insect surveys were conducted both in the hedge and the adjacent crops using three methods: timed observations of floral visitors along transects, timed hand searches for pests and other invertebrates on the oat crop, and 24-hour collection of fauna in pan traps along the hedge. Richness of floral families and floral abundance were also recorded, both in the hedges and the crop. None of the hedge characteristics correlated with adult aphidophagous syrphid abundances, but floral abundance had a positive correlation, suggesting that more floral resource availability attracts and supports more aphidophagous syrphids. Bee and parasitized aphid abundances were also positively correlated with adult aphidophagous syrphid abundance, countering the hypothesis that they would compete for floral and aphid food resources. Aphid abundance was negatively correlated with adult aphidophagous syrphids, which may reflect predator-prey population cycles whereby the high aphid abundance early in the season attracted syrphids, and as aphids were consumed by the syrphid larvae and other predators, aphid abundance declined. Brambles and mustards were some of the flowers most commonly visited by bees and aphidophagous syrphids, and these guilds appeared to share rather than compete for food resources, so it would benefit farmers to encourage these

wildflowers to grow in hedges and along margins in order to support important pollinators and pest controllers.

4.2 Introduction

Provision of habitat for wildlife on farms can enhance ecosystem service delivery, both on the farm and in the wider landscape (Sutter et al., 2018). Insects provide a variety of services, such as pollination, pest control, and waste decomposition, and can be attracted onto farmland by the resources they require to complete their life-cycles (e.g. food that meets diet requirements at different developmental stages, shelter from the elements, etc.). Non-cropped habitats can support various insect ecosystem service providers, which can disperse into nearby crops (Albrecht et al., 2007; Chaplin-Kramer et al., 2011; Dainese et al., 2019; Garratt et al., 2017; Morandin and Kremen, 2013; Ricketts et al., 2008; Van Vooren et al., 2017), though this dispersion effect can decrease as the distance from non-crop habitats increases (Albrecht et al., 2007; Albrecht et al., 2020; Morandin and Kremen, 2013; Ricketts et al., 2008; Saunders and Luck, 2018). A variety of non-cropped habitats can exist on farmland, including aquatic features, non-cultivated margins of sown fields, cultivated buffer and wildflower strips, and stands of trees (Rotchés-Ribalta et al., 2020). Hedges (i.e. lines of woody shrubs and trees forming boundaries separating fields) are another farmland habitat that can provide food in the form of flowers for those that require pollen and nectar (Hannon and Sisk, 2009; Morandin and Kremen, 2013), and prey for predatory insects (Vialatte et al., 2007).

Hedges (as well as small patches of woodlands) are prolific in the Irish landscape, and make up a total of 450,000 hectares, approximately 6.4% of the Ireland’s land area (Green, 2011), and can occur at a density of >10 km hedge per square kilometre of farmland (Bourke et al., 2014). Given that hedges are already common, managing them to enhance attraction of beneficial insects for ecosystem service provision is a more cost-effective approach than creating new non-cropped habitat. Hedge management schedules tend to vary between farms, but Irish hedges are protected from destructive actions by law (Government of Ireland, 2000), and it is recommended that hedges be trimmed every three years, and coppiced or laid every twenty to forty years (Hedgelink, 2013). Effective management needs to be more targeted for the desired ecosystem service in a given setting, however, as different taxa can respond to characteristics of hedges (e.g. physical structure, woody species makeup) in different ways (Bourke et al., 2014; Garratt et al., 2017), which could influence attraction and therefore the services provided.

One highly valued service is pest control, particularly control of aphids, which can decrease crop yields via the transmission of plant diseases (Ng and Perry, 2004) and induce direct damage to crops by feeding on the plants (Larsson, 2005; Rodríguez-Gasol et al., 2020). Several studies have found that non-cropped habitats, including hedges, are effective for attracting aphid consumers into adjacent crops (Garratt et al., 2017; Haenke et al., 2014; Van Vooren et al., 2017). Although the success of relying on natural enemies for pest control varies and depends on several factors, in some cropped systems biological control is sufficient to reduce or discontinue pesticide use (Lechenet et al., 2017; Reganold et al., 2001; Seufert et al., 2012). Understanding how farmland habitats attract predators can inform farmers on management of these habitats in order to maximize ecosystem services, thereby depending less on pesticides, which would cut expenses for farmers and reduce environmental pollution.

We investigated how aphidophagous syrphid abundance (Syrphidae, Diptera) responded to the structure of hedges, food availability (aphids and flowers), and competitors for those food resources. Syrphids have been documented as noteworthy controllers of aphids in crops (Ramsden et al., 2017), and have also shown positive associations with hedges (Bourke et al., 2014; Haenke et al., 2014), particularly in organic systems (Power et al., 2016). In addition, the availability of flowers and aphid prey can have a positive influence on syrphid abundance (Power and Stout, 2011; Ramsden et al., 2015), as flowers provide food for the adult stage, and aphids for the larval stage. However, many other taxa use these sources of food and shelter as well, such as bees, parasitoid wasps, lady birds, and lacewings (Hannon and Sisk, 2009; Morandin and Kremen, 2013; Ramsden et al., 2015), potentially competing for resources with syrphids.

In this study, we assessed the availability of food (flowering plants and aphids) and habitat characteristics (hedges), as well as potential competitors for these resources (other floral visitors and aphid consumers). While we acknowledge that there could be other relationships at play (e.g. hedge relationship with food resources and potential competitors, Figure 4.1), these were not investigated in order to focus on the responses of one target group, that is aphidophagous syrphids. Gaps, hedge dimensions, and the diversity of woody vegetation, have been shown to influence farmland biodiversity under certain circumstances (Garratt et al., 2017; McMahon et al., 2005), and these characteristics have been included in grading systems for determining general hedge quality (Collier and Feehan, 2003; Foulkes et al., 2013), and were thus included in our study. Although there has been much research on aphidophagous pest control services in cereal grain crops, much of it has focused on wheat (Clement et al., 2004; Garratt et al., 2017; Holland et al., 2012; Ramsden et al., 2015; Ramsden et al., 2017; Schmidt et al., 2003; Vialatte et al., 2007; Wilson and Leather, 2012). Although oats only comprised 2.7% of cereals produced in Europe in 2016, the production has seen a modest increase in recent years (Heidorn et al., 2017). Furthermore, oats are a favourable option as a low risk crop for organic production due to their tolerance of weed competition and nutrient poor soils (Bavec, 2014). Given that pesticide use could influence invertebrate populations (Uhl and Brühl, 2019), we focussed on organic oat crops as a model system to test our three hypotheses:

1) The physical and biological attributes of a hedge will relate to the abundance of aphidophagous syrphids.

2) The abundance of food resources (flowers and aphids) will be positively related to the abundance of aphidophagous syrphids.

3) Other guild members (other floral visitors and aphid consumers) will act as competitors and be negatively related to the abundance of aphidophagous syrphids.

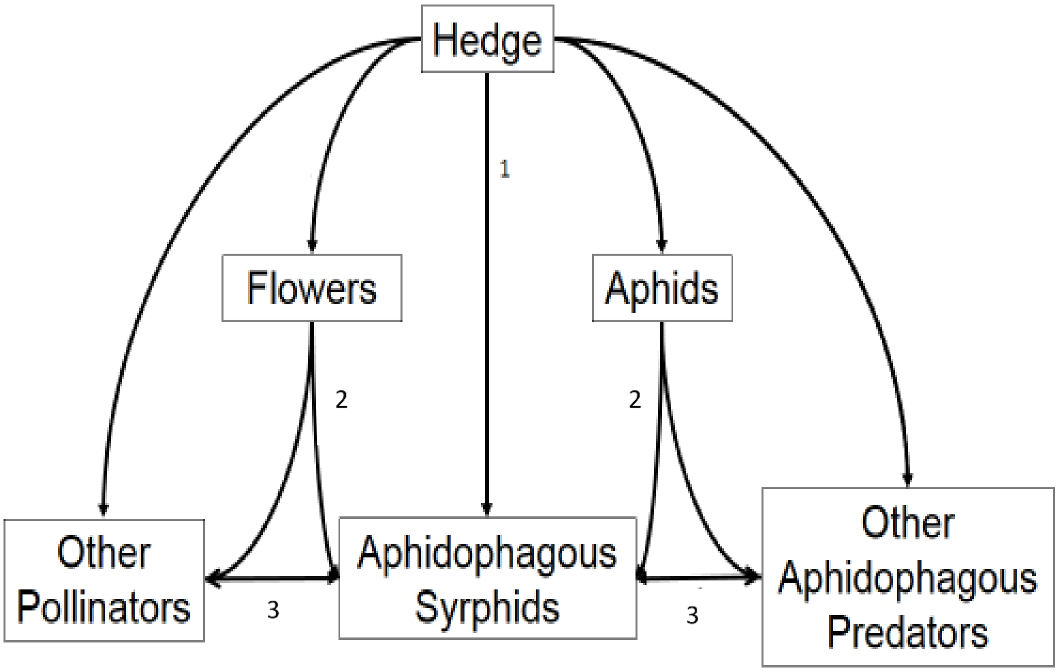

Figure 4.1 Visual representation of hypotheses. Numbers refer to the represented hypothesis.

4.3 Methods

Nine cropped fields were selected in the Ireland because they met the following requirements: they were spring oats, qualified as organic (crops had been managed organically for at least 6 years, including conversion years), were at least three kilometres apart, and had hedges surrounding most of the crop with continuous sections measuring at least 100 metres in length (Figure 4.2). Field sizes ranged from between 2 to 7 hectares, however there was one field at about 16 hectares. All oat crops were sown in either March or April 2017. All surveying occurred between June and August 2017, in three surveying rounds – June 1st to the 18th, June 30th to July 15th, and July 24th to August 11th. All surveying protocols were conducted at each site during each round, except for the hedge assessment which was conducted only once. Surveying was done between 9 am and 8 pm, while weather was dry to a light rain, with sunny skies to overcast with sun breaks, and temperatures exceeding 16 °C. While windy weather was avoided as much as possible, occasionally surveying occurred during strong winds when protocols did not focus on insects in flight (i.e. hand searches, floral abundance), or the strong winds were in bursts rather than continuous, or hedges provided wind breaks.

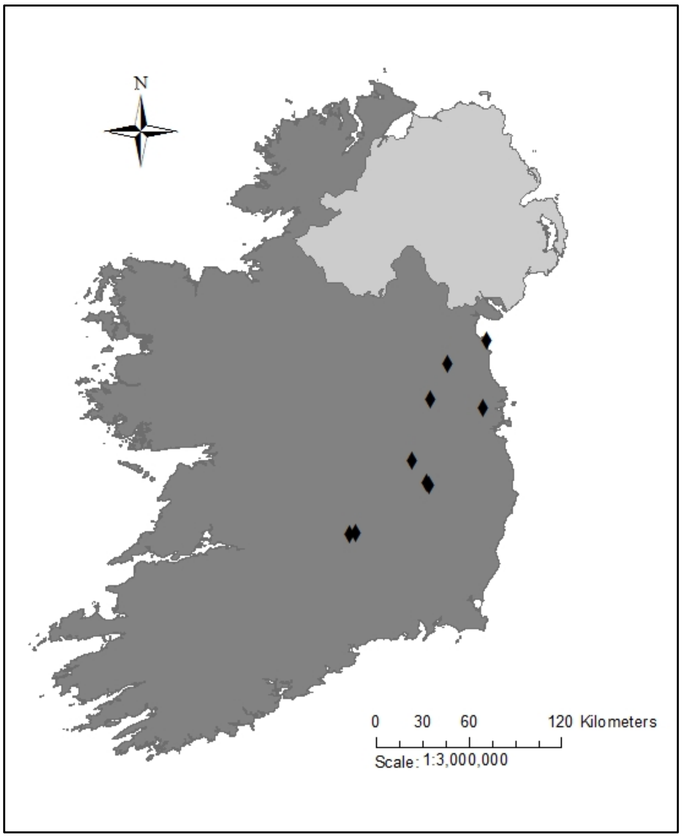

Figure 4.2 The location of the nine field sites (black diamonds) across the Ireland (dark grey).

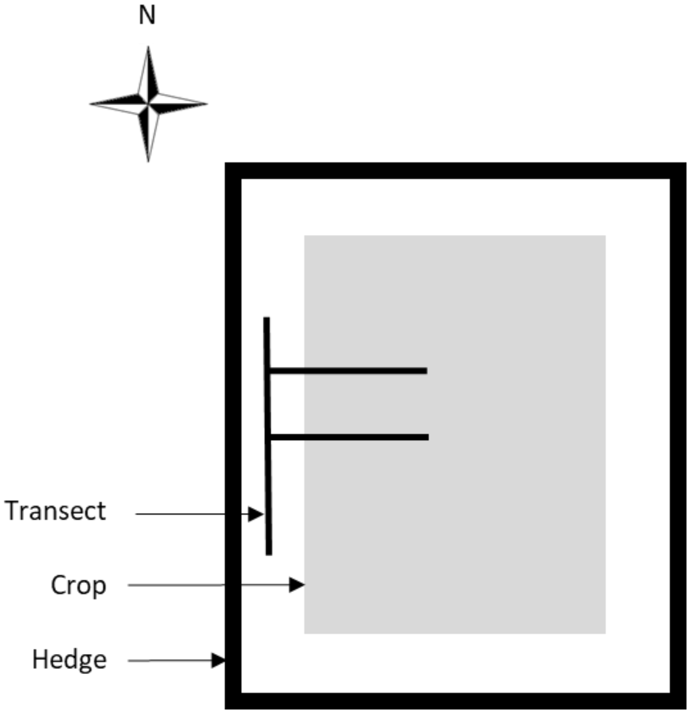

Figure 4.3 Orientation of surveying methods at sites. The thick black rectangle represents the hedge, and the grey box inside is the crop. The three black lines inside the black square are the three 100-metre transects along which all surveying took place. The transect parallel to the hedge marks the 100-metre stretch of hedge assessed.

4.3.1 Hedge Assessment

At each site, not all the hedges around the oat fields were capable of being properly surveyed due to vegetation hindering access, extensive gaps or gates, shortness of length, etc. However, all hedge sides selected either faced South, East, or somewhere in between. A 100-metre transect was laid parallel to the hedge to mark the stretch of hedge that was assessed, and which all other surveying methods were centred around (Figure 4.3). The transect was measured every ten metres with a metre-stick to ensure it was roughly a metre from the hedge. Only shrubs and trees were recorded for the assessment, not forbs or graminoids. The hedge was assessed at each site once during the summer. Data recorded for analysis included the hedge dimensions of average height, average base width, and percent gaps (spaces that allowed one to see through the hedge and into the field on the other side). Height and width were organized into three categories of 1-2 metres, 2-3 metres, and >3 metres. After assessing the hedges, it was found that hedges either had no gaps (<1%), few gaps (1-5%), or many gaps (>60%), and thus we used these categories. Vegetation was also surveyed by woody genera, and quantified either by counts of distinct trees and shrubs in height classes, or in percent linear coverage and average height for trees and shrubs that were continuous rather than distinct individuals. Woody plants were identified with the dichotomous key *Webb's an Irish Flora* (Parnell and Curtis, 2012).

4.3.2 Pan traps

Pan traps were set up at 25, 50, and 75 metres along the transect running along the hedge for 24 hours (Figure 4.3), and they were set out on three separate occasions over the course of the summer. Each location along the transect had a wooden stake placed in the ground for the duration of the summer, a metal clamp attached approximately a metre from the ground, and three bowls painted with one of each of three fluorescent colours (blue, white, yellow). The bowls were filled ½ to ¾ of the way full with water, with a drop of detergent to break the surface tension. We attempted to place the stakes at a consistent half metre from the hedge; however, this was not always possible (dense vegetation, impenetrable ground, etc.). In this case, the stake was placed as close to the hedge as possible, and the distance was recorded. Traps were left out for approximately 24 hours (range 23-28.5 hours due to delays in reaching the field or collecting early due to rain threatening overflow of the bowls). Collected pan trap samples were grouped by site, colour, and sampling round. The traps were drained of water, and the insects stored in vials with a 70% ethanol solution. Lepidopterans and slugs were removed from traps and not recorded, as wing scales and mucus respectively could have damaged other collected specimens. For identification, specimens stored in ethanol were gently patted dry with a tissue and examined under a microscope, and then returned to the tube. For all syrphid identifications, the dichotomous key *British Hoverflies* (Stubbs and Falk, 2002) and the book *Britain's Hoverflies: A Field Guide* (Ball and Morris, 2015) were used. For all bee identifications, the dichotomous key *Field Guide to the Bees of Great Britain and Ireland* (Falk and Lewington, 2017) was used.

4.3.3 Hand-searches for pests and predators

Hand-searches of the oats were conducted along two 100-metre transects that ran into the crops (Figure 4.3), perpendicular to the hedge, and parallel to each other 25 metres apart. Along these transects, 0.5 x 0.5 metre quadrats were placed on the ground every 20 metres, starting at 20 metres from the hedge. In the quadrat, the oats were hand-searched for five minutes to collect all aphids, syrphid larvae and pupae, and all other invertebrates. It was not possible to return to the sites at night in order to sample syrphid larvae when they were most active, and since some of these organic oat fields were very small there were concerns that collecting oat tillers for surveying would cause excessive damage to the crop. Therefore, hand searches were done during the day when all other surveying protocols were conducted. The size of area of oats searched was reduced to 0.5 x 0.5 metres (from 1 x 1 metres for floral abundance surveys) in order to allow for more thorough examination of oats (i.e. repeated searching of the same oat tillers, careful checking of undersides of leaves and where leaves met the stalk, etc.). Collected invertebrates were stored in 70% ethanol for identification in the lab except syrphid juveniles, which were collected live along with aphids outside of the quadrat to rear to the adult stage in the lab. Because this sampling method caused disturbances to the invertebrate communities, the crop transects were moved West or South by one metre each successive round. For all collected juvenile syrphids, they were reared to the adult stage in the lab, and then identified with the same books and keys used to identify specimens caught with pan traps.

4.3.4 Floral abundance

At the same points that quadrats were placed for the hand-searches, as well as 0 metres from the hedge, the floral abundance of plant families was also recorded in a 1 x 1 metre quadrat to maximize standard observation area to a reasonable area. Floral abundance was also recorded along the hedge transect starting at 0 metres, and every twenty metres after that. Along the hedge, the quadrat was placed on the side of the transect closest to the hedge and the flowers were counted within, then it was flipped up to stand vertical and flowers were counted in the hedge that were framed by the quadrat. Floral abundance was measured by counting floral units, where a single unit was defined as flowers from the same plant that were close enough together that insects could walk between the flowers without taking flight (see Appendix Table B.5). Flowering forbs were identified using the same key that was used to identify woody plants in the hedge assessment.

4.3.5 Timed transect walks

All three transects were walked at a steady, timed pace of one minute for every 10 metres, totalling 10 minutes per transect, during which all winged insects were recorded if found on flowers or oat stalks within a 1-metre radius of the observer, and up to a metre from the ground in the hedge. Bees and hover flies were captured for identification, and all other insects were recorded down to at least order, or generally as “insect” if observation was too brief to properly identify. When an insect was caught, the timer was paused while the insect was placed into a tube and labelled. Caught insects were euthanised either by adding 70% ethanol to the tube or placing the tube into a cooling box. Walking into the crop caused disturbance to the dense oat stalks, thus a pace was adopted whereby the observer would walk a few metres, then stop and pause to allow the oat stalks to settle and insects to visit. The direction the transects were walked varied in order to keep the observer's shadow behind them to reduce disruption to insects. Upon returning to the lab, captured specimens were either stored in 70% ethanol or frozen. Frozen specimens were pinned and labelled, and all collected specimens were identified with a microscope either to species for syrphids and bees, or to order for all other invertebrates. For all collected bees and syrphids, they were identified with the same books and keys used to identify the specimens caught with pan traps.

4.3.6 Data Analysis

Analysis was performed using Genstat (v 19 software; VSN Ltd, UK). To test the three hypotheses, three generalized linear mixed models (GLMM) were run using log link functions with Poisson distributions. There were not enough transect replicates to be able to compare observations between field locations (hedge versus crop) or to view trends along crop depth. Therefore, abundance data were pooled to analyse the data by site (nine total) and round (three total), such that there were 27 data points analysed in each model. For each model, site was included as a random variable (as we sampled multiple times (three) from nine sites – a subset of the total number of spring oat crops in Ireland), and abundance of aphidophagous syrphids observed from the floral visit surveys was included as a numerical response variable with a Poisson distribution. The first model tested the hypothesis of a relationship with hedge structure, where explanatory variables were the factor variables of hedge height, width, and % linear gaps, as well as the numerical variable of richness of woody genera. The second model tested the hypotheses of relationships with floral food resources and competitors, where explanatory variables were the numerical variables of floral abundance, floral family richness, and the abundances of bees and non-aphidophagous syrphids observed from the floral visit surveys. The third model tested the hypotheses of a relationships with aphid food resources and competitors, where explanatory variables were the numerical variables of aphid abundance and parasitized aphid abundance.

Aphidophagous syrphids observed from the floral visit surveys were chosen as the response variable because juvenile syrphids were rarely observed in the oat crops, and because flies surveyed visiting flowers were more representative of individuals utilizing the floral food resources than flies surveyed in the pan traps. For the second model, the abundances of only bees and non-aphidophagous syrphids were included to test for a relationship with other floral visitors because – although other invertebrates were observed visiting flowers – bees are well known for their intensive foraging on floral nectar and pollen, and most syrphids utilize floral foods (Ball and Morris, 2015), so it is believed that other members of the same family would likely have an abundance relationship with aphidophagous syrphids. In the third model, the abundance of only parasitized aphids (with and without holes, indicating the parasitoid had emerged and departed) were included to test for a relationship with other aphid consumers because they were the second most abundant invertebrates on the oats (after live aphids), and very few other observed invertebrates were known predators of aphids. It was not possible to identify the sampled aphids to species, which is why in the third model only the abundance of aphids was included and not the richness. However, previous studies have observed that aphid populations in sampled Irish cereal crops comprise of mainly three species (*Metopolophium dirhodum*, *Rhopalosiphum padi*, and *Sitobion avenae*), particularly of *Sitobion avenae*, observed accounting for up to three quarters of aphids on cereals and it has been acknowledged as one of the main pests of Irish cereals (Giller et al., 1995; Kennedy and Connery, 2001 and 2005; Teagasc, 2017b; Walsh et al., 2020b). It was therefore believed abundance would be the most likely variable to have a relationship with aphidophagous syrphids.

4.4 Results

4.4.1 Aphidophagous syrphid communities

The pan trap method and the floral visitor method of surveying adult aphidophagous syrphids produced similar numbers of individuals (145 and 144 respectively), but with slight differences in the species observed (Table 4.1). More species were observed in the floral visitor method (18 species) than the pan trap method (14 species), and while *Platycheirus sp*. far outnumbered any other genus in the floral visitor method, *Syrphus sp*. were observed slightly more often than other genera in the pan traps. However, both methods had the same top four most abundant genera: *Platycheirus*, *Syrphus*, *Melanostoma*, and *Episyrphus* (*balteatus*). Also, with both methods, aphidophagous syrphids outnumbered both bees and non-aphidophagous syrphids. However, this partly may be due to an outlier of aphidophagous syrphid abundance that occurred at the coastal site in round two. Of all the recorded observations in the pan traps (145 individuals), 83% of recordings were of just four species (*Melanostoma scalare, Platycheirus albimanus*, *Episyrphus balteatus*, and *Syrphus ribessii*), with a total of 121 individuals. Of those 121 recordings, 95% (115 recordings) occurred at the north-most coastal site in the second round of early July. This was another reason for choosing the floral visitor data for the analysis, as 24% (34 recordings) of aphidophagous syrphids were observed at the coastal site during round two. Regardless, this outlier should be noted when interpreting the results from the GLMM models (Figure 4.4).

Juvenile syrphids were very rarely seen on the oats from the hand-search surveys. There were 16 recordings of syrphids total, with 5 being adults (escaped before could be identified), and 11 being pupae or larvae. There was an attempt to rear all 11 juveniles to adult stage, but six of them never had an adult eclose from the pupal stage. Of the five that emerged, two were *Episyrphus balteatus*, two were *Platycheirus* sp., and one was *Syrphus vitripennis*. Both *E. balteatus* individuals and the *S. vitripennis* were collected in round one (June), and both *Platycheirus* sp. were collected in round two (July). Despite the high abundance of adult aphidophagous syrphids recorded at the coastal site, it only had two recordings of juveniles.

4.4.2 Hypothesis 1: Hedge structure relationship with aphidophagous syrphids

While there were three surveyed hedges per width category (narrow, medium, wide), there were uneven numbers of hedges in each category of the height and %-gap variables (Table 4.2). For height, most hedges were considered tall (five hedges were >3 metres), and for %-gaps most hedges were considered full (five hedges had <1% gaps). There were 14 genera of woody plants recorded across all nine sites, and individual sites had a maximum of 10 and a minimum of six genera recorded (Table 4.3). *Rubus fruticosus* agg. (brambles) and *Crataegus monogyna* (hawthorn) were recorded at all sites. In the GLMM model, none of the hedge dimensions or the woody richness had a relationship with the abundance of adult aphidophagous syrphids ($P > 0.1$).

4.4.3 Hypothesis 2 and 3: Relationships of floral food resources and other floral visitors with aphidophagous syrphids

There was a higher abundance of floral units recorded in the crops than in the hedges. Most of the floral units in the crops were in the families Polygonaceae (e.g. *Persicaria* spp.- red shank) and Brassicaceae (e.g. *Brassica* and *Sinapis* spp – mustards, see Appendix Figure B.1 and Table B.5). In the hedge, most of the floral units were in the families Urticaceae (e.g. *Urtica* spp - stinging nettle) and Rubiaceae (e.g. *Galium* spp. - cleavers, see Appendix Figure B.1). The floral abundance did not appear to change much when moving further into the crop and away from the hedge, except for large increases in abundance of Polygonaceae and Brassicaceae from 0 to 20 metres from the edge

Table 4.1 Observed species of adult aphidophagous syrphids across all nine sites and three rounds, by surveying method. Ordered from lowest to highest counts.

Pan Trapped Species	Count	Floral Visitor Species	Count
Epistrophe grossulariae	1	*Eupeodes latifasciatus*	1
Eupeodes latifasciatus	1	*Leucozona lucorum*	1
Melanostoma mellinum	1	*Platycheirus angustatus*	1
Platycheirus clypeatus/occultus	1	*Platycheirus rosarum*	1
Platycheirus manicatus	1	*Platycheirus scutatus*	2
Platycheirus granditarsus	2	*Sphaerophoria sp.*	2
Eupeodes luniger	3	*Syrphus torvus*	2
Syrphus vitripennis	4	*Eupeodes corollae*	3
Eupeodes corollae	5	*Platycheirus peltatus/ nielseni*	3
Platycheirus peltatus	5	*Melanostoma mellinum*	4
Melanostoma scalare	10	*Syrphus vitripennis*	4
Platycheirus albimanus	29	*Other*	5
Episyrphus balteatus	32	*Platycheirus clypeatus*	5
Syrphus ribesii	50	*Syrphus ribesii*	7
		Platycheirus manicatus	9
		Episyrphus balteatus	11
		Melanostoma scalare	19
		Platycheirus albimanus	30
		Platycheirus granditarsus	34
TOTAL	145	TOTAL	144

Table 4.2 Structure of the hedges sorted by width variable. For the width variable, categories were Narrow (1-2 m wide), Medium (2-3 m), and Wide (>3 m). For the height variable, categories were Short (1-2 m high), Medium (2-3 m), and Tall (>3 m). For the %-gaps variable, categories were Full (<1% gaps), Gaps (1-5% gaps), and Thinned (>60% gaps).

Site	Width (m)	Height (m)	%-Gaps
4	Narrow	Short	Full
5	Narrow	Medium	Gaps
8	Narrow	Tall	Thinned
1	Medium	Tall	Full
2	Medium	Medium	Full
3	Medium	Tall	Full
7	Wide	Short	Gaps
9	Wide	Tall	Thinned
10	Wide	Tall	Full

Table 4.3 The woody genera recorded in hedges at each site.

Woody Genera	Site 1	Site 2	Site 3	Site 4	Site 5	Site 7	Site 8	Site 9	Site 10
Acer spp.	X			X					X
Crataegus monogyna	X	X	X	X	X	X	X	X	X
Fagus sylvatica					X			X	
Fraxinus excelsior	X		X	X	X	X	X	X	X
Hedera helix	X	X	X		X	X	X	X	
Ligustrum ovalifolium			X		X	X	X		
Lonicera spp.		X	X						
Prunus spp.				X		X			
Prunus spinosa	X	X	X	X	X	X	X	X	
Quercus spp.									X
Rosa spp.		X	X	X	X	X	X		X
Rubus fruticosus	X	X	X	X	X	X	X	X	X
Salix cinerea									X
Sambucus nigra	X	X	X		X		X		X
Symphoricarpos albus									X
Ulmus spp.									X
Unknown							X		

of the crop, with some increases seen in other families as well (Figure 4.5). Total floral abundance, syrphids, and bees also did not change with distance from the crop's edge (Figure 4.6). The flowers in and along the hedge that were most visited by bees and syrphids were in the families Rosaceae (e.g. brambles) and Asteraceae (e.g. *Taraxacum* spp. – dandelions, see Appendix Figure B.2). The flowers in the crop that were most visited by bees and syrphids were in the families Brassicaceae and Polygonaceae (see Appendix Figure B.2). In the GLMM model, floral abundance and bee abundance both had significant, but small positive correlations with adult aphidophagous syrphid abundance (floral abundance effect <0.001, P = 0.014; bee abundance effect = 0.156, P = 0.015). Floral richness (P = 0.090) and the abundance of non-aphidophagous syrphids (P = 0.458) were non-significant.

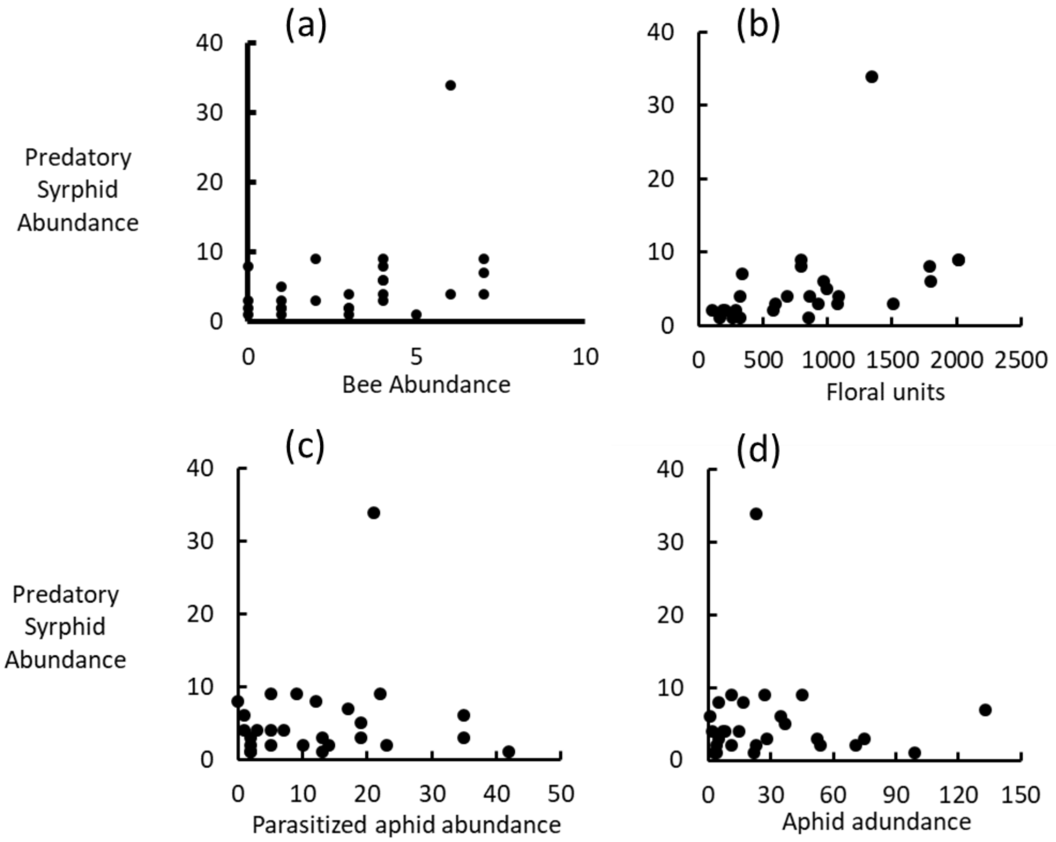

Figure 4.4 Scatterplot comparing aphidophagous syrphid abundance to (a) bee abundance (b) floral units (c) parasitized aphid abundance (d) live aphid abundance. Data was analysed by site (9) and round (3), such that there are 27 data points. Note that if the outlier is removed, only the floral units plot (b) still shows the same trend. The plots of only the variables that had a significant relationship with aphidophagous syrphid abundance are shown.

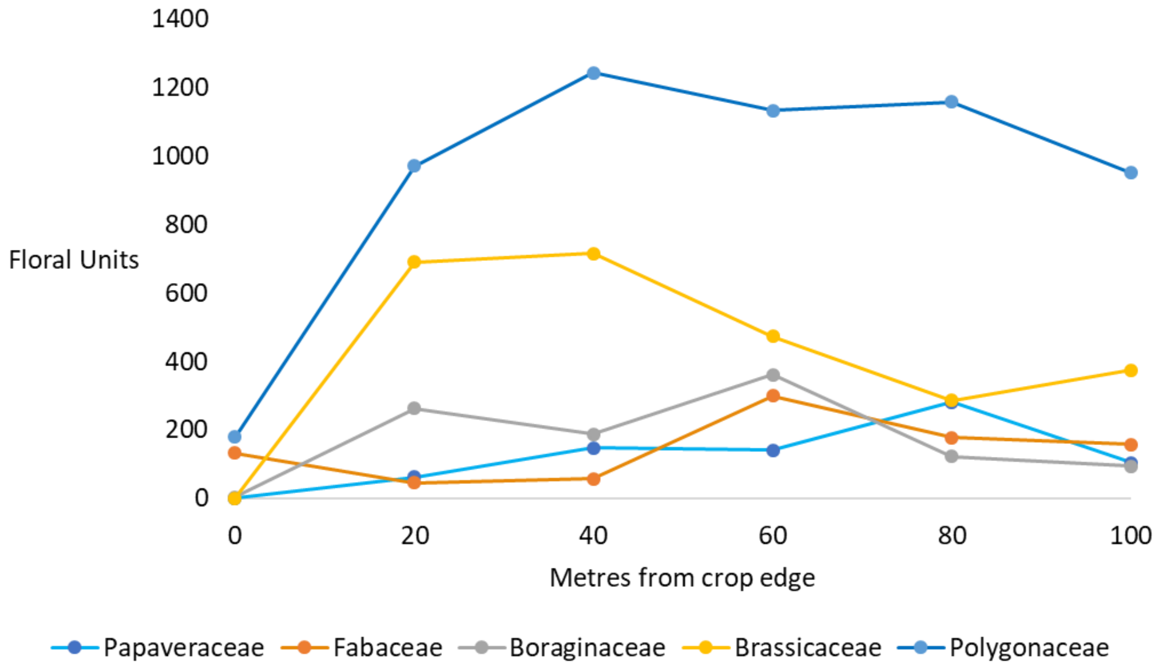

Figure 4.5 Floral abundance of the five most abundant families at different crop depths. Abundances are summed across all nine sites and three rounds. The connecting lines between the data points are to show trends.

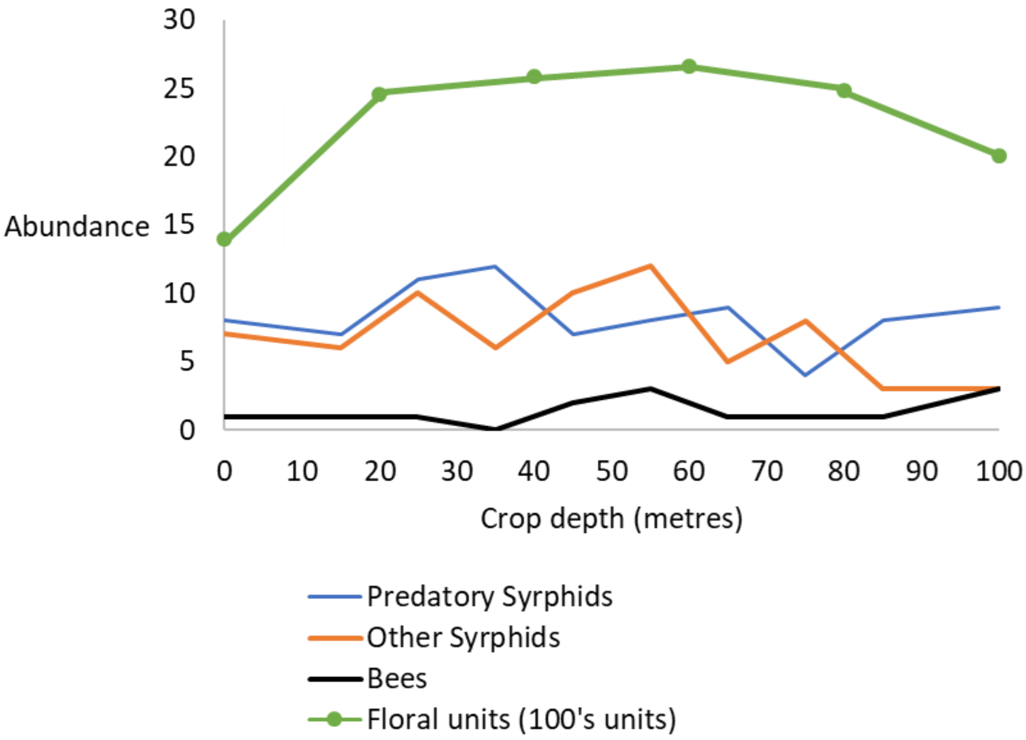

Figure 4.6 Abundances of aphidophagous syrphids, floral food resources, bees and non-aphidophagous syrphids at different crop depths. Syrphids and bees were observed from walking along a 100 metre transect where abundances were recorded between every 10 metres. Floral resources were observed in quadrats placed at 0, 20, 40, 60, 80, and 100 metres. The moving average trendlines included are to show trends. Syrphids and bees included individuals observed on flowers and oats.

4.4.4 Hypothesis 2 and 3: Relationships of aphid food resources and other aphid consumers with aphidophagous syrphids

Of the invertebrate individuals observed on the oats, 34% were live and 29% were parasitized (including individuals with holes, indicating the parasitoid had emerged and departed). The remaining 37% consisted mainly of adult non-syrphid flies and thrips (Thysanoptera), with occasional observations of syrphids, adult Hymenopterans, Coleopterans, Gastropods, etc. The number of live aphids did not appear to change with crop depth, but there was a decline in abundance with each successive round, with the first round in June having much higher abundances than recorded in the second and third rounds in July and August (Figure 4.8). Adult aphidophagous syrphids also did not appear to change with crop depth, but parasitized aphids increased slightly as one moved deeper into the crop (Figure 4.7). Contrary to aphid abundances during the season, though, the abundances of adult aphidophagous syrphids and parasitized aphids peaked in July (Figure 4.9). While parasitized aphid abundances were relatively high in June, aphidophagous syrphid abundances were fairly low (Figure 4.9). In the GLMM, aphid abundance had a significant negative correlation with adult aphidophagous syrphid abundance (-0.013, P = 0.043), and parasitized aphid abundance had a significant positive correlation with adult aphidophagous syrphid abundance (0.044, P = 0.023).

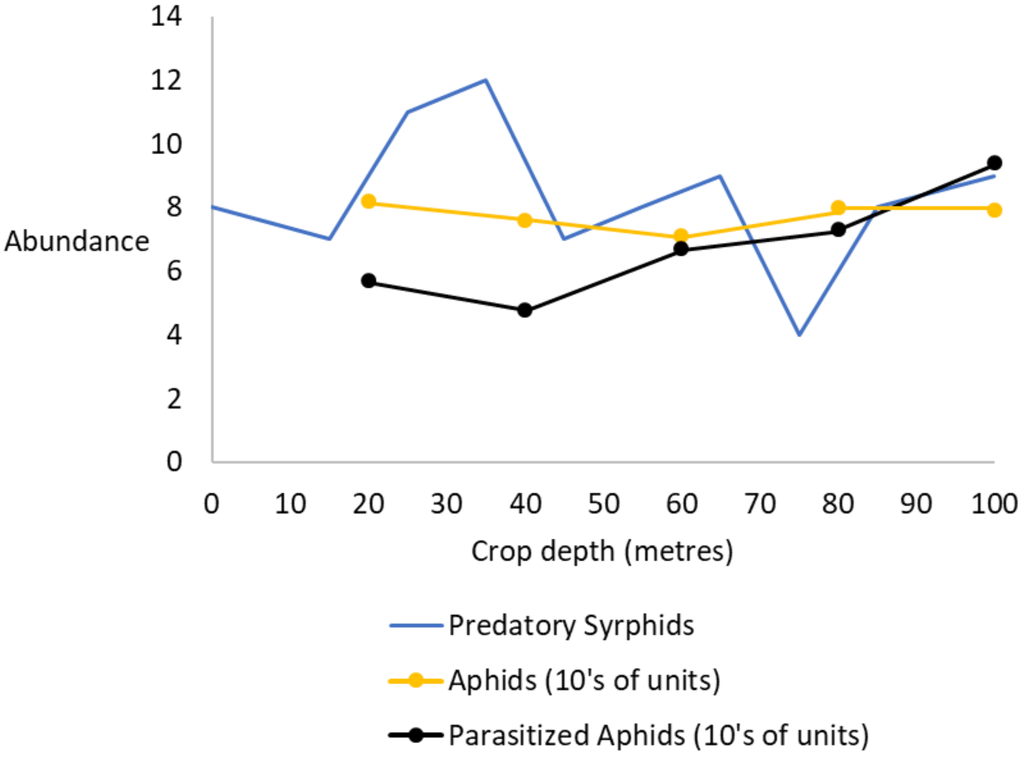

Figure 4.7 Abundances of aphidophagous syrphid adults, aphid food resources, and parasitized aphids at different crop depths. Aphidophagous syrphids were observed from walking along a 100 metre transect where abundances were recorded between every ten metres. Aphids and parasitized aphids were observed in quadrats placed at 20, 40, 60, 80, and 100 metres, the moving average trendlines included are to show trends. Aphidophagous syrphids included individuals observed on flowers and oats.

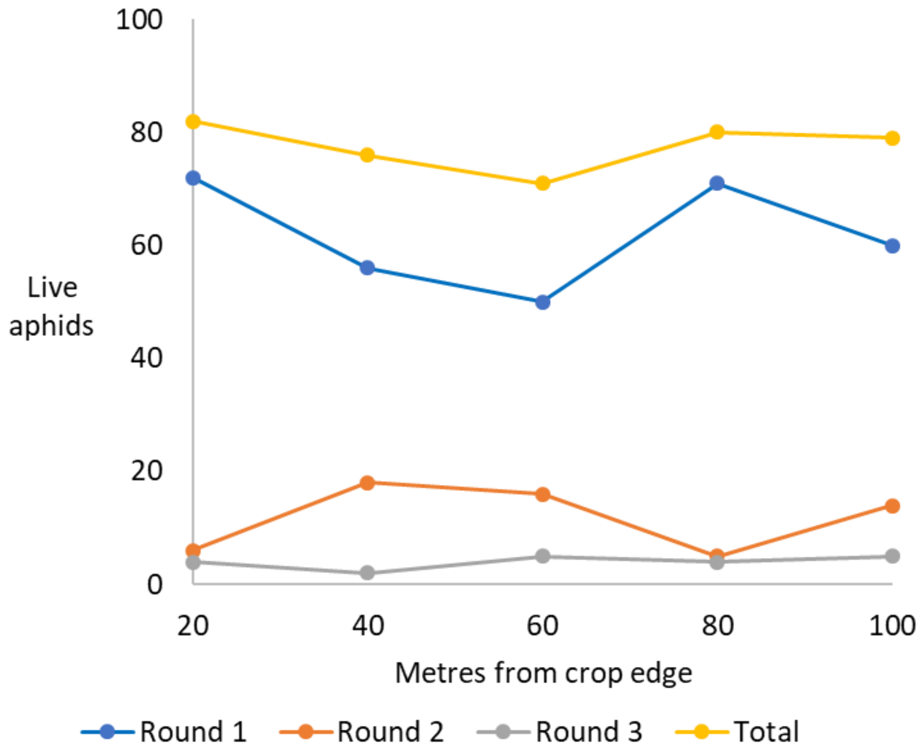

Figure 4.8 Aphid abundance across sites at different crop depths rounds one (early June), two (early July), and three (late July to mid-August).

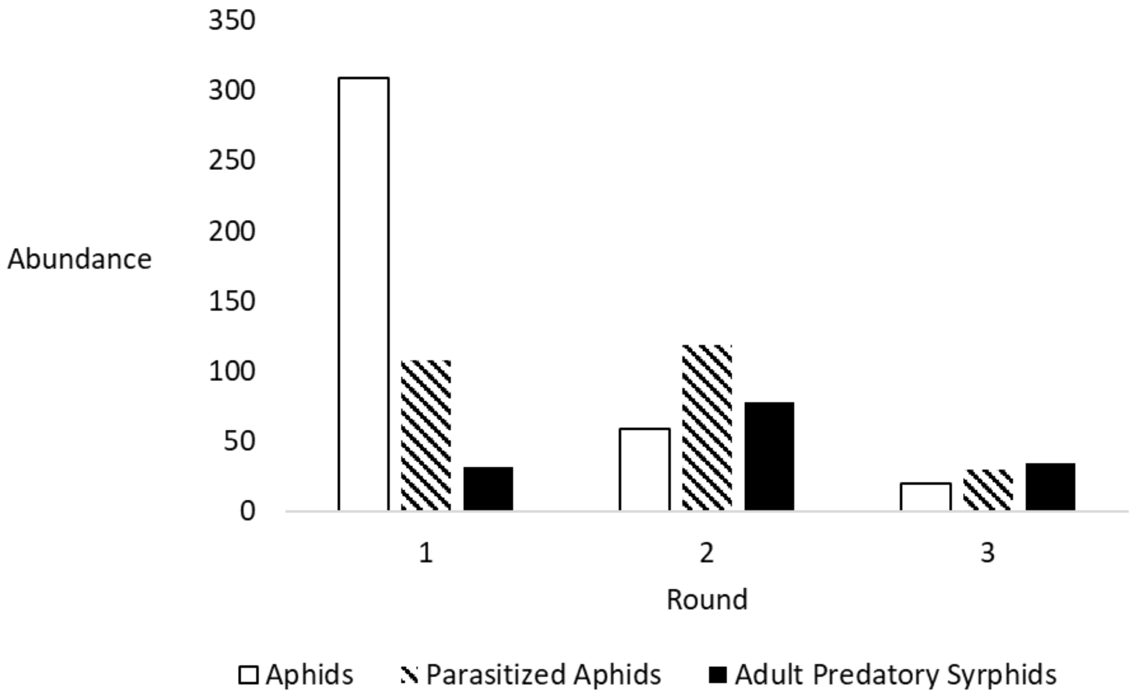

Figure 4.9 Abundances of aphids, parasitized aphids, and adult aphidophagous syrphids during rounds one (June), two (July), and three (late July to mid-August).

4.5 Discussion

Brambles and hawthorn were the most commonly found in the studied hedges, which are excellent floral food resources for syrphids and other insects with nectar and pollen diets (Drabble and Drabble, 1927; Gyan and Woodell, 1987; Power and Stout, 2011; Wignall et al., 2020). The results from the floral surveys reflect this, as the most visited flowers in the hedge were in the Rosaceae family, which includes brambles (*Rubus* spp.) and hawthorn (*Crataegus* spp., though there were no visitations to its flowers, as it was no longer in flower at time of surveying). There was much variability in the structures of the hedges according to the categories we used, but none of the measured hedge characteristics were significantly related to adult aphidophagous syrphids. This could be due to a small sample size, or an over-simplification of quantifying structure parameters. For example, only 100 metres were surveyed of the entire stretch of hedges that commonly surrounded all sides of the crop fields. Furthermore, the categories may not have fully captured the complex structure, which could have influenced the vegetative volume of the hedge, thereby the permeability allowing movement through the hedge and the availability of food and shelter. The biodiversity hedge appraisal system in Ireland, which attempts to rate hedge importance in ecological, historical, and landscape contexts, states that the most favourable hedges would have heights >4 metres, widths >3 metres, and no gaps (the entire stretch of hedge is filled in with woody vegetation), while an unfavourable hedge would have heights <1.5 metres, widths <1 metres, and >10% gaps (Foulkes et al., 2013). By these standards, most hedges in this study had favourable or highly favourable absence of gaps, adequate width or better, and favourable height or better. What these standards mean for habitat suitability for syrphids is still very little understood though. Graham et al. (2018) conducted a literature review of research on hedge structure correlations with biodiversity and found that certain features of hedges like width and gaps correlate with some invertebrate groups, but how they correlate is not the same across all taxa. Baudry et al. (2000) cautioned against too much focus on the hedge structure relationship with biodiversity, particularly in isolation from other contributing factors such as the surrounding landscape features, crop size, and conventional pest management in nearby crops that could include pesticide applications (Haenke et al., 2014; Moquet et al., 2018; Power et al., 2016; Rodríguez-Gasol et al., 2020). This is argued on the basis that no hedge can support all taxa, and that too intensive management of the hedge for this purpose runs the risk of doing the opposite and disturbing and damaging a habitat to no longer be suitable for the taxa that already use it (Baudry et al., 2000). Some actions organic farmers can take to make hedges more attractive to bee and syrphid pollinators without drastically changing the habitat would be to add vegetation that provide floral food resources throughout the year, and restrict disruptive management to November through January when invertebrates are less active (Foulkes, 2016). Lewis (1969) discussed possible ways hedge structure may influence syrphid abundance and distribution in fields, believing that overgrown hedges would be preferred as hibernation sites leading early syrphid abundances to stay close to the hedge, whereas heavily managed and disturbed hedges would support fewer early populations of syrphids and thereby abundances in fields would be more widely distributed. Lewis (1969) also acknowledges that hedges attractive and supportive to beneficial invertebrates can do the same for pests and contribute to infestations, and therefore it is smaller crop fields that are more likely to benefit from pest control of hedge-supported syrphids as their distribution will cover more of the crop (Lewis, 1969). Given that organic crop fields are commonly small, hedge management for predatory syrphids may be effective for pest control despite potential attraction of aphid pests. However, it should be noted for all analyses, that there was an outlier of aphidophagous syrphid abundance at the one coastal site in the second round of surveying (during July), which could be influencing the results of our analyses.

Floral food resources and other consumers of these resources, however, did have a positive relationship with aphidophagous syrphids, though they were small effects. Along the hedge, Rosaceae was the most visited family of flowers by bees and syrphids and one of the most abundant, particularly brambles which occurred at every site. In the crop, Brassicaceae and Polygonaceae were both the most visited by bees and syrphids and the most abundant. Flowers from these families tend to be open, with accessible nectar and pollen, suiting the relatively short mouthparts of syrphid flies. It is possible that the relationship of aphidophagous syrphid abundance with floral abundance may be most influenced by these families, so sites with brambles and hawthorn in the hedges, and wildflower strips and field margins containing open flowers such as mustards, could be the most attractive and supportive of aphidophagous syrphids. The finding of floral abundance being positively correlated with syrphid abundance agrees with other studies as well (Power et al., 2016; van Rijn et al., 2006). There was also a positive correlation between bee and aphidophagous syrphid abundances visiting flowers, which counters the hypothesis that there would be competition for these floral resources. Instead, it appears that habitats that attract and support bees can do the same for aphidophagous syrphids. This is good for farmers who are interested in supporting bees for their pollination services but also want to support aphid predators such as syrphids, which are also capable of pollinating crops. This is also encouraging for conservationists who are interested in habitat management to support various invertebrate wildlife. Interestingly, non-aphidophagous syrphids did not correlate with aphidophagous syrphids. It could be that the non-aphidophagous abundance is driven less by floral resources, and more by the proximity and quality of other nearby habitats besides hedges that would have provided for their larval needs, such as pastures for syrphids with larvae that develop in dung or stagnant waters or leaf piles (Power et al., 2016). This could explain why no relationship was found between the two syrphid types.

Aphids and parasitized aphids were the most abundant invertebrates found from the hand searches of the oats, and both had correlations with the abundance of adult aphidophagous syrphids. Both hypotheses were countered by the model results, where aphid food resources had a negative relationship with aphidophagous syrphid abundance, and other consumers of aphids (shown as parasitized aphids) had a positive relationship with aphidophagous syrphids. However, this could be explained by predator-prey population cycles, where initial prey populations were very abundant (as seen in sampling round one), attracting more predators and parasitoids (in round two), which consumed and diminished the prey food resources. In round three, aphids, predatory syrphids, and parasitized aphids all decreased in abundance from round two, suggesting that as aphid resources are being diminished by consumers and the mature oats become less suitable hosts for the aphids, the declining aphid populations support fewer consumers. As for parasitized aphids having a positive relationship with adult aphidophagous syrphids, it appears that crops that attract and support parasitoids do the same for aphidophagous syrphids. Therefore, the hypothesis that other guilds that utilize the adult food resource of flowers and larval food resources of aphids for aphidophagous syrphids do not apparently compete for these resources, but rather can co-exist and are indicative of a habitat that could support aphidophagous syrphids. Again, however, it should be noted that juvenile syrphids were rarely observed on the oats, which could suggest that the aphidophagous syrphids present were utilizing aphids on other host plants in or near the crops. Aphidophagous syrphids have been acknowledged as important natural enemies of cereal crop pests (Dunn et al., 2020; Rodríguez-Gasol et al., 2020), however, as there have been studies finding numerous juveniles preying on aphids in crops (Ramsden et al., 2017), and studies have shown a single larva can consume hundreds of aphids before reaching the pupal stage both in the lab and under field conditions (Tenhumberg, 1995).

Overall, we found that structure and woody composition of the hedges did not correlate with aphidophagous syrphid abundance, but that could be due to the small sample size and measurements that did not fully capture the complex aspects of the hedges. Future studies should investigate the relationship between syrphids and hedge structure and management, as studies have demonstrated that hedge presence and certain characteristics can play a role in the support and attraction of syrphids (Garratt et al., 2017; Miñarro and Prida, 2013; Power et al., 2016). This study did show, however, that higher floral abundance tends to attract and support adult aphidophagous syrphids. Irish farmers interested in the attraction and conservation of aphidophagous syrphids could potentially use current guidelines for floral resource provision in support of bees (e.g. Fitzpatrick et al., 2017 and Foulkes, 2016) Another reason these guidelines can be referred to is because we observed no apparent abundance restrictions from dietary competition between aphidophagous syrphids and bees. Aphid abundances had a negative relationship with aphidophagous syrphids, where aphid numbers declined from June through August, but syrphid numbers peaked in July, suggesting a natural predator-prey population cycle where syrphids might have contributed to controlling aphid populations. These cycles can be disrupted by the use of agrochemical insecticides (Beers et al., 2016; Geiger et al., 2010; Ricci et al., 2019). Cereal grain farmers could benefit from the pest control services of aphidophagous syrphids by providing floral resources in margins and hedges of the crops, a practice that can support and attract other ecosystem service providers as well.

Chapter 5

General Discussion

5. General Discussion

Most research to date on beneficial insects in agriculture and how to protect, attract, and support them has focused on bees as pollinators, and various predatory invertebrate groups such as beetles, spiders, and parasitoid wasps. Aphidophagous syrphids receive less attention than some of these groups, despite their role as ecosystem service providers of both pollination and pest control (Dunn et al., 2020). In this thesis, I investigated factors that could have a positive (floral resources and aphids) and negative (food resource competitors, insecticides) influence on aphidophagous syrphids, as well as hedge habitat structure, in an Irish farmland context. The effects of insecticides causes contention between farmers, who need to protect their crops from pests and diseases, and conservationists, who are concerned about the decline of biodiversity, with evidence linking declines across taxa to insecticide use (e.g. Hallmann et al. 2014; Yamamuro et al. 2019). The intention of this work was to inform both farmers and conservationists, as well as policymakers, about what can be done to manage crops in a way that supports syrphids. This can have benefits for conservation, as well as for pollination and pest control on farmland. Since there is much concern that insecticides could be harmful to insect populations, which would reduce their performance in controlling pests and pollinating flowers, but little work has been done for syrphids, I investigated insecticides first.

5.1 Insecticidal impacts on and risk assessments of Irish aphidophagous syrphids

5.1.1 Insecticide residues

From my study investigating insecticide residues in aphidophagous syrphid food resources (Chapter 2), while pyrethroids were not detected in any of the samples, neonicotinoids were detected in every sample type (crop aphids, and nectar and stamens of the wildflower *Rubus fruticosus* agg.) and from organic and conventional crops. It should be emphasized that no detections does not mean absence of residues, especially given the small sample size (one sample each of three media types at six sites, giving 18 samples total), and the high detection limits (minimum of 0.49 µg/g permethrin in stamens for pyrethroids, and the one undetected neonicotinoid had a maximum limit of 27.49 ng/g clothianidin in stamens and aphids). These limitations of the study could explain the low detection rates where, out of six samples of each type, only one nectar, three aphid, and four stamens samples had detections of one pesticide each (out of six pesticides investigated). Regardless, the fact that about as many detections were found from organic sites as conventional sites is cause for concern. This is not surprising though, as it is in agreement with multiple studies that have demonstrated persistence and mobility of neonicotinoids in the environment (Casado et al., 2019; Humann-Guilleminot et al., 2019; Silva et al., 2019; Wintermantel et al., 2020).

More investigations should be done on other systemic pesticides as well, such as the fairly new on the market sulfoxaflor and flupyradifurone (Siviter and Muth, 2020). More attention in future studies should also be given to residues occurring in crop pests and herbivorous invertebrates on nearby wild plants, as predators could consume and be impacted by these, working counter to conservation efforts and natural pest control. While aphids that survive exposure to neonicotinoids can experience negative sublethal effects (e.g. decreased reproductivity, feeding activity, and development rate; Daniels et al., 2009; Sarhozaki and Safavi, 2014; Qu et al., 2015), and these effects can decrease pest population size therefore reducing risk of chemical exposure to their predators, the detection of pesticides in live field-collected aphids in my Chapter 2 study suggests that exposure can happen. The severity of the risk posed to pest controllers needs to be understood in terms of palatability and toxicity of pesticide-laced prey, what levels of what types of pesticides are found in their prey, etc. This is particularly important beyond pest controllers to consider pollinators too, as Calvo-Agudo et al. (2019) found that pest honeydew can be a source of pesticide residues to beneficial insects. Future work should be conducted on how much beneficial insects consume and rely on honeydew, from what plant-pest combinations do they feed from, and what are field-realistic concentrations. I attempted to collect honeydew from cereal aphids in my Chapter 2 study for testing of residues, however I was not able to collect enough for analysis. Theoretically, it can be done for cereal grain crop pests, as Ajayi and Dewar (1982) were able to collect over five days an average of 108 µL and 79 µL honeydew per aphid of the species *Sitobion avenae* and *Metopolophium dirhodum* respectively, while the aphids fed on wheat flag leaves. A better understanding of honeydew as a source of food and pesticide exposure is needed to fully understand the harm to invertebrate wildlife, and so I would encourage future studies collect and analyse honeydew separately from the pest if possible.

5.1.2 Effects on syrphids from oral exposure to insecticide residues

Having identified that residues are present in aphidophagous syrphid food resources in Ireland, the next question was what impact these might have on them, which was investigated in Chapter 3. Originally, I attempted to measure the response of *Episyrphus balteatus* adults to consuming residual thiacloprid in nectar, which was the neonicotinoid I detected most frequently in field-collected samples in Chapter 2. However, lethal effects were low and inconsistent, even after consuming sugar-water containing the highest concentration of thiacloprid that is physically possible for water to dissolve (175 ng/µL). Thiamethoxam is more soluble in aqueous mixtures than thiacloprid, which made delivering higher doses, and observing lethal effects, easier for thiamethoxam. However, even at some lower doses of thiamethoxam, an immediate agitated response was observed, suggesting that *E. balteatus* is more sensitive to thiamethoxam than thiacloprid. This is not surprising, as Laurino et al. (2011) saw a similar response in *Apis mellifera*, whereby all bees survived three days or longer from receiving acute exposure thiacloprid at a concentration of 144 ppm, but the 24-hour LC50 for thiamethoxam was 0.134 ppm.

What is surprising, however, is the comparison of the results from Chapter 3 to other studies. For example, Calvo-Agudo et al. (2019) observed high sensitivity of the aphidophagous syrphid *Sphaerophoria rueppellii* to thiamethoxam residues in mealybug honeydew. Though, I would argue this is due to the methodologies used, and therefore much attention should be given to methodology when conducting risk assessments to inform policies on pesticide use. It is not that one of these methodologies is better or worse than the other, but rather that they answer very different questions. Calvo-Agudo et al. (2019) exposed adult syrphids immediately upon eclosion from pupae to a chronic diet of thiamethoxam-laced honeydew, whereas I exposed adult syrphids acutely with a one-off dose of thiamethoxam-laced nectar substitute five days after eclosion, and for the rest of the time the syrphids in my study received non-contaminated food. I would argue that the approach of Calvo-Agudo et al. (2019) would be a worst-case scenario of exposure, whereas mine is a best-case scenario.

What I would criticize about these studies is perhaps the field-unrealistic conditions of the exposure. For example, my acute exposure of neonicotinoid-laced nectar is unrealistic, given what we know of the persistence and mobility of neonicotinoids in the landscape. If an insect can consume neonicotinoid residues from one flower, it is very possible it can consume residues from the flower next to it, within the same flower-patch, within the same field, and sometimes even beyond into adjacent fields or farther – as was observed in my Chapter 2 study, where residues were found in organic fields. Chronic exposure can also be more detrimental to the health of insects, as the lethal dose can be a fraction of what would be observed from acute exposure (Descher and Geusen-Pfister, 1991; Suchail et al., 2001). I decided on acute exposure as I was interested in the exact dose mass of insecticide the syrphids were receiving. Logistically, I could not find a way to observe how much nectar they were consuming in a chronic exposure scenario, in which I would need to know to calculate dose. However, in terms of translating the results of these studies into understanding the risks syrphids face in the field, it would have been more realistic to prioritize chronic exposure over identifying exact dose mass consumed. The mass dose is still useful to know, however, as residue concentrations are unlikely to be the same from flower to flower and in each habitat and landscape, and the mass dose received also depends on how much the individual insect feeds.

For Calvo-Agudo et al. (2019)'s study, I described it as "worst-case scenario" due to the combination of chronic exposure, no-choice food supply, and exposure immediately upon emerging from the pupae, although not all of these would be considered field-unrealistic. As already discussed, chronic exposure is realistic in the field, however no-choice food supply is not. From my observations of individuals eclosing from pupae in Chapter 3, even without food provided within 24 hours or more, *E. balteatus* adults would fly and walk around their 40 cm x 40 cm x 90 cm emergence cage, probing the walls likely in search for food. While I have observed predatory syrphid larvae pupating near the vicinity of the Hemipterans they feed on, and therefore upon emergence it is possible that honeydew from remaining Hemipterans might be their first source of food, it is unlikely that honeydew will be their sole source of food for an extended period of time.

5.1.3 Additional areas for future research of insecticide residues and exposure effects

There are many additional questions that should be investigated in the future to better understand predatory syrphid dietary exposure and susceptibility to pesticide residues in the landscape. In Chapter 2, I found that residues could be found in their adult food resources of flower nectar and pollen-laden stamens, and larval food resource of crop aphids. In Chapter 3, I investigated how adults of one species (*Episyrphus balteatus*) responded to residues of one pesticide (thiamethoxam) in sugar-water (nectar substitute), and I observed mortality, behaviour, and grip ability. There are still numerous syrphid species, pesticides, dietary exposure routes, sublethal effects, and life stages that have yet to be investigated. I argued that one of the reasons why *Sphaerophoria rueppellii* appeared to be so much more susceptible to dietary pesticide exposure was due to methodology, though species differences could also play a part (Calvo-Agudo et al., 2020). Research also needs to be carried out to understand the levels of pesticide residues in the field of syrphids' prey and herbivore honeydew, and more research needs to be done on how all these contaminated food resources (floral nectar and pollen, prey and their honeydew) may influence syrphids and other beneficial insects when consumed. There also needs to be variation in the kinds of consequences investigated. Mortality and lifespan need to be investigated alongside sub-lethal effects, such as behaviour, reproductivity, and motor skills in flight, gripping, walking, and finding and catching prey. The sub-lethal effects of exposed larvae should be investigated beyond the larval stage, investigating whether adults eclose from pupae, and the state of the emerged adults. Furthermore, syrphid reactions to contaminated food should be investigated. A recent study found that the syrphid *Eristalis arbustorum* appeared to be able to detect and avoid neonicotinoid-contaminated floral foods, though this was not the case for *Toxomerus marginatus* (Clem et al., 2020). Whether this discrimination can be observed in ovipositing females, where they avoid contaminated prey to protect their progeny (e.g. *Episyrphus balteatus* females lay fewer eggs on plants treated with pyrethrum, Pineda Gómez, 2008), should also be investigated. While larvae's ability to detect and avoid contaminated prey would also be interesting to investigate, the larval stage is not nearly as mobile as the adult, so even if larvae could detect residues in their prey, they may not have an alternative. Furthermore, studies should also attempt to ask questions in the context of field-realistic conditions, such as how does exposure influence mortality in unfavourable climate conditions, when avoiding predators or infected with bacteria such as Wolbachia, or when food resources are scarce?

5.2 Hedge habitat correlations with aphidophagous syrphids

In Chapter 4, hedge structure was found not to have a correlation with aphidophagous syrphid abundance. This could be due to several factors: small sample size, lack of enough variability between hedges sampled in order to determine a difference in associated syrphid abundances, the methodology used to measure hedge structure, etc. Ahmed et al. (2020) looked at % gaps of hedges as well as presence of adjacent watercourses, and also found no significant relationship with syrphid abundance. Ahmed et al. (2020) did find a significant relationship with sciomyzids (Diptera), where abundances were higher when watercourses were present alone or with an adjacent hedgerow with >50% gaps. Garratt et al. (2017) found only the distance from the hedge influenced syrphid crop abundance, but for linyphiid spiders the quality of the hedge was also influential. My finding that syrphids had no correlation with the measured structure of the hedge could be good for farmers wanting to manage hedges to benefit different kinds of wildlife – they can consider less the needs of the syrphids and focus more on species and wildlife groups that may have more specific needs when it comes to hedge structure, such as birds and spiders (Hatley and Macmahon, 1980; Hinsley and Bellamy, 2000). Perhaps more important considerations of hedge habitats are the management regimes and the time of year hedges are cut, with such work potentially killing individuals residing in the hedge and it could temporarily limit floral and prey food resources. For farmers, it is also important to consider how hedges may harbour and disperse pests into adjacent crops, as well as other potential disservices (e.g. attracting pollinators and predators away from the crop). Recently, Saunders (2020) conducted a literature review of studies investigating ecosystem services and disservices and noted that few studies included both trade-offs and the root causes of the disservices. There was also an issue with studies measuring abundances of potentially harmful wildlife (e.g. hornets and "weeds") without measuring the harm or direct disservice to humans (Saunders, 2020). Such studies of pest impacts on crops associated with hedges should measure the performance of the crop, and not the abundance of pests.

5.3 Food resource and competitor correlations with aphidophagous syrphids

In Chapter 4, aphidophagous syrphid abundance was positively correlated with the abundance of both their hypothesized competitors for food resources (flower-visiting bees and aphid parasitoid activity). This suggests that these sites are attractive to and can support a variety of pollinators and pest controllers. Likewise, however, that would suggest that these insects could also be exposed to pesticides in these food resources. Therefore, depending on the resilience of each taxon to pesticides, the abundances of different groups of ecosystem service providers could be changed, which could translate to diminished biological pest control and pollination services. Greenop et al. (2020), instead of investigating changed abundances, investigated the sub-lethal effect on predation efficiency of individuals after pesticide exposure. From observing different species of Coleoptera, they found the effects on predation performance to vary greatly between species, and translating this to community level responses, Greenop et al. (2020) concluded that more diverse predatory communities are more likely to continue pest control provision after pesticide exposure. This highlights both the importance of ecosystem service provider diversity in crops, and of the inclusion of syrphids in these communities, as my study in Chapter 3 suggests potential of adult syrphids, when adequate food supplies are available, are resilient to at least acute oral exposure of thiamethoxam.

The abundance of floral resources also had a positive correlation with aphidophagous syrphid abundance, but pesticide residues in floral resources could deter some syrphid species (Clem et al., 2020) or cause other sub-lethal effects to behaviour. From Chapter 3, under optimal climate conditions and food availability, and absence of predators and pathogens, acute exposure of active flies to residues in sugar-water resulted in decreased flight and grooming, and increased resting three days after exposure. Although acute doses were unrealistically high, had flies been exposed to less than optimal conditions, the effects seen in this work may have been more pronounced, or effects could have been seen in lower-dose treatments. For example, a decrease in flight and increase in resting would make it more difficult for syrphids to move between and select flowers for foraging. Grip ability was also affected by ingestion of pesticide, whereby dose was negatively correlated with ability to grip surfaces when the surface changed position. This could make it more difficult for syrphids to maintain contact with leaves and flowers if disturbed by wind or passing animals. The positive impacts of floral abundance attraction of aphidophagous syrphids could be hidden by pesticide exposure in conventional crops.

Pesticide exposure could also disguise the pest control benefits of having high abundances of aphidophagous syrphids in crops, not just from killing aphid pests directly but from exposing syrphids as well. In Chapter 4, crop aphid abundance on organic crops negatively correlated with aphidophagous syrphid abundance, though this seemed to be linked to time of year and reflect predator-prey dynamics – aphids had high abundance at the start but then declined throughout the summer, whereas syrphid adults peaked in the middle of the summer. It is unknown whether these syrphid population increases came from matured juveniles in the crop, or from adults migrating in from outside the crop, though this is an important question regarding potential exposure to residues in aphids. In the Chapter 2 study, aphids were collected mostly in June, and from these aphids I detected residues. If syrphid larvae were feeding on contaminated aphids early in the season when syrphid abundances were building up, this could reduce their mid-summer population peak and possibly reflect a change in control of pests throughout the summer. This reduction could be seen for many aphid-consumer abundances in the crop, such as parasitoids whose activity levels showed a similar temporal pattern in my Chapter 4 study as aphidophagous syrphids.

5.4 Additional future directions for research

There are other, lesser-studied ways to attract aphidophagous syrphids and other predators into crops that should be investigated in order to make biological pest control a more economically attractive and feasible method for farmers. For example, studies have suggested that alarm pheromones emitted by either aphids or plants may attract syrphids and other predators to an area searching for prey, and therefore spraying these pheromones may be a safer way to control pests by attracting natural enemies rather than applying indiscriminatory insecticides (Leroy et al., 2010; Riddick, 2020; Vosteen et al., 2016). More research is needed in terms of the efficacy of this approach and to design an application strategy. Another practice is ensuring a variety of wildflowers not just to serve as floral food resources, but to harbour non-crop herbivores to serve as alternative prey to support and create a stock of predators for when crops and their pest prey are not available. Predatory syrphid juveniles can overwinter within and in the margins of cropped fields, which has been linked to reduced crop aphid abundances in the spring (Raymond et al., 2014), and adults are mainly gravid females that shelter in practically anything available, such as trees, caves, and cracks in human-made structures (Rodríguez-Gasol et al., 2020). Research in terms of what forb-herbivore combinations or “banker plant systems” are attractive to crop pest natural enemies and work efficiently in the field could help optimize this approach.

In addition, other deterrents and harmful practices should be investigated as well. This work focused on insecticide chemicals in syrphid diets, which target insects and therefore are of obvious concern to syrphids and other predators. However, other chemicals such as herbicides and fungicides should also be researched for impacts on syrphids, as these have been found to occur in floral food resources (Daniele et al., 2018; David et al., 2016; Main et al., 2020; McArt et al., 2017). Combinations of pesticides should also be investigated for their effects on syrphids, as exposure to multiple pesticides in the field is common (Daniele et al., 2018; David et al., 2016; Main et al., 2020) and can result in synergistic effects (Botías et al., 2020; Pisa et al., 2015; Yang et al., 2017). Also, although aphidophagous syrphid abundance was found to have a positive correlation with bee abundance in Chapter 4, the presence of nearby colonies of forage intensive species such as *Apis mellifera* hives or commercial *Bombus terrestris* nests could have different results. For competitors for aphid food resources in Chapter 4, parasitized aphid activity served as the studied competitor, though there are many other predators to investigate such as ladybirds, lacewings, and spiders. Such studies looking at predatory competitors for a crop pest should also attempt to link predator diversity and richness to pest control. Even if a single or a few predators dominate in a crop, or competition causes some groups to be excluded, pest populations may still be controlled by the pest consumers present.

While hedges and flower strips are considered attractive to invertebrate pollinators and pest controllers, their effectiveness at distributing these invertebrates into the crops is usually concentrated around the edges of the crop nearest these habitats (Albrecht et al., 2020; Garratt et al., 2017; Woodcock et al., 2016b; Zamorano et al., 2020). Smaller crop field sizes or intercropping cereal grain fields may help attract and distribute predators better and the breaks could make aphid movement throughout the crop more difficult. However, there is a cost for the farmer of reduced land to farm and difficulty manoeuvring machinery. Kirchweger et al. (2020) found that even though flowers received fewer visits from pollinators with increasing crop size, the yield loss from this was overshadowed by the total yield of a larger crop. The costs and benefits of this smaller field size approach should be researched, including ways of making it a more attractive method for farmers.

5.5 Conclusion

One of the key findings of this thesis is that neonicotinoid residues have been observed in the food resources throughout the life cycle of aphidophagous syrphids in Irish cereal crops, which includes wildflower nectar and stamens, and crop aphids. While one of the most common aphidophagous syrphids in Irish cereal crops, *Episyrphus balteatus*, appears to be very resilient to acute dietary exposure of thiamethoxam in sugar-water, sub-lethal effects were observed and more research is needed to understand effects under chronic exposure, exposure to other pesticides and mixtures of pesticides, exposure in other food resources and life stages, and exposure alongside other, field-realistic stressors. However, because of the potential resilience of *Episyrphus balteatus*, it is possible that aphidophagous syrphids are one of Ireland's more hardy pollinators and pest controllers, which highlights their importance among other ecosystem service providers that may be more sensitive to conventionally managed crops. Aphidophagous syrphids are also capable of coexisting with other floral visitors and aphid parasitoids in cereal grain crops, allowing for conservation for and ecosystem services from multiple groups. High floral abundance is important for attracting aphidophagous syrphids, which in turn appears to reduce early crop aphid abundances. With proper practices, aphidophagous syrphids can be supported in cereal grain crops, and the crops can reap the pest control benefits from their presence. This body of work added new scientific evidence upon which to base such practices and identified future research directions to strengthen that foundation of evidence.

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7. Appendices

Appendix A. Supplementary data for Chapter 3

Section A.1 Explanation of concentration selected for LD100 honey bee test.

Laurino *et al.* (2010) reported a concentration of 0.5 ppm thiamethoxam in a 25% sucrose solution was 100% lethal to bees when they consumed 35 µL. The unit ppm can mean either ng/µL (35 µL of 0.5 ng/µL would be a 17.5 ng dose) or can mean ng/mg, in which the density of the sucrose solution would need to be considered. In this current study, a 50% sucrose solution with a density of 1.14 mg/µL was used. A 0.5 ng/mg concentration in a sucrose solution with density of 1.14 mg/µL would have a mass per volume concentration of 0.57 ng/µL, and thereby 35 µL would deliver a dose of 19.95 ng pesticide. As Laurino et al. used a 25% sucrose solution, the density of their solution would have been lower than that used in this study, and therefore the concentration per volume would not surpass 0.57 ng/µL, and as a result 20 ng was selected as a sufficient confirmation dose.

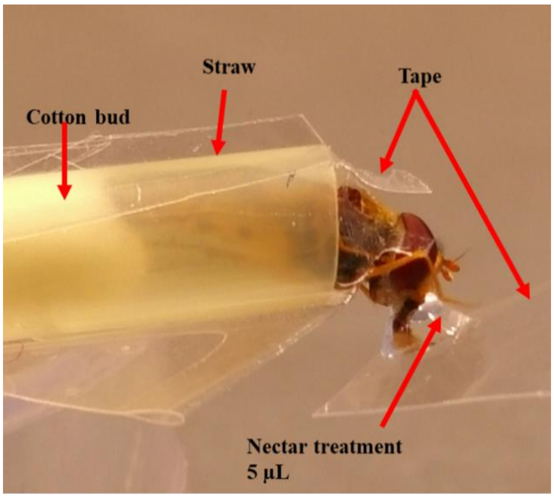


Figure A.1 Feeding harness for hover fly exposure to spiked sugar-water solution treatments.

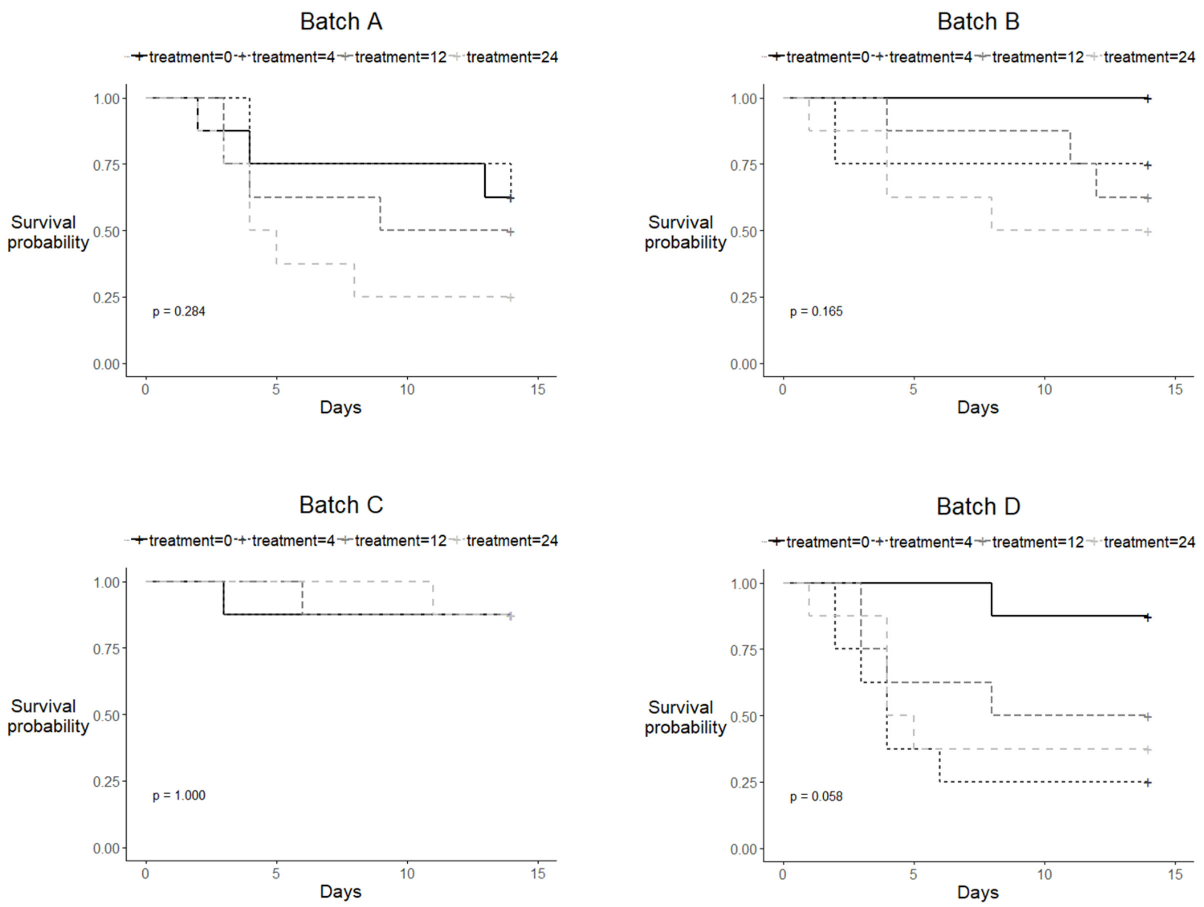


Figure A.2 14-day Kaplan-Meier survival curves of select concentrations (0, 4, 12, and 24 ng/µL) for each individual batch. P-values are for the global log-rank test assessing any differences between the four selected survival curves.

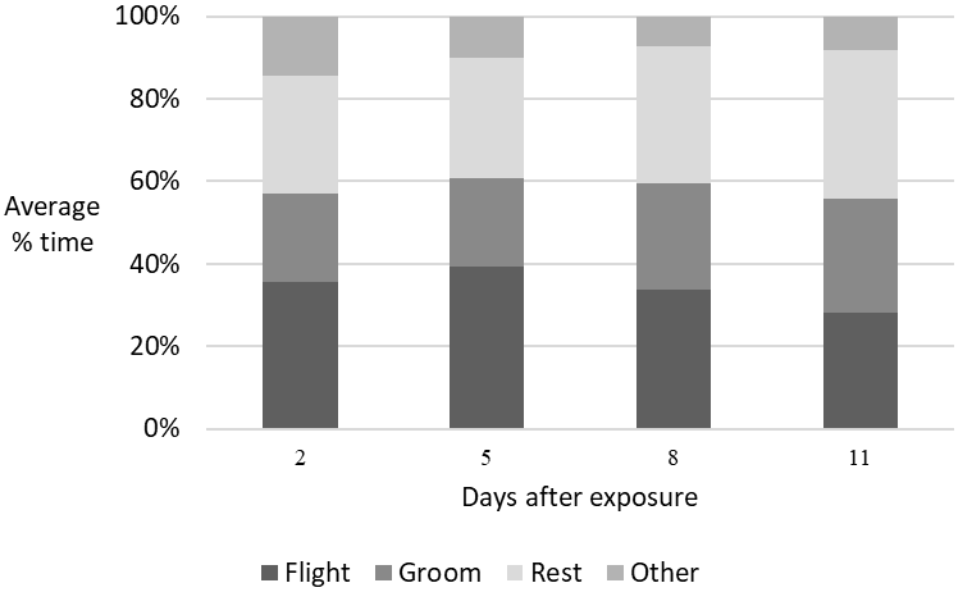
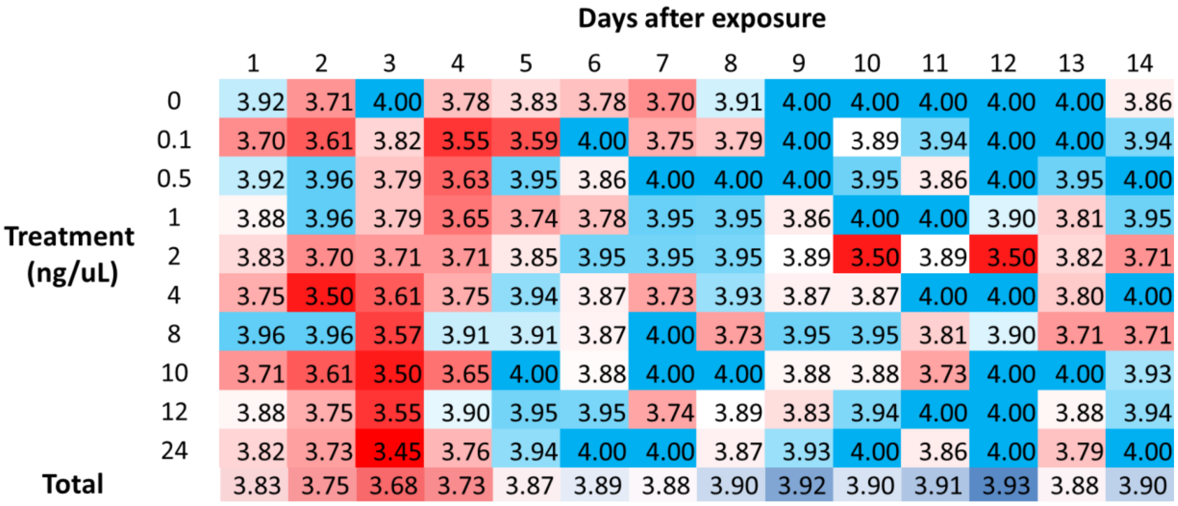


Figure A.3 Average percent time of all surviving flies on any day doing an activity. The other category is the summation of the average percent times of all other activities, which was smaller than the average percent times for each of flight, grooming, and resting.



		Days after exposure													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Treatment (ng/uL)	0	3.92	3.71	4.00	3.78	3.83	3.78	3.70	3.91	4.00	4.00	4.00	4.00	4.00	3.86
	0.1	3.70	3.61	3.82	3.55	3.59	4.00	3.75	3.79	4.00	3.89	3.94	4.00	4.00	3.94
	0.5	3.92	3.96	3.79	3.63	3.95	3.86	4.00	4.00	4.00	3.95	3.86	4.00	3.95	4.00
	1	3.88	3.96	3.79	3.65	3.74	3.78	3.95	3.95	3.86	4.00	4.00	3.90	3.81	3.95
	2	3.83	3.70	3.71	3.71	3.85	3.95	3.95	3.95	3.89	3.50	3.89	3.50	3.82	3.71
	4	3.75	3.50	3.61	3.75	3.94	3.87	3.73	3.93	3.87	3.87	4.00	4.00	3.80	4.00
	8	3.96	3.96	3.57	3.91	3.91	3.87	4.00	3.73	3.95	3.95	3.81	3.90	3.71	3.71
	10	3.71	3.61	3.50	3.65	4.00	3.88	4.00	4.00	3.88	3.88	3.73	4.00	4.00	3.93
	12	3.88	3.75	3.55	3.90	3.95	3.95	3.74	3.89	3.83	3.94	4.00	4.00	3.88	3.94
	24	3.82	3.73	3.45	3.76	3.94	4.00	4.00	3.87	3.93	4.00	3.86	4.00	3.79	4.00
Total		3.83	3.75	3.68	3.73	3.87	3.89	3.88	3.90	3.92	3.90	3.91	3.93	3.88	3.90

Figure A.4 The average number of held flips (from 4) in the grip test for each treatment group on each day after exposure, as well as the average for all flies across treatments at the bottom. Note that the average includes all surviving flies on any given day, meaning the number of individuals included on a day changes on the consecutive days as flies die. Red cells are low grips-held averages, and blue are high.

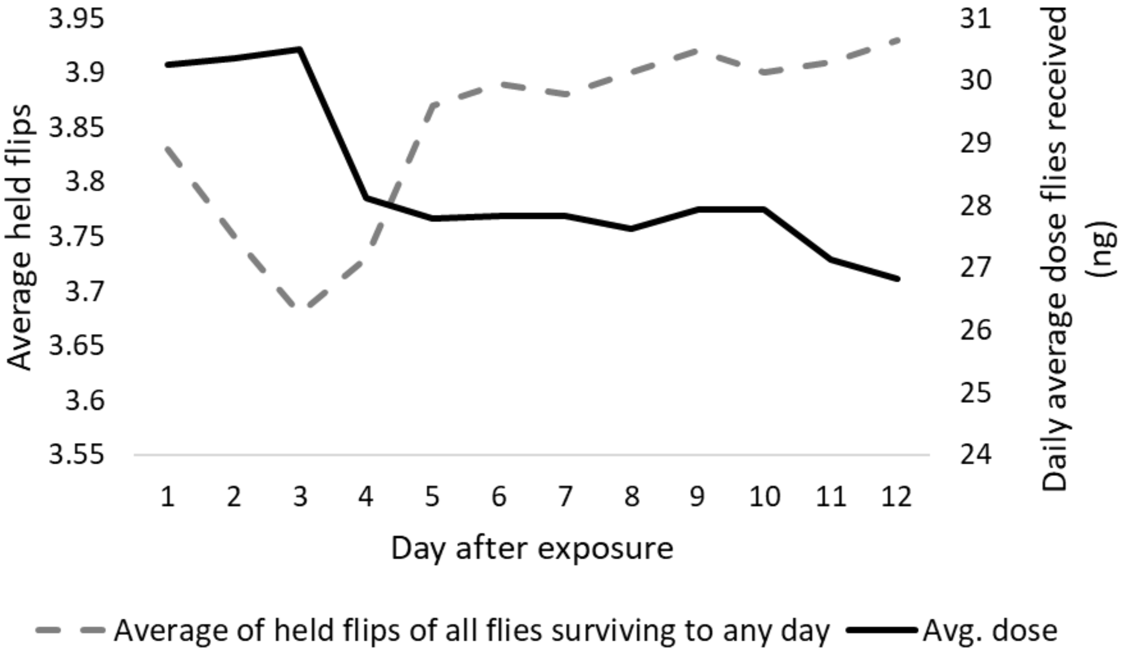


Figure A.5 Two lines depicting average held flips in the grip test and average orally received dose of thiamethoxam for surviving *Episyrphus balteatus* flies and how they change across 12 days after exposure. Graph includes all flies, even ones that did not survive to the end. Therefore, the number of individuals that make up the average on each day changes as flies die over time.

Table A.1 Summary features of the dataset. Number of censored individuals refers to individuals who survived beyond the 14 days of observation.

Batch-Sex	Average Weight (mg)	Variance of weight (mg)	Number of censored individuals / total individuals
Batch A – Male	21	12	18/40
Batch A – Female	19	16	29/40
Batch B – Male	26	15	30/40
Batch B – Female	23	9	31/40
Batch C – Male	21	7	33/39
Batch C – Female	18	5	36/40
Batch D – Male	17	14	27/41
Batch D - Female	15	10	22/40

Appendix B. Supplementary data for Chapter 4

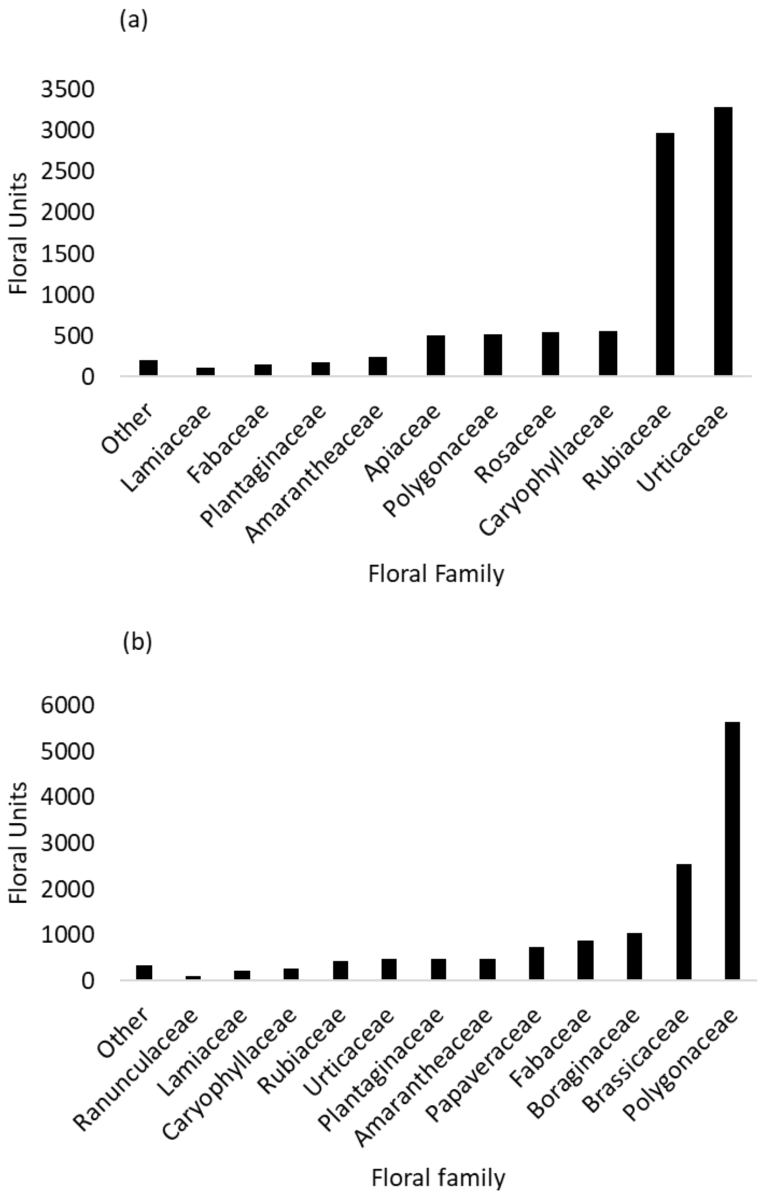


Figure B.1 Floral units (summed across sites and rounds, therefore the whole field season) for each floral family observed in the (a) hedge and (b) crop.

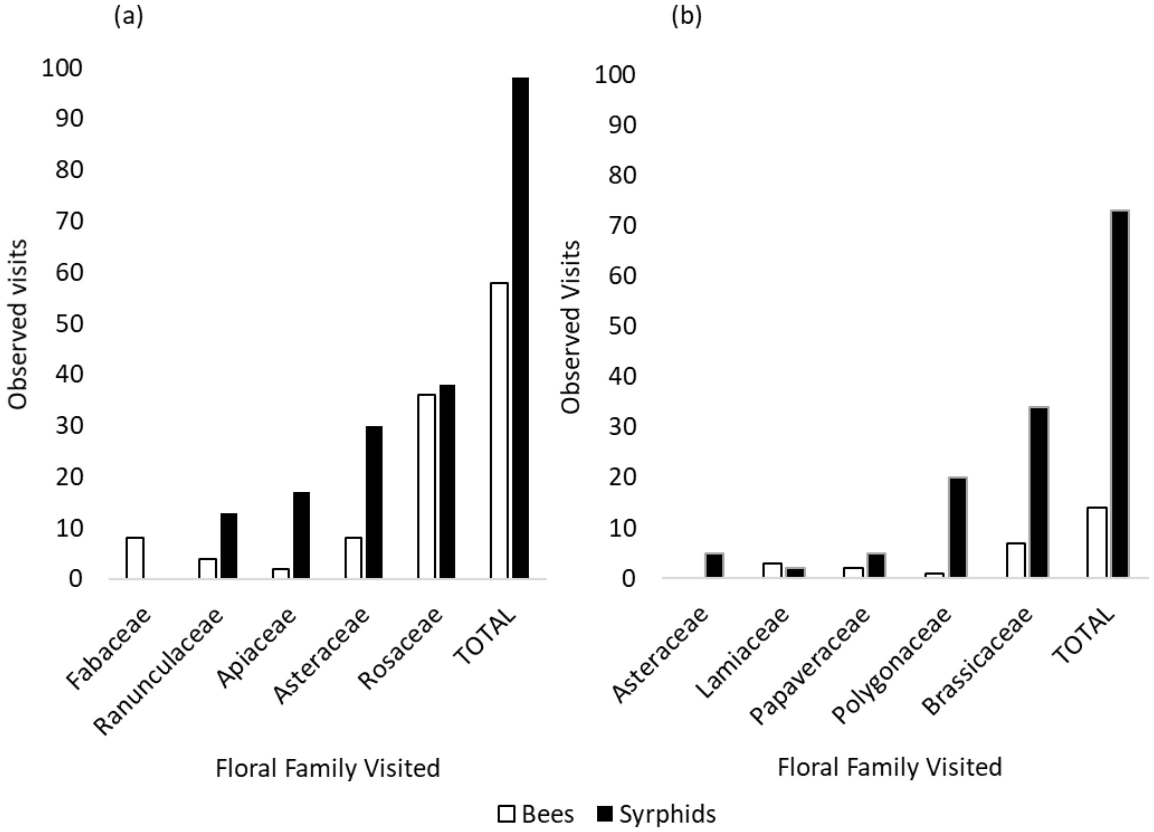


Figure B.2 Top five floral families with most observed visits by bees and syrphids (a) along the hedge and (b) in the crop.

Table B.1 Winged invertebrates observed in pan traps.

Groups	Counts
Diptera	8624
Syrphids	194
Aphidophagous syrphids	145
Thysanoptera	2258
Hymenoptera	1223
Bees	119
Hemiptera	268
Coleoptera	254
Unknown	197
TOTAL	12679

Table B.2 Quantities of woody plants in surveyed hedges at sites, showed as either (a) counts for distinctively individual plants or (b) % cover for plants that appeared continuous.

(a)

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 7	Site 8	Site 9	Site 10
Acer spp.	12			1					3
Fagus sylvaticus					1			17	
Fraxinus excelsior	22		20	7	1	3	13	3	22
Lonicera spp.		4	1						
Prunus spp.				1		1			
Quercus spp.									1
Salix cinerea									2
Sambucus nigra	2	2	1		1		5		2
Ulmus spp.									1
Unknown							5		

(b)

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 7	Site 8	Site 9	Site 10
Crataegus monogyna	70-80%	80-90%	30-40%	<1%	40-50%	1-5%	50-60%	90-100%	70-80%
Hedera helix	30-40%	70-80%	40-50%		5-10%	5-10%	90-100%	23-30%	
Ligustrum ovalifolium			5-10%		70-80%	50-60%	<1%		
Prunus spinosa	70-80%	<1%	1-5%	<1%	1-5%	20-30%	1-5%	40-50%	
Rosa fruticosus		70-80%	40-50%	90-100%	80-90%	<1%	1-5%		<1%
Rubus spp.	70-80%	50-60%	80-90%	90-100%	80-90%	50-60%	70-80%	60-70%	70-80%
Symphoricarpos albus									10-20%

Table B.3 Observed species of adult bees across all nine sites and three rounds, by surveying method. Ordered from lowest to highest counts.

Pan Trapped Species	Count	Floral Visitor Species	Count
Andrena barbilabris	1	*Andrena haemorrhoa*	1
Andrena fucata	1	*Andrena subopaca*	1
Andrena subopaca	1	*Andrena bicolor*	1
Bombus campestris	1	*Bombus cryptarum/magnus*	1
Megachile centuncularis	1	*Bombus horatorum*	1
Sphecodes monilicornis	1	*Bombus pratorum*	1
Andrena bicolor	2	*Halictus rubicundus*	1
Andrena scotica	2	*Lasioglossum albipes*	1
Bombus lapidarius	2	*Bombus lucorum/magnus*	2
Lasioglossum punctatissimum	2	*Bombus lapidarius*	4
Bombus hortorum	4	*Apis mellifera*	15
Bombus pascuorum	4	*Bombus pascuorum*	16
Bombus lucorum/magnus	5	*Bombus terrestris*	16
Lasioglossum albipes	5	*Bombus spp.*	22
Andrena haemorrhoa	6		
Bombus pratorum	6		
Halictus rubicundus	9		
Apis mellifera	27		
Bombus terrestris	42		
TOTAL	122	TOTAL	83

Table B.4 Observed species of adult non-aphidophagous syrphids across all nine sites and three rounds, by surveying method. Ordered from lowest to highest counts.

Pan Trapped Species	Count	Floral Visitor Species	Count
Eristalis abusivus	1	*Eristalis nemorum (interruptus)*	1
Eristalis arbustorum	1	*Helophilus hybridus*	1
Eristalis pertinax	1	*Helophilus spp.*	1
Helophilus hybridus	1	*Neoascia geniculata*	1
Melanogaster hirtella	1	*Volucella bombylans*	1
Myathropa florea	1	*Volucella pellucens*	1
Riponnensia splendens	1	*Anasimyia lineata*	2
Syritta pipiens	1	*Eristalis arbustorum*	2
Volucella bombylans	1	*Eristalis pertinax*	2
Anasimyia lineata	2	*Helophilus pendulus*	2
Lejogaster metallina	4	*Rhingia campestris*	4
Eristalis tenax	6	*Syritta pipiens*	10
Helophilus pendulus	7	*Eristalis tenax*	15
Rhingia campestris	7	*Eristalis spp.*	16
Ferdinandea cuprea	14	Other	59
TOTAL	49	TOTAL	118

Table B.5 Counting methods of floral abundance observations, and examples of observed flowers in observed families. Counting method units were grouped together as one if about one cm apart from each other when plant was at rest, and were not on separate plants or peduncles of greater than 5 cm. Units were always counted as separate if on separate plants or on separate peduncles of greater than 5 cm, regardless of how close they were.

Family	Counting method	Examples of common names of flowers observed	Specific Examples (Scientific names)
Amarantheaceae	Spike/Spear	Orache	*Atriplex* sp.
Apiaceae	Umbel	Umbels	-
Asteraceae	Compound-flower head	Daisy, Dandelions, Thistles	*Bellis perennis*
Boraginaceae	Flower	Borage	*Myosotis* sp.
Brassicaceae	Cluster	Mustard	-
Caprifoliaceae	Cluster	Snowberry	*Symphoricarpos albus*
Caryophyllaceae	Flower	Corny spurry; Chickweeds	*Spergula arvensis*
Fabaceae	Flower	Meadow vetchling	*Lathyrus pratensis*
Fabaceae	Cluster	Black medick; Clover	*Medicago lupulina; Trifolium repens: Trifolium pratense*
Fabaceae	Whorls	Vetch and hairy tare	*Vicia hirsuta*
Lamiaceae	Flower	Lamiums, worts, dead nettle, etc.	-
Papaveraceae	Cluster	NA	*Fumaria* sp.
Papaveraceae	Flower	Poppy	*Papaver* sp.
Plantaginaceae	Spike/Spear	Plantain	*Plantago* sp.
Plantaginaceae	Flower	Speedwell	*Veronica* sp.
Polygonaceae	Cluster	Black bindweed; Knotgrass	*Fallopia convolvulus; Polygonum aviculare*
Polygonaceae	Spike/Spear	Dock; Other	*Rumex sp.; Persicaria* sp.
Primulaceae	Flower	Scarlet pimpernell	*Anagallis arvensis*
Ranunculaceae	Flower	Buttercup	*Ranunculus* sp.
Rosaceae	Flower	Wood aven; Cinquefoil; Bramble	*Geum urbanum; Potentilla sp.; Rubus fruticosus agg.*
Rubiaceae	Cluster	Cleaver	*Galium arvense*
Urticaceae	Spike/Spear	Stinging nettle	*Urtica* sp.