The Colonisation of Vegetable and Salad Crops by Aphids

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Abbreviations

ACC	Acetyl-CoA carboxylase
AChE	Acetylcholinesterase
AHDB	Agriculture and Horticulture Development Board
AI	Active ingredient
ANOVA	Analysis of variance
BADC	British Atmospheric Data Centre
CEDA	Centre for Environmental Data Analysis
cm	Centimetre
CoA	Coenzyme A
Cv.	Cultivar
d.f.	Degrees of freedom
D°	Day-degree
DDT	Dichlorodiphenyltrichloroethane
EC	European Commission
EC.	Emulsifiable concentrate
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPG	Electrical penetration graph
F1	First filial generation
Fig.	Figure
GABA	Gamma-aminobutyric acid
GLM	Generalised linear model
GMT	Greenwich Mean Time
h	Hour(s)
ha	Hectare
IPM	Integrated Pest Management
IRAC	Insecticide Resistance Action Committee
IRAG	Insecticide Resistance Action Group
IRM	Insecticide Resistance Management
IRU	Insect Rearing Unit
kdr	Knock down resistance
kg	Kilogram
km	Kilometre
L	Litre
L:D	Light:dark
L.	Linnaeus
ln	Natural logarithm
log	Logarithm
m	Metre
M	Moles/litre
MACE	Modified acetylcholinesterase

MIDAS	Met Office Integrated Data Archive System
min	Minute(s)
ml	Millilitre
mm	Millimetre
MoA(s)	Mode of action/modes of action
n	Sample size
N/A	Not applicable
nAChR	Nicotinic acetylcholine receptor
Nic-R	Neonicotinoid resistance
nm	Nanometre
No.	Number
PBS-T	Phosphate-buffered saline containing Tween
pH	log10[H+]
PHU	Pesticide Handling Unit
ppm	Parts per million
PTA-ELISA	Plate-trapped antigen enzyme-linked immunosorbent assay
Rdl	Resistance to dieldrin gene
RIS	Rothamsted Insect Survey
r_m	Intrinsic rate of natural increase
S.E.	Standard error
SEM	Standard error of the mean
sp.	Species
spp.	Species (plural)
ssp.	Subspecies
TGM	Tendergreen mustard
TRPV	Transient receptor potential ion channel
Tukey’s HSD	Tukey's Honestly Significant Difference
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
YWT	Yellow water trap
µl	Microlitre

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Declarations

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree. The work presented (including data generated and data analysis) was carried out by the author except in the cases outlined below:

- Suction trap records were provided by the Rothamsted Insect Survey.

Abstract

Aphids are some of the most damaging pests of horticultural crops globally. They cause direct feeding damage to crops and are key vectors of economically-important plant viruses. In the field, aphids are managed largely through the application of synthetic chemical insecticides. The sustainability of crop protection depends on optimising the use of available resources for aphid control. In the UK, this is particularly important due to recent declines in the availability of many insecticides and the increasing number of cases of insecticide resistance.

The project focused on the process of crop colonisation by several pest aphids of horticultural crops. Aphid phenology and disease risk were predicted through the use of forecasting models to warn of crop infestation and periods with potential for virus transmission. For *Cavariella aegopodii, Cavariella pastinaceae, Cavariella theobaldi* and *Myzus persicae* strong relationships were identified between air temperature and the timing of their migration. The models developed improve and/or expand on what is currently available to growers in the UK.

Once in crops, host plant selection by aphids and their responses to insecticides can have implications for the severity of infestations and virus transmission. The host preference behaviour of *M. persicae* was investigated. On Brussels sprout, aphids initiated probing behaviour more quickly, settled and fed more readily compared to carrot and lettuce.

Several synthetic chemical insecticides and compounds based on plant extracts were tested in terms of their effects on the survival and behaviour of alate *M. persicae* and the subsequent transmission of a non-persistent plant virus. Two of the test products induced high levels of mortality in *M. persicae* when applied prophylactically to plant surfaces. The persistence of these insecticides and the rapid knock-down of aphids may benefit the management of plant viruses. Other treatments, including the pyrethroid insecticide, lambda-cyhalothrin, reduced aphid settling behaviour.

Overall, the findings of the project can be used to refine Integrated Pest Management strategies for aphids by (i) targeting the use of insecticide treatments and (ii) informing the selection and sequence of insecticide treatments based on an improved understanding of their effects on aphid survival and behaviour.

Chapter 1 — Introduction

1.1 The importance of sustainable methods for crop protection

By 2050, global food production will need to increase by about 70% in order to meet the demands of a growing population (United Nations, 2015). The sustainable intensification of agriculture is crucial for addressing issues of food security (Godfray *et al.,* 2010; Tilman *et al*., 2011). This will depend on an increase in food production without causing additional environmental damage and/or the loss of biodiversity (Baulcombe *et al.*, 2009; Crist *et al.*, 2017). There is still uncertainty surrounding the exact methods by which this may be achieved (Tilman *et al*., 2002; Pretty and Bharucha, 2014).

Preventing crop losses due to pests and plant diseases is an important component of the sustainable intensification of agriculture. Over the last 50 years there has been a large reliance on synthetic chemical pesticides within many agricultural systems (Chandler *et al.,* 2011; Flint and van den Bosch, 2012). A considerable proportion of these chemicals are applied for the control of arthropod (*e.g.* insect and mite) pests (Garwaithe *et al*., 2017). On average, it is estimated that insecticides have been applied to around 6 million ha of crops each year since 1990 in the UK (FERA, 2016).

Nevertheless, an overreliance on synthetic chemical pesticides has resulted in adverse consequences for the environment, human health and biodiversity; including negative impacts on non-target beneficial organisms (Birch *et al*., 2011; Barzman *et al*., 2015; Wood and Goulson, 2017). In addition, there are concerns regarding the decreasing availability of synthetic chemical pesticides and the increasing cases of pesticide resistance (Clarke *et al*., 2011; Sparks and Nauen, 2015; Foster *et al*., 2017).

The many concerns surrounding an overreliance on pesticides have led to the increasing withdrawal of products from the market (Hillocks, 2012; Goulson, 2018; Scott and Bilsborrow, 2019). Upon the introduction of regulation 1107/2009/EC by the European Union pesticides are now required to meet more stringent environmental, toxicological and safety standards for registration (Jess *et al.*, 2014).

Some of the consequences associated with pesticide use have highlighted the importance for farmers and growers to reduce their reliance on conventional methods of pest control. This calls for more sustainable solutions for crop protection and will involve the employment of alternative and often novel management strategies (Tilman *et al.*, 2011; Hillocks, 2012; Waterfield and Zilberman, 2012; Barzman *et al*., 2015).

1.2 Integrated Pest Management

It is widely reported that Integrated Pest Management (IPM) can provide effective solutions to improve the sustainability of pest control (Tilman *et al.*, 2002; C-IPM, 2016). Described as a systems approach, IPM involves "the careful consideration of all available crop protection methods and the subsequent integration of these tactics to discourage the development of pest populations beyond levels of economic injury" (European Union, 2009). The key components of IPM (Chandler *et al.*, 2011; Flint and van den Bosch, 2012; Barzman *et al*., 2015) include:

- **(i)** Selection of resistant crop cultivars and/or crops bred with total or partial resistance to pests.
- **(ii)** Physical control methods, such as exclusion techniques (*e.g.* insect-proof netting and insect traps).
- **(iii)** Cultural practices including: crop rotation, intercropping, use of trap crops, and companion cropping.
- **(iv)** Biological control provided by natural enemies, such as predatory insects, parasitoids, parasites and microbial pathogens.
- **(v)** Decision support tools including phenological models for pest forecasting and/or pest monitoring in the field.
- **(vi)** Use of natural products for pest control, for example, semiochemicals for mating disruption and/or plant extracts with biocidal properties. In some cases, natural products may form the basis for synthetic chemical pesticides. (Gerwick and Sparks, 2014).
- **(vii)** The use of synthetic chemical pesticides that are target-specific, or classified by regulators as low-risk; alongside the rotation of pesticides with different modes of action (MoAs) to minimise the evolution of heritable insecticide resistance.

Within many IPM programmes prevention techniques (i–iii) and non-chemical methods (iv–vi) of control receive prioritisation. Approaches using synthetic chemical pesticides are employed only when essential (Kogan, 1998; Birch *et al*., 2011; C-IPM, 2016). The reduced input of synthetic chemical pesticides, however, has not adversely affected the productivity of many agricultural systems. In several cases, crop yields are reported to benefit under IPM (Pretty, 2008). For example, in a review of 62 international IPM research and development projects, an increase in crop yield alongside a reduction in the application of synthetic chemical pesticides was outlined in over 60% of the projects. On average, yields increased by 40%, while pesticide usage declined by 60% (Pretty, 2008). Therefore, IPM has the potential to ensure both the economic and ecological sustainability of cropping systems.

In response to the development of pesticide resistance in a range of pests infesting greenhouse crops, IPM approaches have become common practice in protected cropping systems (van Lenteren, 2000). Here, biological control provides the basis of many effective IPM programmes (Pilkington *et al*., 2010). The programmes, however, remain largely marginal in crops grown outdoors (van Lenteren, 2000; Pilkington *et al*., 2010). In the field, IPM approaches face a number of challenges (Lefebvre *et al*., 2015). In contrast to protected crop systems, there is no regulation of environmental conditions and crops are exposed to a wide range of pests and plant pathogens. Furthermore, the retention of biological control agents released in the field cannot be ensured (Macfadyen *et al*., 2015). Annual crops such as field vegetables and salads are particularly challenging, as apart from field boundaries, there is little opportunity to establish stable ecosystems that support beneficial organisms. Released natural enemies are also susceptible to adverse weather conditions and local levels of predation, parasitism and disease (Crawley, 2009).

As outlined by Chandler *et al*. (2011), IPM programmes for outdoor crops tend to concentrate on three main areas (i) crop rotations (ii) choice of cultivar and (iii) the targeted use of pesticides. There is, however, no common solution. Individual crop/pest situations often require IPM programmes that have been developed to meet the specific requirements of growers, in accordance with their local circumstances and pest pressures (Birch *et al*., 2011; Chandler *et al*., 2011).

For field crops grown in the UK, a current challenge is that for many crop/pest situations, there is a lack of effective or economically feasible alternatives to synthetic chemical pesticides (C-IPM, 2016). Greater research effort is therefore required to develop and evaluate IPM approaches that are able to reduce reliance on these compounds, while still maintaining both agricultural productivity and economic stability (Pretty, 2008). Furthermore, IPM research will be critical within the coming years in order to address declining pesticide availability. This will involve the strategic assessment of the key crop/pest situations that are at the greatest risk under new pesticide reduction legislation (Birch *et al*., 2011).

Another challenge will be in encouraging the adoption of whole-crop IPM programmes in agricultural systems, especially in cases where synthetic chemical pesticides currently provide a fast-acting and relatively inexpensive option (Bailey *et al*., 2009; Hillocks, 2012). Birch *et al.* (2011) suggest that on-farm research with participating growers is one of the most convincing ways to demonstrate the potential of IPM in the field.

1.3 Aphids as crop pests

It is estimated that each year arthropod pests are responsible for a global decline of 18–26% in crop yields (Culliney, 2014). Of these species, aphids are considered some the most important pests worldwide (Blackman and Eastop, 2018). This project focused on several important pest aphids of horticultural crops (*Myzus persicae, Cavariella aegopodii, Cavariella pastinaceae* and *Cavariella theobaldi*).

Aphids are small, soft-bodied insects that feed from the phloem cells of plants (Blackman and Eastop, 2017). Approximately 4700 species of aphid have been recorded globally (Remaudière and Remaudière, 1997). Only 450 aphid species, however, are reported to occur on crops and only around 100 of these species are considered to be of notable economic importance (Blackman and Eastop, 2018).

Aphids affect agricultural and horticultural crops in a number of different ways. These include; damage by direct feeding (*e.g.* stunting, discoloration and deformation of plants), the transmission of plant viruses (Blackman and Eastop, 2018) and the

deposition of honeydew, which may promote the growth of sooty moulds and render certain crops unmarketable (Hurej and van der Werf, 1993).

The success of aphids as crop pests can be attributed in part to their method of reproduction. Parthenogenesis (embryonic development without egg fertilisation) and viviparity (internal embryonic development and live birth of progeny) are favoured by many species (Davis, 2012). In aphids, the evolution of these traits has led to a decrease in generation time and an increase in reproductive capacity (Dixon, 1992; Davis, 2012).

Like many pest insects, aphids have been managed historically through the application of synthetic chemical insecticides. Due to their widespread distribution and their importance as crop pests, aphids account for a considerable proportion of insecticide applications made to crops each year (Garthwaite *et al*., 2017). The intense selection pressure resulting from the application of synthetic chemical insecticides has led to the evolution of insecticide resistance in number of important species of aphid (Bass *et al*., 2014; Foster *et al*., 2017).

Furthermore, success in controlling aphid vectors and preventing the spread of plant viruses with synthetic chemical insecticides has been limited (Perring *et al*., 1999). This relates particularly to non-persistent plant viruses (Loebenstein and Raccah, 1980). This highlights the need for integrated control approaches to reduce the number of aphid vectors, especially at crucial times for virus transmission.

1.4 The biology and ecology of *Myzus persicae*

The peach-potato aphid or green peach aphid, *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) is considered one of the most economically-important pest aphids globally (Blackman and Eastop, 2017). This is due to a number of factors relating to its pest status. These include its widespread distribution, polyphagous nature and efficiency as a vector of plant viruses (van Emden *et al*., 1969). *Myzus persicae* also exhibits considerable genetically-based variability in traits such as colour, life cycle, host plant preference and mechanisms of insecticide resistance (van Emden *et al*., 1969; Weber, 1985; Bass *et al*., 2014).

The life cycle of *M. persicae* varies depending on region and temperature during the winter. In the UK, *M. persicae* is predominantly anholocyclic and survives the winter as active adults on herbaceous hosts (van Emden *et al.,* 1969). Alate (winged) adults migrate to their summer hosts from late April to early June, with peak numbers observed in crops during July. Reproduction continues throughout the summer with further alate morphs produced in response to crowded conditions and/or a deterioration in the quality of host plants (van Emden *et al.,* 1969).

The occurrence of a peak in aphid numbers is followed by a population crash. Karley *et al*. (2004) suggest that several ecological factors may be responsible for inducing population crashes, including; deterioration in the nutritional quality of host plants, greater pressure from natural enemies arising late season, and intermittent extreme weather events.

In colder climates, *M. persicae* may overwinter holocyclically as eggs in diapause on *Prunus* species, including the primary host; peach, *Prunus persica* L. (Rosaceae) (Newton *et al*., 1953; Blackman, 1974). In the spring, eggs hatch and *M. persicae* can undergo up to eight generations on its overwintering host. Spring populations can become very dense and alate morphs are produced in response to crowed conditions. Alate adults migrate to summer hosts over a period of several weeks. In the autumn, both male and female alate morphs are produced in response to a change in day length and/or temperature (Hille Ris Lambers, 1966; Blackman, 1971). Parthenogenetic females migrate to winter hosts and give birth to apterous (wingless) egg-laying morphs (oviparae). Oviparae then produce a pheromone that attracts sexual males. Following reproduction, eggs are deposited eggs on winter hosts. Apart from the autumn generation that culminates in egg production, all aphids are parthenogenetic (van Emden *et al*., 1969).

Myzus persicae is known to infest more than 400 secondary host plant species from over 40 different plant families (Blackman and Eastop, 2018). These include many important horticultural crop families, such as Apiaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae and Solanaceae (van Emden *et al.,* 1969; Holman, 2009b). *Myzus persicae* has the ability to adapt rapidly to new host plants. This has allowed for the development of several host races (Bass *et al*., 2014). An

example, is *Myzus persicae* ssp. *nicotianae,* which is specialised on tobacco, *Nicotiana tabacum* L. (Solanaceae) (Blackman, 1987).

Myzus persicae is a major concern to growers because it acts as a vector for over 100 plant viruses, such as potato virus Y (PVY) and potato leafroll virus (PLRV) to the nightshade/potato family, Solanaceae, and various mosaic viruses to several other food crops (Kennedy *et al*., 1962). Both nymphs and adults are able to transmit plant viruses (Namba and Sylvester, 1981), however, alate adults are likely to have a greater propensity for transmission due to their mobility and capacity to disperse. Both persistent and non-persistent viruses are transmitted (Kennedy *et al*., 1962).

For many crops, the control of *M. persicae* relies heavily on the application of synthetic chemical insecticides. Over time this has led to the development of widespread and multiple forms of insecticide resistance. *Myzus persicae* is considered one of the most widely and strongly resistant pest species worldwide (Bass *et al*., 2014). Resistance to organophosphates was first reported in *M. persicae* in 1955 (Anthon, 1955). Since then insecticide resistance has been reported to most major classes of insecticide, including the carbamates, pyrethroids, cyclodienes, and neonicotinoids (Bass *et al.,* 2014). Resistance has occurred through multiple mechanisms (*e.g.* overexpression of esterase, super-kdr genotypes and modification of acetylcholinesterase; MACE) (Bass *et al*., 2014). This has considerably reduced the number of insecticides available for the control of *M. persicae* (Criniti *et al.*, 2008).

1.5 The biology and ecology of *Cavariella aegopodii*

The willow-carrot aphid, *Cavariella aegopodii* (Scopoli, 1763) (Hemiptera: Aphididae) is a widespread pest of members of the Apiaceae family; including economically-important crops such as carrot (*Dacus carota* L.), parsley (*Petroselinum crispum* L.), and parsnip (*Pastinaca sativa* L.) (Gratwick, 1992).

For the most part, it is a concern to growers due to its efficiency in transmitting important plant viruses (Watson *et al*., 1964; Waterhouse and Murant, 1983). It is known to transmit carrot motley dwarf virus (CMD) and parsnip yellow fleck virus (PYFV) to carrots, as well as being a vector of mosaic viruses such as beet mosaic

virus (BtMV) and celery mosaic virus (CeMV) (Watson *et al.,* 1964; Rochow, 1972; Elnagar and Murant, 1976). In addition, *C. aegopodii* can damage crops through direct feeding and the deposition of honeydew which may promote the development of sooty moulds (Goggin *et al*., 2017).

In the UK, *C. aegopodii* overwinters principally as eggs in diapause on willow (*Salix* spp.) (Dunn, 1965). Preferred primary hosts are crack willow (*Salix fragilis* L.) and white willow (*Salix alba* L.). In warmer climates (*e.g.* in the south of the UK) *C. aegopodii* may survive the winter as mobile stages (active adults or immature stages) on herbaceous host plants; which may be wild apiaceous species or crops remaining in the ground or in storage (Gratwick, 1992). In the UK, eggs may hatch as early as February and this is followed by the production of several generations of parthenogenetic apterous females (Gratwick, 1992).

In response to crowding, alate morphs of *C. aegopodii* are produced around May/June. Alate morphs then migrate to summer hosts over a period of five to six weeks. Aphids that have overwintered as active adults are able to produce spring colonies rapidly, with alate morphs potentially appearing earlier than holocyclic colonies developing on *Salix* species. In the UK, Dunn (1965) found that weather variables, such as temperature and rainfall, were important during May for determining the number of *C. aegopodii* that reach their summer host plants. While low rainfall and warm temperatures facilitated the spring/spring migration of *C. aegopodii*, cold and wet weather during this period often prevented colonising aphids from reaching their summer host plants.

In carrot crops*, C. aegopodii* often infests plants at the cotyledon stage, but it may also attack older plants (Dunn, 1969). Crops sown during April/May can incur severe damage, corresponding with the spring migration, whereas crops sown in June may largely avoid losses in yield (Gratwick, 1992).

Summer populations of *C. aegopodii* are supported by many apiaceous crops, including carrot, celery (*Apium graveolens* L.), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgara* Mill.), parsley and parsnip (Dunn, 1965; Dunn and Kirkley, 1966). Reproduction continues throughout the summer with an increase in the production of alate morphs when conditions become crowded. Alate adults may

spread within the crop or disperse to colonise new areas. Peak populations of *C. aegopodii* are observed in crops during June (Dunn and Kempton, 1967). Like *M. persicae,* the peak in *C. aegopodii* numbers is followed by a crash in aphid numbers (Karley *et al*., 2004). Following a crash in numbers, it generally takes populations six to eight weeks to recover (Gratwick, 1992).

During the autumn, male and female alate morphs are produced in response to daylength and/or temperature cues. Aphids then migrate back to their primary host (*Salix* spp.) where oviparous sexual females are produced and mating occurs. Eggs are then deposited on *Salix* species over several weeks (Gratwick, 1992).

Similar to other species of aphid, the development of *C. aegopodii* shares a close relationship with temperature. Dunn (1970) found that the time from birth to apterous adult was approximately sixteen days at 14.5°C, compared with 9.5 days at 22.3°C. Additionally, *C. aegopodii* required 31 days to complete two generations at 14.5°C, whereas aphids could be into their fourth generation during this time at 22.3°C.

The management of *C. aegopodii* is achieved primarily through the application of insecticides (Dunn and Kempton, 1967). Nevertheless, in areas where pest and disease pressures are low, sanitation methods may offer sufficient control; these include the removal of overwintering sources of aphids and virus reservoirs *e.g.* cow parsley, *Anthriscus sylvestris* L. Hoffm. (Apiaceae). There have also been attempts to identify carrot cultivars with resistance to *C. aegopodii*. However, these research efforts have had limited success (Dunn, 1970).

Overreliance on chemical methods for the control of *C. aegopodii* is likely to have implications in terms of the development of insecticide resistance. Resistance to pyrethroid insecticides was discovered recently at Rothamsted Research when screening a sample of *C. aegopodii* collected from cow parsley in North Yorkshire in 2014 (AHDB, 2018).

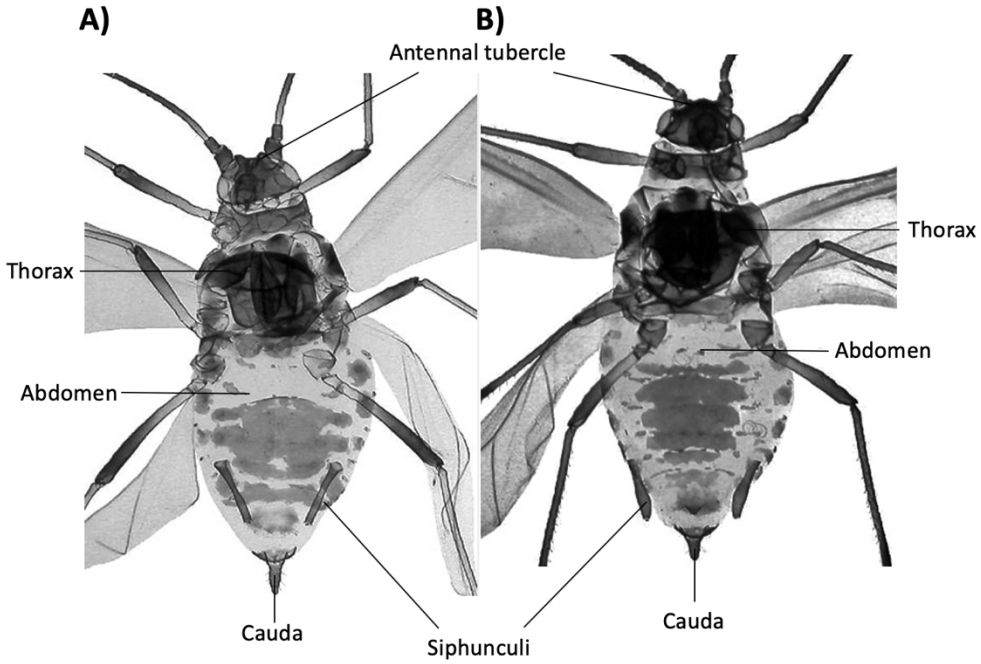

Figure 1.1 – Identification features of **A)** alate *Myzus persicae*, including; black central patch on the upper abdominal surface with lateral extensions and a clear window towards the tail end of the patch, medium length siphunculi that are slightly swollen towards the tips and evenly pigmented, dark cauda tip and well-developed antennal tubercles and **B)** alate *Cavariella aegopodii*, including; abdominal fusion of three or four cross bars, dark siphunculi that are swollen towards the tips and a second small outgrowth on the abdomen above the cauda (supracaudal process). Figure adapted from: Blackman, R. L. and Eastop, V. F. (2000). *Aphids on the World's Crops: An Identification and Information Guide.* John Wiley & Sons Ltd.

1.6 The biology and ecology of *Cavariella pastinaceae* and *Cavariella theobaldi*

The willow-umbellifer or parsnip aphid, *Cavariella pastinaceae* (Linnaeus, 1758) (Hemiptera: Aphididae) and the willow-parsnip aphid, *Cavariella theobaldi* (Gillette and Bragg, 1918) (Hemiptera: Aphididae) are similar in their life cycle and biology to the willow-carrot aphid, *C. aegopodii*. The species alternate between willows (*Salix* spp.) and some Apiaceae, such as hogweed (*Heracleum* spp. L.) and wild parsnip (*Pastinaca* spp. L.) (Holman, 2009b). Partial host alternation has been reported for *C. pastinaceae* and *C. theobaldi* as some colonies may remain on *Salix* spp. throughout the summer (Kundu and Dixon, 1995). Like *C. aegopodii, C. pastinaceae* and *C. theobaldi* infest apiaceous crops of economic importance and may contribute to the transmission of plant viruses. However, unlike *C. aegopodii,* they do not regularly

colonise carrot (or wild hosts, such as cow parsley) in the field (Van Dijk and Bos, 1985).

There is a limited amount of published literature on *C. pastinaceae* and *C. theobaldi*. However, the species differ in their phenology to *C. aegopodii*. Generally, *C. pastinaceae* and *C. theobaldi* begin their migration after *C. aegopodii* and peak populations are usually observed later in the year (Syngenta, 2019).

1.7 Project aims

In 2015/2016 members of the Waitrose Agronomy Group had serious problems with aphid infestation and virus in carrot (Burgess Farms, Produce World Group), lettuce (G's®) and *Brassica* crops. Influenced by these industry concerns, the overall aim of the project was to improve the understanding of the colonisation process of vegetable and salad crops by aphids. The project focused on five main areas and the following chapters will be structured in accordance to these areas. The hypotheses that were tested will be addressed in each chapter.

1) Investigate methods of predicting aphid phenology (*e.g.* forecasting models to predict key events in the life cycle of aphids).

2) Investigate aphid developmental biology and colonisation behaviour on commercially-important host plants.

3) Examine the efficacy and persistence of a range of synthetic chemical insecticides and compounds based on natural plant extracts that are applied for the control of aphids.

4) Determine the effects of a range of synthetic chemical insecticides and compounds based on natural plant extracts on the settling behaviour of aphids on several host plants.

5) Investigate the efficacy of synthetic chemical insecticides and compounds based on natural plant extracts in managing the transmission of a non-persistent plant virus by alate *Myzus persicae*.

Chapter 2 — Monitoring and Forecasting Infestations by Aphids

2.1.1 The monitoring of arthropod pests

The monitoring of arthropod pests is fundamental for establishing successful IPM programmes. A range of monitoring approaches are regularly employed for pest species, including: suction traps, water-pan traps, sticky traps, pheromone traps, light traps and pitfall traps as well as crop inspections of the number of eggs, larvae, pupae and adults (Eastop, 1955; Heathcote *et al*., 1969; Boiteau, 1990). Insect monitoring data serve several purposes, including: (i) ecological studies, (ii) investigating insect migration and monitoring the arrival of pest insects into agroecosystems (iii) timing field scouting and sampling programmes (iv) informing the application of insecticides (v) inferring periods with potential for virus transmission (Prasad and Prabhakar, 2012).

Monitoring at a network of sites is important for determining the spatial distributions of pest insects and identifying the locations of pest hot-spots. It can also provide early warnings of pest infestations to inform management approaches (Ayalew *et al*., 2008; Prasad and Prabhakar, 2012). For species like aphids, suction traps are one of the most effective and reliable methods for monitoring phenology and abundance on a large scale (Harrington and Hullé, 2017). The Rothamsted Insect Survey (RIS) and partners in Scotland, Science and Advice for Scottish Agriculture (SASA), operate a network of sixteen traps across the UK (twelve in England and four in Scotland) (Fig. 2.1). A new suction trap was put into operation at East Malling (Kent) in 2019 to replace the suction trap at Wye (Kent) (1966–2019).

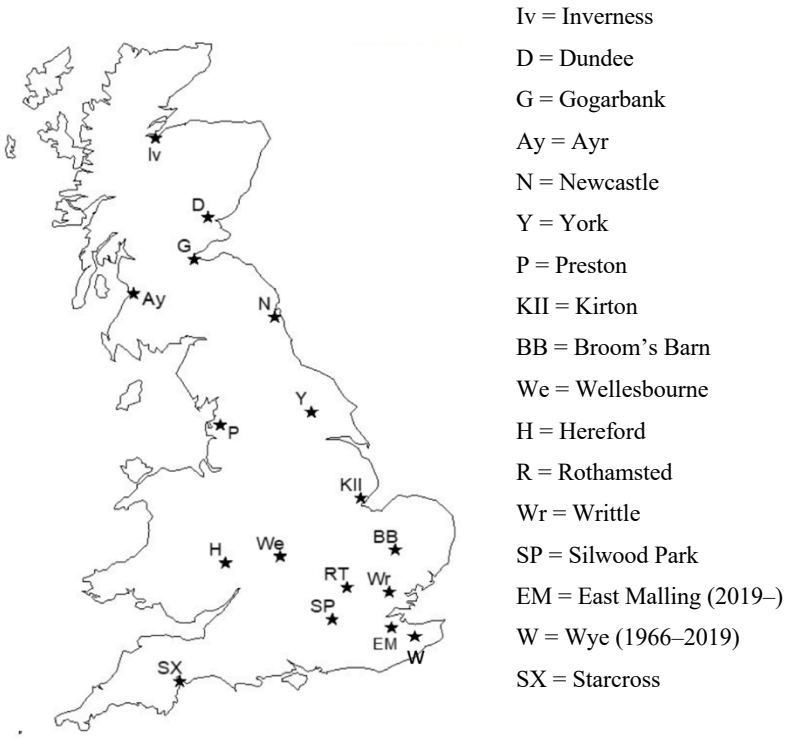

Figure 2.1 – The network of suction traps operated in the UK during 2019 by the Rothamsted Insect Survey (RIS) and partners in Scotland (SASA).

The traps are 12.2 m in height (Fig. 2.2) and sample a constant volume of air (45 m^3 min^{-1}) all year round (Macaulay *et al*., 1988). The aphids captured at this height are likely to be migratory species capable of dispersing considerable distances (Macaulay *et al*., 1988). The area that an individual trap 'represents' varies according to topography. However, samples from a trap are thought to be, on average, representative of the species flying across an area of radius approximately 80 km (Taylor, 1974). Samples are collected daily from the suction traps during the 'aphid season' (April to mid-November) with captured aphids identified at the species, species-group or genus level (Harrington *et al*., 1991). The numbers of aphids caught in each suction trap are reported for the previous week in bulletins issued by the Rothamsted Insect Survey (RIS). The bulletins can be used inform growers on the relative timing and abundance of aphids in their approximate area.

Figure 2.2 – The Rothamsted Insect Survey (RIS) suction trap (12.2 m) operating at Wellesbourne, Warwickshire, UK (2004–present).

The data collected by the Rothamsted suction trap network are useful for monitoring the timing and size of migrating aphid populations at the 'landscape scale', as well as providing the basis for the development and validation of pest forecasting models and decision support systems (Zhou *et al.,* 1995; Harrington and Hullé, 2017).

2.1.2 Models for predicting the phenology and abundance of aphids

Forecasting models are used to predict the development, phenology and abundance of economically-important pest species, such as aphids. They are valuable tools in many IPM systems for optimising the nature, location, and timing of the control strategies employed by growers (Harrington and Hullé, 2017). The models can also be used to infer the risk period for the transmission of certain plant viruses by forecasting the phenology of key virus vectors, such as *M. persicae*. Previously, there was a reliance on calendar dates to time insecticide programmes. This has often resulted in unnecessary applications of insecticides or on occasions left growers unprepared for early aphid migrations during years with warm winters/springs (Morgan, 2004). Additionally, local crop inspections are often insufficient for successfully timing the application of treatments. By the time aphids are detected in crops, it may be too late to manage the transmission of plant viruses effectively (Heathcote *et al*., 1969).

Forecasting systems, particularly those based on weather variables, may offer a solution to these problems.

Previously, several types of forecasting system have been developed to predict aphid infestations (Sigvald, 1992). One of the first successful examples of a forecasting system constructed using long-term aphid data was for the black-bean aphid, *Aphis fabae* (Scopoli, 1763) (Hemiptera: Aphididae), on spring-sown field beans in southern England (Way *et al*., 1981). The spring captures of *A. fabae* in suction traps provided the most accurate prediction of the timing and size of infestations in the summer. Additionally, overwintering egg counts provided the basis for forecasting crop infestations by the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus, 1758) (Hemiptera: Aphididae), in regions where it mostly undergoes a holocyclic life cycle (Kurppa, 1989).

Alternatively, Thacker *et al*. (1997) investigated methods for predicting the summer population densities of *A. fabae*, *M. persicae,* and the pea aphid, *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae). For *A. fabae,* they found that the temperature in May and rainfall during March were more reliable predictors of aphid numbers than egg counts. Relationships between weather data and aphid phenology/abundance, particularly ambient temperature, have also been identified in several other studies (Johnson, 1954; Zhou *et al*., 1995).

2.1.3 Forecasting models for aphids based on temperature

While the development and phenology of aphids depend on several biotic and abiotic factors (Lees, 1966), the most important single factor is often temperature (Campbell *et al*., 1974; Howling *et al*., 1993; Zhou *et al.*, 1995). As poikilothermic organisms, aphids are incapable of regulating their internal body temperature. For this reason, the development and phenology of aphids is governed largely by changes in ambient temperature (Dixon and Hopkins, 2010; Harrington and Hullé, 2017). By large, this is due to an increase in rate of enzymatic reactions with increases in temperature (Higley *et al*., 1986). The rate of aphid development therefore displays a sigmoidal relationship with temperature in which the rate of development increases with temperature until an optimum is reached. After this point, temperature has a minimal or deleterious effect on development of aphids; until extreme temperatures cause death. In temperate

climates, the deleterious effects of temperature are rarely observed as ambient temperatures almost always fall within the linear range of the relationship (Campbell *et al.,* 1974). The linear relationship between development time and temperature can be described by the following equation, in which $D =$ duration of development, $T^{\circ} =$ temperature, $T^{t} =$ lower temperature threshold for development; below which development does not occur (base temperature) and $k =$ the gradient of the line (Campbell *et al.,* 1974). In principle, the base temperature may be estimated by extrapolation from the point where the fitted line intercepts the x-axis.

$$\frac{1}{D} = k(T^{\circ} - T^{t})$$

In the UK, strong statistical relationships have been identified between the timing of spring migrations of aphids and temperature during the winter or early spring, particularly for species that have an anholocyclic life cycle (Wiktelius, 1982; Walters and Dewar, 1986; Harrington *et al*., 1990). These relationships relate to both the survival and development of aphids. Forecasts based on air temperature in the winter/early spring can therefore be useful for predicting the phenology and abundance of aphids (Bale *et al*., 2002; Dixon and Hopkins, 2010).

Day-degree models

Day-degree (or degree-day) models are examples of temperature-based relationships that are commonly used to predict the development and phenology of agricultural pests, such as aphids (Pruess, 1983). The models use 'physiological time' which is expressed and approximated in units called day-degrees (D°).

At the simplest level, a day-degree (D°) is a measurement of accumulated heat units over time. Day-degrees are calculated or estimated from daily maximum and minimum temperatures, and are based on the rate of insect development at temperatures between an upper and lower temperature threshold (Bryant *et al*., 1998). There are a range of approaches used to estimate day-degrees, the simplest of which subtracts the lower temperature threshold (base temperature, T^{t}) for development from the daily mean air temperature (Higley *et al.,* 1986). Here, all negative values are assumed to be zero.

$$\mathrm{D}^{\circ} = 0.5 \times (T_{max} + T_{min}) - T^{t}$$

Other approaches used to estimate day-degrees include the sine and triangle methods (UCIPM, 2003). These methods aim to improve accuracy by attempting to account for the effects of the upper temperature threshold on development. When the upper temperature threshold for development is reached several 'cut-off' methods can be employed, for example (i) vertical cut-off (ii) intermediate cut-off and (iii) horizontal cut-off. These methods assume that when the upper temperature threshold is reached, development (i) ceases, (ii) proceeds at a slow rate and (iii) proceeds at a constant rate (Baskerville and Emin, 1969; UC IPM, 2003).

A method known as 'The Met Office Equation' provides a more accurate estimation of day-degrees (Met Office, 1928). This method attempts to take account of the sinusoidal distribution of temperature over the course of the day, including periods when the minimum temperature is below the base threshold temperature for the development of insects (Table 2.1).

Table 2.1 – Method for calculating day-degrees (D°) as outlined by the Meteorological Office (Met Office, 1928). T_{max} = maximum daily temperature, T_{min} = minimum daily temperature, T_{base} = base temperature for development.

Case	Day-degrees
1. $T_{max} \leq T_{base}$	0
2. $T_{min} \geq T_{base}$	$0.5 * (T_{max} + T_{min}) - T_{base}$
3. $T_{max} - T_{base} > T_{base} - T_{min} > 0$	$0.5 * (T_{max} - T_{base}) - 0.25 * (T_{base} - T_{min})$
4. $0 < T_{max} - T_{base} < T_{base} - T_{min}$	$0.25 * (T_{max} - T_{base})$

Aphid development can be estimated by accumulating day-degrees from a specified starting point. As each species requires a certain amount of heat (between upper and lower temperature thresholds) to complete each stage of its development, in theory, the day-degrees accumulated to a particular event should be constant.

Determining the number of accumulated day-degrees from a defined starting point (biofix) can prove useful for predicting phenological events, such as the beginning of migration (Bryant *et al*., 1998). In the UK, the starting point (biofix) of 1st February is used regularly. This is often the coldest part of the year and it is assumed that when

ambient temperature subsequently increases, insects that have overwintered in diapause will be ready to start post-diapause development (Collier and Finch, 2001).

Compared with other invertebrate groups (*e.g.* lepidoptera), aphids generally have a low temperature threshold (base temperature) for development, often around 4°C (Campbell *et al*., 1974). Whereas the upper temperature threshold, above which development ceases, is between 30 to 35°C for many aphids. For several species of aphid, the upper and lower temperature thresholds for development have been estimated through controlled laboratory or field experiments. For example, Whalon and Smilowitz (1977) examined the development of *M. persicae* at a range of temperatures. They reported the lower and upper temperature thresholds for development to be 4°C and 30°C, respectively.

In terms of validating day-degree models, it is possible to compare the timing of predicted and observed phenological events to estimate the prediction accuracy and error; usually by determining the mean absolute difference between the predicted and observed dates (Knutson and Muegge, 2010).

Several examples of day-degree models have been used to predict the phenology of pest aphids. For example, Ro *et al.* (1998) developed a day-degree model to predict phenological events in the life cycle of holocyclic *M. persicae* on both primary (peach) and secondary (potato) host plants in Washington, USA. Here, the degree-day method, mean-minus-base, was applied. At a base temperature of 4°C, egg hatch, production of the first alate morph, and emigration from primary host plants occurred at 24D°, 332D°, and 509D°, respectively from 1st January. On secondary host plants, immigration of *M. persicae* was first observed at 864D° and peak migration occurred at 1278D°. Similarly, in the UK, a day-degree forecast was developed to predict the start of migration from the primary host (poplar, *Populus* spp.), to lettuce for the lettuce-root aphid, *Pemphigus bursarius* (Linnaeus, 1758) (Hemiptera: Aphididae). The forecast was based on an accumulation of 672D° above 4.4°C from 1st February (Collier *et al.,* 1994). Several other day-degree forecasts have been developed to predict the phenology of key pest aphids.

Statistical models

Statistical phenomenological models (Harrington and Hullé, 2017) or (more simply) statistical models can also be used to forecast infestations by aphids. Regression analysis is often implemented to relate measures of aphid phenology or abundance to weather variables, such as mean air temperature (Harrington *et al*., 1991; Harrington and Hullé, 2017). This assumes a linear relationship between the predicted variable (*e.g.* date of a phenological event) and explanatory variable (*e.g.* winter/early spring temperature). For aphids, higher mean air temperature during the winter/spring often coincides with an earlier onset of migration by aphids (Zhou *et al.,* 1995).

The validity of statistical models for forecasting depends largely on the long-term collection of data using a standardised methodology. Approximately twenty years of data is considered the minimum period required to build reliable forecasts (Harrington and Hullé, 2017). Using large data sets to develop forecasting models sufficiently decreases the probability that the explanatory variables (*e.g.* measures of temperature) for the year being predicted will fall outside the range used to construct the model.

Simulation models

Alternatively, simulation models can be used to forecast aphid infestations and population dynamics (Tenhumberg *et al*., 2009; Thackray *et al*., 2009; Ciss *et al*., 2014). These models are based on detailed knowledge of the biology of the target species and attempt to simulate entire aphid dynamics (Harrington and Hullé, 2017). While these models permit the short-term forecasting of aphids, they can also be used to predict aphid phenology under different situations (*e.g.* climatic scenarios). Simulation models are often designed to account for the interactions between individual variables. However, in practice there is often a vast and complex range of interactions between several biotic and abiotic conditions. For this reason, it can be difficult to study all of the interactions between variables to parameterise the model sufficiently (Harrington and Hullé, 2017).

2.1.4 Forecasting models employed for *Myzus persicae* and *Cavariella aegopodii*

In the UK, scientists at Rothamsted Research predict the start of the migration of *M. persicae* into crops each year, using a forecast based on the mean air temperature from

January to February inclusive. Predicted dates for several locations in the UK are made available through pest bulletins issued by the Agriculture and Horticulture Development Board (AHDB).

For *C. aegopodii*, a forecast was developed based on accumulated day-degrees from 1st February above a base temperature of 4.4°C. It is estimated that the first aphid of the year is captured in a suction trap approximately after 360D° from 1st February (Collier, unpublished data). This represents the start of the spring/summer migration to carrot crops and other secondary hosts. This forecast has been used to warn growers of the onset of the spring migration as part of the AHDB Pest Bulletin series (Syngenta, 2019). Reliable relationships have also been described relating the phenology of *C. aegopodii* to measures of mean air temperature during the winter (Collier *et al*., 2017).

Cavariella pastinaceae and *C. theobaldi* have a similar life cycle to *C. aegopodii.* For this reason, it is possible that they exhibit a similar relationship with temperature. In the UK, there are no forecasting systems in place currently to inform on the phenology or abundance of these species.

While forecasting models are used regularly to warn of the onset of aphid migration, they can be extended to investigate the phenological events that occur after the arrival of the first aphid (*e.g.* capture of 10%, 25%, 50% and 90% of the spring/summer migrants). This information can help growers to monitor local infestations and optimally time management strategies for aphids throughout the crop-growing season.

Additionally, as aphids have short generation times and relatively low temperature requirements for development, they are expected to respond considerably to environmental change (Harrington *et al*., 2007). In the UK, there has been a notable trend in increasing winter/spring temperatures in recent years (Met Office, 2018). On average, species such as *M. persicae* and *C. aegopodii* are arriving earlier in the spring, when crops may be more susceptible to damage (Harrington and Clark, 2010).

Bell *et al*. (2014) investigated trends in the phenology of 55 species of aphid at seventeen suction trap locations in the UK. Using suction trap data, they derived the

first and last records of flight and the total numbers of each species caught at individual sites for each year.

For all species, there was a trend in earlier records of first flight. The majority of species (85%) also showed an increase in the duration of their flight season. In terms of aphid abundance, there was an increase in the log annual count of 54% of the species investigated. *Cavariella aegopodii* was amongst the species that showed all three trends.

To account for these recent trends, there is a need for robust forecasting models that have been developed using aphid capture data and records of air temperature from the last several years.

2.1.5 Monitoring and forecasting infestations by aphids: aims

The aims of the research described in this chapter were to:

- Investigate the relative abundance and relative phenology of *M. persicae*, *C. aegopodii*, *C. pastinaceae* and *C. theobaldi* at several sites in the UK.

- Examine the relationships between temperature variables and the timing of aphid activity. In particular, test the hypothesis that day-degree models or similarly, models based on mean air temperature, can be used to predict the migration of *M. persicae*, *C. aegopodii*, *C. pastinaceae* and *C. theobaldi*.

- Determine whether a day-degree forecast and/or measures of mean air temperature could be used to infer the timing of the spring/summer peak in numbers of *M. persicae*.

- Investigate the relationships between the timing of aphids caught in yellow water traps (YWTs) and captures made by the suction trap at Wellesbourne, Warwickshire, UK.

2.2 Monitoring and forecasting infestations by aphids: Materials and Methods

2.2.1 Investigating the relative abundance and phenology of aphids

Aphid abundance

Patterns in yearly aphid abundance were investigated individually for *M. persicae, C. aegopodii, C. pastinaceae* and *C. theobaldi* caught in the suction traps at six locations in the UK; Broom's Barn (Suffolk), Kirton (Lincolnshire), Preston (Lancashire), Rothamsted (Harpenden), Writtle, (Essex) and Wye (Kent) during 2013–2018. Aphid capture data were supplied by the Rothamsted Insect Survey (RIS).

Additionally, the relationships between the total numbers of each species caught each year (1996–2018) in the suction traps at the six locations were investigated. To normalise the data, aphid counts were first $\log(x+1)$ transformed. Years x sites in which suction traps were inactive for a considerable period (≥ 1 week) during the 'aphid season' (April to mid-November) were not included in the analysis. Linear regressions were fitted to the data, with correlation coefficients estimated in SPSS Statistics (Version 25.0, IBM Corp©, Armonk, USA).

Aphid phenology

The relative phenology of *M. persicae, C. aegopodii, C. pastinaceae* and *C. theobaldi* was investigated at the six suction trap locations. The dates of the first, 10% and 50% capture of the spring/summer migrants of each species were determined in Microsoft® Excel (Microsoft® Office 2016 for Mac, Version 15.0) for recent sets of aphid capture data (2013–2018). Day 240 from 1st January (end of August) was used as a 'cut-off' to separate aphid migrating in the spring/summer from those migrating back to winter host plants in the Autumn. The day of first capture was assumed to indicate the point at which aerial populations of aphids reached the threshold for detection by suction traps; also implying the start of migration from winter hosts, potentially into crops. Trends in the relative timing of the four species of aphid were compared across sites.

Risk periods for infestation and virus transmission by Myzus persicae* and *Cavariella aegopodii

Periods of potential crop infestation/transmission of plant viruses (risk periods) by *M. persicae* and *C. aegopodii* were determined for the six suction trap locations. The earliest capture dates of the first aphid and latest capture dates of 90% aphids were determined for 2013–2018 in Microsoft® Excel (Version 15.0). In addition, a combined risk period for *M. persicae* and *C. aegopodii* was investigated.

2.2.2 Temperature records

Daily maximum and minimum air temperature records, used to develop aphid forecasting models, were accessed from the British Atmospheric Data Centre (BADC), Centre for Environmental Data Analysis (CEDA) Archive – Met Office Integrated Data Archive System (MIDAS) Land and Marine Surface Stations Data (1853-current) (Met Office, 2012). Meteorological stations were selected based on their proximity to each suction trap (Table 2.2) and in most cases these were at the same site as the suction trap.

Table 2.2 – Meteorological stations used for establishing relationships between air temperature data and aphid captures at the different suction trap locations in the UK. * = indicates that the weather station is located at the same site as the suction trap.

Suction trap site	Met. station site	Met. station location	National Grid reference no.	MIDAS Met. Station no.
Broom's Barn	Broom's Barn*	Suffolk	TL 753656	3115
East Craigs	East Craigs*	Midlothian	NT 181737	1636
Hereford	Preston Wynne*	Hereford	SO 564475	4886
Kirton	Kirton*	Lincolnshire	TF 310381	2463
Preston	Moor Park	Lancashire	SD 498401	7245
Rothamsted	Rothamsted No.2*	Hertfordshire	TL 131133	3537
Starcross	Starcross*	Devon	SX 972821	8873
Wellesbourne	Wellesbourne*	Warwickshire	SP 272565	4453
Writtle	Writtle*	Essex	TL 676067	3644
Wye	Wye*	Kent	TR 059470	5375

2.2.3 Forecasting the phenology of *Myzus persicae*

Data preparation of suction trap counts

The Rothamsted Insect Survey (RIS) provided weekly capture data for alate *M. persicae* caught in the suction traps at three sites in the UK: Broom's Barn (Suffolk), Kirton (Lincolnshire) and Rothamsted (Harpenden). Due to differences in the operational time frames of the suction traps, the years with available aphid counts varied between sites (Table 2.3).

Table 2.3 – Operational time frames of the suction traps (used to develop and validate forecasts for *Myzus persicae*) at Broom's Barn, Kirton and Rothamsted.

Site	Years trap active
Broom's Barn	1965 – 2015
Kirton	1981 – 2015
Rothamsted	1965 – 2015

Years in which suction traps were inactive for considerable period of time (*e.g.* longer than one week) during the spring/summer were not included in the analysis. Across the three sites, this allowed for the construction of a primary data set based on 71 sites x years, for which there were reliable suction trap data and weather records between 1965 and 1999.

For each site x year, the weeks of capture of the first, 10%, 25%, 50% and 90% of the spring/summer migrants, up to a specific cut-off point, were calculated in Microsoft® Excel (Version 15.0). These intervals were thought to provide useful monitoring points of aphid infestation during the spring/summer. Week 34 (end of August) was used as the cut-off point to distinguish spring/summer migrants from aphids migrating in the autumn. Years in which fewer than 50 *M. persicae* were caught at a site were not included in the data set as it would not be possible to estimate capture dates accurately when a small number of aphids are caught during a migratory period.

2.2.4 Developing day-degree forecasts for predicting the phenology of *Myzus persicae*

The relationship between the timing of aphid activity and accumulated day-degrees was investigated using the base temperature for *M. persicae* development (4°C). The upper temperature threshold, above which development ceases, has been defined as 30°C for *M. persicae* (Whalon and Smilowitz, 1977). The primary data set was used to establish these relationships (71 sites x years).

Day-degrees were calculated and summed over defined periods of time, for example, from 1st January until the beginning of a particular event (*e.g*. the weeks of first, 10%, 25% 50%, 90% capture of the spring/summer migrants). Days on which the average temperature did not exceed the base temperature (4°C), did not contribute to the accumulated total. The method for calculating day-degrees, as outlined by the Met. Office, is shown in Table 2.1 (see Chapter 2.1.3: p.18).

2.2.5 Developing forecasts based on mean air temperature for predicting the phenology of *Myzus persicae*

The relationships between measures of mean air temperature across defined periods in the winter/spring and the migration of *M. persicae* were investigated across the primary data set (71 sites x years). Linear regressions were fitted to the data set, with correlation coefficients estimated in SPSS Statistics (Version 25.0, IBM Corp©). Multiple linear regression analysis was used to test whether incorporating the effects of site-specific factors, such as site latitude, longitude and altitude, could improve the models.

The models developed were then validated using a separate data set (Broom's Barn, Kirton, Rothamsted: 2000–2010). The fitted equations were used to determine the predicted the weeks of the first, 10%, 25% 50%, 90% capture of the spring/summer migrants each year. The mean absolute differences between the observed and predicted capture dates were then calculated.

2.2.6 Forecasting the timing of the summer peak in *Myzus persicae* numbers

The weeks on which spring/summer peaks in *M. persicae* numbers occurred were determined for each year at the three sites (Broom's Barn, Rothamsted, Kirton). The 'aphid peak' was here defined as the week in which the greatest number of spring/summer migrants was caught within a given year. In all cases the peak was followed by a population crash. The timing of the 'aphid peak' was investigated in relation to both accumulated day-degree sums and measures of mean air temperature. The data set used to investigate these relationships was based on 50 sites x years, for which there were distinct spring/summer peaks (defined as ≥ 50 aphids captured in a week, before the cut-off point of week 34) and reliable temperature records between 1965 and 1999.

The fitted equations were used to determine the predicted weeks of the 'aphid peak' for a separate data set (2000–2010). The mean absolute differences between the observed and predicted weeks of the peak in *M. persicae* numbers were then calculated.

2.2.7 Forecasting the phenology of *Cavariella* spp.

Data preparation of suction trap counts

Daily records of aphid suction trap captures were provided by the Rothamsted Insect Survey (RIS) for *C. aegopodii*, *C. pastinaceae* and *C. theobaldi*. Data on *C. pastinaceae* and *C. theobaldi* have only been recorded since 1996. Data for *C. aegopodii*, however, were available at several sites from 1965. The data set used for analysis consisted of daily capture data at six sites; for which there were both available suction trap data and reliable temperature records. The sites used were: Broom's Barn (Suffolk), Kirton (Lincolnshire), Preston (Lancashire), Rothamsted (Harpenden), Writtle, (Essex) and Wye (Kent). Aphid capture data were available for the majority of sites between 1996–2006.

This allowed the construction of three primary data sets for which there were reliable suction trap data (≥ 50 aphids captured before cut-off point) and weather records.

There were totals of 56 sites x years for *C. aegopodii*, 45 sites x years for *C. pastinaceae* and 35 sites x years for *C. theobaldi.*

For each site x year, the weeks of the first, 10%, 25%, 50% and 90% capture of aphids, up to a specific cut-off point, were calculated for each site x year combination in Microsoft® Excel. Day 240 from 1st January (end of August) was used as the cut-off point to distinguish between the spring/summer migrants and aphids migrating in the autumn.

2.2.8 Developing day-degree forecasts for predicting the phenology of *Cavariella* spp.

Using the primary data sets (1996–2006), the relationships between accumulated day-degrees and the timing of aphid activity were investigated using different base temperatures for development and day-degree accumulation start points for *C. aegopodii*, *C. pastinaceae*, and *C. theobaldi*. Day-degrees were calculated and summed over defined periods of time, for example, from 1st January or 1st February until the beginning of a particular event (*e.g*. the capture dates of the first, 10% and 50% of the spring/summer migrants).

For *C. aegopodii*, the day-degree forecasts were validated using a separate data set based on aphid capture data from eight sites; Broom's Barn (Suffolk), East Craigs (Edinburgh), Hereford (Herefordshire), Kirton (Lincolnshire), Rothamsted (Harpenden), Starcross (Devon), Writtle (Essex) and Wye (Kent) between 1981–1988, for which there were reliable suction trap records and aphid capture date (≥ 50 aphids caught before the cut-off point of Day 240) (55 sites x years). Mean absolute differences were calculated between the predicted dates on which day-degree sums (as indicated by the forecasts) were accumulated and the observed capture dates of aphids.

2.2.9 Developing forecasts based on mean air temperature for predicting the phenology of *Cavariella* spp.

The relationships between measures of mean air temperature across defined periods in the winter/spring and the migration of *Cavariella* spp. were investigated using the

primary data sets (1996–2006) for each species (56 sites x years for *C. aegopodii*, 45 sites x years for *C. pastinaceae* and 35 sites x years for *C. theobaldi*).

Linear regressions were fitted to the data sets, with correlation coefficients estimated in SPSS Statistics (Version 25.0, IBM Corp©). Multiple linear regression analysis was then used to incorporate the effects of site-specific factors (*e.g.* site latitude and longitude) to test whether their inclusion improved the models.

The models developed were then validated using separate data sets. For *C. aegopodii*, the 1981–1988 validation data set (55 sites x years) was used. For *C. pastinaceae* and *C. theobaldi*, validation data sets were comprised of aphid capture data from three sites (Broom's Barn, Kirton, and Rothamsted) between 2008–2015, for which there were reliable suction trap records and aphid capture data (≥ 50 aphids caught before the cut-off point of Day 240). These species were less abundant than *C. aegopodii* and access to weather records was limited after 2010; giving rise to smaller data sets (eighteen sites x years for *C. pastinaceae* and twelve sites x years for *C. theobaldi*).

The fitted equations were used to determine the predicted the weeks of first, 10%, 25% 50%, 90% aphid capture of the spring/summer migrants. The mean absolute differences were then determined between the observed and predicted capture dates.

The data sets used for all forecast development and validation (for the four species of aphid) are outlined in Appendix A.1 (p.262).

2.2.10 Field monitoring of *Cavariella aegopodii*

In 2016–2018, a series of eight yellow water traps (YWTs) (Ringot Flora Insect Trap; Nickerson Brothers Limited, Lincolnshire, UK) were placed approximately 20 cm above the ground in and around carrot (*Dacus carota*) plots at Warwick Crop Centre, School of Life Sciences, Wellesbourne, UK (National Grid reference: SP 27146 56846) (Fig. 2.3). Traps were placed approximately 10 m away from other traps. Traps held approximately 900 ml of water and were used to measure the abundance of *C. aegopodii* and to compare captures over time.

Figure 2.3 – Yellow water trap (YWT) (Ringot Flora Insect Trap, Nickerson Brothers Limited, Lincolnshire) used to catch alate *Cavariella aegopodii* in and around carrot plots (approximately 30 m x 10 m) at Warwick Crop Centre, Wellesbourne, UK in 2016–2018. Traps consisted of a 1.5 m x 10 mm diameter Safi yellow stake, yellow bowl (9 x 26 cm diameter) and adjustable bowl mounting clip so that the bowl can be lifted.

The traps were emptied twice a week (during April–September) with the contents filtered through a plant pot (9 x 14 cm diameter) and the samples collected in muslin cloth. In the laboratory, aphids were removed from the insect samples using Storkbill fine blunt forceps (105 mm, arm length 43 mm, Watkins and Doncaster Ltd., Herefordshire, UK) and preserved in a solution of 95% ethanol 5% glycerol. Alate *C. aegopodii* were later identified under a stereomicroscope (Euromex E series, Holland, x45 magnification).

The captures of *C. aegopodii* in YWTs and in the suction trap at Wellesbourne were compared for each year (2016–2018). Day-degree forecasts previously developed were used to predict the capture dates of the first and 10% of the spring/summer migrants. Predicted dates were compared to the dates when aphids were first caught in the suction trap at Wellesbourne, UK.

2.2.11 Diurnal variation in the flight activity of *Cavariella aegopodii*

During 2016–2018, the numbers of alate *C. aegopodii* caught in YWTs were monitored each hour between 9:00 and 16:00 GMT on days around the summer peak in *C. aegopodii* numbers (late May–mid June). The number of alate *C. aegopodii* caught outside of the intensive monitoring period were also recorded between 16:00 and 9:00 the following day. Monitoring was only carried out on dry days with low wind speed. Across the three years, there were a total of sixteen days (2016; seven days, 2017; two days and 2018; seven days) on which the daily totals of *C. aegopodii* caught were ≥ 20 aphids (Table 2.4). This total was thought to be sufficient to show distinct peaks in the diurnal flight activity of *C. aegopodii*.

Table 2.4 – Total number of monitoring days of the hourly captures of *Cavariella aegopodii* in yellow water traps (YWTs) in and around carrot plots at Wellesbourne, UK (2016–2018) and the total numbers of aphids caught during the intensive monitoring period (9:00–16:00 GMT) across all days.

Year	Total no. of monitoring days	Total *C. aegopodii* caught during intensive monitoring period
2016	7	239
2017	2	83
2018	7	212

At 9:00, YWTs were filled with water and a surfactant (Premiere Savona D2, Premiere Products, Cheltenham, UK; pH 7) to the fill-line (900 ml). Each subsequent hour (from 10:00) YWTs were emptied with the contents filtered through a plant pot (9 x 14 cm diameter) and the samples collected in muslin cloth. Samples were placed into plastic pots (10.6 x 8 cm diameter) that were labelled with the sampling time and number of the YWT (1–8). Traps were then refilled with water/surfactant.

In addition, YWTs were emptied and refilled at 16:00 on each monitoring day. Samples were then taken at 9:00 the following day. This was to determine the number of *C. aegopodii* that were flying outside of the intensive monitoring period.

The samples from YWTs were stored in freezers at –23 ±2°C. Aphids were later removed from the samples and alate *C. aegopodii* were identified under a stereomicroscope (Euromex E series, Holland, x45 magnification).

Statistical analysis

A Kruskal-Wallis H test was carried out in SPSS Statistics (Version 25.0, IBM Corp©) to compare the proportion of the daily catch of *C. aegopodii* between the different time periods. Dunn's Multiple Comparison Test (Dunn, 1961) with Bonferroni correction (Bonferroni, 1936) was carried out to check for statistically significant differences between the 28 pairs of groups. Prior to this the data were checked for normality (Shapiro-Wilk test).

2.3 Monitoring and forecasting infestations by aphids: Results

2.3.1 Investigating the relative abundance and phenology of aphid species

The abundance of aphids

On average, during 2013–2018, *C. aegopodii* and *M. persicae* were most abundant at Broom's Barn, whereas *C. pastinaceae* and *C. theobaldi* were most abundant at Kirton. Across all sites, *C. pastinaceae* and *C. theobaldi* were considerably less abundant than *C. aegopodii* and *M. persicae* (Fig. 2.4).

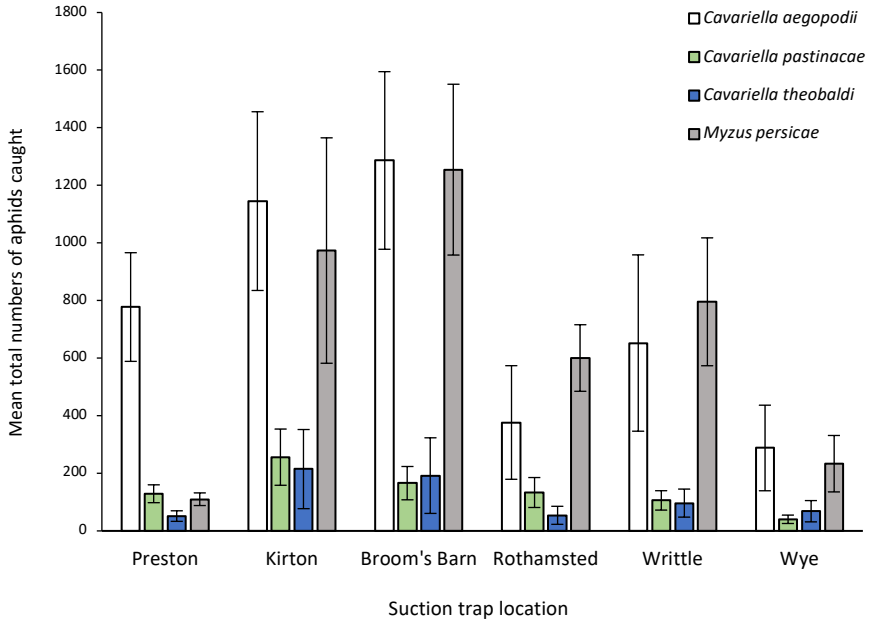

Figure 2.4 – Mean annual numbers (2013–2018) (±1 S.E.) of *Cavariella aegopodii*, *C. pastinaceae, C. theobaldi* and *Myzus persicae* caught at six suction trap locations in the UK. Suction trap locations are ordered by latitude to help visualise patterns in aphid abundance across the UK.

The mean annual number of aphids caught across the six sites were determined for each year (2013–2018). For most years, the mean annual numbers of *C. aegopodii* and *M. persicae* were similar. Mean annual numbers of *C. pastinaceae* and *C. theobaldi* also followed similar patterns each year with fewer than 500 aphids caught (Fig. 2.5). Across all sites, with the exception of *C. pastinaceae,* aphids were most abundant in 2015.

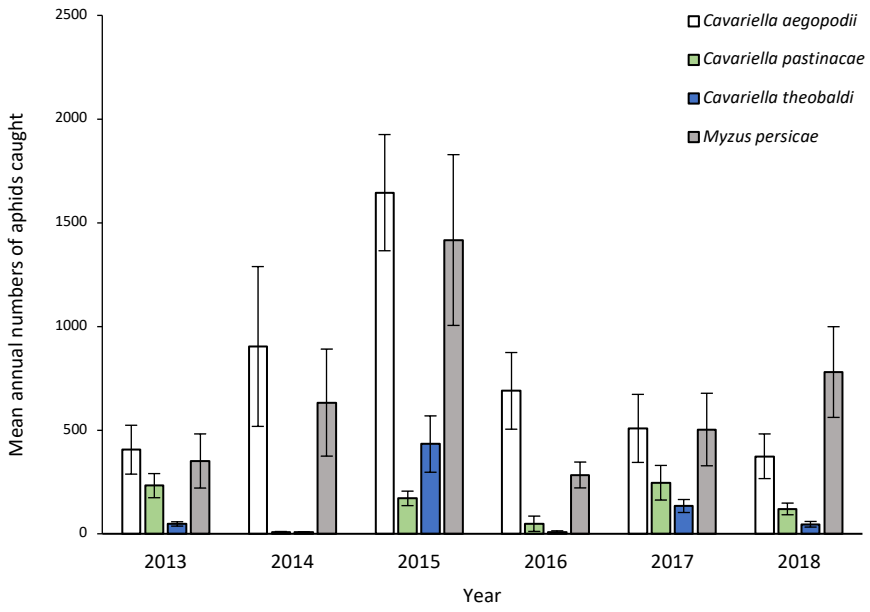

Figure 2.5 – Mean annual numbers (2013–2018) (±1 S.E.) of *Cavariella aegopodii*, *C. pastinaceae, C. theobaldi* and *Myzus persicae* caught at six suction trap locations in the UK.

The relationships between total numbers of *C. aegopodii, C. pastinaceae* and *C. theobaldi* caught each year at six suction trap locations in the UK (Preston, Kirton Broom's Barn, Rothamsted, Writtle and Wye) between 1996 and 2018 were investigated.

The log(x+1) numbers of *C. pastinaceae* and *C. theobaldi* caught were positively correlated ($R^2 = 0.55$, $P < 0.001$). This suggests that the abundance of the two species follows similar patterns at individual sites each year (Fig. 2.6). However, the abundance of *C. aegopodii* was weakly correlated with that of *C. pastinaceae* ($R^2 = 0.14$, $P < 0.001$) and *C. theobaldi* ($R^2 = 0.06$, $P = 0.005$), with a greater amount of scatter produced (Figs. 2.7 and 2.8).

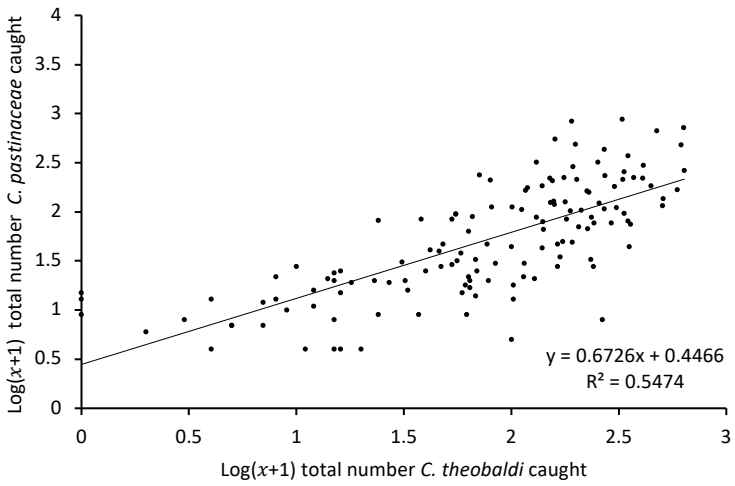

Figure 2.6 – Relationship between log(x+1) transformed total numbers of *Cavariella pastinaceae* and *C. theobaldi* caught each year at six suction trap locations in the UK between 1996 and 2018. The line shows the fitted linear regression (P < 0.001).

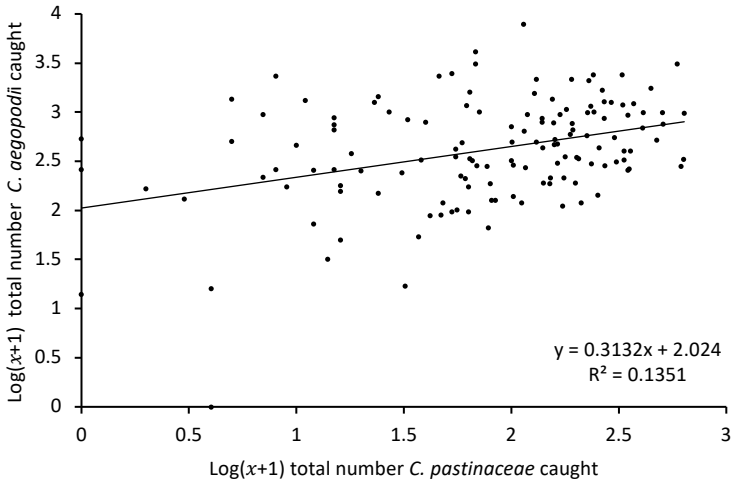

Figure 2.7 – Relationship between log(x+1) transformed total numbers of *Cavariella aegopodii* and *C. pastinaceae* caught each year at six suction trap locations in the UK between 1996 and 2018. The line shows the fitted linear regression (P < 0.001).

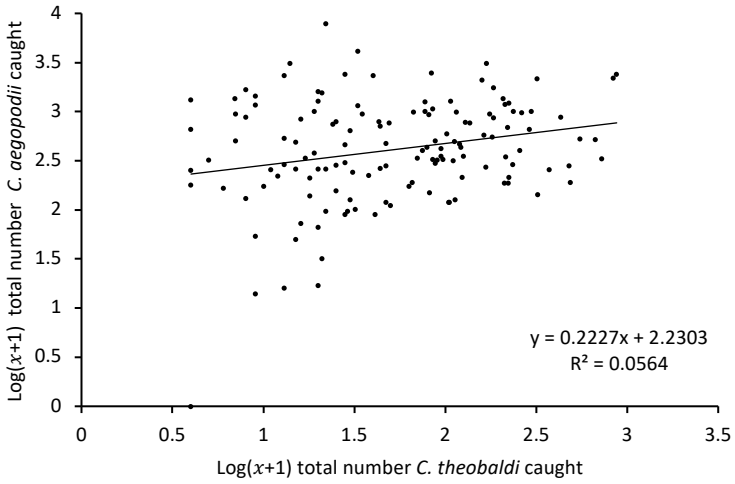

Figure 2.8 – Relationship between log(x+1) transformed total numbers of *Cavariella aegopodii* and *C. theobaldi* caught each year at six suction trap locations in the UK between 1996 and 2018. The line shows the fitted linear regression (P = 0.005).

The phenology of aphids

In order to investigate the relative phenology of the four species of aphid, the mean capture dates (of the first, 10% and 50% aphids of the spring/summer migration) for recent years (2013–2018) were plotted for the six suction trap sites at which the total annual numbers of aphids caught ≥ 50 (SEM and sample sizes for each species at each site are shown in Appendix A.2). The data points for the sites were connected by dashed lines in order to help to visualise trends in aphid phenology; but data points were in no way related and should not be interpreted as such.

With the exception of Wye, the onset of aphid migration (mean capture date of the first aphid) occurred earliest for *C. aegopodii* at sites (Fig. 2.9). Generally, *M. persicae* was the next species to begin its migration. However, at sites in the east and south of the UK, the onset of migration by *C. theobaldi* occurred at a similar time to *M. persicae*. With the exception of Preston, *C. pastinaceae* was the last species to begin its migration at all other sites.

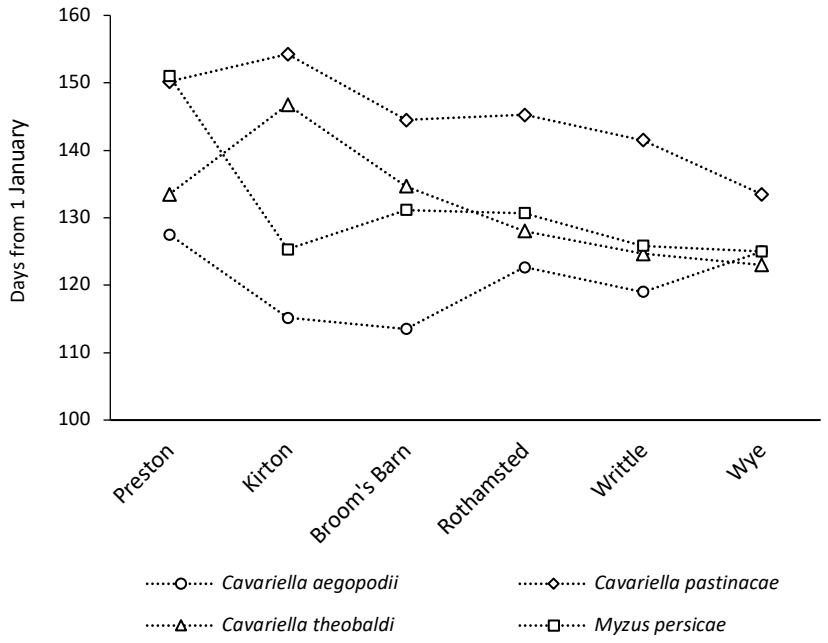

Figure 2.9 – Mean capture date (2013–2018) of the first aphid of the year for each species (onset of migration) at six suction trap locations in the UK. Sites are ordered by latitude.

Similar patterns in the relative timing of the 10% and 50% capture dates were observed for the four species of aphids (Figs. 2.10 and 2.11). All species showed a general decline in capture date (earlier capture dates) at lower latitudes. However, the capture dates of *C. pastinaceae* and *C. theobaldi* were always latest at Kirton.

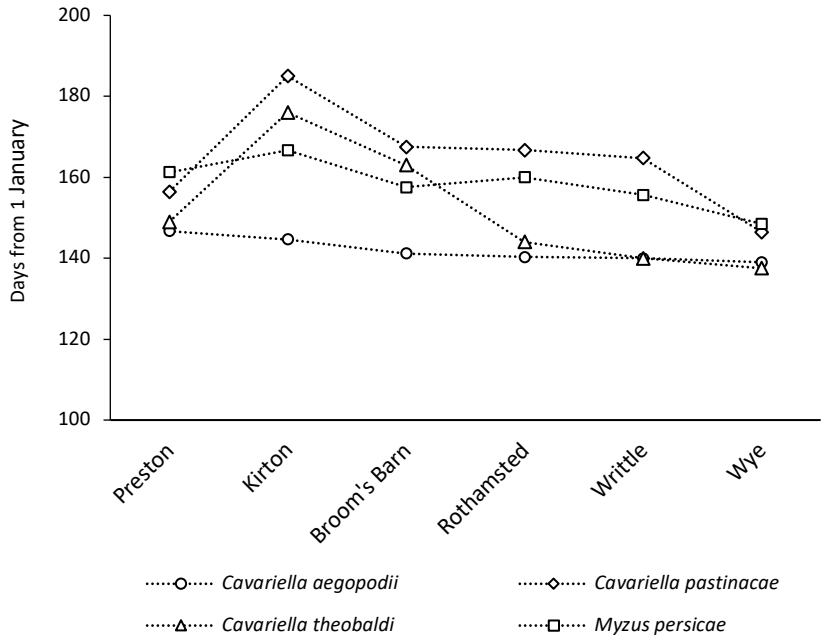

Figure 2.10 – Mean capture date (2013–2018) of 10% of the spring/summer migrants for each species at six suction trap locations in the UK. Sites are ordered by latitude.

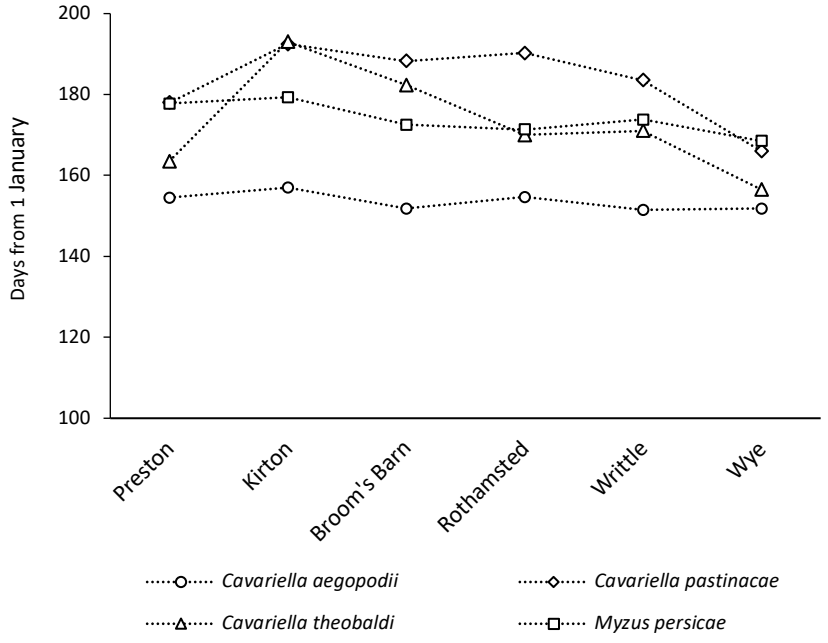

Figure 2.11 – Mean capture date (2013–2018) of 50% of the spring/summer migrants (mid-point of migration) for each species at six suction trap locations in the UK. Sites are ordered by latitude.

Risk periods for infestation and virus transmission by Cavariella aegopodii and Myzus persicae

The dates of the first and 90% capture of *C. aegopodii* and *M. persicae* were determined at individual sites for 2013–2018. For each site, the minimum date of first capture and maximum date of 90% capture (2013–2018) could be used to infer the 'risk period' of aphid infestation and the potential window for virus transmission. A combined 'risk period' was also determined for *C. aegopodii* and *M. persicae*, based on the earliest first capture and latest 90% capture of either species (Table 2.5).

For *C. aegopodii* and *M. persicae*, Kirton had the longest 'risk periods' with mean durations of 54 and 67 days, respectively. In terms of the shortest 'risk period' for *C. aegopodii,* Preston and Wye both had a mean duration of 44 days. Whereas, for *M. persicae,* the shortest 'risk period' was for Preston (mean of days of 35). Similarly, the combined 'risk period' for both species was longest at Kirton (85 days) and shortest at Preston (60 days).

Table 2.5 – Capture dates of the first and (90%) of the spring/summer migrants of *Cavariella aegopodii* and *Myzus persicae* at six suction traps locations (2013–2018) including a combined 'risk period' for both species. The number below is the difference (in days) between the first and 90% capture dates. * = year in which < 50 aphids were caught at a site.

Species	Year	Preston	Kirton	Broom's Barn	Rothamsted	Writtle	Wye
Cavariella aegopodii	2013	146(189) 43	146(197) 51	138(194) 56	148(195) 47	139(190) 51	145(181) 36
	2014	119(151) 32	120(154) 34	114(159) 45	114(174) 60	118(171) 53	*
	2015	128(182) 54	111(180) 69	124(177) 53	113(175) 62	127(173) 46	127(174) 47
	2016	127(163) 36	77(168) 91	82(167) 85	125(167) 42	95(162) 67	108(168) 60
	2017	119(164) 45	114(154) 40	97(151) 54	109(153) 44	112(148) 36	118(149) 31
	2018	126(182) 56	123(163) 40	126(148) 22	127(175) 48	123(151) 28	127(173) 46
	Mean:	**128(172) 44**	**115(169) 54**	**114(166) 52**	**123(173) 50**	**119(166) 47**	**125(169) 44**
	Min (Max):	119(189) 70	77(197) 120	82(194) 112	109(195) 86	95(190) 95	108(181) 73
Myzus persicae	2013	*	167(214) 47	167(212) 45	171(207) 36	165(213) 48	*
	2014	*	76(186) 110	99(164) 65	119(177) 58	107(174) 67	*
	2015	161(193) 32	120(183) 63	127(180) 53	127(185) 58	129(178) 49	129(182) 53
	2016	150(167) 17	127(184) 57	128(168) 40	125(167) 42	93(168) 75	122(176) 54
	2017	132(186) 54	109(176) 67	118(169) 51	118(180) 62	131(184) 53	118(188) 70
	2018	161(196) 35	153(211) 58	148(197) 49	124(194) 70	130(192) 62	131(192) 61
	Mean:	**151(186) 35**	**125(192) 67**	**131(182) 51**	**131(185) 54**	**126(185) 59**	**125(185) 60**
	Min (Max):	132(196) 64	76(214) 138	99(212) 113	118(207) 89	93(213) 120	118(192) 74
Both species	2013	*	146(214) 68	138(212) 74	148(207) 59	139(213) 74	*
	2014	*	76(186) 110	99(164) 65	114(177) 63	107(174) 67	*
	2015	128(193) 65	111(183) 72	124(180) 56	113(185) 72	127(178) 51	127(182) 55
	2016	127(167) 40	77(184) 107	82(168) 86	125(167) 42	93(168) 75	108(176) 68
	2017	119(186) 67	109(176) 67	97(169) 72	109(180) 71	112(184) 72	118(188) 70
	2018	126(196) 70	123(211) 88	126(197) 71	124(194) 70	123(192) 69	127(192) 65
	Mean:	**125(185) 60**	**107(192) 85**	**111(181) 70**	**122(185) 63**	**116(184) 68**	**120(184) 64**
	Min (Max):	119(196) 77	76(214) 138	82(212) 130	109(207) 98	93(213) 120	108(192) 84

2.3.2 Forecasting the phenology of *Myzus persicae*

The mean weekly totals of alate *M. persicae* caught in the suction traps at Broom's Barn, Rothamsted and Kirton were plotted to observe trends in aphid numbers across the sites. *Myzus persicae* was most abundant at Broom's Barn (Fig. 2.12). Similar numbers of *M. persicae* were caught at Rothamsted and Kirton. Generally, peaks in the mean numbers of *M. persicae* caught occurred at similar times across the three sites. The summer peak in mean aphid numbers occurred earliest at Rothamsted (~week 25) and latest at Broom's Barn (~week 28). There were also smaller peaks in the mean numbers of *M. persicae* at Broom's Barn and Kirton during the autumn.

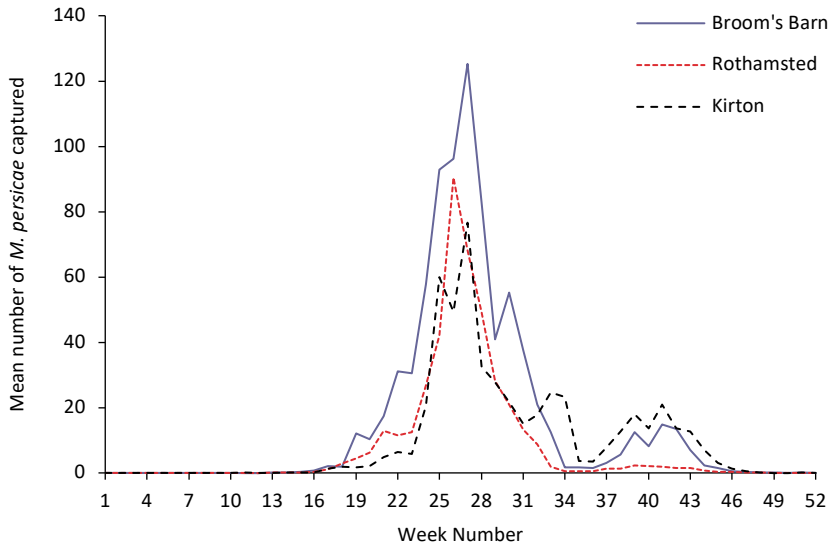

Figure 2.12 – Mean weekly totals of alate *Myzus persicae* caught in the suction traps at Broom's Barn, Rothamsted and Kirton during 1981–2015.

2.3.3 Developing a day-degree forecast for *Myzus persicae*

The relationships between day-degrees accumulated from 1st January, above a base temperature of 4°C and the weeks of the first, 10%, 25%, 50% and 90% capture of the spring/summer migrants of *M. persicae* were investigated across the primary data set (71 sites x years) (Fig. 2.13 A–E).

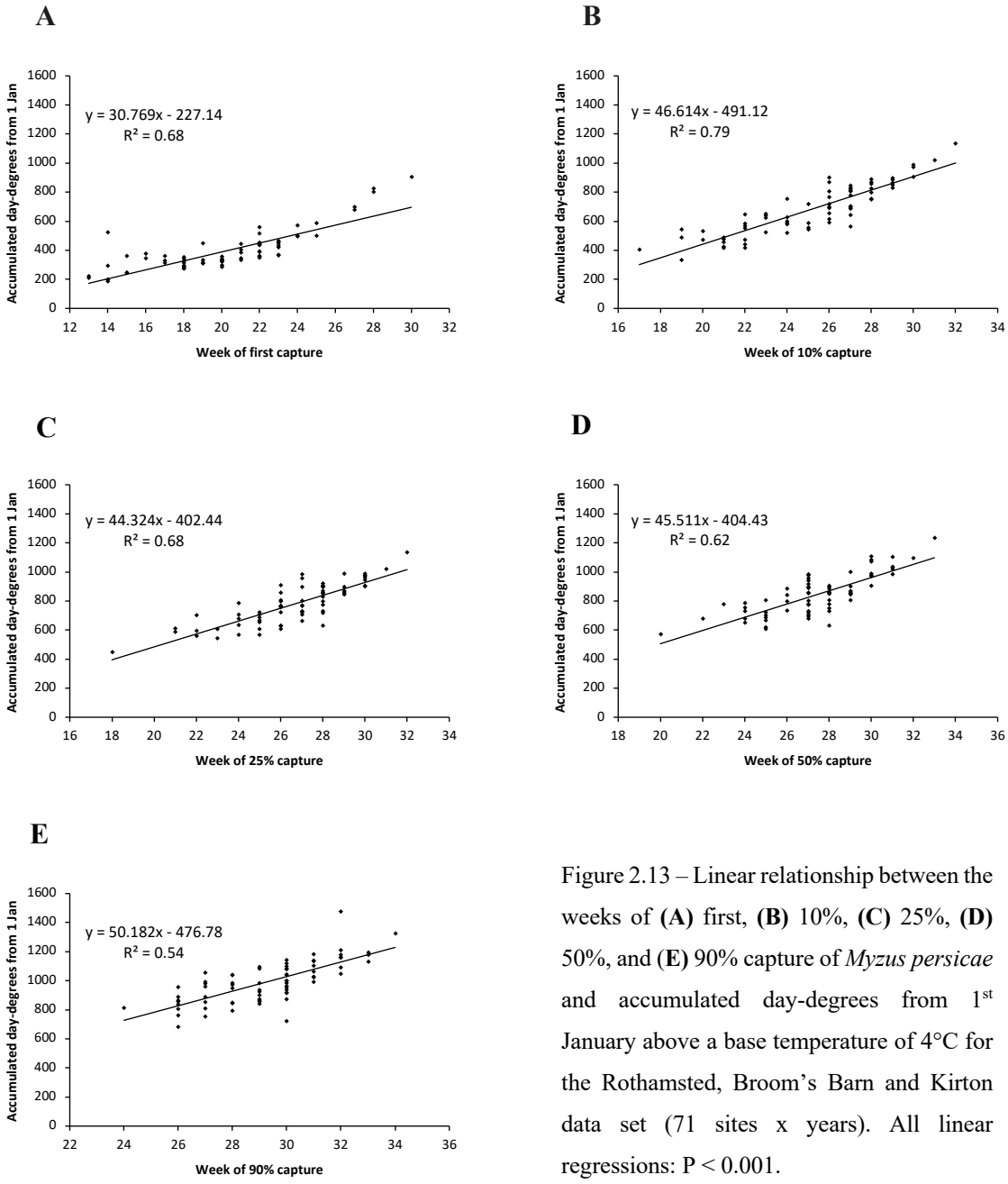

Figure 2.13 – Linear relationship between the weeks of **(A)** first, **(B)** 10%, **(C)** 25%, **(D)** 50%, and **(E)** 90% capture of *Myzus persicae* and accumulated day-degrees from 1st January above a base temperature of 4°C for the Rothamsted, Broom's Barn and Kirton data set (71 sites x years). All linear regressions: $P < 0.001$.

It was not possible to obtain a constant day-degree sum for any of the measures of *M. persicae* activity (Fig. 2.13). Instead, capture weeks were positively correlated with accumulated day-degree sums from 1st January (above a base temperature of 4°C) (*e.g.* the later the week of capture, the higher the accumulated day-degree sum).

2.3.4 Developing a forecast based on mean air temperature for *Myzus persicae*

For all sites x years, the earliest date of the first capture of aphids occurred during week 13 (27th March–2nd April) at Broom's Barn and Rothamsted in 1989. There were no other occasions when the first aphid was caught before the start of April. The earliest capture of 10% aphids occurred on week 17 at Kirton (1990; 23rd–29th April) and the earliest capture of 25% aphids occurred in week 18 at Kirton (1990; 30thApril – 6th May). The capture of 50% aphids was earliest in week 20 at Kirton (1990; 14th–20th May), there was one other occasion when 50% of aphids were captured before the end of May at Broom's Barn in 1998 (25th–31st May). The earliest date of 90% capture of aphids was observed in week 24 (8th–14th June) in 1998 at Broom's Barn.

Figure 2.14 shows the relationships between mean air temperature and the capture weeks of *M. persicae* (for the first, 10%, 25%, 50%, and 90% of the spring/summer migrants) across the primary data set (71 sites x years).

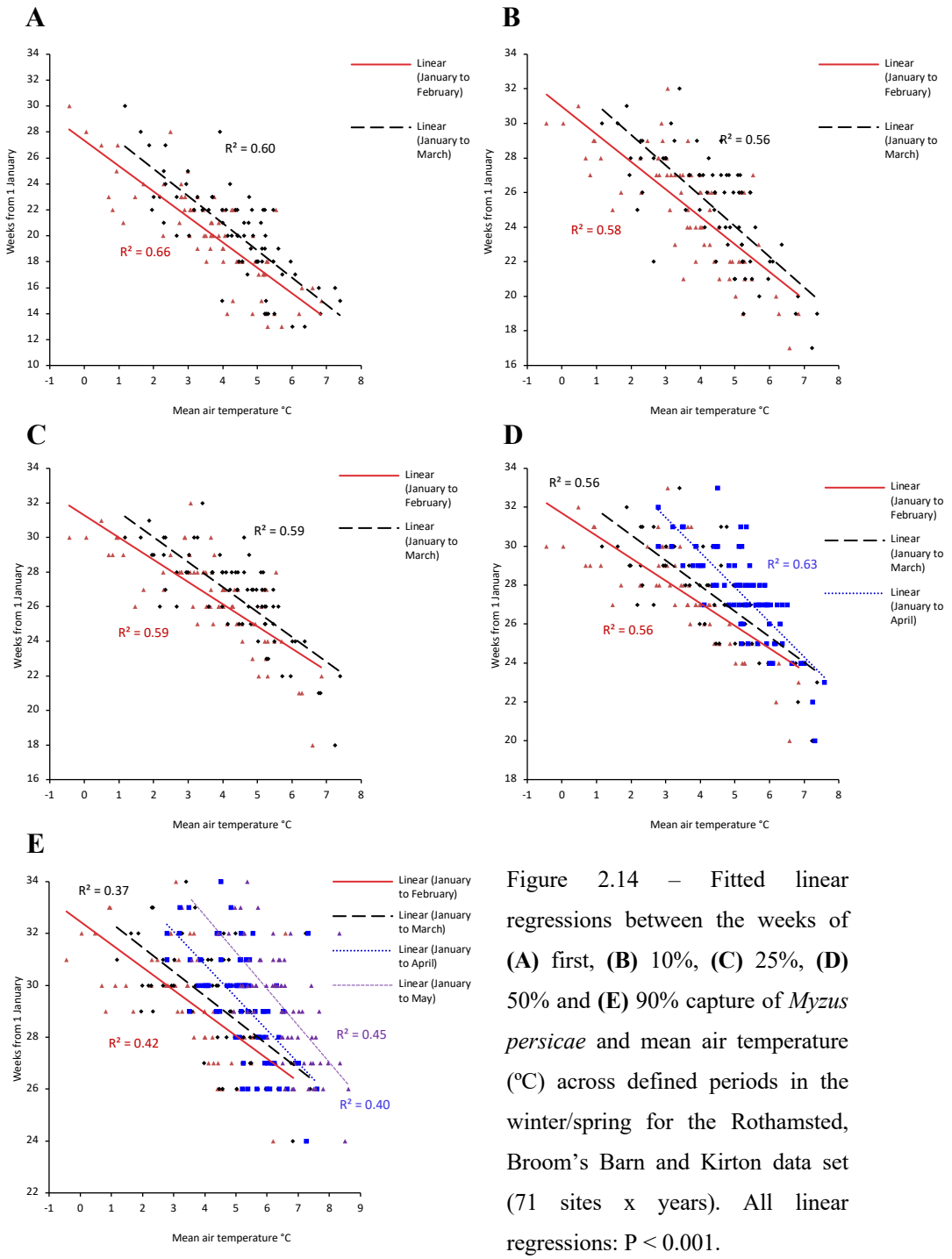

Figure 2.14 – Fitted linear regressions between the weeks of **(A)** first, **(B)** 10%, **(C)** 25%, **(D)** 50% and **(E)** 90% capture of *Myzus persicae* and mean air temperature (ºC) across defined periods in the winter/spring for the Rothamsted, Broom's Barn and Kirton data set (71 sites x years). All linear regressions: $P < 0.001$.

Despite the considerable scatter seen in Figure 2.14, for *M. persicae*, the dates of capture (weeks) were negatively correlated with measures of mean air temperature during the winter/spring. The strongest correlations were produced for the week of first capture and the mean air temperature from January–February ($R^2 = 0.66$, $P < 0.001$).

Linear regression analysis identified statistically significant relationships ($P < 0.001$) between the timing of aphid activity and mean air temperature during the winter/spring. This permitted the development of simple forecasts for predicting the phenology of *M. persicae*. For example, at the end of February it would be possible to predict the weeks of capture of the first, 10%, and 25% of the spring/summer migrants. For predicting the weeks of 50% and 90% capture of the spring/summer migrants, linear regressions gave higher R^2 values when using longer runs of temperature data; with the strongest correlations produced for the January–April range the for the week of 50% ($R^2 = 0.63$, $P < 0.001$) and January–May for the week of 90% capture ($R^2 = 0.45$, $P < 0.001$) (all outputs of the linear regression analyses are shown in Appendix A.3).

By use of multiple linear regression analysis, the effects of site location (*e.g.* latitude, longitude and altitude) were incorporated into the models, alongside measures of mean air temperature. Regression outputs, however, showed these variables were not statistically significant coefficients in the models, individually or in combination ($P > 0.05$).

2.3.5 Validation of forecasts based on mean air temperature for predicting the migration of *Myzus persicae*

The forecasting models based on mean air temperature were validated across the 2000–2010 data set for three sites (24 sites x years: Rothamsted; 9 years, Broom's Barn; 9 years, Kirton; 6 years), with the mean absolute differences (in weeks) calculated between the predicted and observed dates of *M. persicae* activity (Table 2.6).

Table 2.6 – Mean absolute differences (in weeks) between the predicted week and observed weeks of *Myzus persicae* activity (first, 10%, 25%, 50% and 90% dates of capture) for the 2000–2010 validation data set (24 sites x years). The values in parentheses are the number of occasions that forecasts made late predictions.

Forecast	Broom's Barn (*n* = 9)	Rothamsted (*n* = 9)	Kirton (*n* = 6)	Mean
First capture (Jan–Feb)	1.07 (1)	1.87 (1)	1.78 (4)	**1.57**
First capture (Jan–Mar)	1.04 (5)	1.93 (7)	1.60 (2)	**1.52**
10% capture (Jan–Feb)	1.53 (3)	1.98 (0)	2.06 (3)	**1.86**
10% capture (Jan–Mar)	1.30 (5)	2.14 (8)	1.81 (3)	**1.75**
25% capture (Jan–Feb)	1.59 (1)	2.06 (0)	1.85 (3)	**1.83**
25% capture (Jan–Mar)	1.58 (6)	2.26 (7)	1.63 (3)	**1.82**
50% capture (Jan–Feb)	1.50 (1)	2.02 (0)	1.58 (1)	**1.70**
50% capture (Jan–Mar)	1.52 (7)	2.20 (8)	1.52 (5)	**1.75**
50% capture (Jan–Apr)	1.15 (6)	2.29 (9)	0.98 (4)	**1.47**
90% capture (Jan–Feb)	1.68 (0)	2.03 (1)	1.95 (2)	**1.89**
90% capture (Jan–Mar)	1.71 (8)	2.10 (7)	1.88 (4)	**1.90**
90% capture (Jan–Apr)	1.30 (7)	2.10 (8)	1.45 (3)	**1.62**
90% capture (Jan–May)	1.15 (5)	2.15 (8)	1.63 (3)	**1.64**
Mean	**1.39**	**2.09**	**1.67**	
Maximum	**1.71**	**2.29**	**2.06**	
Minimum	**1.04**	**1.87**	**0.98**	

Forecasts performed most accurately for Broom's Barn and least accurately for Rothamsted (Table 2.6), on average predicting the capture of *M. persicae* within 1.41 and 2.1 weeks, respectively. Interestingly, the model for predicting the week of 50% capture of aphids, based on mean air temperature from January–April, yielded the most reliable results across the 2000–2010 data set. Across sites, forecasting models accurately predicted the week of capture of aphids within around two weeks.

Linear regressions were fitted to the error of each model (*i.e.* the difference between the predicted and observed weeks) and the total number of aphids caught by the cut-off point (Day 240 from 1st January). In all cases, no statistically significant relationships were identified ($P > 0.05$).

2.3.6 Predicting the week of the summer peak in numbers of *Myzus persicae*

The timing of the summer peak in numbers of *M. persicae* (caught in suction traps) was investigated in relation to both accumulated day-degree sums and measures of mean air temperature using the primary data set (50 sites x years). Across 50 sites x years, the earliest peak in *M. persicae* numbers occurred during week 22 (Broom's Barn, 1990; 28th May–3rd June). All other peaks were observed after the start of June. The earliest, latest and mean week of the peak in *M. persicae* numbers at the three sites are summarised in Table 2.7.

Table 2.7 – Summary of the week of the summer peak in numbers of *Myzus persicae* captured in the suction traps at Broom's Barn, Rothamsted and Kirton. The years are shown in parentheses.

'Aphid peak'	Broom's Barn	Rothamsted	Kirton
Earliest week	22 (1990)	24 (1989)	24 (1984)
Latest week	31 (1986)	33 (1986)	33 (1996)
Mean week	28	28	29

Statistically significant linear relationships ($P < 0.001$) were found between the timing of the summer peak in numbers of *M. persicae* and measures of air temperature averaged across defined periods in the winter/spring (Fig. 2.15 and Table 2.8).

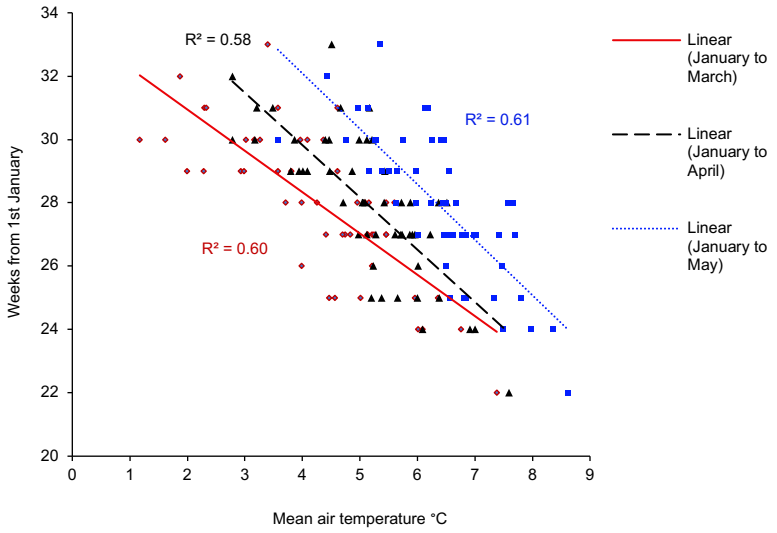

Figure 2.15 – Fitted linear regressions between the timing of the summer peak in numbers of *Myzus persicae* and mean air temperature (ºC) for the Rothamsted, Broom's Barn and Kirton data set (50 sites x years) All linear regressions: $P < 0.001$.

Table 2.8 – Linear regression analysis of the timing of the summer peak in *Myzus persicae* numbers (x) and mean air temperature (y), $y = mx + c$ (50 sites x years).

Mean air temperature	**Parameter**		**S.E.**	**R²**	**P-value**
January–February	c	31.68689	0.54844	0.56030	<0.001
	m	-1.07466	0.14037		<0.001
January–March	c	33.55562	0.69922	0.59634	<0.001
	m	-1.30474	0.15494		<0.001
January–April	c	36.43430	1.05638	0.58408	<0.001
	m	-1.65259	0.20128		<0.001
January–May	c	39.08732	1.30550	0.60908	<0.001
	m	-1.75085	0.20246		<0.001

2.3.7 Validation of forecasts based on mean air temperature for predicting the week of the summer peak in numbers of *Myzus persicae*

The forecasting models based on mean air temperature were validated across the 2000–2010 data set for years with a defined peak in aphid numbers (total of eighteen sites x years). The mean absolute differences (in weeks) were calculated between the predicted and observed weeks of the peak in the numbers of *M. persicae* (Table 2.9).

Forecasts performed most accurately for Kirton and least accurately for Rothamsted (Table 2.9). The model for predicting the 'peak week', based on the mean air temperature from January–April, yielded the most reliable results across the three sites.

Table 2.9 – Mean absolute differences (in weeks) between the predicted weeks and observed weeks of the peak in numbers of *Myzus persicae* for the 2000–2010 validation data set (eighteen sites x years).

Forecast	Broom's Barn	Rothamsted	Kirton	Mean
Peak (Jan–February)	1.87	2.39	1.70	**1.99**
Peak (Jan–March)	1.40	2.69	1.49	**1.86**
Peak (Jan–April)	1.19	2.66	0.93	**1.59**
Peak (Jan–May)	1.22	2.82	1.34	**1.79**
Mean	**1.42**	**2.64**	**1.37**	
Maximum	**1.87**	**2.82**	**1.7**	
Minimum	**1.19**	**2.39**	**0.93**	

2.3.8 Developing a day-degree forecast for *Cavariella* spp.

For *C. pastinaceae* and *C. theobaldi* capture dates were positively correlated with accumulated day-degrees using a range of base temperatures and day-degree accumulation start points. This indicated that the later the capture date, the higher the number of accumulated day-degrees. As examples, the relationships between accumulated day-degrees from 1st January (above a base temperature 4°C) and the date of 10% capture are shown for *C. pastinaceae* and *C. theobaldi* in Figure 2.16.

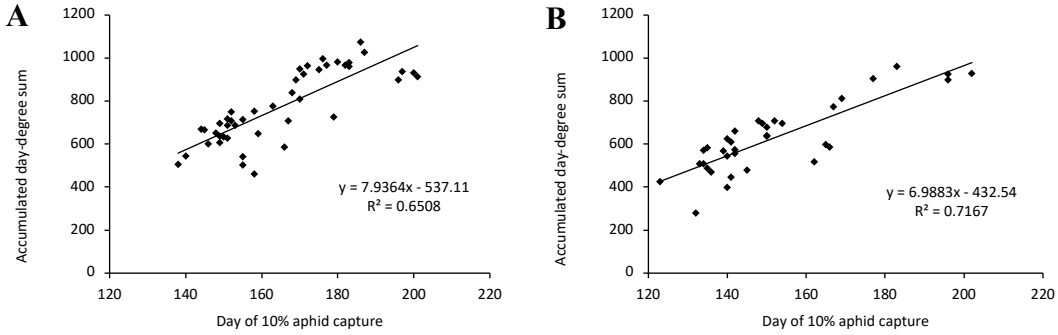

Figure 2.16 – Linear relationship between the date of **(A)** 10% capture of *Cavariella pastinaceae* and accumulated day-degrees from **1st January** above a base temperature of **4°C** ($P < 0.001$) and **(B)** 10% capture of *Cavariella theobaldi* and accumulated day-degrees from **1st January** above a base temperature of **4°C**. All linear regressions: $P < 0.001$.

For *C. aegopodii*, relatively constant accumulated day-degree sums were found for the dates of first capture and 10% capture of aphid. This was observed at base temperatures of 4°C and 4.4°C, with day-degrees accumulated from either the start of January or the start of February (Fig. 2.17).

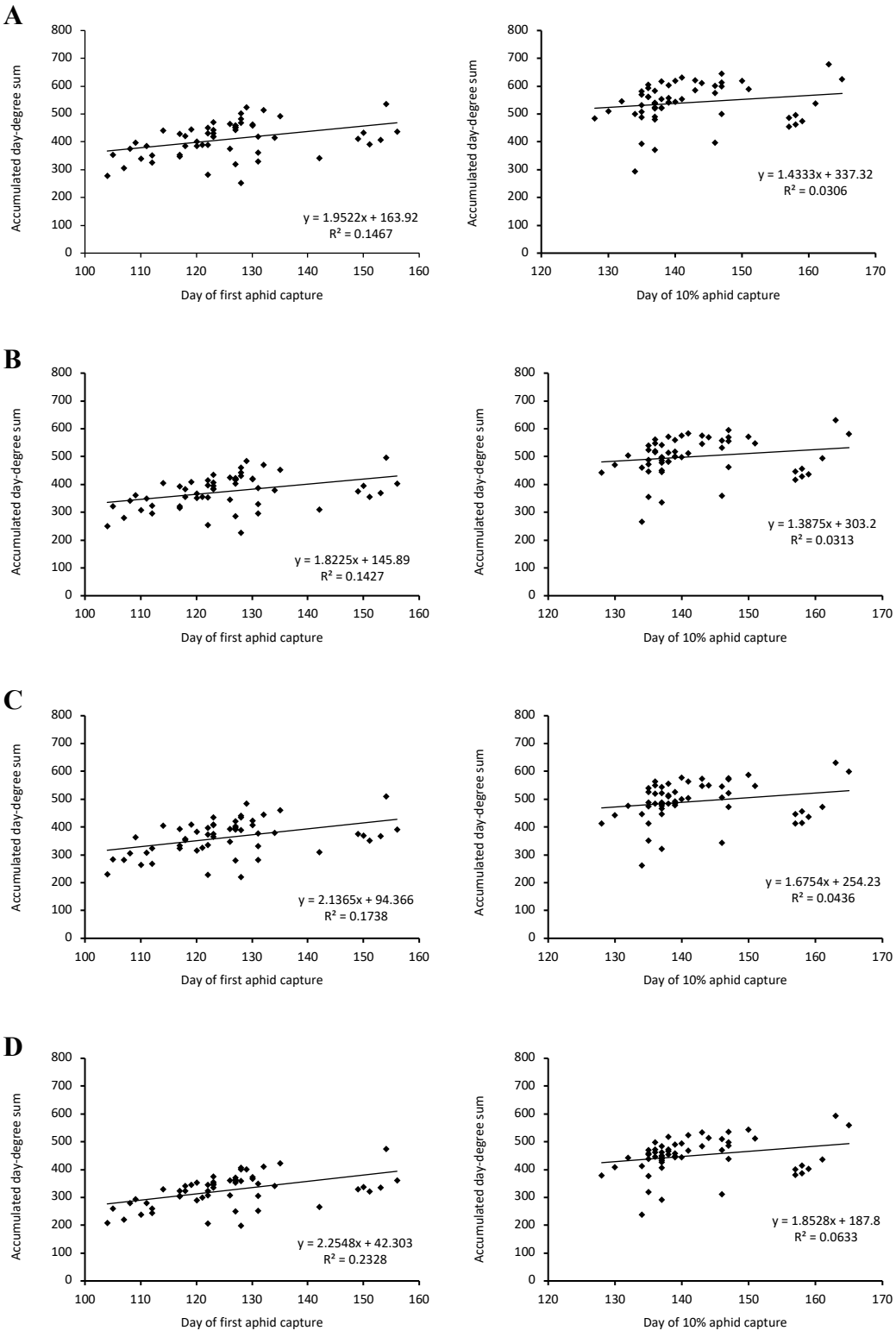

Figure 2.17 – Linear relationship between week of **(A)** first and 10% capture of *Cavariella aegopodii* and accumulated day-degrees from **1st January** above a base temperature of **4°C, (B)** first and 10% capture of *C. aegopodii* and accumulated day-degrees from **1st January** above a base temperature of **4.4°C, (C)** first and 10% capture of *C. aegopodii* and accumulated day-degrees from **1st February** above a base temperature of **4°C** and **(D)** first and 10% capture of *C. aegopodii* and accumulated day-degrees from **1st February** above a base temperature of **4.4°C** across the 1996–2006 data set.

The mean accumulated day-degree (D°) sum was determined for each forecasting model developed for predicting the first and 10% capture dates of *C. aegopodii* (Table 2.10).

Table 2.10 – Mean accumulated day-degree (D°) sums for the forecasting models developed to predict the first and 10% dates of capture of *Cavariella aegopodii.*

Day-degree forecast	**Mean accumulated D° sum**	**±1 S.E.**
First capture (Base 4°C) from 1st January	409	8.34
First Capture (Base 4.4°C) from 1st January	375	7.89
First Capture (Base 4°C) from 1st February	362	8.39
First Capture (Base 4.4°C) from 1st February	325	7.64
10% Capture (Base 4°C) from 1st January	541	9.59
10% Capture (Base 4.4°C) from 1st January	500	9.18
10% Capture (Base 4°C) from 1st February	492	9.39
10% Capture (Base 4.4°C) from 1st February	451	8.62

2.3.9 Validation of day-degree forecasts for predicting the migration of *Cavariella aegopodii*

Each day-degree forecast developed for *C. aegopodii* was validated using the 1981–1988 data set (based on 55 sites x years). Fitted equations were used to calculate the predicted dates of first and 10% capture. The mean absolute differences between the observed and predicted dates were calculated for the individual sites (Tables 2.11 and 2.12).

Table 2.11 – Mean absolute difference (in days) between the observed and predicted dates of the first capture of *Cavariella aegopodii* in suction traps across the 1981–1988 data set for each of the day-degree forecasts developed. Numbers in parenthesis are the mean absolute difference (in days) between the observed and predicted dates using the forecast that is currently used in the UK (360D° from 1st February above the base temperature of 4.4°C).

Site	1st Jan (4°C)	1st Jan (4.4°C)	1st Feb (4°C)	1st Feb (4.4°C)	Mean
Broom's Barn	6.6	7.5	4.6	6.8 (6.1)	**6.4**
East Craigs	3.8	4.2	3.4	3.2 (6)	**3.7**
Hereford	7.1	7.1	7.9	8.4 (7)	**7.6**
Kirton	6.3	6.6	7.3	7.6 (8.6)	**7.0**
Rothamsted	7.4	7.6	7.1	6.3 (8)	**7.1**
Starcross	7.7	7.8	7.0	6.7 (11.6)	**7.3**
Writtle	2.9	2.7	3.6	3.9 (3.9)	**3.3**
Wye	4.5	4.3	6.1	6.6 (4.6)	**5.4**
Max:	**7.7**	**7.8**	**7.9**	**8.4 (11.6)**	
Min:	**2.9**	**2.7**	**3.4**	**3.2 (3.9)**	
Mean:	**5.8**	**6.0**	**5.9**	**6.2 (7.0)**	

Table 2.12 – Mean absolute difference (in days) between the observed and predicted dates of the 10% capture of *Cavariella aegopodii* in suction traps across the 1981–1988 data set for each of the day-degree forecasts developed.

Site	1st Jan (4°C)	1st Jan (4.4°C)	1st Feb (4°C)	1st Feb (4.4°C)	Mean
Broom's Barn	4.5	4.1	2.9	5.0	**4.1**
East Craigs	4.6	5.2	4.2	3.8	**4.5**
Hereford	4.3	4.1	3.1	2.7	**3.6**
Kirton	2.9	2.6	3.3	3.1	**3.0**
Rothamsted	8.1	8.1	6.6	6.1	**7.2**
Starcross	6.0	5.8	5.5	5.5	**5.7**
Writtle	4.0	4.0	2.1	2.4	**3.1**
Wye	4.8	4.6	3.3	3.5	**4.1**
Max:	**8.1**	**8.1**	**6.6**	**6.1**	
Min:	**2.9**	**2.6**	**2.1**	**2.4**	
Mean:	**4.9**	**4.8**	**3.9**	**4.0**	

When validating the day-degree forecasts, the different models yielded very little difference in terms of the mean absolute difference (in days) between the observed and predicted dates for the first and 10% capture of *C. aegopodii*. For predicting the

onset of aphid migration (first capture), on average, all models developed were accurate within six days and improved forecast accuracy compared with the day-degree forecast that is used currently in the UK (accurate within seven day). Forecasts performed most accurately for Writtle with a mean absolute difference of 3.3 days between the observed and predicted dates of capture and the least accurately for Hereford with a mean absolute difference of 7.6 days between the observed and predicted dates of capture. Predicting the capture date of 10% *C. aegopodii*, using an accumulation start point of 1st February improved the accuracy of the model by roughly one day, compared to accumulating day-degrees from 1st January. Forecasts performed the most accurately for Writtle with a mean absolute difference of 3.1 days and the least accurately for Rothamsted with a mean absolute difference of 7.2 days.

2.3.10 Developing a forecast based on mean air temperature for predicting the migration of *Cavariella aegopodii*

For all sites x years, the earliest date of first capture of aphids occurred on day 104 (13th April) at Kirton in 2000. There were seventeen other occasions when the first aphid was caught before the start of May. The earliest capture of 10% aphids occurred on day 128 (8th May) at Preston in 1998; there were 47 other occasions when the capture 10% aphids occurred before the start of June. The earliest capture of 25% aphids occurred on day 135 (14th May) at Broom's Barn in 2000. There were 44 other occasions when the capture 25% aphids occurred before the start of June. The earliest capture of 50% aphids occurred on day 140 (20th May) at Broom's Barn in 1997 and there were eighteen other occasions when 50% were captured before the start of June. The earliest date of 90% capture of aphids occurred on day 157 (6th June) at Preston in 1998. There were 37 other occasions when the capture of 90% aphids occurred before the start of July.

Linear regressions were fitted to the dates of first, 10% and 50% capture of *C. aegopodii* and measures of mean air temperature during the winter/spring across the primary data set (55 sites x years). In all cases, capture dates were negatively correlated with mean air temperature; indicating that an earlier capture of *C. aegopodii* occurred after warmer winters/springs (Fig. 2.18). The strongest correlations were produced for the capture of the first aphid and mean air temperature during January–March inclusive ($R^2 = 0.48$, $P < 0.001$).

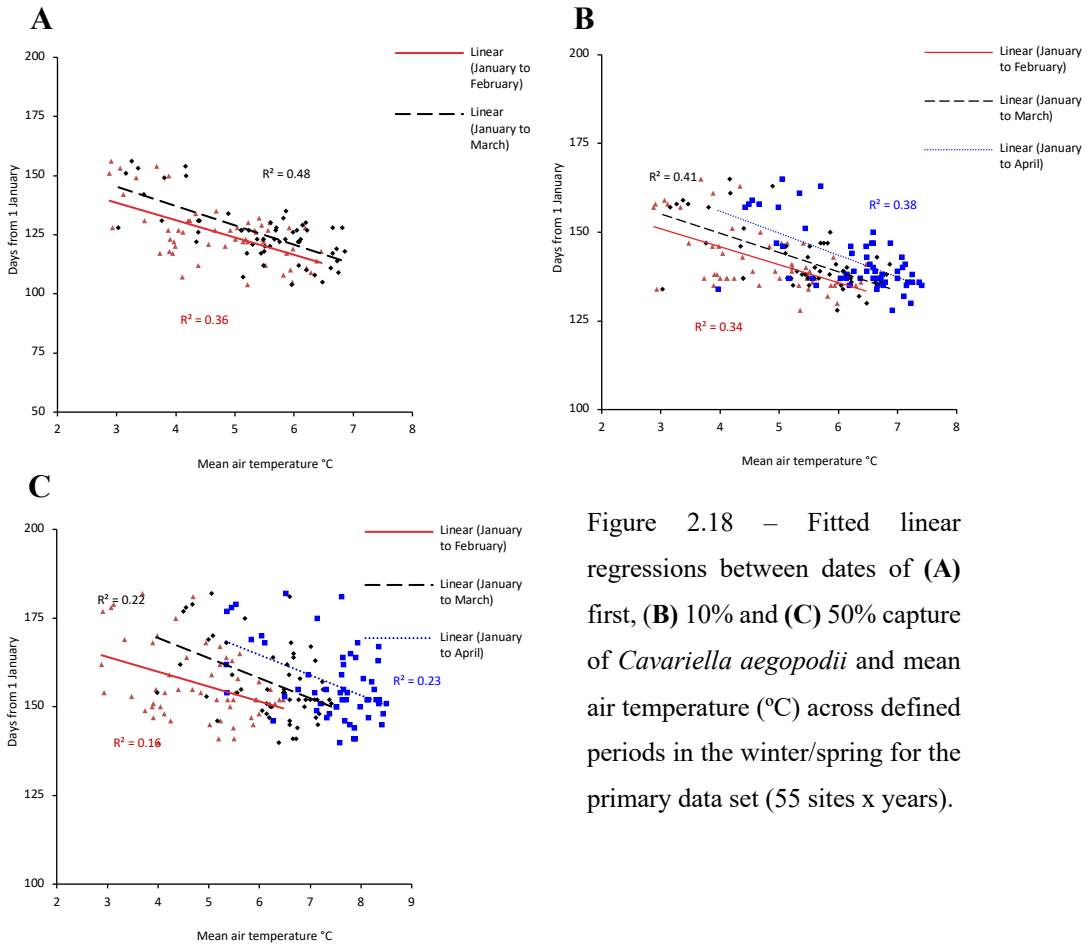

Figure 2.18 – Fitted linear regressions between dates of **(A)** first, **(B)** 10% and **(C)** 50% capture of *Cavariella aegopodii* and mean air temperature (ºC) across defined periods in the winter/spring for the primary data set (55 sites x years).

Table 2.13 – Linear regression analysis of the dates of first, 10% and 50% capture of *Cavariella aegopodii* (x) and mean air temperature (y), $y = mx + c$ (55 sites x years).

Capture	Mean air temperature	Parameter		S.E.	R^2	P-value
First	January–February	c	160.476	6.48317	0.36039	<0.001
		m	-7.32833	1.32855		<0.001
First	January–March	c	169.87921	6.43287	0.4776	<0.001
		m	-8.18362	1.16471		<0.001
10%	January–February	c	166.12458	4.73039	0.3351	<0.001
		m	-5.05701	0.96937		<0.001
10%	January–March	c	171.41011	4.89277	0.4099	<0.001
		m	-5.42551	0.88586		<0.001
10%	January–April	c	180.67969	6.83452	0.37714	<0.001
		m	-6.19064	1.08263		<0.001
50%	January–February	c	176.60695	6.44309	0.15694	<0.001
		m	-4.18617	1.32034		<0.01
50%	January–March	c	181.75277	6.87248	0.2043	<0.001
		m	-4.63318	1.244		<0.001
50%	January–April	c	192.0299	9.27638	0.21578	<0.001
		m	-5.66414	1.46943		<0.001

2.3.11 Model validations of forecasts based on mean air temperature for predicting the migration of *Cavariella aegopodii*

The forecasting models based on mean air temperature were validated across the 1981–1988 data set (55 sites x years) with the mean absolute differences (in days) calculated between the predicted and observed dates of *C. aegopodii* activity (Table 2.14).

Table 2.14 – Mean absolute differences (in days) between the predicted dates and observed dates of *Cavariella aegopodii* activity (first, 10% and 50% capture of aphids) for the 1981–1988 validation data set (55 sites x years).

Site	First Jan–Feb	First Jan–Mar	10% Jan–Feb	10% Jan–Mar	10% Jan–Apr	50% Jan–Feb	50% Jan–Mar	50% Jan–Apr	Max:	Min:	Mean:
Broom's Barn	9.40	9.88	7.02	7.23	6.63	15.22	14.33	14.31	15.22	6.63	**10.66**
East Craigs	9.23	7.80	8.35	7.67	6.60	17.90	17.14	15.70	17.90	6.60	**11.59**
Hereford	6.51	8.48	5.78	7.24	6.34	7.89	9.09	7.35	9.09	5.78	**7.45**
Kirton	6.85	6.63	8.98	9.18	8.95	15.75	15.76	15.17	15.76	6.63	**11.49**
Rothamsted	10.95	10.58	5.81	5.75	3.96	9.03	9.01	7.16	10.95	3.96	**7.78**
Starcross	8.91	8.23	5.30	5.45	4.68	8.55	8.28	7.09	8.55	4.68	**6.80**
Writtle	8.53	7.97	9.75	9.83	7.65	10.00	9.24	8.12	10.00	7.65	**8.94**
Wye	8.99	8.11	9.00	9.24	7.97	10.98	10.35	10.16	10.98	7.97	**9.40**
Max:	10.95	10.58	9.75	9.83	8.95	17.9	17.14	15.7			
Min:	6.51	6.63	5.30	5.45	3.96	7.89	8.28	7.09			
Mean:	**8.67**	**8.46**	**7.5**	**7.7**	**6.60**	**11.92**	**11.65**	**10.63**			

Forecasts performed the most accurately for Starcross and least accurately for East Craigs. In all cases, using longer runs of temperature data yielded more accurate predictions of the capture dates of *C. aegopodii*.

2.3.12 Developing forecasts based on mean air temperature for predicting the migration of *Cavariella pastinaceae* and *Cavariella theobaldi*

For *C. pastinaceae* and *C. theobaldi*, statistically significant relationships ($P < 0.001$ or $P < 0.01$) were identified between mean air temperature in the winter/spring and measures of aphid activity (Tables 2.15 and 2.16).

Table 2.15 – Linear regression analysis of the dates of first, 10% and 50% capture of spring/summer *Cavariella pastinaceae* migrants (x) and mean air temperature (y), $y = mx + c$ (45 sites x years).

Capture	Mean air temperature	Parameter		S.E.	R^2	P-value
First	January–February	c	168.2584	6.12226	0.29808	<0.001
		m	-5.3684	1.25629		<0.001
First	January–March	c	176.20329	6.16985	0.41602	<0.001
		m	-6.17884	1.1164		<0.001
10%	January–February	c	194.02465	10.97558	0.14551	<0.001
		m	-6.09445	2.25219		<0.01
10%	January–March	c	205.29671	11.52731	0.22786	<0.001
		m	-7.43008	2.08579		<0.001
10%	January–April	c	218.42449	16.15359	0.20593	<0.001
		m	-8.56742	2.56561		<0.01
50%	January–February	c	223.85927	9.37677	0.25113	<0.001
		m	-7.3065	1.92411		<0.001
50%	January–March	c	228.75766	10.26057	0.26543	<0.001
		m	-7.31833	1.85658		<0.001
50%	January–April	c	244.74433	14.14819	0.26857	<0.001
		m	-8.9289	2.2471		<0.001

Table 2.16 – Linear regression analysis of the dates of first, 10% and 50% capture of spring/summer *Cavariella theobaldi* migrants (x) and mean air temperature (y), $y = mx + c$ (35 sites x years).

Capture	Mean air temperature	Parameter		S.E.	R^2	P-value
First	January–February	c	157.66504	7.13896	0.28048	<0.001
		m	-5.21538	1.45413		<0.01
First	January–March	c	160.61583	7.25535	0.31966	<0.001
		m	-5.20902	1.32287		<0.001
10%	January–February	c	192.00709	12.89237	0.23805	<0.001
		m	-8.43193	2.62604		<0.01
10%	January–March	c	195.57346	13.306	0.257	<0.001
		m	-8.19659	2.42608		<0.01
10%	January–April	c	210.33703	19.04824	0.22733	<0.001
		m	- 9.53305	3.05949		<0.01
50%	January–February	c	228.60224	12.97905	0.34712	<0.001
		m	-11.07365	2.64369		<0.001
50%	January–March	c	227.90742	13.96536	0.30803	<0.001
		m	-9.75941	2.5463		<0.001
50%	January–April	c	248.40746	19.7777	0.29575	<0.001
		m	-11.82579	3.17666		<0.001

2.3.13 Model validations of forecasts based on mean air temperature for predicting the migration of *Cavariella pastinaceae* and *Cavariella theobaldi*

Models were validated using separated data sets (2008–2015) based on eighteen sites x years for *C. pastinaceae* and twelve sites x years for *C. theobaldi.* The mean absolute differences (in days) between the predicted dates and observed dates of aphid activity (*e.g.* first, 10% and 50% capture of aphids) were calculated for each validation data set (Tables 2.17 and 2.18).

For both *C. pastinaceae* and *C. theobaldi*, forecasts performed the most accurately for Broom's Barn (accurate within seven to ten days). Forecasts were least reliable for *C. pastinaceae* at Kirton (accurate within seventeen days) and *C. theobaldi* at Rothamsted (accurate within eighteen days).

Models for predicting the capture date of 50% aphids yielded the most accurate results for both species, based on the mean air temperature for January–March for *C.*

pastinaceae (accurate within seven days) and for January–February for *C. theobaldi* (accurate within ten days).

Table 2.17 – Mean absolute differences (in days) between the predicted dates and observed dates of *Cavariella pastinaceae* activity (first, 10% and 50% capture of aphids) for the 2008–2015 validation data set (eighteen sites x years).

Site	First Jan–Feb	First Jan–Mar	10% Jan–Feb	10% Jan–Mar	10% Jan–Apr	50% Jan–Feb	50% Jan–Mar	50% Jan–Apr	Max	Min	Mean
Broom's Barn	7.73	8.61	2.41	5.47	8.66	10.57	7.87	7.24	10.57	2.41	**7.32**
Kirton	11.17	8.82	15.49	16.66	16.77	8.63	8.76	9.81	16.77	8.63	**12.01**
Rothamsted	16.09	16.00	11.18	8.76	10.46	5.81	3.94	5.98	16.09	3.94	**9.78**
Mean:	**11.66**	**11.14**	**9.69**	**10.30**	**11.96**	**8.34**	**6.86**	**7.68**			

Table 2.18 – Mean absolute differences (in days) between the predicted dates and observed dates of *Cavariella theobaldi* activity (first, 10% and 50% capture of aphids) for the 2008–2015 validation data set (twelve sites x years).

Site	First Jan–Feb	First Jan–Mar	10% Jan–Feb	10% Jan–Mar	10% Jan–Apr	50% Jan–Feb	50% Jan–Mar	50% Jan–Apr	Max	Min	Mean
Broom's Barn	7.72	9.67	6.13	9.48	13.24	7.88	13.56	16.82	16.82	6.13	**10.56**
Kirton	23.38	22.07	21.01	19.03	21.28	8.09	9.14	11.69	23.38	8.09	**16.96**
Rothamsted	9.26	11.79	16.25	21.34	25.43	13.47	19.85	25.14	25.43	9.26	**17.81**
Mean:	**13.45**	**14.51**	**14.46**	**16.62**	**19.88**	**9.81**	**14.18**	**17.88**			

2.3.14 Field monitoring of *Cavariella aegopodii*

The numbers of alate *C. aegopodii* caught in the suction trap at Wellesbourne were plotted for 2016, 2017 and 2018 (Fig. 2.19). *Cavariella aegopodii* was always caught earlier in the YWTs than in the suction trap in 2016 (one week earlier), 2017 (two weeks earlier) and 2018 (two weeks earlier). For the three years, peak numbers of *C. aegopodii* caught in YWTs were always observed one week after peak numbers were caught in the suction trap.

The day-degree forecasts developed (Table 2.10) were used to predict the dates of first and 10% capture of *C. aegopodii* for each year. Vertical lines on Figure 2.19 represent the predicted week of the first capture of aphids (red dashed line: based on an accumulation of 409 D° from 1st January above a base temperature of 4°C) and the predicted week of the 10% capture of aphids (blue dashed line: based on an accumulation of 541 D° from 1st January, above a base temperature of 4°C).

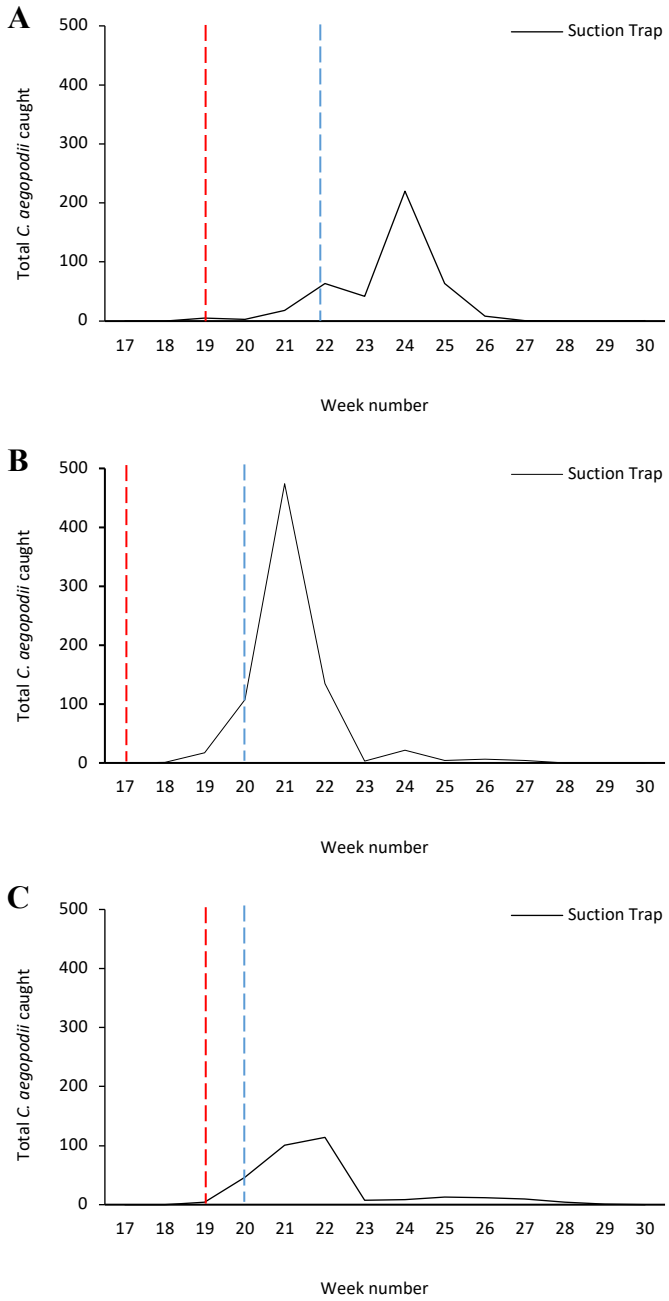

Figure 2.19 – The numbers of alate *Cavariella aegopodii* caught in the suction trap at Wellesbourne, Warwickshire, UK in **(A)** 2016, **(B)** 2017 and **(C)** 2018. Red dashed lines and blue dashed lines display the predicted dates (from day-degree forecasts developed) of the first and 10% capture of *Cavariella aegopodii*, respectively.

Day-degree forecasts performed most accurately in 2016 when predicting the capture date of 10% *C. aegopodii* (mean absolute difference of 1.8 days). Generally, forecasts that accumulated day-degrees from 1st January yielded the most accurate predictions across the three years (mean absolute difference of 3.3 days). There was no difference and little difference (0.4 days) observed in the accuracy of forecasts when accumulating day-degrees at base temperature of either 4°C or 4.4°C from the 1st January and 1st February, respectively (Table 2.19).

Table 2.19 – Difference (in days) between the predicted and observed dates of first and 10% capture of *Cavariella aegopodii* in the suction trap at Wellesbourne, Warwickshire, UK. Numbers are preceded by (+) and (–) to indicate late and early predictions, respectively. Mean absolute differences (in days) are shown for each day-degree forecast (across 2016–2018 and for individual each years).

Year (capture)	**1st Jan (4°C)**	**1st Jan (4.4°C)**	**1st Feb (4°C)**	**1st Feb (4.4°C)**	**Mean absolute difference:**
2016 (**first**)	+6	+6	+9	+8	7.3
2017 (**first**)	–4	–4	–7	–10	6.3
2018 (**first**)	–4	–4	–3	–4	3.8
2016 (**10%**)	0	0	+4	+3	1.8
2017 (**10%**)	–2	–2	–3	–4	2.8
2018 (**10%**)	+4	+4	+6	+5	4.8
Mean absolute difference:	3.3	3.3	5.3	5.7	

2.3.15 Diurnal variation in the flight activity of *Cavariella aegopodii*

The mean proportion of the daily catch of *C. aegopodii* was determined for each time period across days in which the daily total was ≥ 20 aphids caught for 2016–2018 (Fig. 2.20). The greatest proportion of *C. aegopodii* were caught in YWTs between 11:00–12:00 (35%), followed by 12:00–13:00 (29%). The lowest proportion of *C. aegopodii* were caught in YWTs between 9:00–10:00 (2%). Few *C. aegopodii* were flying outside of the intensive monitoring period (16:00–9:00; 3%).

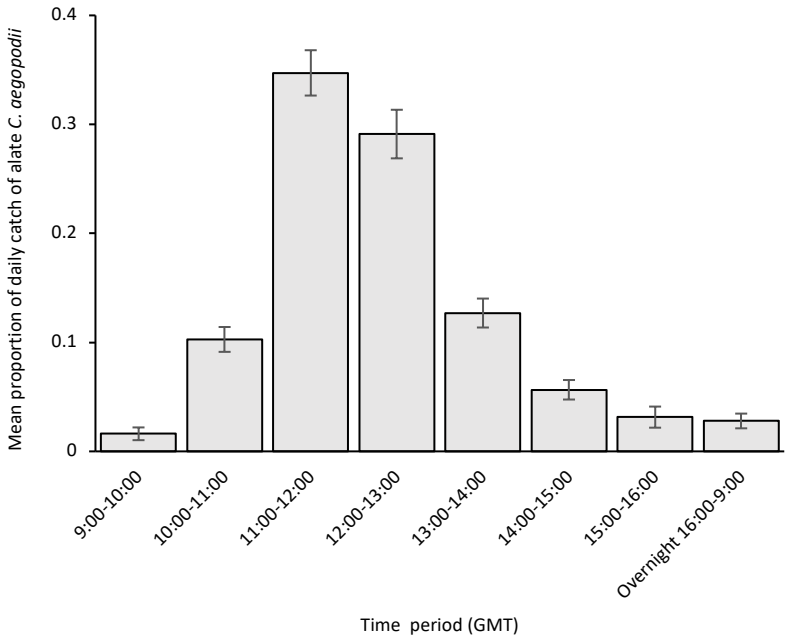

Figure 2.20 – The mean proportion (±1 S.E.) of the daily catch of alate *Cavariella aegopodii* in yellow water traps (YWTs) each hour across sixteen monitoring days (2016–2018) in and around carrot plots at Wellesbourne, Warwickshire, UK.

There was a statistically significant difference between the proportion of the daily catch of *C. aegopodii* in YWTs for the different times of day (Kruskal-Wallis H test: $H = 100.668$, d.f. = 7, $P < 0.001$).

Dunn's multiple comparison test was carried out for the 28 pairs of groups. There was very strong evidence ($P < 0.001$, adjusted with Bonferroni correction) of a difference between the 11:00–12:00 time period and the 9:00–10:00, 14:00–15:00, 15:00–16:00 and 16:00–9:00 (overnight) time periods. A statistically significant difference was also identified between the 11:00–12:00 and 10:00–11:00 time periods (adjusted $P = 0.019$). The 11:00–12:00 period was not statistically significantly different to the 12:00–13:00 (adjusted $P = 1.0$) and 13:00–14:00 time periods (adjusted $P = 0.2$).

2.4 Monitoring and forecasting infestations by aphids: Discussion

2.4.1 The relative abundance and phenology of aphid species

Aphid abundance

Differences were observed in the mean numbers of the four species of aphid (*M. persicae*, *C. aegopodii*, *C. pastinaceae* and *C. theobaldi*) caught at different suction trap locations in the UK during 2013–2018. With regard to *C. aegopodii,* the largest numbers were caught in the east (Broom's Barn, Suffolk) and the East Midlands (Kirton, Lincolnshire). In part, this could be due to the warmer winter/spring temperatures experienced in these regions compared with the north of the country. These temperatures are expected to improve aphid survival over the winter and result in the earlier migration of aphids to support the establishment of dense spring colonies. To an extent, the distribution of host plants is also likely to influence the abundance of aphids. For *C. aegopodii,* while the distribution of winter hosts, *Salix* species (Salicaceae), remains relatively uniform across the UK (National Biodiversity Network, 2019), there are regional differences in the distribution of summer hosts. Carrots, for example, have been traditionally grown in sandy/sandy loam soils in the east of the country (*e.g*. Suffolk, Norfolk and Cambridgeshire). However, since the adoption of 'strawing' in the 1990s, which allows the protection of carrots during the winter, Nottinghamshire (East Midlands) has also become an important production area (AHDB, 2013).

Most *M. persicae* were also caught in the east (Broom's Barn, Rothamsted and Writtle) and East Midlands (Kirton). As these sites are largely in arable regions, it is likely that an abundance of food resources (*e.g. Brassica* crops and potato) favours the proliferation of spring/summer colonies of *M. persicae* (AHDB, 2013). Cocu *et al.* (2005) found that the annual numbers of *M. persicae* caught in suction traps were positively correlated with the area of agricultural land. This was particularly the case for areas with a high proportion of oilseed rape, *Brassica napus* (Linnaeus, 1758), which is a host plant and an important overwintering site for aphids. *Myzus persicae* was considerably less abundant in the north west (Preston, Lancashire) compared with *C. aegopodii*. In part, this may be due to the regional production of carrots in Lancashire which may support the development of large summer colonies of *C.*

aegopodii. In addition, as *M. persicae* is predominantly anholocyclic in the UK, it is possible that the survival of active adults over the winter may be reduced in colder region of the UK, compared to that of *C. aegopodii*, which mostly overwinters as eggs that tend to be more tolerant of lower temperatures.

Cavariella pastinaceae and *C. theobaldi* were much less abundant at all sites during 2013–2018, compared with *M. persicae* and *C. aegopodii*. Most *C. pastinaceae* and *C. theobaldi* were recorded at Kirton and Broom's Barn. Again, this is likely to be due to a combination of warmer spring/summer temperatures and the regional distribution of host Apiaceous crops (*e.g.* parsnips) in the east and East Midlands.

Relationships were found between the numbers of *C. aegopodii*, *C. pastinaceae* and *C. theobaldi* caught at individual sites each year (1996–2018). The strongest correlations were produced for the numbers of *C. pastinaceae* and *C. theobaldi*. This is to be expected as the two species share very similar life cycles and biology. The numbers of *C. aegopodii* captured were less correlated with those of *C. pastinaceae* and *C. theobaldi*, however, statistically significant relationships were still identified. Again, this is may be explained by the similar life cycles of the species. The reasons for the differences identified in the abundance relationships between *C. aegopodii* and the two species of 'parsnip aphid' are not clear. It is possible that they could relate to several factors; including subtle differences in host range, varying susceptibility, particularly in terms of their development, to abiotic factors such temperature and rainfall, and biotic factors such as the relative levels predation, parasitism and disease.

Aphid phenology

Trends were identified in the relative timing of the four species of aphid. At all sites the earliest mean capture dates of the first, 10% and 50% aphid(s) during 2013–2018 were observed for *C. aegopodii*. Generally, *M. persicae* and *C. theobaldi* were the next species to be captured. At most sites, *C. pastinaceae* was the last species to begin its migration.

The different phenology of *C. pastinaceae* and *C. theobaldi* could suggest that these species have slightly different temperature requirements for development compare to *C. aegopodii.* This would explain why their capture in suction traps is consistently

later, for example, if they require higher temperatures to begin their development. The lower temperature thresholds (base temperatures) for the development of *C. pastinaceae* and *C. theobaldi* have not yet been defined.

It is also possible that the observed timings of the migrations of *C. pastinaceae* and *C. theobaldi* may relate to their relative abundance. For example, in all years considerably fewer *C. pastinaceae* and *C. theobaldi* were caught at sites compared with *C. aegopodii* and *M. persicae.* It is likely that smaller aphid populations in the early spring would not be detected as early by the suction traps. This is due to the time required for the production of a sufficient number of alate adults (aerial populations) to reach the threshold for detection by the suction traps (Harrington *et al*., 1991). This would likely result in later dates of capture for 10% and 50% of the spring/summer migrants.

Additionally, as a larger number of *C. pastinaceae* and *C. theobaldi* were caught in the late part of the migratory period, their migration patterns were skewed to the right (*i.e*. towards the end of the spring/summer migration period). For this reason, it is expected that the dates of the earlier captures (*e.g*. 10% aphids) would be later than they would have been if the same number of aphids followed a normal distribution. The opposite is true for *C. aegopodii* and *M. persicae*, as more aphids were caught in the early part of their migratory periods. A left skew in their patterns of migration would therefore advance the 50% capture date of aphids compared to a normally distributed pattern of migration.

For all species, Preston and Kirton generally had the latest dates of first, 10% and 50% capture. This may be because spring temperatures are slower to rise the further north of the country. Cocu *et al*. (2005) found that the capture of the first *M. persicae* by suction traps is explained mostly by climate, with warmer temperatures resulting in earlier first captures and increases in rainfall delaying captures.

Risk periods for Myzus persicae and Cavariella aegopodii

By determining the minimum dates of first capture and the maximum dates of 90% capture for the spring/summer migrants of *C. aegopodii* and *M. persicae* during 2013–2018, the 'risk periods' (the period of time with potential for aphid infestation/virus transmission in crops) for aphid infestation and virus transmission in crops could be inferred for individual sites. Due to the considerably lower abundance of *C. pastinaceae* and *C. theobaldi* during these years, it is unlikely that these species would be a major concern for the transmission of plant viruses.

The mean duration of the 'risk period' at sites ranged from 44–54 days (from the end of April to mid-June) for *C. aegopodii*, and 35–67 days (from the beginning of May to mid-July) for *M. persicae*. Interestingly, the longest 'risk periods' were observed for Kirton and then Broom's Barn, where there was a relatively high abundance of *C. aegopodii* and *M. persicae*. The warm spring temperatures experienced in these regions may permit the rapid development of dense spring colonies and, providing that the temperature does not exceed the upper temperature thresholds for development, would favour reproduction throughout the summer. Preston had the shortest 'risk period' for both species of aphid, with later first catches and earlier 90% catches. Later first catches are likely to be a reflection of the lower spring temperatures in north of the country. However, the reason for the earlier catches of 90% of aphids is unclear. At lower temperatures, a prolonged migratory period may be expected due to slower rates of aphid development and reproduction. However, if air temperature becomes too cold, aphid flight may be inhibited.

The earliest date of the capture of the first aphid was used as an indication of the onset of migration into crops and the potential for the transmission of plant viruses. However, it is possible that in the south/east of the country, *C. aegopodii* may have overwintered as active adults. This may add some variability to the capture dates of this species. Additionally, it is possible that these aphids are important for virus transmission. For example, a major virus infecting carrot crops in the UK is parsnip yellow fleck virus (PYFV). The transmission of PYFV to carrots depends on a 'helper' virus (anthriscus yellows virus, AYV) which does not infect carrot. For this reason, PYFV infection comes from outside the crop; almost entirely from wild species of

Apiaceae (*e.g.* cow parsley) (Morgan, 2004). This may allow the progeny of anholocyclic aphids that are deposited on wild apiaceous hosts, to acquire the virus from their first feed. For aphids that have overwintered as eggs in diapause, willow is not known to be a host of PYFV and thus for transmission to carrot to occur, aphids are first required to feed on an infected apiaceous host (Fox *et al.,* 2015).

For establishing 'risk periods', the dates of 90% capture of the spring/summer migrants (until the specified cut-off point: Day 240, end of August) were selected over the dates of 90% capture of the total annual numbers. For *C. aegopodii,* the autumn peak in aphid numbers generally relates to aphids returning to winter hosts to begin egg-laying. These aphids are unlikely to have an impact in terms of virus transmission that year. However, for *M. persicae* it is possible that the second peak may relate to the redistribution of aphids within crops or movement to herbaceous hosts. In terms of virus transmission, it possible that this second peak may have some importance.

2.4.2 Day-degree forecasts

Near constant accumulated day-degree sums were not obtained for the capture weeks of *M. persicae* at the published upper and lower temperature thresholds for development, 4°C and 30°C, respectively (Whalon and Smilowitz, 1977). This may be due to the anholocyclic life cycle that *M. persicae* generally undergoes in the UK. The onset of migration by aphids that overwinter as active adults may occur after potentially lower accumulated day-degree requirements than for aphids that overwinter as eggs in diapause; which often do not respond to small increases in temperature earlier on in the year and instead require a set amount of heat to complete diapause (Leather *et al*., 1995; Harrington and Clark, 2010). For this reason, a greater amount of inherent variability would be expected in the timing of migration by *M. persicae.* Nevertheless, in locations where populations of *M. persicae* are predominately holocyclic, robust day-degree forecasts have been developed, *e.g*. in Washington, USA (Ro *et al*., 1998). Further north of the UK, the frequency of holocyclic clones of *M. persicae* is thought to increase (Zhou *et al*., 1995). It is possible therefore, that in these regions the development/phenology of *M. persicae* may exhibit a stronger relationship with accumulated day-degrees.

Cavariella aegopodii, *C. pastinaceae* and *C. theobaldi* generally overwinter in the UK as eggs in diapause on woody host plants (Dunn, 1965). For this reason, it is possible that upon the completion of diapause, the relationship between their rate of development and temperature may be described in terms of accumulated day-degrees.

For *C. aegopodii*, a relatively constant number of accumulated day-degrees (above the base temperatures of 4°C and 4.4°C) were found from either the start of January or the start of February to the dates of first and 10% capture. Validation of the day-degree forecasts found that consistent estimates of the dates of first and 10% capture could be obtained for the different suction trap sites, of within two to eight days.

For the capture of the first *C. aegopodii*, there was little difference between the start points (1st January and 1st February) investigated for the accumulation of day-degrees. For many insect species the 1st February is used as a biofix date in the UK. This follows the coldest part of the year and it is assumed that when air temperature subsequently increases, insects that have overwintered in diapause will be ready to begin post-diapause development (Collier and Finch, 2001). However, as the end point of egg diapause is unknown for *C. aegopodii,* it is possible the accumulation of day-degrees from the start of January could be important for development. Additionally, for species that overwinter as active aphids in the south of the UK, both survival and development depend on temperature over the course of winter (Bale, 1991; Zhou *et a*l., 1995). However, for the capture of 10% of the spring/summer *C. aegopodii* migrants, using an accumulation start point of 1st February for day-degrees improved the accuracy of forecasts by one day.

The base temperature for the development of *C. aegopodii* has not been determined. The day-degree forecasts developed here displayed relatively little difference in terms of their reliability at base temperatures of 4°C and 4.4°C. In part, this could be due to the fact that accumulated day-degrees calculated by all methods are highly correlated, even when different temperature thresholds are used (Pruess, 1983). To clarify the base temperature for the development of *C. aegopodii*, forecasts could be validated using a data set larger than the 55 sites x years used here. Additionally, laboratory

experiments could be carried out to investigate the development of *C. aegopodii* at a range of temperature thresholds.

From the day-degree forecasts, predictions for the capture of the first *C. aegopodii* were less accurate than for predictions of the capture of 10% of the spring/summer migrants. In part, this may be explained by the inherent variability associated with the capture of the first aphid by suction traps; which is more prone to stochastic sampling error by suction traps (Harrington *et al*., 1991). For this reason, there is likely to be some discrepancy between the first aphid to begin migration and the first aphid to be captured by suction traps.

Additionally, in warmer regions of the UK (*e.g.* southern England), there is the chance that the first aphid to be caught by suction traps may be one that has overwintered in an anholocyclic manner. As previously mentioned, the onset of migration by active adults may occur at potentially lower accumulated day-degree requirements, than would be expected for aphids that have overwintered holocyclically (Leather *et al*., 1995; Harrington and Clark, 2010). This may add extra variability to predicting the date of first capture of *C. aegopodii* when using a day-degree forecast.

The day-degree forecast that is currently used to predict the start of migration by *C. aegopodii* in the UK is based on an accumulation of 360D° at the base temperature of 4.4°C from 1st February (Collier, unpublished data). Here, the mean accumulated sum (from 1st February at base temperature of 4.4°C) to the capture of the first aphid was estimated at 325D°. Differences in the accumulated sums may have arisen through the data sets used to establish these relationships. For example, the current day-degree model (360D°) was developed using data from two sites (Wellesbourne and Kirton) whereas forecasts here were developed using data from six suction trap locations across the UK (Broom's Barn, Kirton, Preston, Rothamsted, Writtle and Wye).

A relatively constant day-degree sum was not obtained for the 50% capture date of *C. aegopodii*. At both base temperatures, the date of 50% capture displayed strong positive correlations with accumulated day-degrees. While some variation was expected, due to the random nature of sampling by suction traps, the strong linear correlations that were produced suggested that other factors may influence the timing of 50% capture. Similar results were found by Worner *et al.* (1995) when using day-

degree methodologies to predict the timing of 50% migration of *Phorodon humuli* (Schrank, 1801), damson–hop aphid (Hemiptera: Aphididae) at two sites in the UK.

The exact reasons why the date of 50% capture of *C. aegopodii* and accumulated day-degrees were positively correlated are unclear. For other species of aphid (*e.g. A. fabae*) it has been suggested that a lack of linearity in the relationship between temperature and the rate of development could result in inconsistent day-degree sums (Collier, 2014). However due to the relatively constant day-degree sums that were obtained for capture dates of first and 10% of *C. aegopodii*, this is unlikely to be the reason.

As *C. pastinaceae* and *C. theobaldi* have a similar life cycle to *C. aegopodii* (*e.g.* holocyclic and mostly host alternating) it was possible that these species might share a similar relationship with day-degrees. However, for both species, constant day-degree sums to aphid capture dates were not obtained. Instead, accumulated day-degrees were positively correlated with aphid capture dates. As previously mentioned, it is possible that these species have different temperature requirements for development compared with *C. aegopodii.* To test this hypothesis a wider range of base temperatures (lower temperature thresholds) for development could be investigated.

To some extent, discrepancies in the accuracy of the day-degree predictions may relate to their limitations as forecasting models. Day-degree models do not account for the inherent variation in the development times of many insects (Shaffer, 1983; Phelps *et al*., 1993). For example, accumulated day-degree requirements for a particular event (*e.g.* start of migration) are usually based on the mean or median of a population and therefore do not consider the variation between individual insects.

2.4.3 Forecasts based on mean air temperature

Strong linear relationships were observed for *M. persicae* using measures of mean air temperature in the winter/early spring. Firstly, warmer winters were associated with earlier migrations (first capture dates) of *M. persicae*. This relationship has been observed in previous studies (Harrington *et al*., 1989, 1990; Howling *et al*., 1993; Harrington and Clark, 2010), particularly for species that overwinter in an

anholocyclic manner (Walters and Dewar, 1986; Harrington *et al.,* 1990). Harrington *et al.* (1995) suggested that an increase of 1°C in mean air temperature during January–February can advance the onset of migration by *M. persicae* by two weeks in the spring.

For the capture of the first, 10% and 25% of the spring/summer migrants, relationships were strongest with the mean air temperature during January–February inclusive. Interestingly, when validating the forecasts, slight improvements were observed when using the mean air temperature from January–March inclusive. However, this also increased the number of late predictions. When predicting the capture dates of 50% and 90% *M. persicae*, using longer runs of mean air temperature data (*e.g*. January–April inclusive) improved the accuracy of forecasts.

The close relationships with mean air temperature in winter/early spring observed for *M. persicae* were not unexpected. Aphids that have largely anholocyclic life cycles the UK, like *M. persicae*, are more responsive to temperature in the winter than overwintering eggs in diapause. For this reason, they are able develop and reproduce as temperature permits (Harrington *et al*., 1991). Similar studies, have outlined the close relationship between mean air temperature and the phenology of aphids (Harrington *et al*., 1990; Howard and Dixon, 1990; Howling *et al*., 1993; Zhou *et al*., 1995). For example, Howling *et al*. (1993) found that February was the single most important month for determining the timing of the spring migration of *M. persicae* at Rothamsted. However, they found that the models could be improved by the inclusion of additional periods of mean air temperature data (*e.g.* January–March inclusive).

In terms of their accuracy, forecasts based on mean air temperature performed similarly across the three sites (Broom's Barn, Kirton, Rothamsted). This could be explained by the relatively close proximity of the suction traps, which are located in the East and East Midlands of the country, and therefore similar relationships with mean air temperature and the phenology of *M. persicae* would be expected.

Additionally, the forecasts developed to predict the week of the summer peak in the numbers of *M. persicae* yielded reliable predictions for the three sites. Strongest correlations were produced using the mean air temperature from January–May

inclusive. When validating the forecasts, there was very little difference from using mean air temperature across different periods in the winter/spring. Other studies have identified a relationship between 'peaks' in the number of aphids and mean air temperature. For example, using a sub-set of data from 86 potato fields in the UK sampled during 1994–1996, Parker (1998) found that the timing of peak populations of *M. persicae* could be predicted using mean air temperature from January–March. However, he found that mean air temperature in May was also important.

It is possible that the forecasts based on mean air temperature could be improved by incorporating capture data from additional suction trap sites, particularly those located in different regions of the UK (*e.g*. the north and south of the country) into the regression models to account for any regional differences in the phenology of *M. persicae.* Additionally, models could be developed using daily capture data, opposed to weekly data (which in this project were supplied by the Rothamsted Insect Survey). This may improve forecast accuracy by allowing predictions to be made to the nearest day.

The relationship between the activity of *C. aegopodii* and mean air temperature, across defined periods in the winter/spring, was also explored, with forecasting models developed. Similar to *M. persicae*, results for *C. aegopodii* were generally indicative of the warmer the spring, the earlier the start of the spring/summer migration. Using longer runs of temperature data to develop forecasts, generally improved the accuracy of their predictions.

Comparisons could be made on validity of using forecasts based on mean air temperature and forecasts based on day-degree, as a common validation data set was used (1981–1988: 55 sites x years). Forecasts based on mean air temperature were generally less reliable than the day-degree forecasts developed for predicting the capture of the first and 10% *C. aegopodii*, with mean absolute differences of 2–3 and 3–4 days, respectively. However, while constant day-degree sums were not identified for the date of 50% capture of *C. aegopodii*, forecasts based on mean air temperature provided relatively a reliable option; on average predicting the date of 50% capture within eleven to twelve days.

The difference in accuracy between using a day-degree model or model based on mean air temperature to predict the phenology of *C. aegopodii* may in part be due to its predominantly holocyclic life cycle in the UK. For example, Harrington *et al.* (1991) carried out linear regression analysis with data from Rothamsted to relate mean air temperature to the date of the first capture of fourteen species of aphid. Strong relationships with mean winter temperature were found for species that undergo largely anholocyclic life cycles in the UK, but relatively poor relationships were identified for holocyclic species.

For *C. pastinaceae* and *C. theobaldi*, statistically significant relationships ($P < 0.01$) were found between aphid capture dates (first, 10% and 50%) and mean air temperature in the winter/spring. This permitted the development of simple forecasts. Compared to those developed for *C. aegopodii*, these forecasts performed less accurately, predicting capture dates within seven to twelve days for *C. pastinaceae* and ten to twenty days for *C. theobaldi*.

The reduced accuracy of predictions may be explained by a number of different factors. Firstly, smaller data sets were used to develop these forecasts (*C. pastinaceae*: 45 sites x years, *C. theobaldi*: 35 sites x years), as there were fewer usable years in which the total number of aphids caught before the cut-off point (Day 240) was ≥ 50. Secondly, forecasts were only validated over twelve sites x years for three sites. This was due to limited access to reliable temperature records for recent years to validate the forecasting models. Thirdly, it is possible that the relationship between temperature and their phenology differs between *C. aegopodii* and *C. pastinaceae*/*C. theobaldi*.

2.4.4 The influence of latitude, longitude and altitude on forecasting models

The inclusion of latitude and longitude as additional explanatory factors in the multiple regression analysis, did not significantly improve the forecasting models based on mean air temperature for the four species of aphid. This result was unexpected. In previous studies, effects of latitude and longitude on the phenology of *M. persicae* have been observed (Harrington *et al*., 1995; Zhou *et al*., 1995; Alford *et al*., 2012). For example, Zhou *et al*. (1995) reported that latitude had a minor effect on the

phenology *M. persicae*, whereas longitude advanced the capture dates of aphids. Additionally, Harrington *et al*. (1995) reported that the onset of migration by *M. persicae* is delayed at higher latitudes and this effect could not be accounted for by the relationship between latitude and temperature alone. In terms of altitude, while it may influence the phenology of aphids via its effect on temperature, the locations of the suction traps used for analysis were in lowland agricultural regions, with a range in altitude of 3 m (Kirton) to 119 m (Rothamsted). For this reason, any differences are expected to have been small.

2.4.5 The field monitoring of *Cavariella aegopodii*

In 2016–2018, comparison of the relative timing of *C. aegopodii* caught in the suction trap and yellow water traps (YWTs) at Wellesbourne, UK, showed that aphids were always caught first in YWTs. Firstly, it is possible these were from anholocyclic clones that had overwintered in carrot. However, some of the variability between the methods of trapping is likely to have arisen through the sampling error associated with trapping, which is essentially a random process. Additionally, as suction sampling takes place at 12.2 m above the ground, it is possible that the numbers of aphids captured at this height would not provide an entirely accurate representation of what is happening at the crop level. For this reason, there may be some discrepancy between the predicted numbers (from models developed using suction trap data) and the actual numbers of aphids in crops. Yellow water traps may therefore provide a more accurate representation of the number of aphids on a local scale.

In all years, the peak in *C. aegopodii* numbers was observed one week earlier in the suction trap compared to in YWTs. To some extent, this shows that aphid captures by suction traps can be used to estimate when peaks will occur in crops.

Day-degree forecasts for predicting the capture of the first and 10% *C. aegopodii* were validated using temperature data for Wellesbourne (2016–2018). Day-degree forecasts provided considerably reliable predictions of aphid capture dates for all three years of within zero to ten days.

Day-degree forecasts were most accurate in 2016 for predicting the capture date of

10% *C. aegopodii*. Overall, forecasts that accumulated day-degrees from 1st January yielded the most accurate predictions across the three years. Again, there was little difference observed in the accuracy of forecasts using a base temperature of either 4°C or 4.4°C. However, as the forecasts were only validated across three years, no strong conclusions can be drawn.

2.4.6 Diurnal variation in the flight activity of *Cavariella aegopodii*

The greatest proportion of *C. aegopodii* were caught in YWTs during the time periods of 11:00–12:00 and 12:00–13:00. There is relatively little information on the daily flight activity of *C. aegopodii* in the literature. However, for many species of aphid it has been reported that daily patterns in flight depend largely on the availability of flight-mature adults, their urge to fly and their light intensity, temperature and wind-speed thresholds for take-off (Johnson and Taylor, 1957; Johnson *et al*., 1957; Lewis and Taylor, 1965; Dixon, 2012).

A bimodal periodicity in daily flight has been observed for several species of aphid. For example, from hourly captures of *A. fabae* by suction traps, Johnson *et al*. (1957) observed two peaks in flight during the course of the day. The first peak occurred during the morning (~9:00 GMT). This was attributed to aphids that had developed and become flight-mature overnight, but were prevented from flying immediately due to low light intensity and temperature. Once light intensity and temperature had risen above given thresholds the following morning, *A. fabae* was able to initiate flight. The second peak in flight activity occurred later during the day (~18:00 GMT) and corresponded to the appearance of newly moulted aphids in the morning, that had completed their teneral development by the afternoon. Into the evening, numbers declined as the levels of light intensity and temperature fell below the necessary thresholds for initiating flight.

However, bimodal periodicity would not be expected if aphids are capable of flying for extended periods (Lewis and Taylor, 1965). For example, the lime aphid, *Eucallipterus tiliae* (Hemiptera: Aphididae) (Linnaeus, 1758) does not autolyse its wing muscles. As an adult, the species flies throughout its life and has a single peak

in flight activity around midday. In this case, the timing of flight is likely determined by light intensity and temperature thresholds (Dixon, 2012).

In these field experiments, a single peak in the flight activity of *C. aegopodii* was observed around midday. This may relate to several factors; including the teneral development of *C. aegopodii* and the light intensity and temperature thresholds for its flight. It is possible that flight may also be inhibited at times with low temperature. This would explain why relatively few *C. aegopodii* were caught during the morning (9:00–10:00 GMT). Additionally, the number of aphids flying outside of the intensive monitoring period (9:00–16:00 GMT) was low. Again, this is likely a reflection of low temperatures and, in this case, low light intensity overnight.

Furthermore, while several previous studies investigated aerial populations of aphids using suction traps, here YWTs were used to sample the diurnal variation in aphid flight activity. While YWTs are likely to capture aphids that are migrating into crops, they are also likely to capture aphids that redistribute themselves within crops during the day. At the crop level, it is possible that this trivial movement may have some effect on the number of aphids caught in YWTs at certain times of the day.

A limitation to the interpretation of the data from this experiment was that factors such as hourly temperature, light intensity and wind speed were variable, and the experiment was not conducted over a sufficiently large number of occasions to take these into consideration in a meaningful way. Monitoring was only undertaken on warm days with low wind speeds during May–June (around the 'peak' in numbers of *C. aegopodii*) to increase the likelihood of capturing a sufficient number of aphids in YWTs. While the experiments in this project help to determine the pattern of flight activity by *C. aegopodii,* they do not elucidate the factors that cause this pattern.

Information on the diurnal variation in the flight activity of *C. aegopodii* could be used to manage the control options that applied in the field. For example, this knowledge could be used to inform decisions regarding the best time(s) to apply treatments or to remove crop covers for weeding, which is particularly important for organic carrot production. This information could also supplement the use of forecasting models by providing an estimate of the time of day that aphids are likely to migrate into crops.

2.4.7 Forecasting advice for growers

Going forward, there is likely to be considerable interest in forecasting the phenology and abundance of aphids. This is due to the potential it offers growers in terms of optimising the timing of their control approaches and reducing their expenditure. Additionally, due to more stringent pesticide legislation, is it likely that growers will have fewer insecticides to rely on for pest control (Hillocks, 2012; Goulson, 2018; Scott and Bilsborrow, 2019).

Forecasting models, like those developed in this project, could be used to inform and improve the control options that are applied for control of aphids in the UK. The models could be employed as regional forecasts or used on a local scale. For example, it would be possible to provide growers with the relevant fitted equations to be used with temperature data from their site in software packages, which may be as simple as Microsoft® Excel. Going forward, exploring the benefit of developing forecasting models using data from individual sites could be worthwhile. This would test whether the accuracy of predictions could be improved by using forecasts that are specific to a grower's location.

Additionally, knowing when large infestations by aphids can be expected would be of great use to growers. However, the methods developed for predicting abundance so far have not received general acceptance and few forecasting systems for aphid abundance are in use currently (Kindlmann and Dixon, 2010). This is likely to relate to the additional factors, other than temperature, that increase the complexity of predicting the size of aphid populations. For example, the severity of infestations by aphids may be affected by intrinsic factors, including: fecundity, mortality rate and migration rate (Kindlmann and Dixon, 2010) as well as external biotic and abiotic factors (Harrington and Clark, 2010). Of these external factors, the abundance of natural enemies (Fidelis *et al*., 2019), levels of parasitism (Parker, 1998), the distribution and abundance of winter and summer host plants (Bell *et al*., 2012) and other climatic conditions (*e.g*. rainfall and extreme weather events) are likely to be important (Thacker *et al*., 1997; Harrington and Clark, 2010).

The number of aphids caught by suction traps are published in weekly bulletins by the Rothamsted Insect Survey (RIS) during the 'aphid season' for the previous week.

However, it is likely that when high numbers are reported to growers the aphid peak may have already occurred in crops. This emphasises the need for robust forecasting models that can provide early and reliable warnings of infestations by aphids.

2.4.8 Limitations and further research

A major limitation to the analysis carried out in this chapter was the access to reliable weather records from the Met Office for several sites after 2010. It is possible that existing forecasting models could be refined by incorporating additional data into the regression models (*e.g*. data from recent years) as this would allow for recent trends in winter/spring temperatures to be taken into account.

Additionally, there are still important gaps in knowledge of the biology and phenology of aphids, particularly with regard to *C. pastinaceae* and *C. theobaldi.* Increasing the understanding of these species, their population dynamics and their potential as virus vectors will undoubtedly help to improve forecasting models and to interpret the variation/error associated with their use.

2.4.9 Conclusions

Predicting the timing of infestations by aphids with accuracy can be difficult due to the complex range of intrinsic and external factors affecting aphid populations. Overall, the relationships outlined in this chapter between aphid phenology and measures of temperature (*e.g*. accumulated day-degree sums and mean air temperature) hold despite the influences of other abiotic and biotic factors. It is likely that these additional factors, however, lead to part of the variation in the relationships identified.

- Strong relationships were found between air temperature and aphid phenology.

- Forecasts based on mean air temperature, averaged over defined periods in the winter/early spring can be used to predict the timing of the spring/summer migrations of *M. persicae, C. aegopodii*, *C. pastinaceae* and *C. theobaldi.* These forecasts, however, vary in terms of the accuracy of their predictions.

- Day-degree forecasts can be employed to predict the dates of first and 10% capture dates of *C. aegopodii* by accumulating day-degrees from the start of January and start of February, respectively. However, there is still some uncertainty surrounding the precise base temperature for the development of *C. aegopodii*. In terms of predicting the onset of migration by *C. aegopodii*, the day-degree model that was developed in this project improves on the model that is employed currently in the UK.

- There is diurnal variation in the flight activity of alate *C. aegopodii*. Aphids were most active around midday. This could have implications for timing/management of the control options that are applied in the field.

3.1.1 Host plant selection by aphids

Aphids share close associations with their host plants. The knowledge of these relationships is important for understanding the ecology of aphids and their ability to spread to colonise new areas (Holman, 2009a). Biological traits that allow aphids to find and exploit their host plants largely determine their status as pests (Powell *et al*., 2006). While the term host preference refers to behavioural features of insects that lead to the accumulation of more individuals on some plants compared to others (Singer, 2000); host acceptance often relates to the sustained feeding and/or reproduction of insects on plants. A clear indication of host acceptance by aphids is the onset of reproduction, in particular the deposition of nymphs by viviparous adults (Powell *et al*., 2006).

In aphids, host plant selection is determined by a range of visual, mechanical and chemical stimuli (Bernays and Chapman, 1994; Troncoso *et al*., 2005; Döring, 2014). Aphids employ a number of mechanisms to locate and recognise host plants; these include the examination of a plant's volatile chemicals, surface waxes, intercellular polysaccharides and contents of the mesophyll and phloem (Niemeyer, 1990; Troncoso *et al*., 2005). In the literature, these behavioural events are regularly divided into sequential stages (Vargas *et al*., 2005; Powell *et al*., 2006), for example, (i) pre-alighting behaviour, (ii) exploration of the leaf surface and probing of the subepidermal tissues, (iii) deep probing of plant tissues, and (iv) assessment of sap from the phloem. The recognition of a plant as a host or non-host may occur at any of these stages (Nottingham and Hardie, 1993; Powell and Hardie, 2000; Vargas *et al*., 2005; Powell *et al*., 2006).

Stage 1: Pre-alighting behaviour

Prior to landing, alate adults respond to the light wavelengths reflected by plant surfaces. Host-specific information, however, is unlikely to be provided by visual cues alone (Kennedy, 1976). For example, when landing, many insects do not discriminate between green-coloured objects, for example, the leaves of host plants ('appropriate landings') and those of non-host plants ('inappropriate landings') (Finch and Collier, 2000).

Landing is also influenced by plant volatiles that are detected by an aphid's antennal olfactory sensilla (Chapman *et al*., 1981; Hardie *et al*., 1994a; Visser *et al*., 1996; Webster, 2012; Döring, 2014).

Stage 2: Leaf surface exploration and probing of subepidermal tissues

Once landed, aphids detect volatiles at the plant's surface (Storer *et al*., 1996; Hardie and Powell, 2000). In order for the recognition of host plants, it has been proposed that insects require certain blends of ubiquitous plant volatiles, rather than relying exclusively on the presence or absence of highly specific volatiles (Viser, 1986; Bruce *et al*., 2005; Webster, 2012). The detection of specific blends of volatiles by aphids may initiate feeding behaviour (Powell *et al*., 1995). Additionally, epicuticular waxes (Powell *et al*., 1999; Shepard *et al*., 1999), leaf texture (Powell *et al*., 1999), topology (Ibbotson and Kennedy, 1959), and colour (Pelletier, 1990; Döring, 2014) can be important for host selection prior to initiating probing behaviour (Powell *et al*., 2006).

Following the detection of these stimuli, aphids initially probe the epidermis for short periods of time (*e.g.* < 30 seconds). Alate adults often alight on the upper leaf surface and after a brief period of probing they may either reject a plant and take flight (Powell and Hardie, 2000) or accept a plant and often relocate to the lower surface of the leaf (Calabrese and Edwards, 1976). As the stylet and labium do not have external contact chemoreceptors (Wensler, 1977), instead an epipharyngeal gustatory organ assesses the ingested plant sap at this stage (Wensler and Filshie, 1969).

Stage 3: Deep probing of plant tissues

Deep probing of plant tissues usually involves periods of probing that last 30 s to 1 min (Bradley *et al*., 1962a; Powell *et al*., 2006). Here, ingested plant sap from the mesophyll and parenchyma tissues and the contents of the phloem are assessed (Powell *et al*., 2006). Following stylet penetration of the phloem, aphids inject saliva which aids to inhibit plant defensive mechanisms such as the sealing of the phloem (Prado and Tjallingii, 1994; Miles, 1999). Additionally, plant condition can be important at this stage. For example, Kennedy (1958) found that *M. persicae* and *A. fabae* had a preference for growing or senescing leaves and attributed it to their higher levels of soluble nitrogen compounds.

Stage 4: Assessment of sap from the phloem

Phloem acceptance may be inferred by periods of probing/feeding that exceed 10 min (Powell *et al*., 2006). These periods can last for several hours while aphids intake phloem sap (Tjallingii, 1994; Dinant *et al*., 2010). At this stage nutritional cues have a crucial role in plants being accepted or rejected by aphids.

3.1.2 Host plant acceptance by aphids

Parturition by aphids on a particular plant is often a clear sign of host acceptance (Powell *et al*., 2006). After stylet insertion, aphids are reported to probe/feed for at least 30 min before initiating parturition (Blackman, 1990; Kobayashi and Ishikawa, 1993; Tosh *et al*., 2002).

It was first assumed that prolonged feeding from sieve elements in the phloem was a prerequisite for reproduction by aphids (Blackman, 1990; Kobayashi and Ishikawa, 1993). Nevertheless, several studies have since investigated the relationship between stylet penetration and the onset of reproduction (Tosh *et al*., 2002, 2003; Powell *et al*., 2004). These studies reported that chemical signals in the peripheral plant tissues may induce reproduction, prior to an aphid contacting the phloem and undergoing significant feeding and sampling the nutrients of a host plant (Powell *et al*., 2004).

3.1.3 Host plant selection and acceptance by specialist and generalist species of aphid

The majority of aphids are specialists (Stern, 2008), utilising one or several closely related species of plant, *e.g*. the willow-carrot aphid, *C. aegopodii,* whose hosts are members of the Apiaceae. Generalist species of aphid (*e.g*. *M. persicae*), however, are considered to be some of most damaging pests. This is due to their ability to locate and exploit hosts from many different plant families and spread diseases between them. The time taken for many phytophagous insects to either accept or reject a plant is thought to be related to host range, with specialists generally requiring less time to make decisions compared to generalists (Bernays and Funk, 1999; Powell *et al*., 2006).

This trend has been observed in studies conducted with aphids. Funk and Bernays (2001) evaluated two races of *Uroleucon ambrosiae* (Thomas, 1878) (Hemiptera: Aphididae) with different host ranges; one specialised on giant ragweed, *Ambrosia trifida* L. (Asteraceae) and the other fed across several genera of Asteraceae. Results from 'choice' and 'no choice' experiments and electrical penetration (EPG) studies found differences in the behavioural mechanisms for detecting feeding stimuli between the two races. Compared to the generalist, the specialist race was faster to locate its host plant and access the phloem. Additionally, Vargas *et al*. (2005) examined host selection behaviours in the generalist aphid, *M. persicae* and its subspecies specialised on tobacco, *M. persicae* ssp. *nicotianae*. The specialist subspecies was shown to undertake more direct selection and acceptance behaviours compared to the generalist at several stages of host selection, including: prior to landing, the responses to host volatiles and the exploration of the leaf surface and sub-epidermal tissues.

For many species of aphid there is a reliance on plant secondary metabolites during the host selection process (Wensler, 1962; Powell and Hardie, 2000). Metabolites from non-host plants may act to prevent feeding/parturition, whereas those from host plants may stimulate these events, especially in the case of specialist species of aphid (Powell *et al*., 2006). Nevertheless, while specialists may select a host plant on the basis of one or a few metabolites, the process of host selection by generalist species of aphid is often more complex. Compared to specialists, it is thought that generalist species of aphid require a range of plant traits for host selection; for example, both primary and secondary metabolites (Powell *et al*., 2006). However, the identification of the plant metabolites that have a role in host preference and acceptance for generalist species is a major research challenge.

3.1.4 Host preferences of *Myzus persicae*

Myzus persicae is a highly adaptable and polyphagous species; feeding on more than 400 plant species from over 40 different plant families (van Emden *et al*., 1969; Weber, 1986). It has also evolved strategies to respond to a wide range of defensive chemicals produced by plants (Francis *et al*., 2006). Due to its polyphagous nature, *M. persicae* is a species that is particularly well suited for examining host preference and acceptance behaviours between different plants.

It has been shown in several studies that *M. persicae* displays considerable genetic variation with regard to host-plant adaptation. Weber (1985) investigated over 1000 clones, mostly originating on potato, *Solanum tuberosum* L. (Solanaceae) and sugar beet, *Beta vulgaris* L. (Chenopodiaceae). While these clones performed better on hosts from which they originated, *M. persicae* still displayed a high level of adaptability to new hosts. Weber (1985) suggested that the high phenotypic plasticity and the genetic variability of *M. persicae* are likely to contribute to its polyphagous nature.

3.1.5 The development of *Myzus persicae* on selected host plants

The success of *M. persicae* as a pest of crops can be attributed in part to factors relating to its development. Viviparous *M. persicae* can develop rapidly in mild climates; requiring around ten days to complete a single generation (Horsfall, 1924; Weed, 1927; Barlow, 1962). Furthermore, it is reported that as many as twenty generations can be produced in a year (Horsfall, 1924).

The developmental rate of aphids varies with temperature. For the majority of aphid species, optimal developmental rates and fecundity occur between 20 and 30°C (Barlow, 1962; Dean, 1974; Tsitsipis and Mittler, 1976). This is also the case for *M. persicae*. The upper and lower temperature thresholds reported for *M. persicae* are 4°C and 30°C, respectively, with optimal survival occurring at 20°C (Whalon and Smilowitz, 1979; Liu and Meng, 1999). Reproduction by *M. persicae* ceases at temperatures above 30°C and thermal death is reported at 38.5°C (Broadbent and Hollings, 1951). Several studies have investigated the development time of *M. persicae* on different host plants at a range of temperatures (Table 3.1).

Table 3.1 – Summary of studies investigating the development time, in days, of *Myzus persicae* on a range of host crop plants. The numbers in parentheses are the temperatures (°C) used for each study. * = study carried out in an insectary in Pennsylvania with seasonal variation in temperature.

Author(s)	Host	Development time in days (temperature)				
		0–10°C	**11–20°C**	**21–23°C**	**23–26°C**	**27°C+**
Horsfall (1924)	Radish		10.8*			
Weed (1927)	Spinach	20.8 (10)	11.4 (16)	8.3 (21)	6.5 (24)	7.2 (28)
Lal (1950)	*Brassica*			7.0 (18)	6.5 (26)	4.3 (30)
MacGillivray and Anderson (1958)	Potato			9.1 (20)		
Barlow (1962)	Tobacco	16.8 (10)	6.3 (15)	4.9 (20)	4.2 (25)	

Horsfall (1924) investigated the development of viviparous *M. persicae* on radish, *Raphanus raphanistrum* ssp. *sativus* L. (Brassicaceae) in an insectary (Pennsylvania) with seasonal variation in temperature. In this study four instars were reported, lasting on average: 2.0, 2.1, 2.3, and 2.0 days, respectively. Reproduction was recorded six to seventeen days after birth, with the mean age of first birth being 10.8 days. On average, females deposited 1.6 nymphs per day. The duration of parturition varied considerably but on average lasted for 14.8 days. The mean longevity was 23 days. These data were collected under caged conditions with the exclusion of predators and pathogens.

However, at 20°C MacGillivray and Anderson (1958) recorded five instars of *M. persicae* on potato with a mean development time of 2.4, 1.8, 2.0, 2.1, and 0.7 days, respectively. Additionally, they observed a mean reproductive period of twenty days, a mean fecundity of 75.5, a mean generation time of 9.1 days and a mean longevity of 41.1 days.

The development of *M. persicae* may be influenced by the 'choice' of host plant. By measuring reproductive and mortality rates of *M. persicae* in glasshouse trials, Heathcote (1962) investigated the suitability as hosts of a range of commercially-important crops. It was found in the study that *M. persicae* develops well on *Brassica* species, including Brussels sprout (*Brassica oleracea gemmifera*). However, while *M. persicae* was found on lettuce leaves (*Lactuca sativa var. capitata*) at all stages of the plant's development, aphids reproduced at a much slower rate than when feeding on *Brassica* species.

3.1.6 Host preference and selection behaviour of *Myzus persicae*: aims

The aims and hypotheses of the research described in this chapter were to:

- Investigate the development of *M. persicae* (clones MP1S and 2050A) on three commercially-important host crops (Brussels sprout, carrot and lettuce) which have considerable problems with infection by plant viruses and a non-host species (sugar maple).

- Conduct a series of 'choice' and 'no choice' experiments to investigate whether the host preference and selection behaviour of alate *M. persicae* differs between the three plant species. This will test the hypothesis that alate *M. persicae* selects host plants based on their ability to support aphid development.

- Examine whether the host preference and selection behaviour of alate *M. persicae* is influenced by previous host experience *i.e.* whether the plant species that aphids are reared on affects their subsequent host plant preference/selection behaviour.

3.2 Host preference and selection behaviour of *Myzus persicae*: Materials and Methods

3.2.1 Insect and plant material

Parthenogenetic colonies of two clones of *Myzus persica*e (2050A and MP1S) were reared at 18°C, under a photoperiod of 16L:8D in the Insect Rearing Unit (IRU) at Warwick Crop Centre, School of Life Sciences, Wellesbourne. Both clones were maintained on Brussels sprout (*Brassica oleracea* var. *gemmifera* cv. Doric F1), carrot (*Dacus carota* cv. Nairobi F1) and lettuce (*Lactuca sativa* cv. Lobjoits Green Cos) in rearing cages (47.5 x 47.5 x 47.5 cm) with fine nylon mesh sides (Bugdorm-44545; Watkins and Doncaster Ltd., Herefordshire, UK). All plants were grown initially at 20°C, under a photoperiod of 16L:8D in pots (9 x 9 x 8 cm) containing M2 compost (Levington® medium grade sphagnum moss peat: Everris Limited, Ipswich, UK: pH 5.3–6.0; N = 192, P = 98, K = 319 mg/L).

All aphids were reared on host plants for at least two generations prior to use in bioassays, unless stated otherwise. Three days before starting the experiments, alate adults were removed from the rearing cages. Newly-emerged alate adults were then collected using a fine paintbrush from the inner walls and ceiling of the rearing cages for use in the bioassays.

3.2.2 The development of *Myzus persicae*

The development of *M. persicae* clones MP1S and 2050A was investigated on three host plants; Brussels sprout cv. Doric F1 (Brassicaceae), carrot cv. Nairobi F1 (Apiaceae), lettuce cv. Lobjoits Green Cos (Asteraceae) (3–4 weeks after sowing) and a non-host species; sugar maple, *Acer saccharum* Marshall (Sapindaceae) using a method adapted from Awmack *et al*. (1997). The decision to use sugar maple as non-host was firstly because it was not listed as a host plant of *M. persicae* by the Centre for Agriculture and Bioscience International (CABI, 2019). Secondly, other members of the genus *Acer* (*e.g*. silver maple, *Acer saccharinum* L.) have been recorded as non-host plants of *M. persicae* (Annis *et al*., 1982).

Alate *M. persicae* (0–3 day old) were placed singly onto plants (total of twelve alate adults for each plant species; six of clone MP1S and six of clone 2050A across two replicates in time) using a fine paint brush. All aphids that were introduced onto the plants were alate adults, as this is the form that would locate a new host in the field during the process of host selection (van Emden *et al*., 1969). Alate adults had been reared on their respective host for at least one generation. For the development studies with the non-host (sugar maple) alate adults were collected in equal numbers from aphid colonies reared on either Brussels sprout, carrot or lettuce.

Individual plants were kept in 200 x 500 mm micro-perforated polypropylene bags (Cryovac®, New Jersey, USA) at 20°C under a photoperiod of 16L:8D. Sugar maple leaves were first placed into damp OASIS® floral foam (OASIS® Ideal Floral Foam Maxlife Brick, Smithers Oasis Ltd., Washington, USA) inside a plant pot (9 x 9 x 8 cm).

Once alate adults deposited apterous nymphs, the adults were removed from the plants. Individual nymphs were then observed each day until they begin to reproduce. This provided an indication of their development time (D) on each plant species. Each day the number of nymphs deposited by the apterous adults was recorded and nymphs were removed from plants in order to prevent overcrowding, which may have adversely influenced reproduction.

The intrinsic rate of natural increase (r_m) for each individual aphid was then calculated using the formula proposed by Wyatt and White (1977):

$$r_m = (\ln Md) \times c/D$$

Here, Md is the number of nymphs produced by the adult in a time equivalent to its development time, D, (birth to onset of reproduction), and c is a constant with a value of 0.738.

3.2.3 The host preference and selection behaviour of alate *Myzus persicae*: 'choice' and 'no choice' experiments

Leaf disc 'arena' system

A series of leaf disc 'choice' and 'no choice' experiments (Table 3.2) were carried out to determine the feeding preferences of *M. persicae* (clone 2050A) between three commercially-important host crops; Brussels sprout cv. Doric F1, carrot cv. Nairobi F1 and lettuce cv. Lobjoits Green Cos. The feeding behaviour of *M. persicae* was also investigated on sugar maple, *Acer saccharum* Marshall (Sapindaceae). Alate adults used in the non-host (sugar maple) 'no choice' experiment were collected in equal numbers from aphid colonies reared on either Brussels sprout, carrot or lettuce using a fine paintbrush.

Table 3.2 – The different combinations of plant species investigated during 'choice' and 'no choice' experiments with alate *Myzus persicae* 2050A. Letters in parentheses indicate the host plant alate *Myzus persicae* was reared on: BS = Brussels sprout, C = carrot, L = lettuce.

'Choice'	'No choice'
Brussels sprout *vs.* carrot (BS, C)	Brussels sprout (BS, C, L)
Brussels sprout *vs.* lettuce (BS, L)	Lettuce (BS, L)
Lettuce *vs.* carrot (BS)	Carrot (BS, C)
Sugar maple *vs.* Brussels sprout (BS)	Sugar maple (BS, C and L)
Sugar maple *vs.* carrot (C)	
Sugar maple *vs.* lettuce (L)	

For Brussels sprout, lettuce and sugar maple, leaf discs were excised using a cork borer (23.75 mm diameter; size 16) and placed onto 1.2% water agar (Agar Technical No. 3, Oxoid, USA) in 9 cm plastic Petri dishes (SARSTEDT, Germany). In order to make all leaf discs comparable, the area of carrot leaves was determined using Image-J 1.x software (Schneider *et al*., 2012). A similar area to the other plant species was used. All leaves from the host plant species used in the bioassays were removed from plants (grown at 20°C) around three–four weeks after sowing.

Two holes (23.75 mm diameter) were made in filter paper (90 mm diameter) (Whatman® Qualitative Filter Paper No.1; GE healthcare, UK) at approximately an equal distance from the centre. The filter paper was then placed onto the agar (around the leaf discs) to allow the alate adults to move freely between the two discs. For 'choice' experiments each 'arena' contained a 'choice' of two leaf discs from different plants. 'No choice' 'arenas' (Fig. 3.1) contained two leaf discs from the same plant and were referred to as the 'left leaf disc' and the 'right leaf disc'.

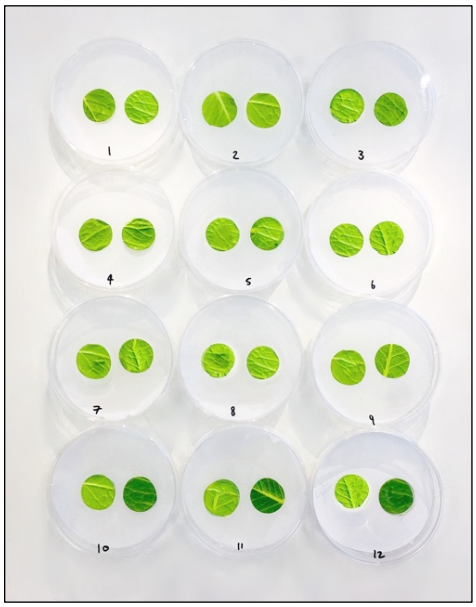

Figure 3.1 – Leaf disc 'arena' system with Brussels sprout (⌀ 23.75 mm).

Alate adults (0–3 days old) were selected at random from the inner walls and ceilings of rearing cages and removed using a fine paintbrush. Alate adults were used as they are adapted to disperse and locate new host plants. Alate adults initially underwent an hour-long starvation period in an empty 9 cm Petri dish (SARSTEDT, Germany), before being placed individually into leaf disc 'arenas'. The starvation period was an estimation of the time aphids might spend in flight prior to landing.

Staggered observations of behaviour were recorded hourly using a stereomicroscope (Euromex E series, Holland, x45 magnification) in the laboratory under uniform lighting. Initially, a total of twelve alate adults were monitored individually for 5 min immediately after infestation (0 h); in order to capture the major types of behaviour exhibited during the initial exploratory period. The twelve alate adults were then observed for one minute each hour for the remaining four hours of the experiment.

For each ‘choice’ and ‘no choice’ combination with the three species of host plant, experiments were replicated twice in time; giving a total observation time of 216 min with 24 alate adults monitored for each combination (Table 3.2).

Alongside recording the position of the alate adult, the time spent conducting activities such as ‘walking’, ‘resting’ and ‘probing’ was monitored with a stopwatch. An alate adult was considered to be ‘probing’ if immobile with the rostrum touching the substrate (Fig. 3.2) and ‘resting’ if immobile but without the rostrum touching the substrate.

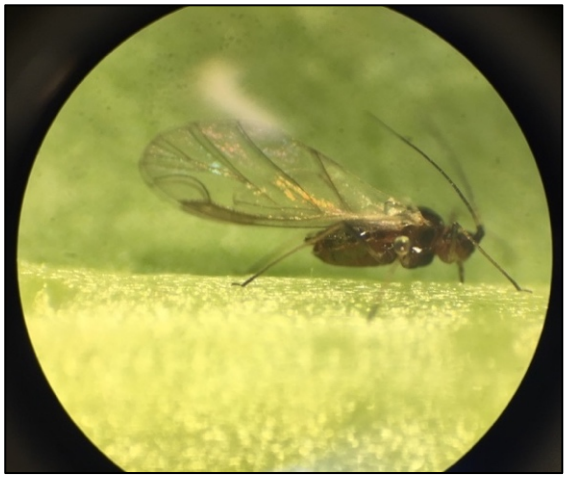

Figure 3.2 – Alate *Myzus persicae* 2050A probing/feeding on a leaf disc of Brussels sprout cv. Doric F1 under a stereomicroscope (Euromex E series, Holland, x45 magnification).

Cage system

Additionally, a cage system (Fig. 3.3) was employed to examine aphid colonisation behaviour by focusing on the accumulation of alate *M*. *persicae* on different species of plant. A series of ‘choice’ and ‘no choice’ experiments were carried out with *M. persicae* (clone 2050A) at 18°C.

Figure 3.3 – Cage system ‘no choice’ experiment with Brussels sprout cv. Doric F1.

In ‘choice’ experiments, individual cages contained four plants; two of each plant species being investigated. The plants were positioned at the corners of the cage with the arrangement alternated between replicates to take account of the different spatial orientations (Fig. 3.4). In the ‘no choice’ experiments, the two individual cages each contained four plants of the same species. For the non-host species, sugar maple, leaves of a comparable surface area to the foliage of host plants were placed into damp blocks of OASIS® floral foam (7.7 x 11 x 8 cm).

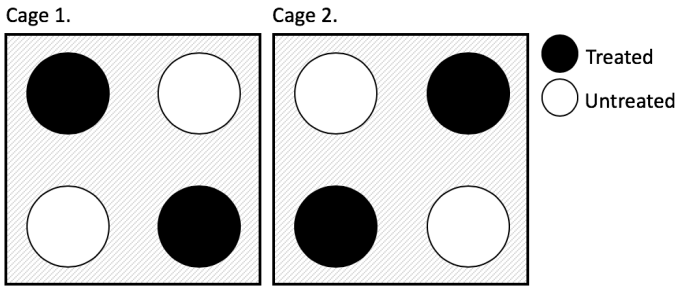

Figure 3.4 – Arrangements of the plant species between replicates to account for the different spatial orientations of plants within the cages during ‘choice’ experiments with alate *Myzus persicae*.

Twenty alate adults (0–3 days old) were selected at random from the inner walls and ceilings of rearing cages and removed with a fine paintbrush. After a 1 h starvation period in empty 9 cm plastic Petri dishes (SARSTEDT, Germany), the twenty alate adults were distributed evenly across the two cages (ten alate adults per cage). Alate

adults in Petri dishes were placed into the centre of each cage and the Petri dish lids were then removed.

Hourly observations of the number of alate adults on each plant were carried out for 5 h after infestation; the first observation taking place 1 h post-infestation. Aphids were also recorded if they were 'off plant'; *i.e.* on the cage ceilings/walls, plant pots and/or remaining in the Petri dish. If an aphid was not found it was recorded as being 'off' plant. Each 'choice'/'no choice' experiment was repeated on a separate occasion. A total of 40 alate adults was monitored for each 'choice'/'no choice' experiment.

3.2.4 Statistical analysis

All statistical analyses were carried out in SPSS Statistics (Version 25.0, IBM Corp©, Armonk, USA).

Leaf disc 'arena' system – 'choice' and 'no choice' experiments

A binomial generalised linear model (GLM) with logit link function was used to assess how the proportion of time aphids spent conducting a particular behaviour (*e.g.* probing/feeding) varied on the different plant species for the leaf disc 'choice' and 'no choice' experiments. Data were classified using the type of behaviour as the response factor and a particular plant species as the explanatory factor, with the differences between individual aphids as a blocking source of variation. The proportions of time aphids spent conducting different behaviours were simplified to follow a binomial distribution. This allowed one particular type of behaviour (*e.g.* probing/feeding) to be focused on in contrast to all others.

Leaf disc 'arena' system – settling time

The time until 'lock-in' by aphids can provide an indication of host preference. Here, 'lock-in' was defined as the selection by *M. persicae* of a leaf disc, on which the aphid subsequently remained to feed for the remainder of the experiment. The time until 'lock-in' was analysed using a Kaplan-Meier estimator for each of the leaf disc 'no choice' experiments. A log-rank (Mantel-Cox) test (Kleinbaum and Klein, 2012) was carried out during this analysis to determine whether there was a statistically significant difference between the time until 'lock-in' on the different plant species.

Leaf disc 'arena' system – probing behaviour

Sustained periods of probing/feeding may be used to infer host acceptance by aphids; conversely, multiple short duration probes may indicate 'restless' behaviour. This may have implications in terms of the transmission of plant viruses. The number of individual short duration probes (< 1 min) was assessed during the leaf disc 'no choice' experiments and compared using a Kruskal-Wallis H test for the different species of plant. Prior to this the data were checked for normality (Shapiro-Wilk test).

Cage system – 'no choice' and 'choice' experiments

For cage 'no choice' experiments the mean number of aphids accumulated on each species of plant and those remaining off plants 5 h after infestation were analysed using one-way analysis of variance (ANOVA). Prior to this the data were checked for normality (Shapiro-Wilk test). Tukey's honestly significant difference (HSD) test was used to compare means between the groups.

For cage 'choice' experiments it was not possible to compare population means with a one-way ANOVA test as the alate adults were from the same population (*i.e.* in the same cages). For alate *M. persicae* that had accumulated on plants, it was assumed that an equal distribution would be expected between the plant species in 'choice' experiments if there was not a clear host preference. To test the null hypothesis (no preference) the numbers of alate adults on particular plant species at the end of the 5 h study period were compared using a Chi-square (χ^2) goodness of fit test for each 'choice' experiment.

Cage system – settling time

The time taken by alate *M. persicae* to settle on plants in the cage 'no choice' experiment was analysed using a Kaplan-Meier estimator. A log-rank test (Kleinbaum and Klein, 2012) was carried out during this analysis to determine whether there were statistically significant differences in settling time of alate *M. persicae* on the different plants.

3.3 Host preference and selection behaviour of *Myzus persicae*: Results

3.3.1 Development of *Myzus persicae* on different host plants

The mean development time and the mean daily rate of reproduction did not differ significantly between the two clones of *M. persicae* (MP1S and 2050A) on each of the host plant species (one-way ANOVA: development time: Brussels sprout: $F = 0.372$, d.f. = 11, $P = 0.556$, carrot: $F = 0.77$, d.f. = 11, $P = 0.787$, lettuce: $F = 0.125$, d.f. = 11, $P = 0.731$; daily rate of reproduction: Brussels sprout: $F = 1.268$, d.f. = 11, $P = 0.286$, carrot: $F = 0.639$, d.f. = 11, $P = 0.443$, lettuce: $F = 2.673$, d.f. = 11, $P = 0.113$). This allowed for pooling of data.

However, the development of *M. persicae* varied on the four plant species that were investigated (Table 3.3) (one-way ANOVA: $F = 22.099$, d.f. = 35, $P < 0.001$). The mean development time was shortest on Brussels sprout plants (7.42 days) followed by on carrot plants (7.92 days) and no statistically significant difference was found between these two host plants (Tukey's HSD: $P = 0.746$). Lettuce appeared to be less favourable for supporting the development of *M. persicae,* with a mean development time of 11.58 days compared with Brussels sprout and carrot (Tukey's HSD: $P < 0.001$ and $P < 0.001$). For both clones, alate adults did not reproduce on sugar maple (non-host) and all aphids died three to four days after the start of the experiment.

Statistically significant differences were also found for the daily rate of reproduction of *M. persicae* on the three species of host plant (one-way ANOVA: $F = 115.958$, d.f. = 35, $P < 0.001$). Aphids deposited more nymphs per day on Brussels sprout compared to carrot (Tukey's HSD: $P < 0.001$) and lettuce plants (Tukey's HSD: $P < 0.001$). A statistically significant difference was also found between carrot and lettuce plants (Tukey's HSD: $P = 0.002$) with lettuce being less favourable as a host (Table 3.3).

Table 3.3 – Mean development time in days, mean daily rate of reproduction (number of nymphs per day) and the intrinsic rate of increase r_m (see Chapter 3.2.2 pp.87–88 for method of calculation) of *Myzus persicae* on three host plants and a non-host plant (sugar maple). Data are shown ± standard error of the mean. All experiments were carried out at 20°C.

	Plant species			
	Brussels sprout	**Carrot**	**Lettuce**	**Sugar maple**
Mean development time	7.42 ±0.4	7.92 ±0.3	11.58 ±0.7	–
Mean daily rate of reproduction	1.73 ±0.1	0.75 ±0.1	0.24 ±0.03	–
r_m	0.26 ±0.01	0.16 ±0.01	0.06 ±0.01	–

3.3.2 The host preference and selection behaviour of alate *Myzus persicae*: 'choice' and 'no choice' experiments

Leaf disc 'arena' system: 'choice' experiments

In 'choice' experiments *M. persicae* preferentially probe/feed on Brussels sprout leaf discs (> 40%) compared with leaf discs of carrot (18.9%) and lettuce (8.5%) (Fig. 3.5A and B). When given the 'choice' of leaf discs of carrot or lettuce, *M. persicae* probed/fed for a greater amount of time on carrot (28.3%) compared with lettuce (7.8%) (Fig. 3.5C). However, in the carrot *vs.* lettuce experiment *M. persicae* spent the majority of the observation time off the leaf discs (61%), either resting (36%) or walking (25%) in the 'arenas'. This suggested a disruption in settling behaviour.

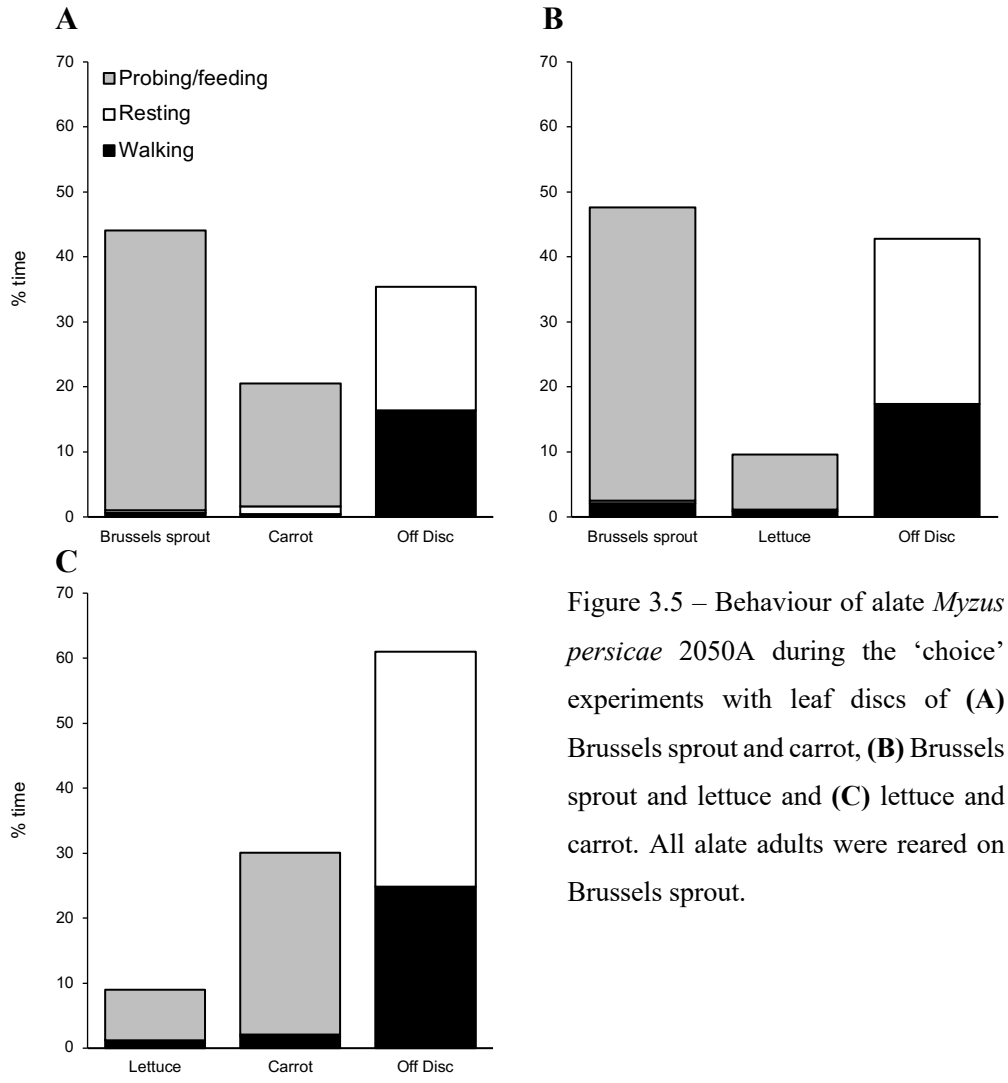

Figure 3.5 – Behaviour of alate *Myzus persicae* 2050A during the 'choice' experiments with leaf discs of **(A)** Brussels sprout and carrot, **(B)** Brussels sprout and lettuce and **(C)** lettuce and carrot. All alate adults were reared on Brussels sprout.

Leaf disc 'arena' system: 'no choice' experiments

In 'no choice' experiments *M. persicae* probe/feed for the greatest amount of time on leaf discs of Brussels sprout (68.5%) (Fig. 3.6A) compared to on leaf discs of carrot (31.8%) and lettuce (24.3%) (Fig. 3.6B) (Fig. 3.6C). *Myzus persicae,* however, spent a considerable amount of time off the leaf discs walking and resting in the carrot (32.8%) and lettuce (40%) 'no choice' experiments, suggesting 'restless' behaviour.

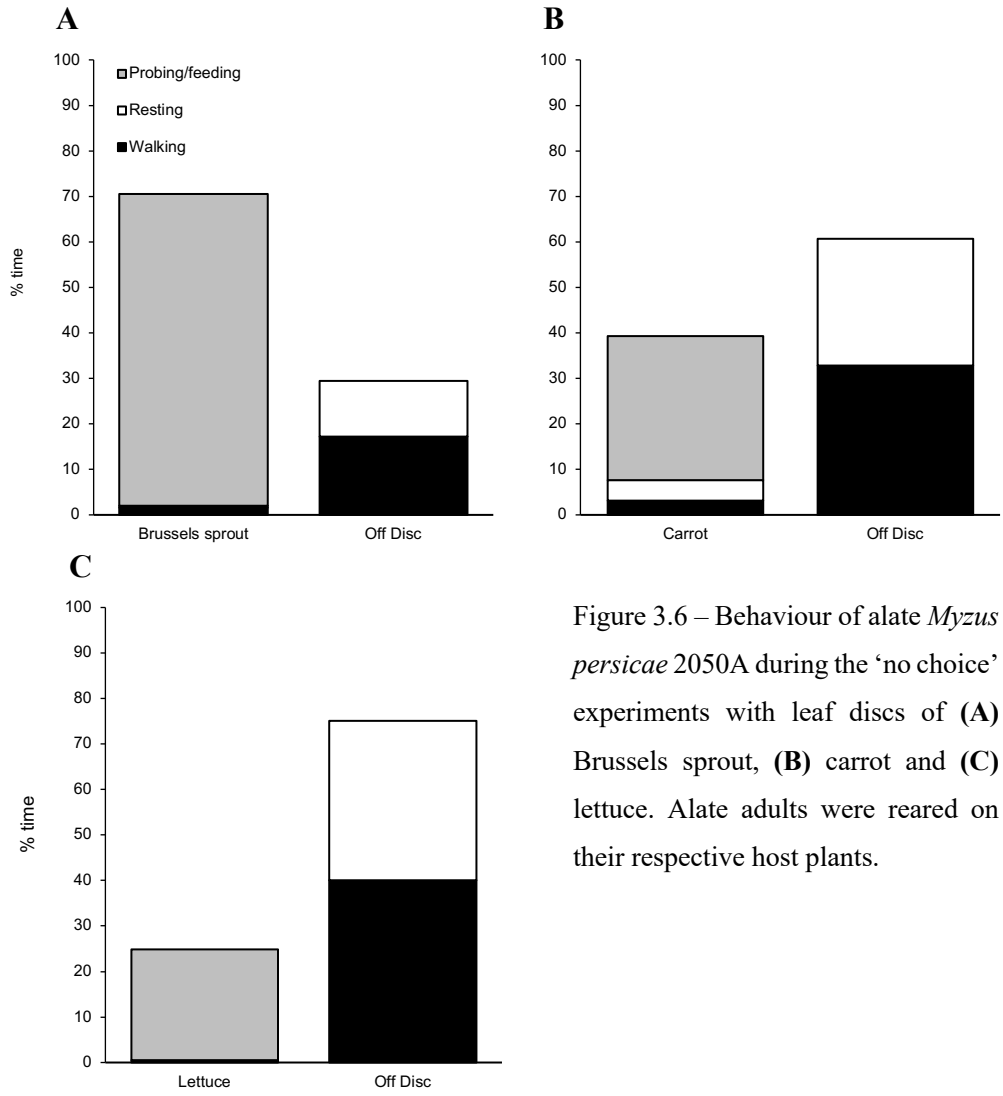

Figure 3.6 – Behaviour of alate *Myzus persicae* 2050A during the 'no choice' experiments with leaf discs of **(A)** Brussels sprout, **(B)** carrot and **(C)** lettuce. Alate adults were reared on their respective host plants.

The proportion of time alate *M. persicae* spent probing/feeding or walking during the leaf disc 'choice' and 'no choice' experiments was compared to the 'no choice' experiment with leaf discs of Brussels sprout (preferred host) with a binomial GLM (logit link function).

In the ‘no choice’ ‘arenas’ containing carrot or lettuce, alate *M. persicae* spent significantly less time probing/feeding (Table 3.4) and more time walking (Table 3.5) compared to the ‘arenas’ containing only Brussels sprout. This was observed for all ‘no choice’ experiments regardless of the previous host plant experience of alate *M. persicae.*

In the ‘choice’ experiments, when alate adults were presented with a leaf disc of Brussels sprout and a leaf disc of a ‘less-preferred’ host (either carrot or lettuce), no statistically significant differences were found for time spent probing/feeding (Table 3.4) or walking (Table 3.5) compared to the Brussels sprout ‘no choice’ experiment. This suggests that the behaviour of alate *M. persicae* does not differ significantly when given the choice of a leaf disc of a ‘preferred’ or ‘less-preferred’ host; with alate adults probing/feeding preferentially on the leaf disc of Brussels sprout.

Compared to *M. persicae* reared on Brussels sprout, no statistically significant differences were found for the time spent probing/feeding (Table 3.4) and walking (Table 3.5) in the Brussels sprout ‘no choice’ ‘arenas’ by aphids reared on either carrot or lettuce, suggesting that previous host plant experience did not have a major effect on the host preference behaviour of alate *M. persicae*.

Table 3.4 – Analysis (binomial GLM: logit link function) of the time alate *Myzus persicae* 2050A spent probing/feeding in the leaf disc 'choice'/'no choice' experiments compared with the Brussels sprout 'no choice' experiment.

Experiment	Species reared on	Coefficient	Std. error	95% Wald Confidence Interval		Wald chi-square	d.f.	P
				Lower	Upper			
Brussels sprout ('no choice')	Carrot	-0.28	0.2811	-0.831	0.271	0.994	1	0.319
Brussels sprout ('no choice')	Lettuce	-0.487	0.3091	-1.093	0.119	2.484	1	0.115
Carrot ('no choice')	Brussels sprout	-1.594	0.3629	-2.305	-0.883	19.287	1	**<0.001**
Carrot ('no choice')	Carrot	-1.541	0.34	-2.208	-0.875	20.56	1	**<0.001**
Lettuce ('no choice')	Brussels sprout	-2.417	0.3641	-3.131	-1.704	44.065	1	**<0.001**
Lettuce ('no choice')	Lettuce	-1.913	0.3713	-2.641	-1.186	26.555	1	**<0.001**
Brussels sprout *vs.* carrot ('choice')	Brussels sprout	-0.29	0.2975	-2.641	0.293	0.948	1	0.33
Brussels sprout *vs.* carrot ('choice')	Carrot	-0.254	0.2661	-2.641	0.268	0.908	1	0.341
Brussels sprout *vs.* lettuce ('choice')	Brussels sprout	-0.633	0.3236	-2.641	0.001	3.823	1	0.051
Brussels sprout *vs.* lettuce ('choice')	Lettuce	-0.536	0.3206	-2.641	0.093	2.793	1	0.095

Table 3.5 – Analysis (binomial GLM: logit link function) of the time alate *Myzus persicae* 2050A spent walking in the leaf disc 'choice'/'no choice' experiments compared with the Brussels sprout 'no choice' experiment.

Experiment	Species reared on	Coefficient	Std. error	95% Wald Confidence Interval		Wald chi-square	d.f.	P
				Lower	Upper			
Brussels sprout ('no choice')	Carrot	0.188	0.214	-0.232	0.607	0.77	1	0.38
Brussels sprout ('no choice')	Lettuce	0.417	0.2244	-0.023	0.857	3.45	1	0.063
Carrot ('no choice')	Brussels sprout	0.68	0.2401	0.209	1.151	8.019	1	**0.005**
Carrot ('no choice')	Carrot	0.848	0.2606	0.338	1.359	10.602	1	**<0.001**
Lettuce ('no choice')	Brussels sprout	1.34	0.2154	0.918	1.762	38.734	1	**<0.001**
Lettuce ('no choice')	Lettuce	1.164	0.2187	0.736	1.593	28.351	1	**<0.001**
Brussels sprout *vs.* carrot ('choice')	Brussels sprout	0.02	0.2169	-0.405	0.445	0.009	1	0.925
Brussels sprout *vs.* carrot ('choice')	Carrot	0.389	0.2165	-0.035	0.814	3.233	1	0.072
Brussels sprout *vs.* lettuce ('choice')	Brussels sprout	0.208	0.1951	-0.175	0.59	1.132	1	0.287
Brussels sprout *vs.* lettuce ('choice')	Lettuce	0.106	0.2068	-0.299	0.512	0.264	1	0.607

Additionally, there was no statistically significant difference found between the time *M. persicae* spent probing/feeding (binomial GLM: d.f. = 1, P = 0.38) and walking (binomial GLM: d.f. = 1, P = 0.266) in the 'no choice' 'arenas' with either leaf discs of carrot or lettuce when aphids had been reared on their respective host plant.

Leaf disc 'arena' system: 'choice' and 'no choice' experiments with a non-host plant

In all 'choice' experiments with the non-host plant species (sugar maple) alate *M. persicae* spent < 3% of the time observed on leaf discs of sugar maple (Fig. 3.7). During the 'choice' experiment with Brussels sprout and sugar maple (Fig. 3.7A), while *M. persicae* still spent the majority of time probing/feeding on Brussels sprout (51%), the presence of sugar maple significantly reduced this time compared with the Brussels sprout 'no choice' experiment (68.5%) (Fig. 3.6A) (binomial GLM: d.f. = 1, P = 0.011). For carrot and lettuce, a similar amount of time was spent probing/feeding in the 'choice' experiments with sugar maple (Figs 3.7B and C) and the respective 'no choice' experiments (Figs. 3.6B and C) (binomial GLM: carrot: d.f. = 1, P = 0.075, lettuce: d.f. = 1, P = 0.331). No statistically significant differences were identified for the time spent walking in the 'choice' experiments with sugar maple and the 'no choice' experiments with the three host plants when aphids were reared on their respective hosts (binomial GLM: Brussels sprout: d.f. = 1, P = 0.299, carrot: d.f. = 1, P = 0.203, lettuce: d.f. = 1, P = 0.644).

In the 'no choice' experiment with two leaf discs of sugar maple, aphids spent < 4% of the time observed probing/feeding and 56.8% of the time walking (Fig. 3.7D). Statistically significant differences were found for the proportion of time aphids spent probing/feeding and walking in 'no choice' experiments with the host plants (Fig. 3.6) (binomial GLM: probing/feeding; Brussels sprout: d.f. = 1, P < 0.001, carrot: d.f. = 1, P = 0.001, lettuce: d.f. = 1, P = 0.004; walking; Brussels sprout: d.f. = 1, P < 0.001, carrot: d.f. = 1, P <0.001, lettuce: d.f. = 1, P = 0.008).

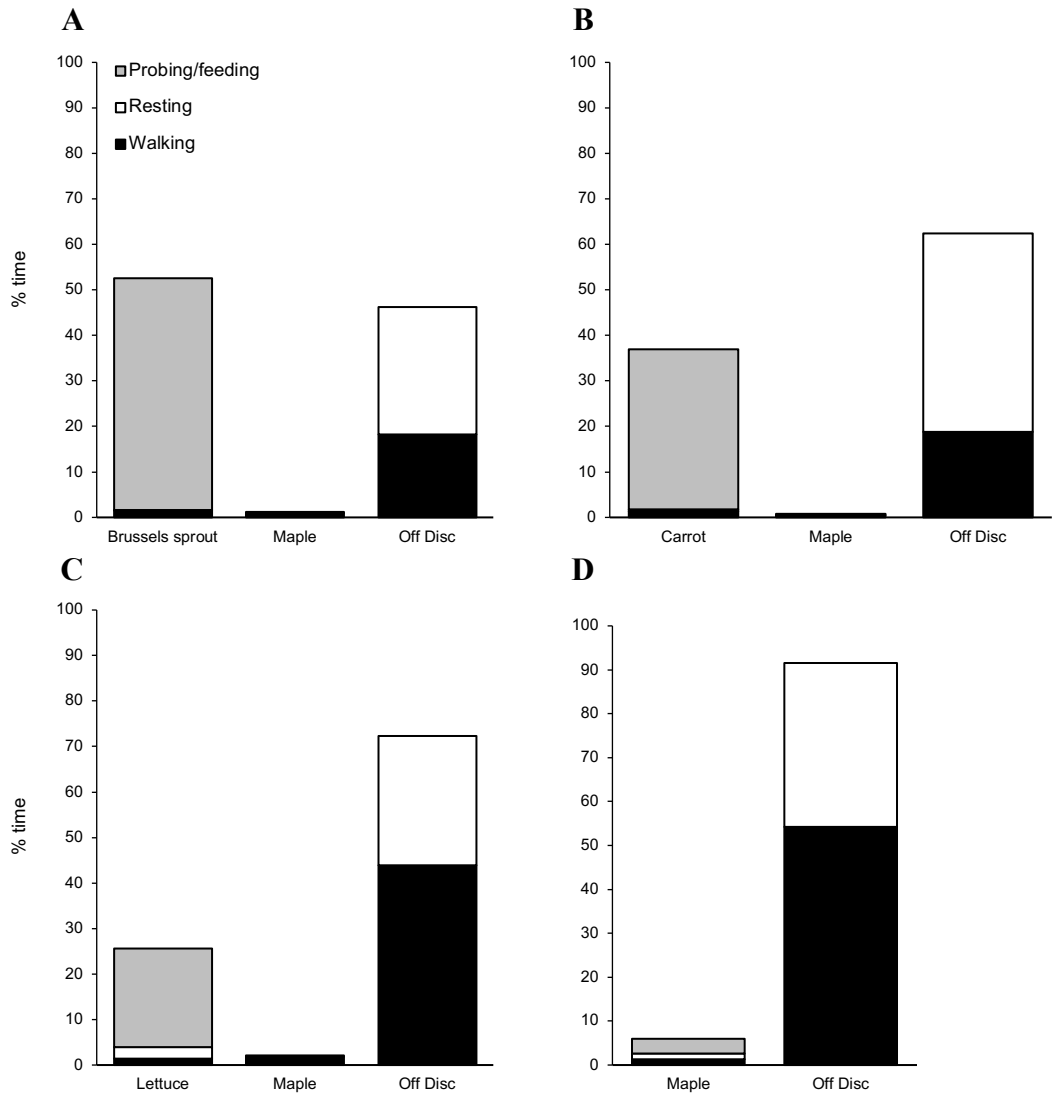

Figure 3.7 – Behaviour of alate *Myzus persicae* 2050A during the 'choice' experiments with leaf discs of **(A)** Brussels sprout and sugar maple, **(B)** carrot and sugar maple, **(C)** lettuce and sugar maple and **(D)** the 'no choice' experiments with leaf discs of sugar maple. In 'choice' experiments aphids were reared on the respective host plant. In the sugar maple 'no choice' experiment some of the aphids were reared on each of the three host plants.

Analysis of time until 'lock-in' on leaf discs by alate Myzus persicae

In order to assess host preference behaviour, the time until 'lock-in' on different plant species was monitored during the 'no choice' experiments with alate *M. persicae* (Fig. 3.8). One hour after being placed into leaf disc 'arenas' that contained Brussels sprout leaf discs, 67% *M. persicae* had 'locked-in' and did not subsequently leave the leaf discs. By the end of the 'no choice' experiment with Brussels sprout, 96% alate *M. persicae* had 'locked-in' to feed. During the carrot and lettuce 'no choice' experiments ≤ 50% of alate *M. persicae* had 'locked-in' on leaf discs to feed by the end of the 5 h study period (carrot = 50%, lettuce = 38%). No alate adults 'locked-in' to feed on leaf discs of the non-host species, sugar maple.

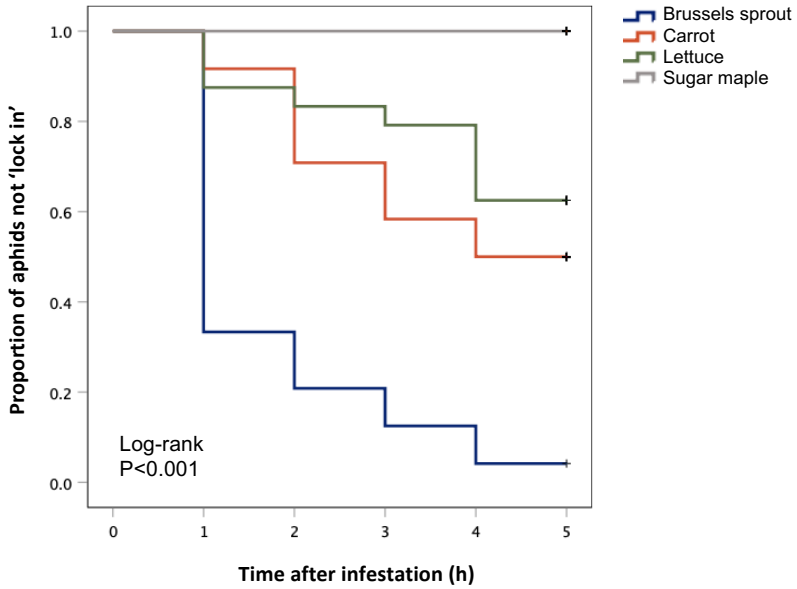

Figure 3.8 – Kaplan-Meier plot showing the effect of plant species on the time until 'lock-in' during the leaf disc 'no choice' experiments with alate *Myzus persicae* 2050A.

Pairwise comparisons of the 'lock-in' curves for the 'no choice' experiments were carried out for the different plant species using a log-rank test (Table 3.6). Time until 'lock-in' differed significantly in 'no choice' experiments with Brussels sprout compared to those with carrot and lettuce. However, the time until 'lock-in' did not differ significantly in lettuce and carrot 'arenas'.

Table 3.6 – Log-rank test pairwise comparisons of 'lock-in' curves for the leaf disc 'no choice' experiments with alate *Myzus persicae* 2050A. P-values were adjusted with Bonferroni correction.

'Lock-in' curves	Chi-square	d.f.	P-value
Brussels sprout and carrot	19.169	1	<0.001
Brussels sprout and lettuce	26.073	1	<0.001
Brussels sprout and sugar maple	50.400	1	<0.001
Carrot and lettuce	0.909	1	0.34
Carrot and sugar maple	15.856	1	<0.001
Lettuce and sugar maple	10.863	1	0.001

The probing behaviour of alate Myzus persicae

The mean number of short duration probes (< 1 min) performed by alate *M. persicae* at each monitoring point was determined for the leaf disc 'no choice' experiments (Table 3.7). In all cases, most short duration probing occurred immediately after placing alate adults into the leaf disc 'arenas'. The total number of short duration probes (< 1 min) performed by alate *M. persicae* during each 'no choice' experiment was assessed using a Kruskal-Wallis H test. There was no statistically significant difference in the number of short duration probes on the different plant species (χ^2 = 6.995, d.f. = 3, P = 0.072).

Table 3.7 – Probing behaviour of alate *Myzus persicae* 2050A during leaf disc 'no choice' experiments. Values in the table show the mean number of short duration probes (< 1 min) each hour. Numbers in parentheses are the total number of aphids that were probing/feeding for the full minute observed.

Time after infestation (h)	Brussels sprout	Carrot	Lettuce	Sugar maple
0	2.1 (0)	2.5 (0)	5 (0)	0.6 (0)
1	0.2 (16)	1.2 (6)	0.8 (3)	0.2 (1)
2	0.2 (20)	0.2 (11)	0.5 (5)	0.3 (1)
3	0 (21)	0.3 (10)	0.8 (5)	0.3 (1)
4	0 (23)	0.2 (12)	0.4 (9)	0 (0)
Mean total no. probes (< 1 min)	2.5	4.4	7.5	1.4

Cage system: 'choice' and 'no choice' experiments

The settling behaviour of alate *M. persicae* (2050A) varied in the cage experiments with the different combinations of host plants. In the 'no choice' experiments with Brussels sprout > 50% of aphids had settled on plants 3 h after infestation and 80% had settled 5 h after infestation (Fig. 3.9A). Aphids took longer to settle in the 'no choice' experiments with carrot and lettuce, with only 55 ±6.5% and 50 ±4.1% on the plants 5 h after infestation, respectively (Figs 3.9B and C). In 'no choice' cages with the non-host species (sugar maple) only 12.5 ±2.5% of aphids had accumulated on plants 5 h after infestation (Appendix B.1).

The mean number of aphids that had settled on plants 5h after infestation in the 'no choice' experiments were compared using a one-way ANOVA test ($F = 48.097$, d.f. = 15, $P < 0.001$). The number of aphids on Brussels sprout differed significantly to those on carrot (Tukey's HSD: $P = 0.004$) and on lettuce (Tukey's HSD: $P = 0.001$). In the 'no choice' experiments with either carrot or lettuce plants; however, there was no statistically significant difference between the mean number of aphids on plants after 5 h (Tukey's HSD: $P = 0.815$). In all cases, the mean numbers of aphids on sugar maple were lower than those accumulated on the three host plants after 5 h (Tukey's HSD: $P < 0.001$).

In the 'choice' experiments with Brussels sprout and carrot plants (Fig. 3.9D), 5 h after infestation more aphids had settled on Brussels sprout (60 ±4.1%) compared to carrot (25 ±2.9%) (Chi-square test: $\chi^2 = 8$, d.f. = 1, $P = 0.005$). Similarly, aphids preferentially settled on Brussels sprout opposed to lettuce, with 62.5 ±4.8% and 15 ±2.9% of aphids on the plants, respectively (Chi-square test: $\chi^2 = 11.645$, d.f. = 1, $P < 0.001$). In 'choice' experiments with carrot and lettuce plants (Figs. 3.9E and F), 32.5 ±4.8% aphids had settled on carrot 5 h after infestation compared to 25 ±2.9% on lettuce (Chi-square test: $\chi^2 = 0.391$, d.f. = 1, $P = 0.532$). However, a considerable proportion of aphids (42.5 ±2.5%) had not made a 'choice' by this time and remained off the plants. Previous host experience did not significantly affect the plants aphids accumulated on in all cage experiments ($P > 0.05$ for all comparisons). For example, aphids reared on either carrot or lettuce plants showed a similar level of preference for these hosts compared to the aphids reared on Brussels sprout.

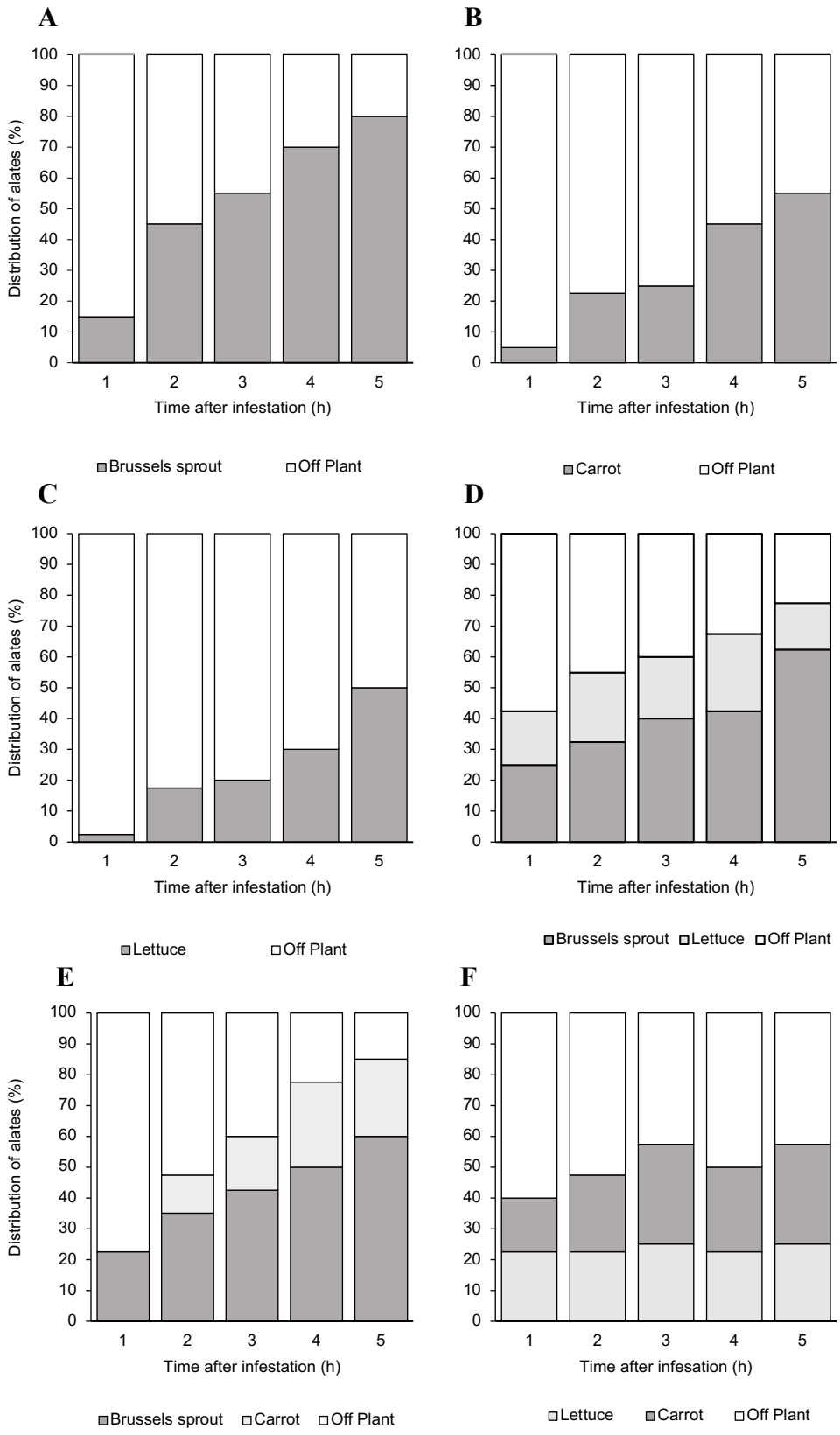

Figure 3.9 – Settling preference (distribution %) of alate *Myzus persicae* 2050A on host plants during cage 'no choice' and 'choice' experiments. Aphids were all reared on Brussels sprout cv. Doric F1.

Cage system: the settling behaviour of alate Myzus persicae

The settling behaviour of alate *M. persicae* on plants during the 'no choice' cage experiments was assessed using a Kaplan-Meier estimator (Fig. 3.10).

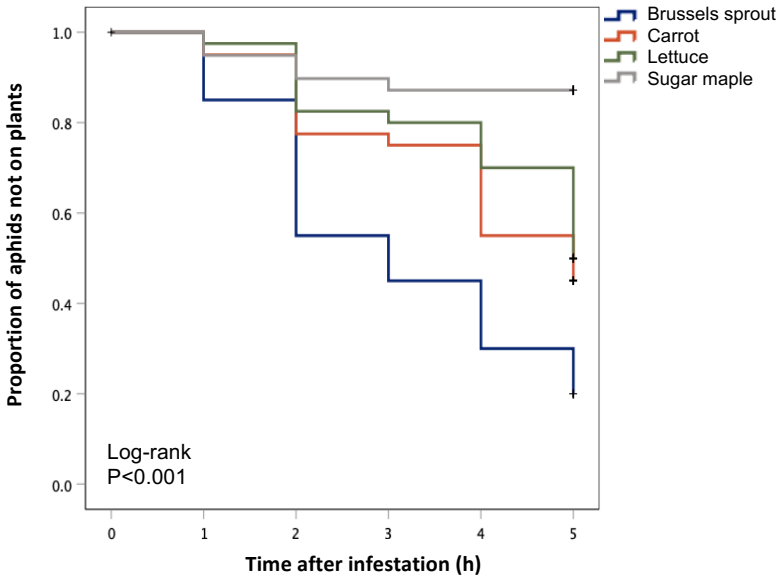

Figure 3.10 – Kaplan-Meier plot showing the effect of plant species on the settling time of alate *Myzus persicae* 2050A during the cage 'no choice' experiments.

Pairwise comparisons of the individual settling curves for the 'no choice' cage experiments were carried out using a log-rank test (Table 3.8). The settling time of alate *M. persicae* differed significantly in cages with Brussels sprout plants compared to in cages with carrot or lettuce plants, but did not significantly differ between cages of carrot and lettuce. In cages with the non-host species, sugar maple, settling time differed significantly to the three host species; with considerably fewer alate adults settling on sugar maple over the 5 h period.

Table 3.8 – Log-rank test pairwise comparisons of the settling curves on the cage 'no choice' experiments with alate *Myzus persicae* 2050A. P-values were adjusted with Bonferroni correction.

Settling curves	Chi-square	d.f.	P-value
Brussels sprout and carrot	7.660	1	0.006
Brussels sprout and lettuce	12.111	1	0.001
Brussels sprout and sugar maple	34.017	1	<0.001
Carrot and lettuce	0.473	1	0.492
Carrot and sugar maple	13.667	1	<0.001
Lettuce and sugar maple	10.740	1	0.001

3.4 The host preference and selection behaviour of *Myzus persicae*: Discussion

3.4.1 Development of *Myzus persicae* on different host plants

There was considerable variation in the development and host selection/preference behaviour of *M. persicae* on the plant species investigated. Firstly, it was important to determine whether the selected cultivars of Brussels sprout (cv. Doric F1), carrot (cv. Nairobi F1) and lettuce (cv. Lobjoits Green Cos) supported the development of *M. persicae.* On the different host plants, no statistically significant differences were found between the development of two clones of *M. persicae* (MP1S and 2050A). The development of *M. persicae*, however, varied considerably on the different plant species.

The mean development time of *M. persicae* was the shortest on Brussels sprout plants (7.42 ±0.4 days) and the longest on lettuce plants (11.58 ±0.7 days). *Myzus persicae* also had a higher mean rate of daily reproduction on Brussels sprout plants, compared with on carrot and lettuce. As expected, aphids did not reproduce or survive for very long (3–4 days) on the non-host species, sugar maple. The intrinsic rates of increase (r_m) were: 0.26 ±0.01, 0.16 ±0.01 and 0.06 ±0.01 on Brussels sprout, carrot and lettuce plants, respectively. For aphids, even small differences in the intrinsic rates of increase on different host plants can be important for their survival, particularly as these differences are amplified through rapid parthenogenesis (Nikolakakis *et al*., 2003). In the field, it is therefore important for aphids to locate and exploit the optimal host to support their development.

The suitability of Brussels sprout for supporting the development of *M. persicae* was not unexpected. Previously, Heathcote (1962) reported that *M. persicae* developed well on species of *Brassica* when investigating the suitability of eleven cultivated crop varieties. Conversely, he found that *M. persicae* reproduced at a much slower rate on lettuce and suggested that the aphid was poorly adapted to this particular host plant. Several other studies (Cook and Sylvester 1961; Lowe, 1973) have also reported the suitability of *Brassica* species for supporting the development of *M. persicae*.

The success of *M. persicae* on Brussels sprout plants is likely to relate to a combination of factors; including good nutritional quality (Cole, 1997) and the presence of specific plant cues that act to initiate feeding and reproductive responses (Powell *et al*., 2006).

3.4.2 Host preference and selection behaviour of alate *Myzus persicae*

'Choice' and 'no choice' experiments were indicative of a preference for Brussels sprout in alate *M. persicae* (clone 2050A). In both the leaf disc and cage 'choice' experiments, alate *M. persicae* preferentially probe/fed or accumulated on Brussels sprout compared to the carrot and lettuce; regardless of the plant species that aphids had been reared on. Alate adults were relatively quick to settle and 'lock-in' to feed on Brussels sprout leaf discs in 'no choice' experiments, with 67% of aphids settled within 1 h. Aphids were slower to settle on leaf discs of either carrot or lettuce, with only 50% and 38% 'locked-in' 5 h after infestation, respectively. Similarly, in cage 'no choice' experiments a considerable proportion (> 50%) of aphids had settled on Brussels sprout plants 3 h after introduction into the cages and 80% had settled by the end of the 5 h study period. The inhibition of the take-off reflex on Brussels sprout plants provided a clear indication of host preference. However, considerably fewer alate adults had settled on carrot and lettuce plants 5 h after infestation in the cage 'no-choice' experiments, with only 55% and 50% on plants, respectively.

The preference of *M. persicae* for Brussels sprout plant material identified in these experiments is likely to result from a range of physical and chemical plant characteristics. For example, *Brassica* species have distinctive chemical compositions (*e.g*. glucosinolates and isocyanates). These compounds may stimulate settling and feeding behaviour by aphids during host selection (Bruce *et al.,* 2005). It is also possible that the host preference of *M. persicae* for Brussels sprout plants may relate to their high nitrogen content. In terms of development, nitrogen is often a limiting resource for insects. Additionally, the nitrogen content of plants is used regularly by aphids as an indicator of host quality (Ahmed *et al*., 2019).

With regard to carrot and lettuce, there was little difference in terms of aphid preference for these two host plants. Despite this, the results from the leaf disc and cage 'choice' experiments suggest a slight preference for carrot over lettuce. However, the restlessness of *M. persicae* on the 'less-preferred' hosts was apparent in these

experiments. Compared to the leaf disc 'no choice' experiment with Brussels sprout plants, *M. persicae* spent more time off the carrot and lettuce leaf discs, walking in the 'no choice' 'arenas'. Together with the findings of the cage 'no choice' experiments, this suggests a reduction in the settling behaviour by *M. persicae* when presented with these host plants, either individually or in combination. Several factors may explain this result. For example, physical and chemical plant characteristic (*e.g*. nutritional stimuli and/or primary and secondary plant metabolites) are likely to influence the settling behaviour of alate *M. persicae.* The detection of these characteristics would allow aphids to make 'decisions' on selecting the optimal host plant to support their development. Interestingly, the order of feeding preference for the different host plants was the same as development performance, with *M. persicae* performing the best on Brussels sprout and the worst on lettuce. This provides further evidence to suggest that *M. persicae* preferentially selects host plants to support its development and maximise clonal fitness.

3.4.3 The effect of a non-host on the host selection and preference behaviour of alate *Myzus persicae*

While alate *M. persicae* performed the greatest number of short duration (< 1 min) probes on leaf discs of lettuce, this was not statistically different from the number of probes performed on the other plant species. Interestingly, the smallest number of short duration probes occurred on the non-host plant (sugar maple); mostly during the initial 5 min when aphids were first placed into the leaf disc 'arenas'. This suggests that aphids were able to quickly recognise sugar maple as a non-host species. This is supported by the fact that *M. persicae* spent < 4% of the time probing/feeding on sugar maple during the leaf disc 'choice' and 'no choice' experiments, and during the leaf disc 'choice' experiments aphids consistently selected the host plants to settle on. This is likely due to the detection of an arrestant stimulus after brief periods of probing on the non-host, which acted to prevent feeding/parturition (Powell *et al*., 2006). It also possible that volatiles from sugar maple, to some extent, inhibited the probing/feeding behaviour of alate *M. persicae* during the 'choice' and 'no choice' experiments. Non-host volatiles have been shown to have repellent and/or deterrent properties for a number of insects, including aphids (Nottingham *et al*., 1991; Hardie *et al*., 1994b; Agelopoulos *et al*., 1999).

Interestingly, in 'choice' experiments with leaf discs of Brussels sprout, sugar maple significantly reduced the time aphids spent probing/feeding. This was not the case in leaf disc 'arenas' containing the 'choice' of sugar maple and either carrot or lettuce. It is possible that the difference may relate to the relative time that alate *M. persicae* spent probing/feeding on the leaf discs of each host plant. Alate adults often made quicker decisions to feed on leaf discs of Brussels sprout during the 'no choice' experiments. For this reason, it is possible that volatiles from the non-host species (sugar maple) could have disrupted initial settling behaviour in 'choice' 'arenas' with Brussels sprout. However, alate adults generally took longer to 'lock-in' and feed on leaf discs of carrot or lettuce in 'no choice' experiments. Therefore, by the time alate adults settled to feed on carrot or lettuce in the 'choice' experiments with sugar maple there may have been some habituation to the volatiles of the non-host species. This may explain why the presence of sugar maple did not significantly affect the time alate *M. persicae* spent probing/feeding in the carrot *vs.* sugar maple and lettuce *vs*. sugar maple 'choice' experiments.

3.4.4 The effect of previous host plant experience on the host selection and preference behaviour of alate *Myzus persicae*

The host plant on which *M. persicae* was reared on did not significantly affect its subsequent probing/feeding behaviour in 'choice' and 'no choice' experiments. Aphids reared on carrot or lettuce, still preferentially fed on Brussels sprout leaf discs in 'choice' experiments. Additionally, rearing history had no impact on the time aphids spent probing/feeding in the various leaf disc 'no choice' experiments. However, previous studies have reported that the host preference of aphids can be influenced by the experience of a previous host plant (*e.g*. Lushai *et al*., 1997). Preconditioning effects were observed by Russell (1966), who found that *M. persicae* settled more readily on sugar beet if taken from cultures of this host rather than from cultures on Chinese cabbage. Additionally, for some races of *A. pisum,* changes in host preference have been observed after rearing aphids for extended periods on a particular host plant (Auclair, 1966; Markkula and Roukka, 1970).

In these experiments, it is possible that the number of generations aphids were reared on carrot or lettuce prior to use in the bioassays was insufficient to allow a pre-

conditioning effect to occur (minimum of two generations). If aphids had been reared for longer periods on these hosts it may have allowed further acclimation to the new nutritional environment. Subsequently, this may have influenced the preference behaviour of *M. persicae* in the 'choice' and 'no choice' tests.

Despite this, Nikolakakis *et al*. (2003) found that the performance of *M. persicae* on tobacco and pepper, in terms of rate of increase (r_m), age at first reproduction, fecundity and longevity, was improved by the second parthenogenetic rearing generation compared with the first generation. Improved performance on 'less-preferred' hosts following several generations of parthenogenesis has also been observed for other aphid species such as *Dysaphis anthrisci* (Börner) (Hemiptera: Aphididae) and *A. fabae* (Shaposhnikov, 1961; Markkula and Roukka, 1970). Nevertheless, Via (1991) found no evidence to suggest that the performance of *A. pisum* on alfalfa, *Medicago sativa* L. (Fabaceae) and clover, *Trifolium pratense* L. (Fabaceae) was improved by previous rearing experience.

Additionally, clone 2050A of *M. persicae* originated on Brussels sprout (field-collected in 1996) (S. Foster, personal communication) and has subsequently been maintained long term on cultivars of *Brassica oleracea* in the Insect Rearing Unit (IRU), Warwick Crop Centre, School of Life Sciences, Wellesbourne. For this reason, it is possible that the host preference of this clone may be largely genetically determined. Studies by Weber (1985) and Nikolakakis *et al*. (2003) reported better performance of *M. persicae* clones on the hosts from which they originated.

3.4.5 Limitations and further research

A limitation to these experiments was the use of a single cultivar of each plant species to investigate the development and host selection/preference behaviour of *M. persicae*. It is likely that different cultivars may vary in terms of their suitability for *M. persicae.* For example, Ahmed *et al*. (2019) found that *M. persicae* performed better on cabbage cultivars that had higher levels of nitrogen. In addition, they found that cultivars with higher nitrogen content also had higher total volatile emissions and overall were more attractive to *M. persicae*. Therefore, amongst other factors, nutritional differences in cultivars may have some influence on host preference. Additionally, only a single

Brassica species was examined in these experiments (Brussels sprout). It is possible that different species of *Brassica* may vary considerably in terms of their desirability to aphids.

Another limitation was the number of replicates used to investigate the development of *M. persicae* on different plant species (total of twelve replicates per plant species). Ideally, more aphids would have been followed from birth to reproductive maturity. The effect of temperature on aphid development could also be taken into consideration. In these development studies a single temperature (20°C) was used; which is warmer than average spring/summer temperatures in the UK.

The systems developed to investigate host selection and preference of alate *M. persicae* in this chapter were simple and practical, allowing for the examination of aphid behaviour in a standardised and easily reproducible manner. Leaf disc experiments permitted the behaviour of alate adults to be monitored and provided insights into the major behaviour types exhibited by aphids over time. However, it is possible that an aphid's response to a leaf disc may differ to how it responds to a whole plant.

Alternatively, it would have been possible to examine the host selection/preference behaviour of *M. persicae* using an electrical penetration (EPG) system (Gabrys *et al*., 1997; Margaritopoulos *et al*., 2005). Nevertheless, attaching a wire tether to aphids for EPG recording inevitably restricts their behaviour (Powell *et al*., 2006). In these experiments it was important to capture the movement of *M. persicae* (*e.g*. time spent walking) in order to infer the effects of host plants on settling behaviour. While EPG signals can provide detailed accounts of the time aphids spend probing on plants and the type of probing behaviour they perform, Powell and Hardie (2001) found that freely moving aphids display similar probing activity to tethered individuals during EPG studies, when examined under the same conditions.

3.4.6 Conclusions

The findings of this chapter provide key information on the development and behaviour of *M. persicae* on cultivars of Brussels sprout, carrot and lettuce. This information will be useful when investigating the effects of insecticides on the development and behaviour of alate *M. persicae* in Chapters 4 and 5.

- At 20°C the development of *M. persicae* varied on the three species of host plant that were tested. The intrinsic rates of increase (r_m) were; 0.26 ±0.01, 0.16 ±0.01 and 0.06 ±0.01 on Brussels sprout, carrot and lettuce plants, respectively.

- On Brussels sprout, alate *M. persicae* initiated probing behaviour more quickly, settled and fed more readily, and was less 'restless' than on carrot and lettuce plants.

- There was little difference in terms the time alate *M. persicae* spent probing/feeding on carrot and lettuce plant material. However, slightly more alate *M. persicae* accumulated on carrot material in 'choice' experiments compared to lettuce.

- On 'less-preferred' host plants (*e.g.* carrot and lettuce) there is a tendency for alate *M. persicae* to exhibit restlessness behaviour and to take longer to settle.

Chapter 4 — Investigating the Efficacy and Persistence of Insecticides for the Control of *Myzus persicae*

4.1.1 The chemical control of arthropod pests

Prior to the introduction of DDT (Dichlorodiphenyltrichloroethane) in the 1940s, biological or 'natural' insecticides provided the main form of pest control (Bate, 2007; Oberemok *et al*., 2015). Many of these compounds were natural plant extracts or products mined from the earth (Coats, 1994). Over the last 50 years, synthetic chemical insecticides have replaced many natural products as the standard method of controlling pest insects (Flint and van den Bosch, 2012). While synthetic chemical insecticides have brought a new order of pest control, they have also raised new concerns (Coats, 1994).

The injudicious use of synthetic chemical insecticides has adversely affected the environment and biodiversity (Birch *et al*., 2011; Barzman *et al*., 2015; Wood and Goulson, 2017). There are also issues regarding the decreasing availability of synthetic chemical insecticides and the increasing cases of pest resurgence, secondary pest problems and insecticide resistance (Hardin *et al*., 1995; Clarke *et al*., 2011; Sparks and Nauen, 2015). These factors highlight the need for the development of selective insecticides that have little impact on the environment (Knowles, 2008). Nevertheless, the rate at which new insecticides are developed has decreased over recent years. This is due to factors including: a decline in the discovery rate of new active molecules, the rising costs of registration and more stringent pesticide legislation (Chandler *et al*., 2011; Hillocks, 2012). Substantial data relating to a product's efficacy, toxicity, persistence and metabolism are required before marketing a new product (Foster *et al*., 2011).

4.1.2 Maintaining insecticide efficacy through Insecticide Resistance Management (IRM)

In many cases, an overreliance on insecticides has led to the natural selection of resistant forms of pest insects and the consequent evolution of populations with heritable resistance (Metcalf, 1989). There are several examples in which cross-

resistance has developed to several insecticides through a common resistance mechanism (McCaffery and Nauen, 2006). Resistance has already been reported for important pest insects, such as *M. persicae*, to the major classes of insecticides (Bass *et al*., 2014).

To minimise the negative impacts associated with insecticide use, it is important to limit the amount applied to crops and where possible rotate insecticides, selecting those with alternative modes of action (MoA). Insecticide Resistance Management (IRM) aims to prevent or delay the development of resistance, or in certain cases, regain susceptibility to insecticides by informing their use (Sparks and Nauen, 2015).

Key components of IRM include: 'alternations, sequences or rotations' of insecticides from different MoA groups. These practices help to reduce the reliance on treatments with a single MoA. Insecticide applications can be ordered into MoA spray windows that are informed by the stage of crop development and/or the biology of the pest insect (Sparks and Nauen, 2015). Provided that there are treatments available, determining the properties of insecticides, such as efficacy and persistence, can be useful for establishing successful insecticide rotation programmes.

4.1.3 The action of insecticides

The selection and application of plant protection products require an understanding of their biological action on and in plants. Insecticides can have a combination of contact, systemic and translaminar action, with several (*e.g*. the neonicotinoid, imidacloprid) possessing properties of all three (Elbert *et al*., 1991). Insecticides with contact action are applied to the surface of plants. For control to be achieved, they require direct contact between insects and the active ingredient. For this reason, thorough coverage is crucial. These insecticides enter through the insect cuticle when directly sprayed onto the insect or the insect walks over the active ingredient (Sánchez-Bayo *et al*., 2013). Any surface not covered will not produce insecticidal effects; including new shoots and leaves that develop after insecticide application. The effects of contact insecticides are greatest directly after they are applied (Sánchez-Bayo *et al*., 2013). Several contact insecticides, such as pyrethroids, are also reported to have repellent properties (Ruscoe, 1977; Dewar and Denholm, 2017).

Conversely, insecticides with systemic action move through the xylem and/or phloem vessels to traverse the entire plant; including into new shoots and leaves, *e.g.* the neonicotinoids; imidacloprid and thiamethoxam (Kleier, 1994; Sicbaldi *et al*., 1997; Brück *et al*., 2009; Prabhaker *et al.*, 2011). Systemic treatments can require time for translocation and control is provided only when insects are probing/feeding where the active ingredient is present (Sánchez-Bayo *et al*., 2013).

Unlike treatments with only contact action, translaminar insecticides move into the leaf tissue after their application. For this reason, they often have longer persistence than contact insecticides (Cloyd *et al*., 2011). However, unlike systemic treatments, translaminar insecticides do not move far past their point of application.

4.1.4 The use of biopesticides in Integrated Pest Management (IPM) systems

While there is no standard definition of a biopesticide, it was described by Chandler *et al*. (2011) as "a mass-produced agent manufactured from a living microorganism or a natural product and sold for the control of plant pests". There are four main types of biopesticide: (i) microorganisms (*e.g*. bacteria and fungi); (ii) biochemicals (*e.g.* plant extracts); (iii) semiochemicals (*e.g.* pheromones) and (iv) other novel alternative products.

The properties of some biopesticides make them well suited for use in IPM programmes. These may include: improvements in environmental safety, target-specificity, biodegradability and considerably lower costs for development compared with synthetic chemical insecticides (Chandler *et al*., 2011).

Generally, biopesticides have a slower knock-down of pest insects compared with synthetic chemical insecticides. They can be less persistent in the environment and more susceptible to environmental conditions (Copping and Menn, 2000). As the efficacy and persistence of biopesticides are often lower than synthetic chemical insecticides, they are rarely used as stand-alone treatments (Chandler *et al*., 2011). The selectivity and safety of some biopesticides, however, make them good candidates for pesticide rotation programmes. Their application can be beneficial at certain stages of crop growth or in the life cycle of the pest insect and may also provide sufficient control in areas with low pest pressure.

4.1.5 The control of aphids with chemical insecticides

Over the last 50 years, synthetic chemical insecticides have been used widely for the control of aphids (Dewar and Denholm, 2017). Table 4.1 shows the main classes of insecticides applied for aphid control in the UK, with their mode of action (MoA) and Insecticide Resistance Action Committee (IRAC) MoA classification group (IRAC, 2019).

Table 4.1 – Modes of action (MoAs) for the main classes of insecticides used for the control of aphids over the last 50 years in the UK.

Sub-group or exemplifying active ingredient (AI)	Mode of action (MoA)	IRAC group
Carbamates	Acetylcholinesterase (AChE) inhibitors	1A
Organophosphates	Acetylcholinesterase (AchE) inhibitors	1B
Cyclodienes/organochlorines	GABA-gated chloride channel blockers	2A
Pyrethroids	Sodium channel modulators	3A
Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators	4A
Pyridine azomethine derivatives *e.g.* pymetrozine	Chordotonal organ TRPV channel modulator	9B
Spirotetramat	Inhibitor of acetyl-CoA carboxylase	23
Diamides	Ryanodine receptor modulators	28
Flonicamid	Modulation of chordotonal organ function	29

Considerable progress has been made in the discovery and development of compounds with insecticidal activity. Organophosphates and carbamates were amongst the first synthetic chemical insecticides to be developed (Kolbezen *et al*., 1954; Casida, 1963). These compounds interfere with the transmission of nerve impulses and cause the paralysis of insects by inhibiting the breakdown of the neurotransmitter, acetylcholine (ACh) (Casida, 1963).

Pyrethroids were the next major class of insecticides to be developed. Pyrethroids affect nerve impulse transmission by preventing the closure the voltage-gated sodium channel of insects, which regulate the movement of sodium ions into nerve cells (Soderlund and Bloomquist, 1989). Some pyrethroids are also reported to have repellent activity against colonising aphids which may help to reduce the transmission of non-persistent plant viruses (Ruscoe, 1977; Dewar and Denholm, 2017). In the UK, the pyrethroid insecticide, lambda-cyhalothrin has been applied widely for the control of aphids in a number of crops (Garwaithe *et al.*, 2017).

Neonicotinoids were discovered and developed during the 1970/80s; however, they were not marketed for use as crop protection products until 1991, with the introduction of imidacloprid (Elbert *et al*., 1991). As several resistance mechanisms had evolved to older chemistries (organophosphates, carbamates, cyclodienes and pyrethroids) neonicotinoids became the main option for the control of aphid species, such as *M. persicae* (Nauen and Denholm, 2005). Neonicotinoids are now the most widely used insecticides globally (Jeschke *et al*., 2010; van Lexmond *et al*., 2015). As neurotoxins, they bind to insect nicotinic acetylcholine receptors (nAChR) in the central nervous system to cause paralysis and death (Nauen *et al.*, 2001). Neonicotinoids are broad-spectrum and many have excellent insecticidal activity, systemic action and high levels of persistence; making them ideal for the control of sucking-pests (Nauen *et al.*, 2001; Elbert *et al*., 2008; Dewar and Denholm, 2017). Additionally, neonicotinoids have a range of application methods; including with irrigation water in drip or drench systems, as seed treatments or as foliar-sprays (Elbert *et al*., 2008).

While many of the insecticides mentioned above have offered effective and persistent solutions to pest problems, several have poor specificity and cause toxic effects on beneficial insects and/or the environment (Inglesfield, 1989; Grue *et al*., 1997; Whitehorn *et al*., 2012). Many of these treatments (*e.g.* a number of organophosphates and carbamates) have been withdrawn under pressure from the environmental lobby (Dewar and Denholm, 2017).

In recent years, the outdoor use of neonicotinoids in the UK has been a contentious issue and has received scrutiny from pesticide regulatory bodies (European Food Safety Authority, 2018a). Due their high persistence in the environment, neonicotinoids can accumulate in soils and are prone to leaching into waterways

(Bonmatin *et al.*, 2015). They may also have non-target effects on a range of taxa, including important pollinator species (Goulson, 2013; Pisa *et al.*, 2015; Rundlöf *et al.,* 2015). In 2018, the European Union withdrew the outdoor use of three key neonicotinoids: clothianidin, imidacloprid and thiamethoxam (European Food Safety Authority, 2018a, 2018b, 2018c). With a lack of alternative and effective solutions available, there are major concerns for the UK crop production industry.

The loss of key insecticides highlights the demand for more selective treatments with lower risk to the environment and non-target organisms. This has led to the development of novel insecticide groups; several of which have properties that are well suited to the control of aphids (Dewar and Denholm, 2017). Two examples are spirotetramat and cyantraniliprole, which have been applied in the UK for the control of aphids over the last few years (Garthwaite *et al.,* 2017).

Spirotetramat (a tetramic acid derivative) is described as having 'unique two-way systemic action' (Nauen *et al*., 2008). After uptake spirotetramat is transported within the plant's vascular system, moving upwards and downwards in the xylem and phloem, respectively, to provide protection throughout the plant (Nauen *et al*., 2008). As an inhibitor of the enzyme acetyl-CoA carboxylase (ACC), spirotetramat affects lipid biosynthesis (Bretschneider *et al*., 2007; Nauen *et al*., 2008). For this reason, spirotetramat is particularly effective against the juvenile stages of sucking-pests, *e.g.* aphid nymphs (Nauen *et al*., 2008). Spirotetramat can also considerably reduce the fecundity and fertility of aphids (Gong *et al*., 2016).

Cyantraniliprole (an anthranilic diamide) moves through xylem vessels and has root systemic and foliar translaminar action against a range of sucking-pests (Lahm *et al*., 2005). The insecticide acts by targeting ryanodine receptors (a class of intracellular calcium channels) to cause a loss of muscle function, followed by paralysis and often death (Lahm *et al*., 2005; Selby *et al*., 2013).

There are a range of insecticides approved for outdoor use against aphids in the UK. Table 4.2 outlines the active substances authorised for outdoor use on at least one crop in the UK (as of 1st March 2020); including synthetic chemical insecticides and insecticides based on natural compounds (FERA, 2020).

Table 4.2 – The range of active ingredients (AIs) authorised for outdoor use against aphids on at least one crop grown in the UK, as of 1st March 2020. Information sourced from: LIAISON (FERA Science Ltd.).

Active ingredient (AI)	Sub-group	IRAC group
Oxamyl	Carbamate	1A
Pirimicarb	Carbamate	1A
Alpha-cypermethrin	Pyrethroid	3A
Cypermethrin	Pyrethroid	3A
Deltamethrin	Pyrethroid	3A
Esfenvalerate	Pyrethroid	3A
Etofenprox	Pyrethroid	3A
Lambda-cyhalothrin	Pyrethroid	3A
Tau-fluvalinate	Pyrethroid	3A
Tefluthrin	Pyrethroid	3A
Zeta-cypermethrin	Pyrethroid	3A
Pyrethrins	Pyrethrin	3A
Acetamiprid	Neonicotinoid	4A
Thiacloprid	Neonicotinoid	4A
Tebufenpyrad	Mitochondrial complex I electron transport inhibitor	21A
Spirodiclofen	Tetronic acid derivative	23
Spirotetramat	Tetramic acid derivative	23
Chlorantraniliprole	Diamide	28
Cyantraniliprole	Diamide	28
Flonicamid	Flonicamid	29
Fatty acids C7–C20	Fatty acid	–
Maltodextrin	Plant-derived starch	–

4.1.6 The control of *Myzus persicae* with chemical insecticides

For many field crops, the control of *M. persicae* relies almost entirely on the application of synthetic chemical insecticides (Foster *et al*., 2000; Bass *et al*., 2014). Through intensive applications, the efficacy of many of these treatments has been lost due to the development of widespread and multiple forms of insecticide resistance in *M. persicae* (Table 4.3). To date, at least seven different mechanisms of resistance have been identified for this aphid (Bass *et al*., 2014).

Table 4.3 – Mechanisms of resistance reported in *Myzus persicae* to the major sub-groups of insecticides.

Sub-group	Mechanism(s) of resistance	Resistance reported in the UK
Carbamates	**1)** Modified AChE (MACE)	Yes
	2) Overproduction of carboxylesterases	Yes
Organophosphates	**1)** Overproduction of carboxylesterases	Yes
Cyclodienes	**1)** Duplication and mutation of the GABA-*Rdl* receptor	Yes
Pyrethroids	**1)** Knock-down mutations (kdr and super-kdr) in voltage-gated Na^+ channel	Yes
Neonicotinoids	**1)** Overproduction of the cytochrome P450	No
	2) Reduced penetration through insect cuticle	No
	3) Mutation of the nicotinic acetylcholine receptor (nAChR): Nic–R^+ and Nic-R^{++}	No

The first mechanism of insecticide resistance to be discovered in *M. persicae* was the overproduction of the carboxylesterases, E4 and FE4, which hydrolyse and sequester insecticides before they reach their target sites (Devonshire and Moores, 1982). This mechanism conferred broad-spectrum resistance to organophosphate, mono-methyl carbamate and pyrethroid insecticides, although the latter group to a much lesser extent (Devonshire and Moores, 1982; Bass *et al*., 2014).

Resistance to dimethyl carbamates was the next mechanism to be discovered in *M. persicae.* Conferred through an insensitivity of the target site; the enzyme, acetylcholinesterase (AChE), modified AChE (MACE) caused a specific > 100-fold insensitivity to pirimicarb (Moores *et al*., 1994). In the UK, MACE resistance is reported in ~70% of the samples analysed at Rothamsted Research (IRAG, 2019).

Resistance to many pyrethroids then arose through a mutation in voltage-gated sodium channels. Termed `knock-down resistance' or kdr (Martinez-Torres *et al.,* 1999), this mechanism was first reported in *M. persicae* in 1997 (Martinez-Torres *et al*., 1997). More recently, an alternative super-kdr variant (M918L) was identified in *M. persicae* on oilseed rape (*Brassica napus* L.) in France and was linked to resistance to lambda-cyhalothrin (Fontaine *et al*., 2011; Bass e*t al*., 2014). Is it thought that these mechanisms were present in populations of *M. persicae* for some time before their discovery (Bass *et al*., 2014). Currently, kdr resistance in *M. persicae* is less common in the UK (found in 20% of the samples analysed at Rothamsted Research) compared to super-kdr resistance (~70% of the samples) (IRAG, 2019).

For cyclodiene insecticides, the prolonged use of endosulfan, which was used as a rotation option in resistance management programs for *M. persicae*, resulted in the development of resistance through target-site mutations in the GABA receptors (Bass *et al*., 2014). This mutation inhibited the generation of new action potentials (ffrench-Constant *et al*., 2000).

Resistance to neonicotinoid insecticides was first described in *M. persicae* in 2007. A clone of the tobacco subspecies, *M. persicae* ssp. *nicotianae* (5191A) from Greece exhibited 30–60-fold resistance to a range of neonicotinoids compared to a susceptible strain (Philippou *et al*., 2010; Puinean *et al*., 2010). This resistance was linked to the overexpression of the cytochrome P450 CYP6CY3, which detoxifies certain

neonicotinoids (Bass *et al*., 2013). However, these levels of resistance were unlikely to affect the efficacy of neonicotinoids in the field provided that treatments were applied at the recommended rates (Bass *et al*., 2014). Further studies indicated an additional mechanism involved in the resistance of *M. persicae* clone 5191A to neonicotinoids (Puinean *et al*., 2010). The 5191A clone exhibited reduced penetration of neonicotinoids through the cuticle. The mechanism behind this resistance has not been determined fully, but may have resulted from changes to the structure and/or composition of the aphid cuticle (Puinean *et al*., 2010; Bass *et al*., 2014).

In 2009, a clone of *M. persicae* (FRC) was collected from peach in Southern France, where there had been a loss in the efficacy of neonicotinoid insecticides. The resistance of the FRC clone was linked to a point mutation (R81T) in the β1 subunit of the nicotinic acetylcholine receptor (nAChR), which inhibited the binding of neonicotinoids (Bass *et al*., 2011). This was the first proven case where a target-site mutation had rendered neonicotinoids ineffective in the field.

Since the discovery of the R81T mutation, this form of resistance has extended from southern Spain, through southern France to northern and central Italy (Slater *et al*., 2011; Panini *et al*., 2014). This likely relates to the widespread distribution of peach and other members of the *Prunus* genus. However, it is also possible that a switch from holocycly in the south of Europe to obligate anholocycly in the north may have constrained the spread of the mutation in populations of *M. persicae* (Bass *et al*., 2015). More recently, strong resistance in *M. persicae* (Nic–R^{++} types) to neonicotinoids has been reported in northern Africa on secondary herbaceous host plants (*e.g*. potato, tomato and pepper) and in Europe, as far north as Belgium (IRAG, 2019).

In the UK, there are low frequencies of *M. persicae* with low resistance to neonicotinoids (Nic-R types). However, these levels of resistance are unlikely to reduce the efficacy of treatments when they are applied at the recommended rates (Foster and Denholm, 2008). As of April 2019, still no Nic–R^{+} and Nic-R^{++} types have been reported in the UK (IRAG, 2019).

Other mechanisms of insecticide resistance have been suggested for *M. persicae*. For example, behavioural 'avoidance' (see Chapter 5.1.3: p.153) has been proposed as an additional mechanism of resistance to neonicotinoids (Fray *et al*., 2014).

To avoid further losses in insecticidal efficacy, insecticide rotation programmes are required to combat the development of resistance in *M. persicae*. However, with a limited arsenal of chemicals available for aphid control this is an ongoing challenge.

4.1.7 Investigating the efficacy and persistence of insecticides for the control of *Myzus persicae*: aims

Two major factors to consider when selecting a product for pest control are efficacy and persistence. In many cases, the knowledge of these factors can help to avoid injudicious applications of insecticides. This information is also important for timing insecticide applications to control aphid populations and for successfully managing the transmission of plant viruses.

The central aim of this chapter was to explore the efficacy and persistence of a range of synthetic chemical insecticides and compounds based on natural products applied on a prophylactic basis for the control of aphid colonisers (alate *M. persicae*). It was expected that treatments with different modes of action (MoA) would differ in terms of their effects on the survival and reproduction of alate *M. persicae*. To test this hypothesis experiments were carried out with treated and untreated (control) Brussels sprout plants.

4.2 Investigating the efficacy and persistence of insecticides for the control of *Myzus persicae*: Materials and Methods

4.2.1 Insect and plant material

Monoclonal parthenogenetic colonies of *Myzus persicae* (2050A) were reared at 18°C, under a photoperiod of 16L:8D in the Insect Rearing Unit (IRU) at Warwick Crop Centre, School of Life Sciences, Wellesbourne. Aphid cultures were maintained on Brussels sprout (*Brassica oleracea* var. *gemmifera* cv. Doric F1) in rearing cages (47.5 x 47.5 x 47.5 cm) with fine nylon mesh sides (Bugdorm-44545; Watkins and Doncaster Ltd., Herefordshire, UK). All plants were grown initially in pots (9 x 9 x 8 cm) containing M2 compost (Levington® medium grade sphagnum moss peat: Everris Limited, Ipswich, UK: pH 5.3–6.0; N = 192, P = 98, K = 319 mg/L) at 20°C, under a photoperiod of 16L:8D.

Three days prior to starting the experiments, alate adults were removed from the rearing cages. Newly-emerged alate adults (0–3 days old) for use in the bioassays were then collected from the inner walls and ceiling of the rearing cages using a fine paint brush.

Resistance to pyrethroid insecticides (Esterase-R_3/kdr-SR) has been reported in the 2050A clone of *M. persicae* (Foster *et al*., 2002). When examining the effects of the pyrethroid insecticide, lambda-cyhalothrin (Hallmark Zeon® 120EC.; Syngenta, Basel, Switzerland), a susceptible clone of *M. persicae* (MP1S) was used instead. The susceptible (MP1S) clone was reared in the same conditions as the resistant (2050A) clone.

4.2.2 Insecticide application

Six foliar-applied insecticides: lambda-cyhalothrin (Hallmark Zeon® 120EC.; Syngenta, Basel, Switzerland), spirotetramat (Movento®; Bayer CropScience, North Carolina, USA), Plant Extract 1, Plant Extract 2, Systemic Insecticide 1 and Systemic Insecticide 2 (coded due to commercial sensitivity) (Table 4.4) were applied to Brussels sprout cv. Doric F1 plants at the 4–5 true leaf stage. For each insecticide, a

spray emulsion was prepared at the field dose recommended by the manufacturers as indicated on the insecticide labels: *e.g.* Hallmark Zeon® (lambda-cyhalothrin) at 0.1 L/ha and Movento® (spirotetramat) at 0.5 L/ha. All insecticides were sprayed to runoff in 300 L/ha water using a knapsack sprayer with 02F110 nozzles in the Pesticide Handling Unit (PHU) at Warwick Crop Centre, School of Life Sciences, Wellesbourne.

Table 4.4 – Characteristics of the insecticides tested. Several insecticides are coded as they were not authorised for outdoor use in the UK as of 1st March 2020.

Commercial or coded name	Active ingredient	IRAC group	Sub-group	Action
Hallmark Zeon®	lambda-cyhalothrin	3A	Pyrethroid	Contact/ feeding
Movento®	spirotetramat	23	Tetramic acid derivative (ketoenole)	Systemic
Plant Extract 1	–	N/A	–	Contact
Plant Extract 2	–	N/A	–	Contact
Systemic Insecticide 1	–	–	–	Systemic (with contact/feeding action)
Systemic Insecticide 2	–	–	–	Systemic (with contact/feeding action)

4.2.3 Efficacy and persistence of insecticides for the control of *Myzus persicae* bioassays

Alate adults of *M. persicae* were starved initially in empty plastic 9 cm Petri dishes (SARSTEDT, Germany) for 1 h before using them to infest plants. Brussels sprout plants cv. Doric F1 (4–5 true leaf stage) were then each infested with ten alate adult *M. persicae* either zero, three, or seven days after the plants had been treated by spraying them with the test products at the recommended rates. On each infestation day (zero, three, and seven) alate adults were transferred onto the upper leaf surface of plants using a fine paintbrush. On the day of spraying, aphids were transferred 1 h after the application of the test product, to allow time for the leaf surfaces to dry. For each insecticide, a total of three treated and three untreated (control) plants were infested zero, three, or seven days after application of the test products. Each experiment was then replicated (blocked) once more in time.

Alate adults were confined to individual plants with 200 x 500 mm micro-perforated polypropylene bags (Cryovac®, New Jersey, USA) (Fig. 4.1). Plants were then maintained at 18°C, under a photoperiod of 16L:8D.

Figure 4.1 – Insecticide-treated Brussels sprout cv. Doric F1 infested with alate *Myzus persicae.*

To allow alate adults time to settle, the first observation was made an hour after infestation. The total number of surviving alate adults on each plant was monitored hourly between 12:00–17:00 GMT on the day of treatment and then monitored once a day for two weeks. If any alate adults were not found (alive or dead) on a particular day and numbers did not subsequently increase on a following day, it was assumed that the missing aphid had died. Reproduction was also monitored, with the numbers of nymphs recorded if they were produced.

4.2.4 Statistical analysis

A Kaplan-Meier estimator was carried out in SPSS Statistics (Version 25.0, IBM Corp©, Armonk, USA) to produce survival plots. Kaplan-Meier curves were then compared with the log-rank tests (Kleinbaum and Klein, 2012) to determine statistically significant differences between the cumulative survival of alate *M. persicae* on the treated plants and the untreated (control) plants.

Additionally, the mean numbers of nymphs per surviving alate adult on treated and untreated (control) plants were compared at specific time points after the plants were infested (*e.g.* seven and fourteen days after infestation) using independent samples t-tests. For some treatments, no nymphs were recorded on the plants during the experiments. In these cases, one sample t-tests were used to test whether the number of nymphs per surviving alate adult on untreated control plants significantly differed to zero. Prior to this, data were checked for normality (Shapiro-Wilk test) and the assumption of homogeneity of variance was tested using Levene's Test of Equality of Variances.

Apart from the Kaplan-Meier survival plots, all graphs were made using the sciplot package (Version 1.1-1) (Morales, 2017) in R (Version 3.5.1).

4.3 Investigating the efficacy and persistence of insecticides for the control of *Myzus persicae*: Results

4.3.1 The effects of insecticides on the survival of alate *Myzus persicae* and their relative persistence.

To assess the efficacy and persistence of the six test products, the survival of alate *M. persicae* was monitored on Brussels sprout plants that had been infested zero, three and seven days after spraying plants with the test products at the recommended rates.

Lambda-cyhalothrin

On plants infested on the day of spraying (Day 0), lambda-cyhalothrin caused a mean mortality of 50% one day after exposure to the treatment (Fig. 4.2A). Less than 10% mean mortality was observed on the untreated control plants at this stage. By Day 14, around 80% mean mortality was achieved on the treated plants compared to 50% on the untreated control plants. The mean % mortality, however, varied considerably between replicates, as indicated by the wide error bars (±1 S.E.) for the treated plants (Fig. 4.2A). Lambda-cyhalothrin showed moderate to low levels of persistence on plants that were infested three and seven days (Fig. 4.2B and C) after spraying, with means of ~40% and 30% mortality observed respectively, one day after exposure to the treatment. After the first day mean % mortality proceeded at a similar rate to that of the untreated control.

Spirotetramat

After the application of spirotetramat there was a lag period of around three days before there was a noticeable difference in the mean % mortality of *M. persicae*, compared to the untreated control plants (Fig. 4.2D). Similar lag periods were observed on the plants infested three and seven days after spraying (Fig. 4.2E and F). On Day 0 plants, 100% mortality was achieved nine days after the aphids were exposed to the insecticide (Fig. 4.2D). On plants that were infested three days after spraying, 100% mortality was reached after eleven days (Fig. 4.2E). Plants infested seven days after spraying still displayed good levels of insecticide persistence, with a mean of 80% mortality observed seven days after exposure (Fig. 4.2F); a comparable level to the seven-day stage on Day 0 and Day 3 plants (Fig. 4.2D and E).

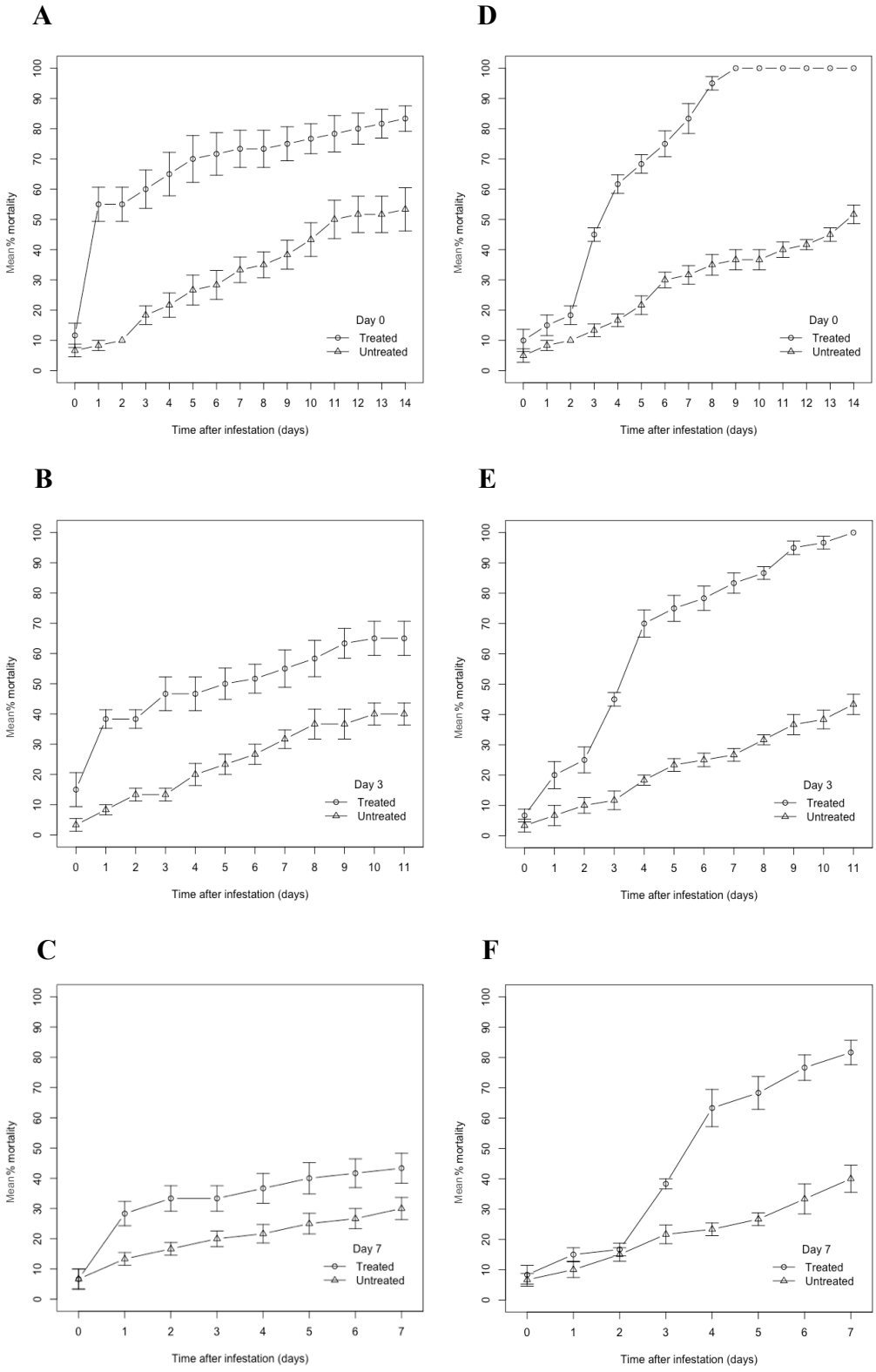

Figure 4.2 – Mean (±1 S.E.) % mortality of alate *Myzus persicae* on Brussels sprout cv. Doric F1 plants treated with **lambda-cyhalothrin** infested **(A) zero days** after application, **(B) three days** after application and **(C) seven days** after application and with **spirotetramat** infested **(D) zero days** after application, **(E) three days** after application and **(F) seven days** after application and on the respective untreated (control) plants.

Plant Extracts 1 and 2

Overall, the mean % mortality of *M. persicae* on plants treated with Plant Extract 1 and Plant Extract 2 was similar to that observed on the untreated control plants (Fig. 4.3). Compared to the untreated control plants, there was a slight increase in mortality (~10%) one day after exposure to Plant Extracts 1 and 2 for plants infested on the day of spraying (Fig. 4.3A and D). After the first day, however, the patterns of mortality did not differ from the untreated controls. Very similar mean % mortality was observed on the treated plants and the untreated control plants infested three (Fig. 4.3B and E) and seven (Fig. 4.3C and F) day after spraying.

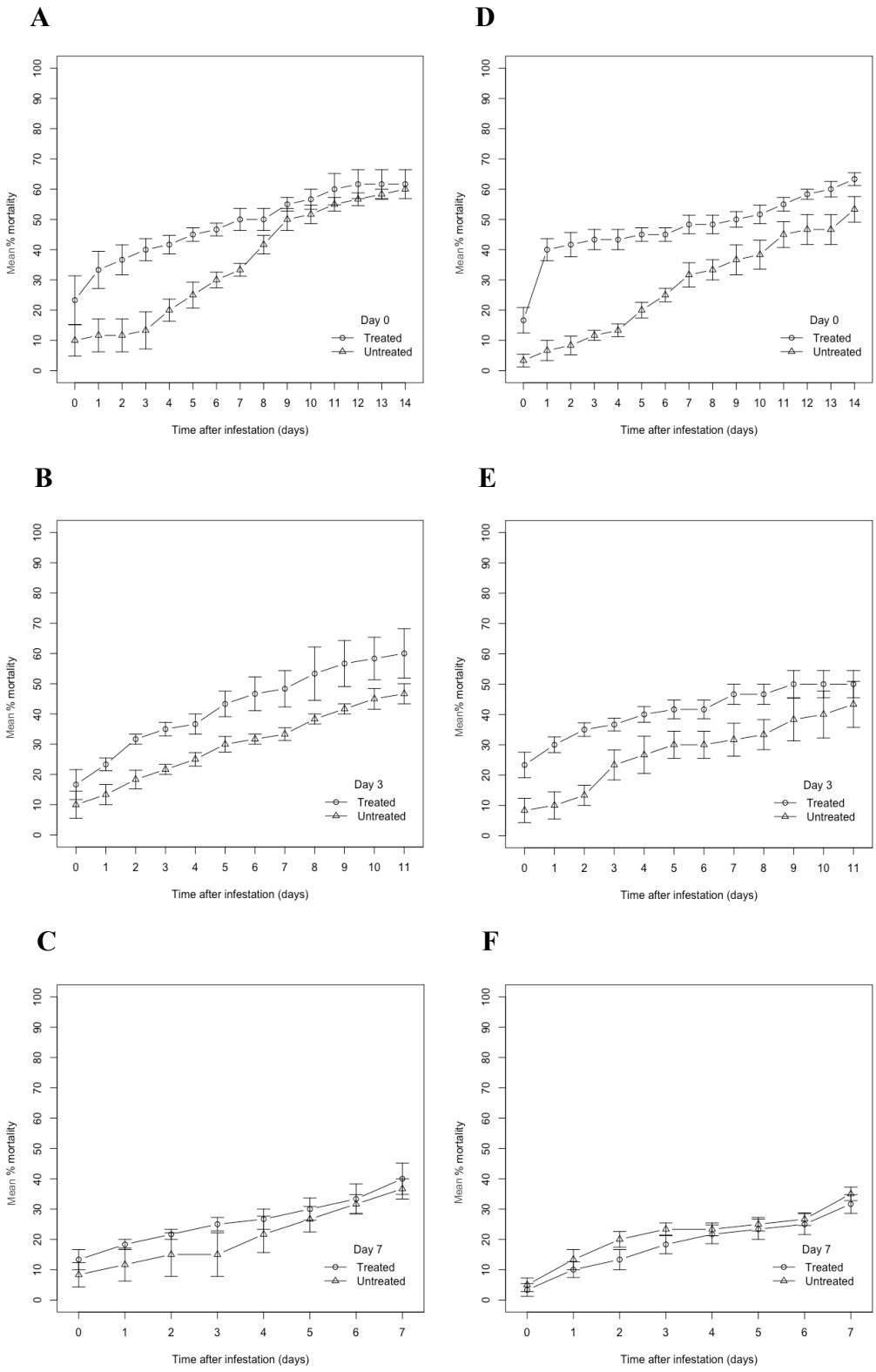

Figure 4.3 – Mean (±1 S.E.) % mortality of alate *Myzus persicae* on Brussels sprout cv. Doric F1 plants treated with **Plant Extract 1** infested **(A) zero days** after application, **(B) three days** after application and **(C) seven days** after application and with **Plant Extract 2** infested **(D) zero days** after application, **(E) three days** after application and **(F) seven days** after application and on the respective untreated (control) plants.

Systemic Insecticides 1 and 2

Systemic Insecticide 1 had high levels of efficacy and persistence. In all cases (Fig. 4.4A, B and C), a mean of > 30% mortality was observed 5 h after exposure to the insecticide and a mean of between 65–80% mortality was observed one day after infestation. Overall, 100% mortality was achieved four days after exposure when the plants were infested on the day of spraying (Fig. 4.4A) and five days on plants infested three (Fig. 4.4B) and seven (Fig. 4.4C) days after spraying.

Systemic Insecticide 2 also showed high levels of efficacy and persistence. In all cases, around 70–80% mortality was achieved one day after exposure to the treatment and 90% mortality after two to three days (Fig. 4.4D, E and F). Similar to Systemic Insecticide 1, 100% mortality was observed four days after exposure to Systemic Insecticide 2 on Day 0 plants (Fig. 4.4D) and on Day 3 plants (Fig. 4.4E) and five days after exposure to the treatment on Day 7 plants (Fig. 4.4F).

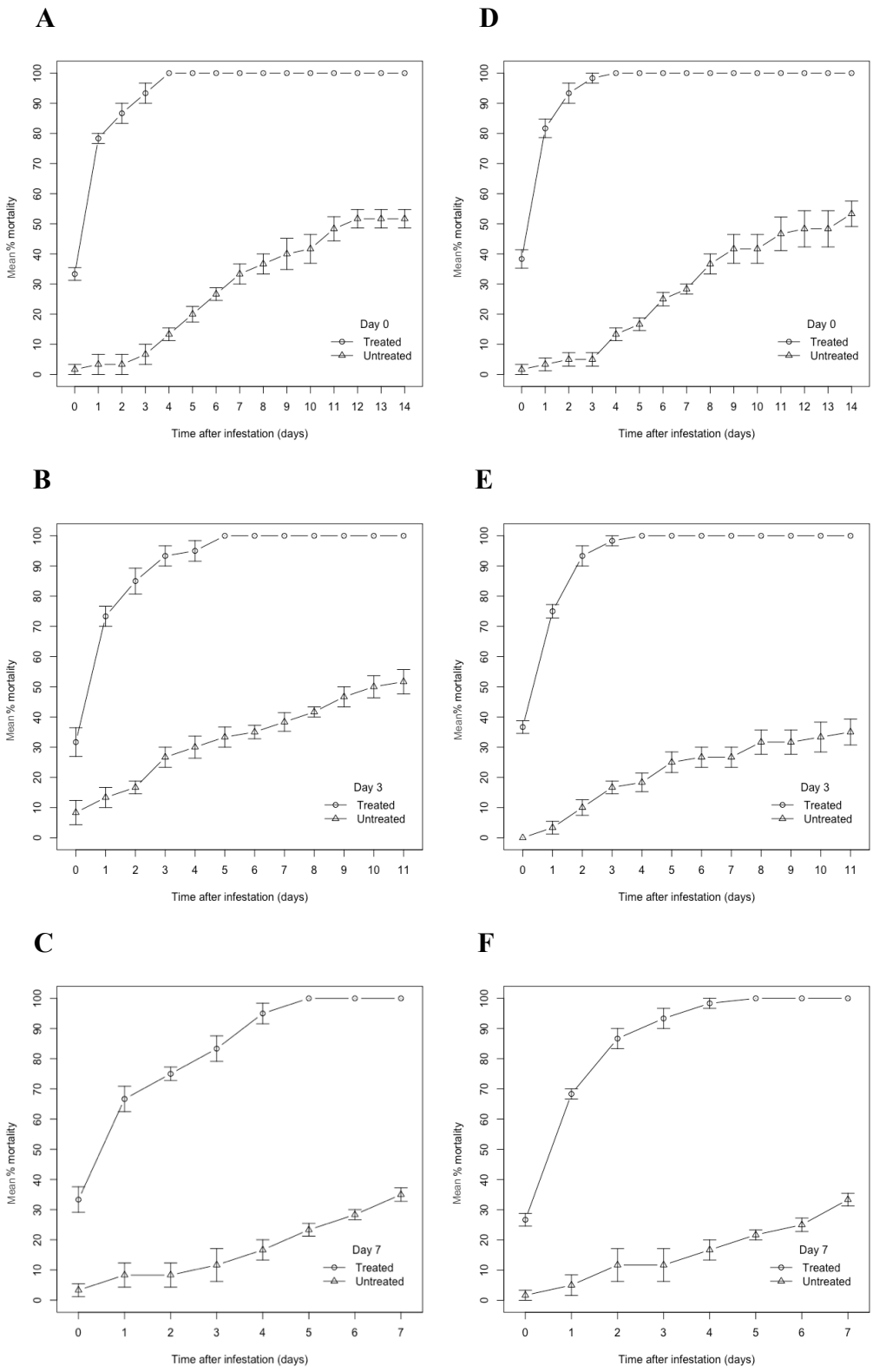

Figure 4.4 – Mean (±1 S.E.) % mortality of alate *Myzus persicae* on Brussels sprout cv. Doric F1 plants treated with **Systemic Insecticide 1** infested **(A) zero days** after application, **(B) three days** after application and **(C) seven days** after application and with **Systemic Insecticide 2** infested **(D) zero days** after application, **(E) three days** after application and **(F) seven days** after application and on the respective untreated (control) plants.

4.3.2 Kaplan-Meier survival plots for alate *Myzus persicae*

Untreated (control) plants

Initially for each insecticide and the respective untreated control, the replicates (six individual plants) were compared using a Kaplan-Meier estimator with a log-rank test. No statistically significant differences were found between individual replicates, in terms of the survival time of alate adults, on the treated plants or the untreated control plants ($P > 0.05$). This allowed for pooling of data from the replicates.

The Kaplan-Meier survival curves for the six replicate control experiments for the three infestation occasions (zero, three and seven days after spraying) were compared using a log-rank test. This was to test whether patterns in cumulative survival significantly deviated between different batches of *M. persicae*. In all cases, no statistically significant differences ($P > 0.05$, d.f. = 5) were found. For the experiments with lambda-cyhalothrin, a different clone of *M. persicae* (MP1S) was used (Fig. 4.5: control 1). However, the survival curve for the MP1S clone did not differ significantly from those of the 2050A clone, that was used in all other cases. Cases were censored (accounted for during Kaplan-Meier analysis) when alate adults survived to the end of the study, as information about their survival time was unknown.

Lambda-cyhalothrin

There were statistically significant differences in the cumulative survival of alate *M. persicae* between plants infested zero and three days after spraying with lambda-cyhalothrin and the untreated control plants; with lower cumulative survival observed on the treated plants (Fig. 4.6A and B). However, cumulative survival did not differ significantly on plants infested seven days after spraying and on the untreated control plants (Fig. 4.6C).

Spirotetramat

For spirotetramat, the cumulative survival of alate *M. persicae* was significantly lower ($P < 0.001$) on the treated plants compared to the untreated control plants on all infestation occasions (Fig. 4.6D, E and F). Compared to the untreated controls, clear differences in cumulative survival were not evident until three days after exposure to the treatment.

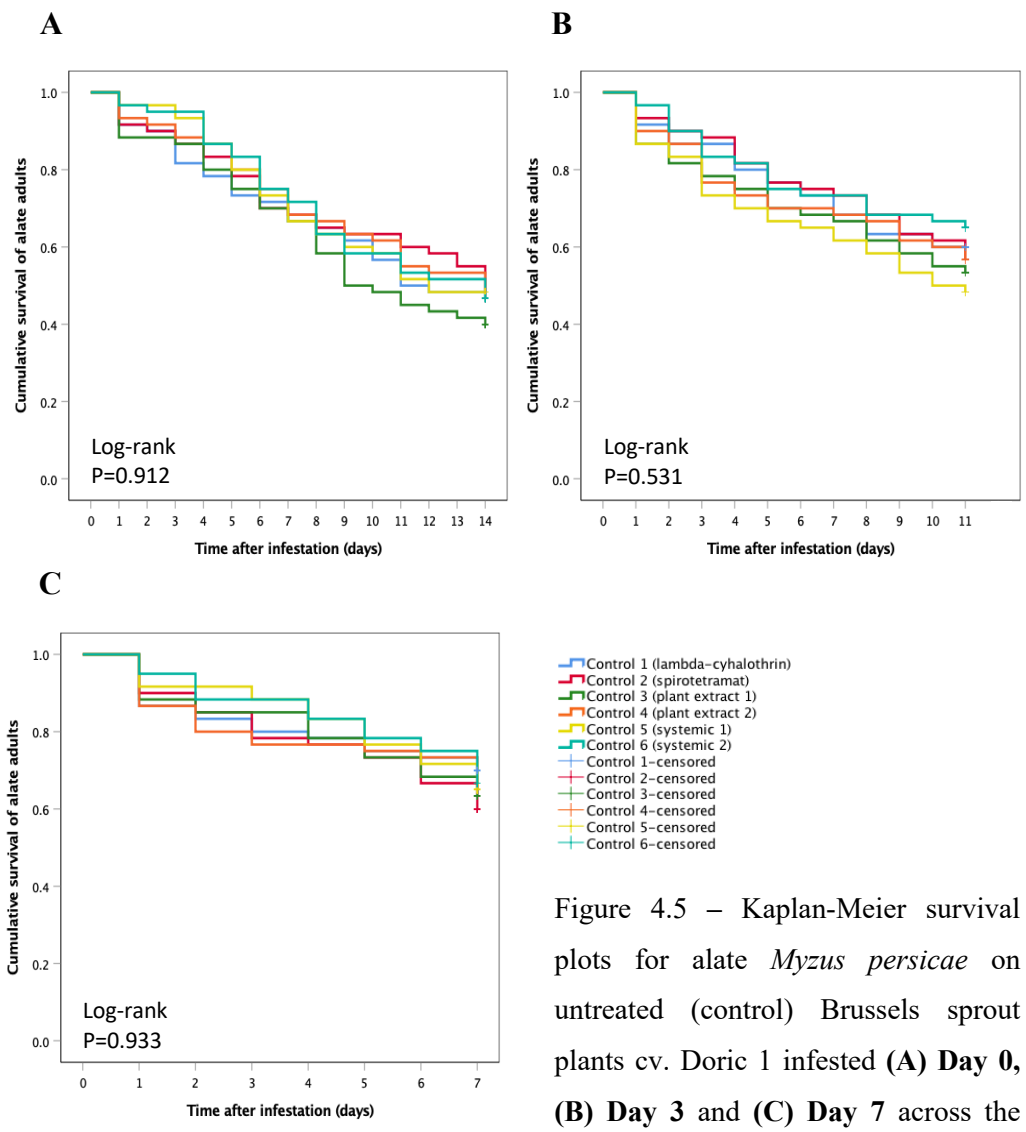

Figure 4.5 – Kaplan-Meier survival plots for alate *Myzus persicae* on untreated (control) Brussels sprout plants cv. Doric 1 infested **(A) Day 0, (B) Day 3** and **(C) Day 7** across the insecticide efficacy and persistence experiments.

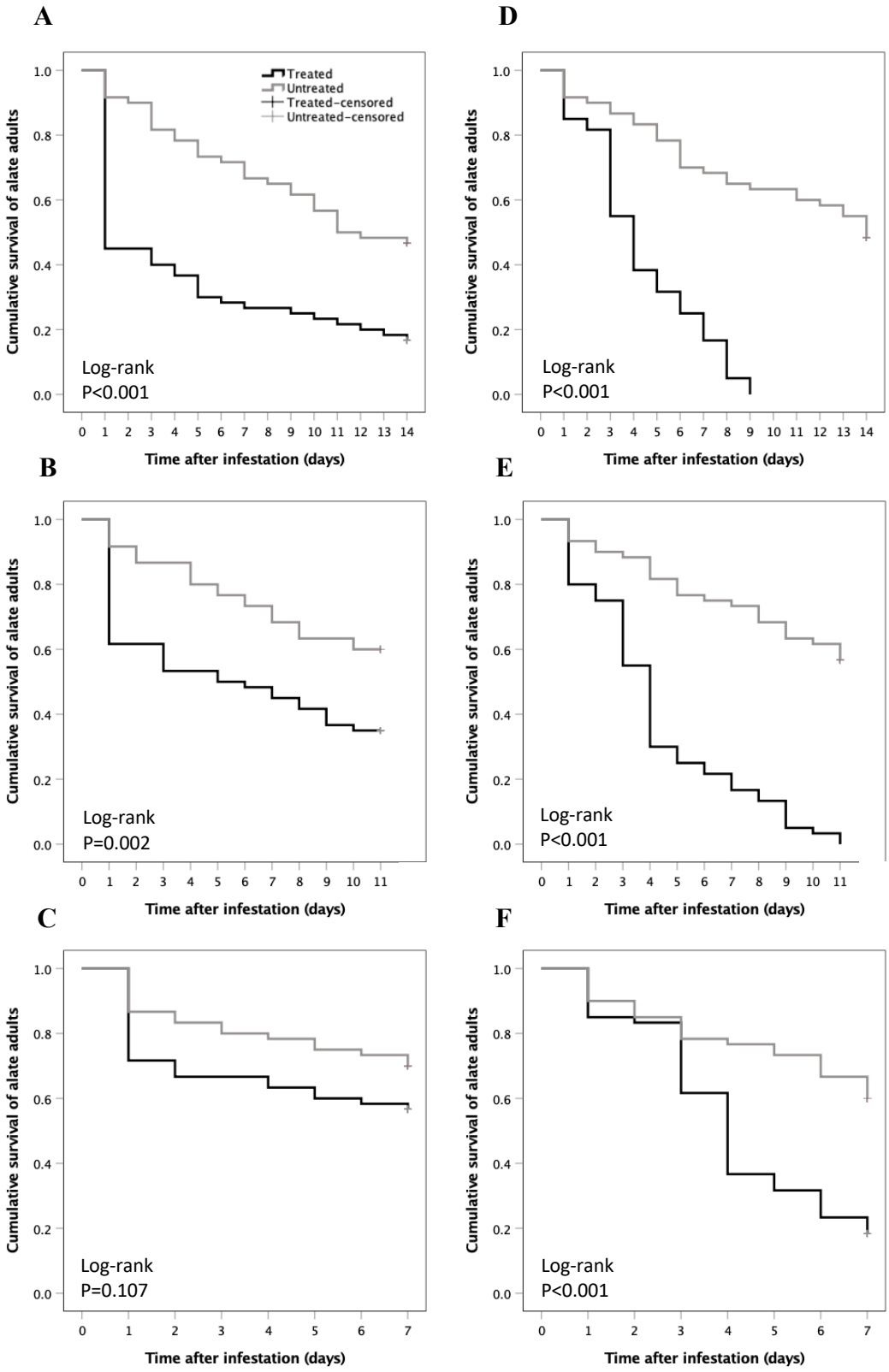

Figure 4.6 – Kaplan-Meier survival plots for alate *Myzus persicae* on Brussels sprout cv. Doric F1 plants treated with **lambda-cyhalothrin** infested **(A) zero, (B) three** and **(C) seven days** after application and **spirotetramat** infested **(D) zero, (E) three** and **(F) seven days** after application.

Plant Extracts 1 and 2

No statistically significant differences ($P > 0.05$) were found between the cumulative survival of alate *M. persicae* on plants treated with Plant Extracts 1 or 2 and the respective untreated control plants for all infestation occasions (Fig. 4.7). Interestingly, in the experiments with Plant Extract 1 control mortality was higher on Day 0 plants compared with on the Day 3 plants (Fig. 4.7A and B). The difference in control mortality may explain why a higher P-value ($P = 0.417$) was obtained for the Day 0 plants.

Systemic Insecticides 1 and 2

For all infestation days, the cumulative survival of *M. persicae* was significantly lower on plants treated with Systemic Insecticides 1 and 2 compared to on untreated control plants (Fig. 4.8). In all cases, 100% mortality was reached four to five days after exposure to the treatments.

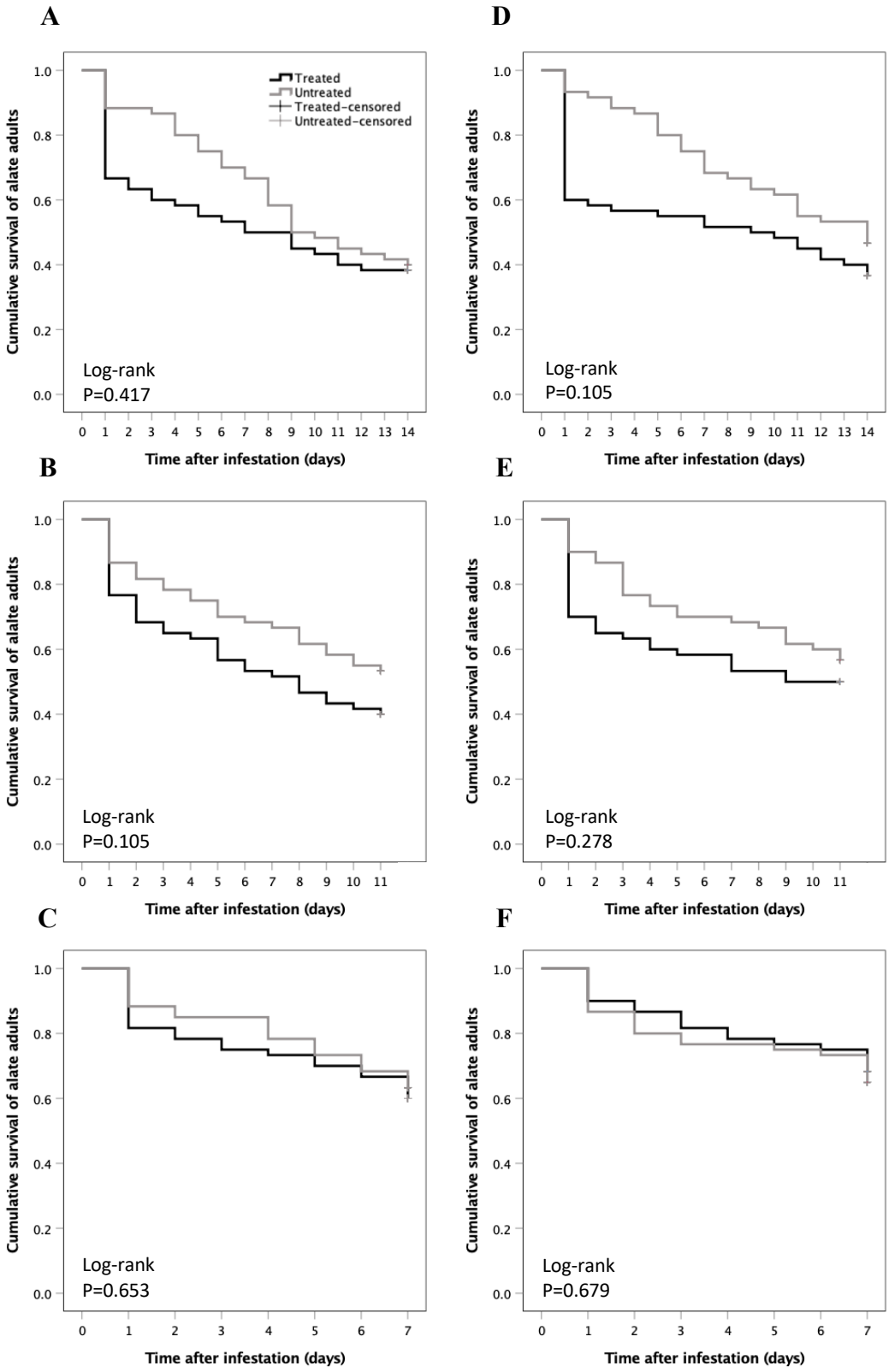

Figure 4.7 – Kaplan-Meier survival plots for alate *Myzus persicae* on Brussels sprout cv. Doric F1 plants treated with **Plant Extract 1** infested **(A) zero, (B) three** and **(C) seven days** after application and **Plant Extract 2** infested **(D) zero, (E) three** and **(F) seven days** after application.

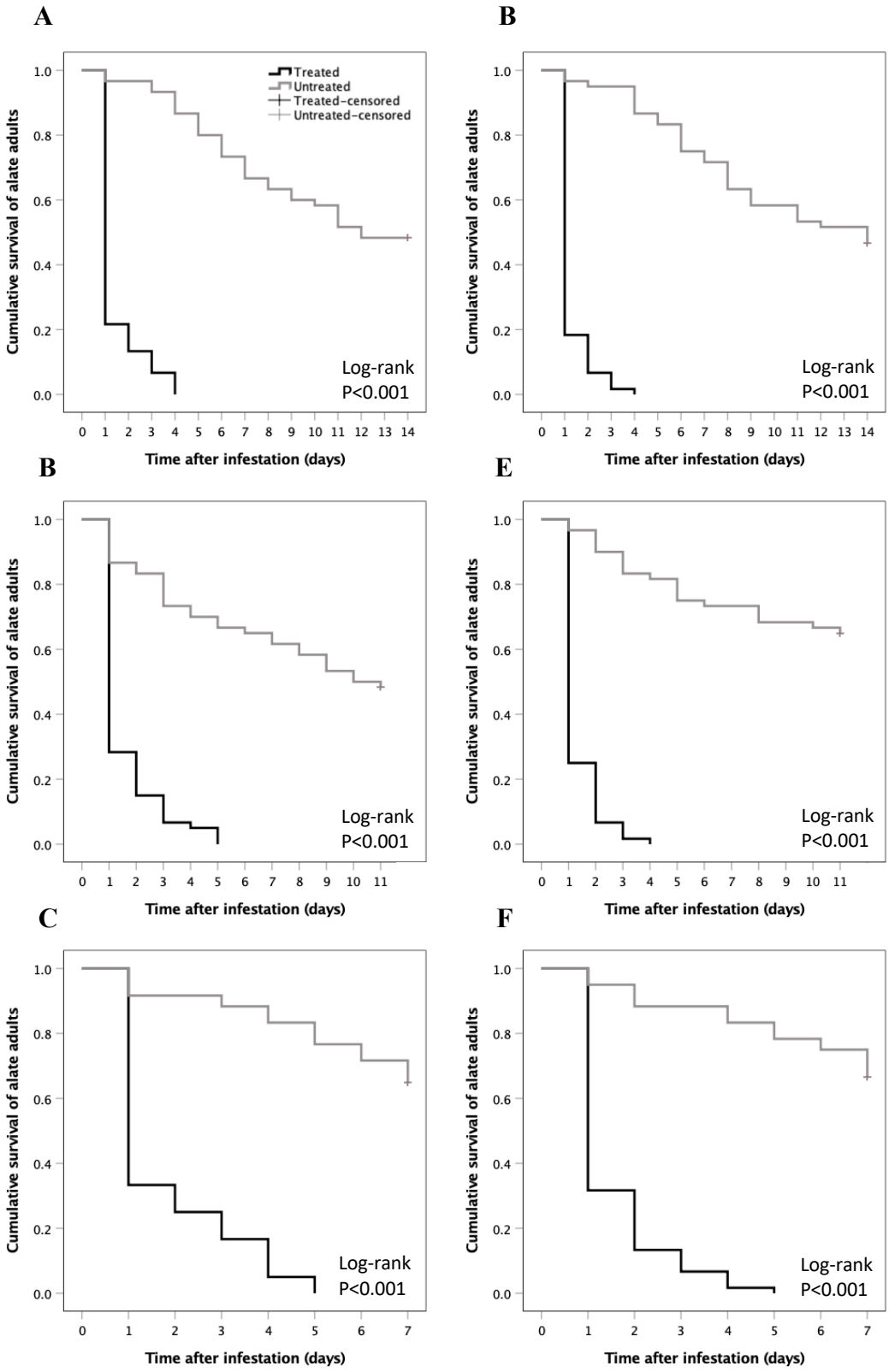

Figure 4.8 – Kaplan-Meier survival plots for alate *Myzus persicae* on Brussels sprout cv. Doric F1 plants treated with **Systemic Insecticide 1** infested **(A) zero, (B) three** and **(C) seven days** after application and **Systemic Insecticide 2** infested **(D) zero, (E) three** and **(F) seven days** after application.

4.3.3 The effects of insecticides on the reproduction of *Myzus persicae*

The mean numbers of nymphs produced per surviving alate adult were compared between each insecticide and the respective untreated control on seven and fourteen days after infestation (Fig. 4.9). Independent samples t-tests were used to identify any statistically significant differences between the treated and untreated plants. Where no nymphs were recorded on the treated plants, one sample t-tests were used to investigate whether the number of nymphs deposited on the respective untreated control plants differed significantly from zero.

Compared to the untreated controls, no statistically significant differences in the number of nymphs deposited were identified for plants treated with lambda-cyhalothrin, Plant Extract 1 or Plant Extract 2 ($P > 0.05$), despite, in all cases, there being slightly fewer nymphs per surviving adult on the treated plants (Fig. 4.9A, C and D). For three of the treatments; spirotetramat and Systemic Insecticides 1 and 2, no nymphs were recorded on the treated plants during the experiments (Fig. 4.9B, E and F).

Between plant replicates, in some cases there was considerable variation in the number of nymphs produced per surviving adult. The greatest variation in nymph production (as indicated by the wide error bars ±1 S.E.) was seen on plants treated with Plant Extract 1 followed by plants treated with lambda-cyhalothrin, both fourteen days after infestation (Fig. 4.9A and D).

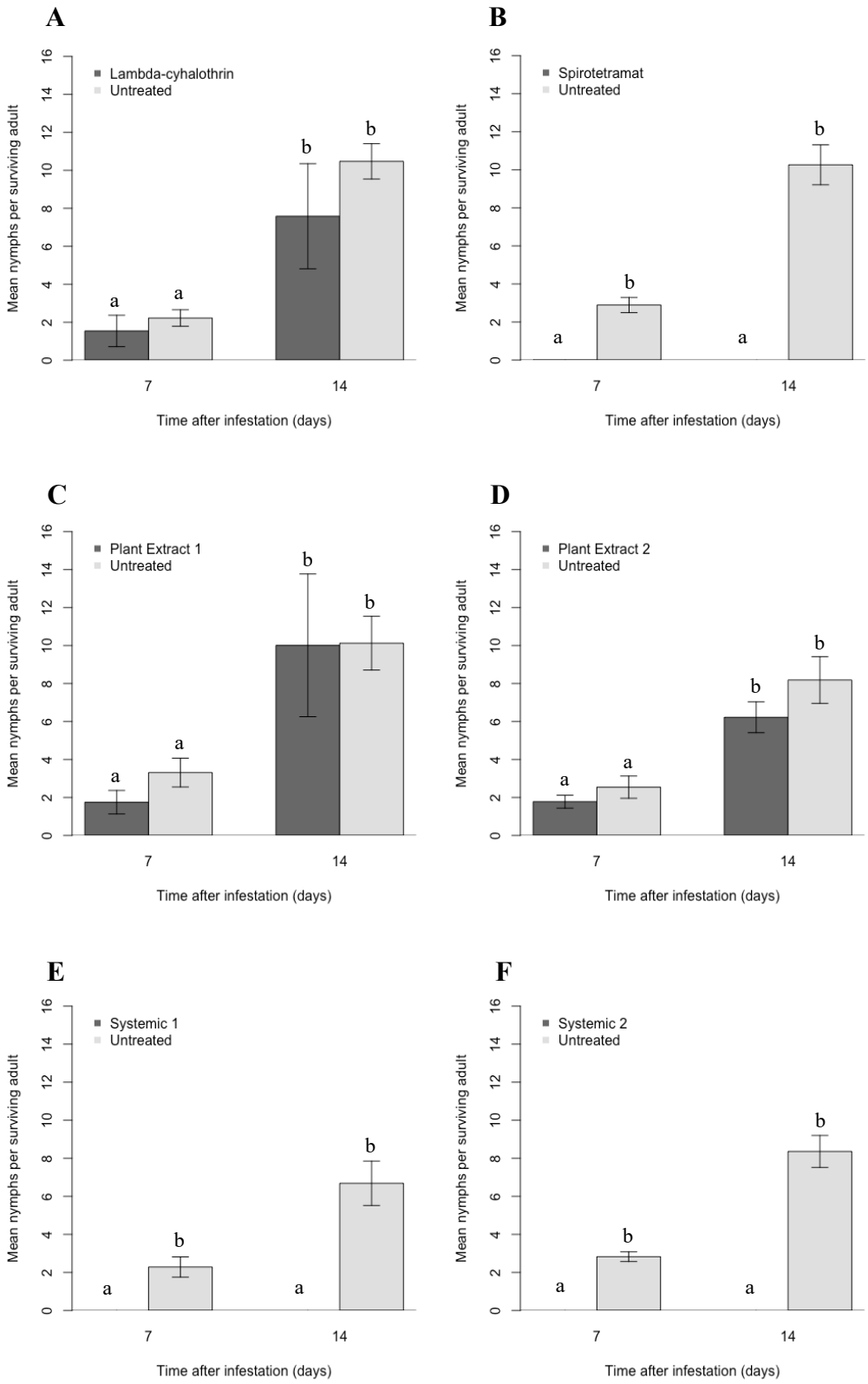

Figure 4.9 – Mean (±1 S.E.) number of nymphs of *Myzus persicae* per surviving alate adult on Brussels sprout cv. Doric F1 plants treated with **(A) lambda-cyhalothrin, (B) spirotetramat, (C) Plant Extract 1, (D) Plant Extract 2, (E) Systemic Insecticide 1** and **(F) Systemic Insecticide 2** (all insecticides freshly applied) and the respective control on seven and fourteen days after infestation. Different letters above the bars indicate statistically significant differences ($P < 0.05$).

4.4 Investigating the efficacy and persistence of insecticides for the control of *Myzus persicae*: Discussion

4.4.1 Developing a system for investigating the efficacy and persistence of insecticides for the control of *Myzus persicae*

There are several established methods for investigating the susceptibility of *M. persicae* and other aphid species to insecticides *e.g.* Insecticide Resistance Action Committee Susceptibility Test Methods Series method No: 001 *Myzus persicae* (IRAC, 2009). Many of these methods involve placing aphids onto insecticide-treated leaf discs or individual leaves in Petri dishes or similar containers. Mortality is then assessed after a specified period of time. Most of these methods have been designed to monitor apterous aphids, but could be adapted to monitor alate adults.

In the experiments carried out here, a system using Brussels sprout plants (4–5 true leaf stage) was designed to test the efficacy and persistence of insecticides for the control of *M. persicae*. An advantage of using whole plants over leaf discs is that the insecticidal effects observed are more likely to resemble those in the field. Additionally, individual leaves or excised leaf discs have limited viability for use in bioassays; as they are prone to wilting and lose turgor pressure over time. For this reason, they do not allow aphid survival to be assessed over longer periods.

In terms of insecticide coverage, however, leaf discs offer greater uniformity between replicates compared with plants. Leaf discs are a uniform size and can be dipped in an insecticide solution or may be sprayed using a Potter tower air atomising sprayer (Potter, 1952). These techniques can improve the accuracy of treatment. Plants often vary in size and surface area and may differ in the amount of an active ingredient present on the plant surface. However, this can be mostly avoided by selecting plants of a similar growth stage/size (*e.g.* 4–5 true leaf stage) and ensuring a consistent spraying technique.

4.4.2 The effects of insecticides on the survival of *Myzus persicae* and their persistence

Two insecticides (lambda-cyhalothrin and spirotetramat) applied widely for the control of *M. persicae* in outdoor crops in the UK and four novel insecticides were investigated in terms of their levels of efficacy and persistence.

Efficacy and persistence of lambda-cyhalothrin

On plants infested on the day of spraying, lambda-cyhalothrin caused a considerable reduction (~50%) in the mean survival of alate *M. persicae* MP1S (susceptible clone) after one day of exposure to the treatment. Comparisons of Kaplan-Meier survival curves found a statistically significant difference between the treatment and the untreated control (Fig. 4.6A). The fast action of lambda-cyhalothrin was not unexpected. Like many pyrethroid insecticides, the contact action of lambda-cyhalothrin rapidly disrupts the functioning of the insect nervous system by targeting sodium channels in nerve cell membranes (IRAC, 2019). Similar studies, have outlined the fast action of lambda-cyhalothrin for other species of aphid. For example, Boquel *et al*. (2015) showed that when exposed to lambda-cyhalothrin (Matador®, Syngenta 15 g AI/ha) on potato plants (*Solanum tuberosum* L.) strong intoxication was induced in *R. padi* alate adults, beginning as early as 20 min after exposure to the treatment.

The insecticidal effects of lambda-cyhalothrin persisted on plants that were infested three days after spraying with ~40% mortality observed one day after exposure to the treatment. For plants infested seven days after spraying, the Kaplan-Meier survival curves for the treatment and the untreated control were not significantly different. This suggests that on Brussels sprout plants, the major insecticidal effects of lambda-cyhalothrin persist for between three to seven days after application. However, the half-life reported for lambda-cyhalothrin on plant surfaces is five days (Knisel, 1993). In a similar study with lambda-cyhalothrin, Boquel *et al*. (2015) did not observe any symptoms of intoxication in *A. fabae, M. euphorbiae* and *R. padi* on potato plants ten to thirteen days after treatment.

Across individual replicates, there was considerable variation in the mean % mortality of alate *M. persicae* on plants treated with lambda-cyhalothrin compared to on plants treated with the other five test products. For this treatment, direct contact or ingestion is required for insecticidal effects to be observed. If lambda-cyhalothrin had been sprayed directly onto aphids it is possible that there would have been more consistency between replicates, as well as potentially higher levels of mortality. This relates to the "Zeon technology" of lambda-cyhalothrin, (Hallmark Zeon®) which encapsulates the active ingredient (AI) in microcapsules with thin walls. Microencapsulation of the AI is reported to improve contact accuracy to enable the rapid knock-down of pest insects (Wege *et al.,* 1999; Knowles, 2008). However, this effect relates to aphids that are present on plants before the insecticide is applied. In these experiments, alate adults were released onto pre-treated plants, as the central aim was to investigate the efficacy and persistence of prophylactic treatments on plant colonisation by aphids. Additionally, it is possible that alate adults only made contact with/fed on new leaves that developed after treatment, or rapidly relocated to these leaves on the Day 7 plants. It is unlikely that these aphids would have experienced considerable insecticidal effects.

As a large proportion of the field populations of *M. persicae* in the UK are now resistant to pyrethroids (IRAG, 2019), the application of lambda-cyhalothrin is unlikely to provide a sustainable control option.

Efficacy and persistence of spirotetramat

Spirotetramat had good levels of efficacy and persistence for the control of *M. persicae.* When freshly applied, spirotetramat caused 100% mortality in nine days. This is not dissimilar to the results obtained by Wang *et al.* (2016), who found that at the recommended field dose, 100% mortality of adult *M. persicae* occurred 8.5 days after exposure to the treatment on leaf discs of cabbage. The insecticidal effects of spirotetramat persisted on plants that were infested three and seven day after spraying. This result is consistent with the findings of previous studies, where the long-lasting activity of spirotetramat for aphid control has been demonstrated (Nauen *et al*., 2008).

For spirotetramat, there was a three to four-day lag period before insecticidal effects were observed on plants infested zero, three and seven days after spraying.

Spirotetramat requires time for activation of the product (hydrolysis to enol form) and like other systemic treatments, the subsequent translocation throughout the plant (Nauen *et al*., 2008). However, as similar lag periods were observed on plants infested three and seven days after spraying, the delay in insecticidal activity is most likely due to the mode of action of this insecticide, which interrupt lipid biosynthesis by inhibiting the enzyme, ACC.

While spirotetramat has excellent systemic and translaminar activity, it is reported to have little/no contact action (Nauen *et al*., 2008). Compared to the untreated control, this could explain why there was a relatively small difference in the survival of *M. persicae* on the treated plants for the first two days after they were sprayed. The contact action of spirotetramat could be investigated further by spraying aphids directly and then placing them onto untreated plants to monitor their survival. Due to the lack of contact activity and its slow-acting nature, spirotetramat is unlikely to provide effective management of the spread of non-persistent plant viruses.

Efficacy and persistence of Plant Extracts 1 and 2

In terms of the control of alate *M. persicae*, Plant Extracts 1 and 2 had low levels of efficacy and persistence. The Kaplan-Meier survival curves for alate *M. persicae* did not differ significantly between the treatments and the respective untreated controls. As both insecticides have only contact action, their low efficacy and persistence may in part be explained by a lack of systemic activity. The low levels of persistence, however, may also be attributed to high volatility and/or the rapid breakdown of the active ingredients.

In certain cases, the use of Plant Extracts 1 and 2 could still benefit resistance management programmes. Potential for resistance to these insecticides is likely to be low due to their physical modes of action. For this reason, they could be used with traditional chemistries as alternate MoAs. In addition, the application of Plant Extracts 1 and 2 in tank mixtures with other insecticides may allow for some initial knock-down while traditional chemistries take effect.

Efficacy and persistence of Systemic Insecticides 1 and 2

Systemic Insecticides 1 and 2 displayed excellent levels of insecticide efficacy and persistence. Both treatments were fast-acting, with ~80% mortality observed one day

after alate *M. persicae* were exposed to the treatments, compared to ~10% mortality on the untreated control plants. Systemic Insecticides 1 and 2 also led to some knock-down of alate *M. persicae* within the first five hours of exposure to the treatments, suggesting rapid contact/feeding action. For both insecticides, 100% mortality was achieved within 4–5 days for plants that had been infested zero, three and seven days after application. Insecticides with rapid action can useful for the control of vectors of non-persistent viruses, particularly in terms of managing the secondary spread of virus in crops (Gibson and Cayley, 1984; Lowery and Boiteau, 1988). The application of Systemic Insecticides 1 and 2 may be useful before aphids first migrate into crops to prevent or reduce infestation. Alternatively, their application may be advisable when peak numbers of *M. persicae* are expected in crops. In the case of non-persistent plant viruses, it is unlikely that these treatments would prevent transmission fully. However, the fast knock-down of virus vectors combined with the high persistence of these products may contribute to an overall reduction in virus spread within crops. For Systemic Insecticide 1, this hypothesis will be tested in Chapter 6.3.2 (pp.204-205).

4.4.3 The effects of insecticides on the reproduction of *Myzus persicae*

The six insecticides tested differed in terms of their effects on the reproduction of alate *M. persicae* and/or the survival of deposited nymphs. In these experiments, it was difficult to determine whether the test products affected the reproductive process of *M. persicae*, induced toxicity in deposited nymphs or had a combination of both effects. However, based on the knowledge of the MoA of each insecticide, it may be possible to infer these effects.

When freshly applied, lambda-cyhalothrin and Plant Extracts 1 and 2 did not contribute to a statistically significant reduction in *M. persicae* reproduction and/or nymph survival. For Plant Extracts 1 and 2, this is likely to be explained by the low efficacy and persistence of these compounds. In the case of lambda-cyhalothrin, the residual efficacy on Brussels sprout plants lasted between three to seven days. However, most nymphs were deposited by surviving alate adults between seven to ten days after infestation; when the residual efficacy of this insecticide is likely to be low. For mortality to occur, direct contact between the treatment and deposited nymphs is required.

During the experiments, no nymphs were recorded on plants treated with spirotetramat for all infestation occasions. Previously, spirotetramat has been shown to cause considerable reductions in the fecundity and fertility of *M. persicae* at relatively low concentrations (Nauen *et al*., 2008; Wang *et al*., 2016). Adult *M. persicae* exposed to spirotetramat have also been shown to produce non-viable nymphs that are unable to extend their legs or feed (Wang *et al*., 2016). In cases where viable nymphs were deposited on plant surfaces treated with spirotetramat, they died within 24 h due to the high susceptibility of nymphs to the treatment's mode of action (ACC inhibitor/interruption of lipid biosynthesis) (Nauen *et al*., 2008).

Similarly, no nymphs were observed on plants treated with Systemic Insecticides 1 and 2. This is most likely due to the rapid knock-down of the majority of the alate adults before they were able to reproduce. However, if any nymphs were deposited, it is likely that they would have been very susceptible to the effects of these insecticides due to their small size and died prior to their detection on the plants. This could be tested by repeating the experiments with nymphs being placed onto treated plants.

4.4.4 Conclusions

The six test products varied considerably with regard to their effects on the survival of alate *M. persicae*.

- On Brussels sprout plants infested on the day of spraying, lambda-cyhalothrin, spirotetramat, and Systemic Insecticides 1 and 2 led to statistically significant reductions in the number of live alate adults over time, compared to the untreated controls. The treatments, however, differed in their levels of persistence.

- While there were some initial reductions in the survival of alate *M. persicae* on plants treated with Plant Extracts 1 and 2, the Kaplan-Meier survival curves for these insecticides did not significantly differ to those of the untreated controls.

- Spirotetramat and Systemic Insecticides 1 and 2 led to considerable reductions in the reproduction of *M. persicae* and/or nymph survival.

5.1.1 The effects of insecticides on the behaviour of pest insects

Insecticides provide the basis for many pest management programmes. These compounds are specifically selected or designed for their ability to kill pest insects (Haynes, 1998). However, prior to death, or through the effects of sub-lethal doses, insecticides can interfere with the behaviour of insects. This is to be expected, as many of these compounds (*e.g*. carbamates, organophosphates, pyrethroids, neonicotinoids) target specific sites within the nervous system of insects (IRAC, 2019).

In addition to neurotoxic effects, some insects may modify their behaviour in response to their sensory perception of insecticides. These behavioural changes can reduce the efficacy of treatments and over time may accelerate the evolution of insecticide resistance through behavioural mechanisms (Haynes, 1988; Fray *et al*., 2014). Separating neurotoxicity from the sensory perception responses of insects, however, can prove difficult. This is reflected in the literature, as there is a lack of clear terminology used to describe the effects of insecticides on insect behaviour. For example, the terms ‘repellent’, ‘deterrent’, ‘anti-feedant’, ‘stimulant’, ‘excitant’ and ‘arrestant’ are all used to describe the effects of insecticides on insect behaviour, without a clear distinction between neurotoxic and sensory perception responses (Haynes, 1988).

Investigating the effects of insecticides on the behaviour of insects is important for several reasons. These include: (i) elucidating the mode of action of synthetic chemical insecticides (ii) identifying changes in behaviour that may contribute to or interfere with the control of pest populations and/or the transmission plant viruses by insects (iii) highlighting the potential for the evolution of behavioural resistance to insecticides (Von Keyserlingk, 1985; Haynes, 1988).

5.1.2 The effects of insecticides on the behaviour of aphids

The behaviour-modifying effects of insecticides have been reported in studies carried out with aphids (Lowery and Boiteau, 1988; Nauen, 1995; Nauen and Elbert, 1997; Boquel *et al*., 2015). The behaviour of aphids can be affected at both lethal and sub-lethal doses; the latter often arising through poor spray coverage and/or the decrease in the efficacy of insecticide residues after application. The types of behaviour modified by insecticides include aphid settling/dispersal, host selection, probing and feeding. At sub-lethal doses, reproductive behaviour may also be affected and there is more potential for the development of insecticide resistance through changes in behaviour (Moriarty 1969; Haynes, 1988; Fray *et al*., 2014).

With regard to neurotoxic effects, insecticides may stimulate or depress the locomotory behaviour of aphids, such as walking or flight. Insecticides may also disrupt coordination, lead to convulsion/tremors and/or cause paralysis of aphids (Lowery and Boiteau, 1988; Collar *et al*., 1997). These symptoms of intoxication may in turn affect aphid behaviour including; host-finding, dispersal, migration, probing/feeding and reproduction (Haynes, 1988).

Several studies have highlighted neurotoxic effects of insecticides on the behaviour of aphids. For example, Collar *et al*. (1997) assessed the behaviour of *M. persicae* on insecticide-treated pepper plants, *Capsicum annuum* L. (Solanaceae) and the subsequent transmission of potato virus Y (PVY). They found that cypermethrin (pyrethroid, 3A) reduced probing/feeding behaviour considerably by inducing paralysis within 2.5 min of exposure to the treated plants. Due to paralysis, aphids were unable to subsequently transmit PVY to healthy plants.

Neurotoxic effects on aphid behaviour were also reported by Zhu *et al.* (2010) for the novel insecticide, sulfoxaflor (Sulfoximine: 4C). Prior to death, the treatment caused excitatory symptoms in *M. persicae* such as tremors and paralysis. The rapid induction of intoxication would be likely to reduce the time aphids spend probing/feeding on treated plants. This may benefit the management of certain plant viruses.

5.1.3 The effects of insecticides on the settling and dispersal behaviour of aphids

The initial settling behaviour of aphids consists a series of brief probes on the upper leaf surface. If the plant is accepted, aphids often relocate to the lower leaf surface to probe/feed for prolonged periods of time. However, if the plant is rejected aphids will disperse after a few exploratory probes (Powell *et al*., 2006).

In several studies, aphids have been shown to actively modify their settling and dispersal behaviour in response to insecticides (Nauen, 1995; Nauen and Elbert, 1997). For example, Nauen (1995) found that *M. persicae* exhibited a significant migratory response away from leaves of cabbage, *Brassica oleracea* L., treated with imidacloprid at low concentrations (neonicotinoid, 4A) onto untreated leaves. Once on the untreated leaves aphids fed and deposited nymphs. Aphids that moved to imidacloprid-treated leaves from the untreated leaves settled only for a short duration before returning to untreated leaves. Fray *et al*. (2014) also observed behavioural 'avoidance' in *M. persicae* in response to neonicotinoids. They found that a neonicotinoid-resistant clone (FRC) spent less time on insecticide-treated leaves, compared with a susceptible clone (US1L). They suggested that increased dispersal away from treated plant material may confer some of the resistance of FRC aphids to neonicotinoids.

Similar avoidance responses have been observed for aphids exposed to pyrethroid insecticides, including *M. persicae* and *Aphis nasturtii* (Kaltenbach, 1843) (Hemiptera: Aphididae) to deltamethrin and fenvalerate (Lowery and Boiteau, 1988) and *Macrosiphum euphorbiae* (Thomas, 1878) (Hemiptera: Aphididae), *Rhopalosiphum padi* (Linnaeus, 1758) (Hemiptera: Aphididae), and *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) to lambda-cyhalothrin (Boquel *et al.,* 2015).

Conversely, Boiteau *et al*. (1985) found that when aldicarb (carbamate, 1A) was applied at a sub-lethal dose (LD50) to potato plants, there was a reduction in the dispersal behaviour of alate *M. persicae* compared with aphids that were not exposed to the treatment. Boiteau *et al*. (1985) speculated that a reduction in flight activity would affect the ability of *M. persicae* to transmit plant viruses. Insecticides that reduce settling behaviour and/or increase aphid dispersal, however, may limit the success of the management of plant viruses.

5.1.4 The effects of insecticides on the probing and feeding behaviour of aphids

The probing and feeding behaviour of aphids can result in acquisition and subsequent transmission of viruses from infected to susceptible plants (Pirone and Harris, 1977; Haynes, 1998; Fereres and Moreno, 2009). It is possible, however, to interfere with uptake and transmission of plant viruses by influencing the probing/feeding behaviour of aphids with insecticides and/or chemicals with anti-feedant properties (Haynes, 1988; Harrewijn and Kayser, 1997). Many studies have investigated the effects of insecticides on the probing and feeding behaviour of aphids. Several of these studies have reported a reduction, on treated plant material, in the time aphids spend probing and feeding (Lowery and Boiteau, 1988; Morita *et al*., 2007; Boquel *et al*., 2015). In some cases, a reduction in probing/feeding behaviour may help to manage the spread of non-persistent viruses by aphids; provided that insecticides do not disrupt settling behaviour and increase movement by aphids (Castle *et al*., 2009).

In some cases, a reduction in probing/feeding behaviour of aphids can be attributed to an increase in dispersal away from plants treated with insecticides (Lowery and Boiteau, 1988; Nauen, 1995; Nauen and Elbert, 1997; Fray *et al*., 2014; Boquel *et al*., 2015). Nevertheless, insecticides may reduce probing/feeding by aphids without modifying settling/dispersal behaviour.

For example, Lowery and Boiteau (1988) showed that the pyrethroid insecticides, deltamethrin and fenvalerate, reduce the duration that alate and apterous morphs of *M. persicae* and *Aphis nasturtii* feed on potato plants, by up to 77% compared with on the untreated plants. However, tests investigating the colonisation of pyrethroid-treated and untreated leaf discs found no repellent action on *M. persicae,* with similar numbers of aphids recorded on the treated and the untreated leaf discs.

Additionally, Morita *et al*. (2007) investigated the effect of the pyridine organic compound, flonicamid, on the feeding activity of five species of aphid; *M. persicae, Aphis gossypii* (Glover, 1877) (Hemiptera: Aphididae), *R. padi, Schizaphis graminum* (Rondani, 1852) (Hemiptera: Aphididae) and *Lipaphis erysimi* (Kaltenbach, 1843) (Hemiptera: Aphididae). For all species of aphid, there was a strong inhibition of salivation and phloem feeding after exposure to the insecticide. Cho *et al*. (2011) also examined the feeding behaviour of *M. persicae* in response to flonicamid applied to

Chinese cabbage (*Brassica rapa* L. cv. Chunchujeonguk) at a range of doses. At low doses, the insecticide did not have a notable impact on probing/feeding behaviour. However, higher doses of flonicamid inhibited phloem ingestion leading to starvation in *M. persicae*. Similarly, Boquel *et al*. (2015) showed that flonicamid reduced the proportion of *A. fabae* performing probing behaviour and instead increased the proportion of aphids resting or walking on potato plants.

Harrewijn and Kayser (1997) investigated the effect of pymetrozine (pyridine azomethine compound) on the feeding behaviour of several species of aphid (*A. fabae, M. euphorbiae, M. persicae* and *A. gossypii*) on various host plants. Initial 'choice' experiments did not find any deterrent or anti-feedant action for this treatment. Results from electrical penetration graph (EPG) studies, however, showed a disruption in the patterns of salivation/ingestion in response to pymetrozine, ultimately leading to an inhibition in probing behaviour. Instead of inducing general toxicity in aphids, Harrewijn and Kayser (1997) concluded that pymetrozine causes starvation by interfering with the nervous regulation of feeding behaviour in a selective manner.

5.1.5 The effects of insecticides on the behaviour of *Myzus persicae*: aims

The aims and hypotheses of this chapter were:

- Investigate the effects of insecticides on the initial settling and probing/feeding behaviour of alate *M. persicae* to test the hypothesis that insecticides with different modes of action differ in terms of their effects on aphid behaviour.

- Determine whether alate *M. persicae* has similar behavioural responses to insecticides when they are applied to three commercially-important host plants (Brussels sprout, carrot and lettuce).

- Examine whether the initial colonisation choice behaviour of alate *M. persicae* is affected on insecticide-treated surfaces in relation to the untreated control experiment.

- Identify treatments that may reduce or exacerbate the transmission of non-persistent plant viruses in relation to aphid settling behaviour.

5.2 The effects of insecticides on the behaviour of *Myzus persicae*: Materials and Methods

5.2.1 Insect and plant material

Parthenogenetic colonies of two clones of *Myzus persica*e (2050A and MP1S) were reared at 18°C, under a photoperiod of 16L:8D in the Insect Rearing Unit (IRU) at Warwick Crop Centre, School of Life Sciences, Wellesbourne. Both clones were maintained on Brussels sprout (*Brassica oleracea* var. *gemmifera* cv. Doric F1), carrot (*Dacus carota* cv. Nairobi F1) and lettuce (*Lactuca sativa* cv. Lobjoits Green Cos) in rearing cages (47.5 x 47.5 x 47.5 cm) with fine nylon mesh sides (Bugdorm-44545; Watkins and Doncaster Ltd., Herefordshire, UK). All plants were grown initially at 20°C, under a photoperiod of 16L:8D in pots (9 x 9 x 8 cm) containing M2 compost (Levington® medium grade sphagnum moss peat: Everris Limited, Ipswich, UK: pH 5.3–6.0; N = 192, P = 98, K = 319 mg/L).

All aphids were reared on host plants for at least two generations prior to use in the bioassays, unless stated otherwise. Three days before starting the experiments, alate adults were removed from the rearing cages. Newly-emerged alate adults were then collected using a fine paintbrush from the inner walls and ceiling of the rearing cages for use in the bioassays.

5.2.2 Insecticide application

For each insecticide (Table 5.1), whole plants of Brussels sprout cv. Doric F1, carrot cv. Nairobi F1 and lettuce cv. Lobjoits Green Cos (3–4 weeks after sowing) were dipped in an emulsion (300 ml) prepared at the field recommended dose as indicated by the manufacturers on the insecticide labels *e.g.* Hallmark Zeon® (lambda-cyhalothrin) at 0.1 L/ha (Brussels sprout and lettuce) or 0.15 L/ha (carrot) and Movento® (spirotetramat) at 0.5 L/ha (Brussels sprout and lettuce) or 0.3 L/ha (carrot) at concentrations of: Hallmark Zeon®: 0.3 ml/L (Brussels sprout and lettuce) 0.5 ml/L (carrot); Movento®: 1.7 ml/L (Brussels sprout and lettuce), 1 ml/L (carrot). Treatments were applied in the Pesticide Handling Unit (PHU) at Warwick Crop Centre, School of Life Sciences, Wellesbourne.

Table 5.1 – Characteristics of the insecticides tested in the 'choice' and the 'no choice' behaviour experiments with alate *Myzus persicae*.

Commercial or coded name	Active ingredient	IRAC group	Sub-group	Action
Hallmark Zeon®	lambda-cyhalothrin	3A	Pyrethroid	Contact/ feeding
Movento®	spirotetramat	23	Tetramic acid derivative (ketoenole)	Systemic
Plant Extract 1	–	N/A	–	Contact
Plant Extract 2	–	N/A	–	Contact
Systemic Insecticide 1	–	–	–	Systemic (with contact/feeding action)
Systemic Insecticide 2	–	–	–	Systemic (with contact/feeding action)

Plants were dipped, rather than sprayed, in order to provide an even coverage of the product on the plant surface. After the application of the test products, treated and untreated (control) plants (dipped in an equal amount of water) were left to dry for 1 h prior to starting the bioassays.

Resistance to pyrethroid insecticides (Esterase-R_3/kdr-SR) has been reported for the 2050A clone of *M. persicae* (Foster *et al*., 2002). When examining the effects of the pyrethroid insecticide, lambda-cyhalothrin (Hallmark Zeon®), a susceptible clone of *M. persicae* (MP1S) was used instead. No statistically significant differences were found between the behaviour of the MP1S clone and the 2050A clone (used in all other cases) on untreated plant material of Brussels sprout, carrot and lettuce in the leaf disc and cage experiments.

5.2.3 The effect of insecticides on the behaviour of *Myzus persicae*: 'choice' and 'no choice' experiments

Leaf disc 'arena' system

A series of leaf disc 'choice' and 'no choice' experiments (Table 5.2) were carried out to determine the settling and feeding behaviour of alate *M. persicae* (0–3 days old) with insecticide-treated and untreated leaf discs of the same host. Three host plants were investigated: Brussels sprout cv. Doric F1, carrot cv. Nairobi F1 and lettuce cv. Lobjoits Green Cos. To allow time for drying, all leaf discs were excised 1 h after application of the test product or after dipping plants in an equal amount of water in the case of the untreated (control) experiments. Following this, the method employed was similar to the *Leaf disc 'arena'* system: Chapter 3.2.3 (pp.89–91).

Table 5.2 – The different insecticides investigated during the choice' and 'no choice' experiments with alate *Myzus persicae.*

'Choice'	'No choice'
Lambda-cyhalothrin *vs.* untreated	Lambda-cyhalothrin
Spirotetramat *vs.* untreated	Spirotetramat
Plant Extract 1 *vs.* untreated	Plant Extract 1
Plant Extract 2 *vs.* untreated	Plant Extract 2
Systemic Insecticide 1 *vs.* untreated	Systemic Insecticide 1
Systemic Insecticide 2 *vs.* untreated	Systemic Insecticide 2
	Untreated

Alongside recording the main behaviour types exhibited by alate *M. persicae* (*e.g.* probing/feeding, resting or walking) intoxication and death were also recorded if they occurred during the experiments. Intoxication was separated into two distinct categories: (i) walking intoxicated (*i.e.* erratic movement of aphids or with the wings held abnormally/perpendicular to the abdomen) (ii) immobile intoxicated (*i.e.* unable to move due to paralysis or convulsion/tremors).

For each combination of insecticide and host plant, the total observation time was 216 min with 24 alate adults monitored (Table 5.2). 'No choice' control experiments with untreated leaf discs of the three plant host species were also carried out.

Cage system

Additionally, a series of cage 'choice' and 'no choice' experiments were carried out (Table 5.2) to examine aphid colonisation behaviour by focusing on the accumulation of alate *M. persicae* (0–3 days old) on insecticide-treated or untreated plants (Brussels sprout cv. Doric F1, carrot cv. Nairobi F1 and lettuce cv. Lobjoits Green Cos). The method used was similar to *Cage system*: Chapter 3.2.3 (pp.91–93), however, in these experiments 'choice' cages contained two insecticide-treated and two untreated plants of the same host species. The arrangement of the plants was alternated between replicates to take account of the different spatial orientations (Fig. 5.1).

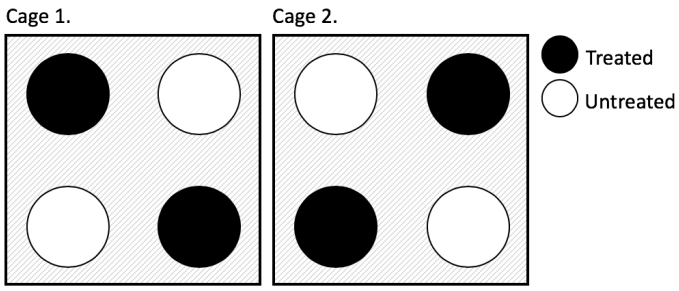

Figure 5.1 – Treatment layouts for 'choice' experiments examining the effects of six insecticides on the initial colonisation behaviour of alate *Myzus persicae* on three host plants (Brussels sprout, carrot and lettuce).

For each host plant, separate 'no choice' experiments were carried out with cages containing four treated plants. A total of 40 alate *M. persicae* was monitored during all 'choice' and 'no choice' experiments for each combination of the six insecticides and the three host plants.

5.3 The effects of insecticides on the behaviour of *Myzus persicae*: Results

5.3.1 Leaf disc 'choice' and 'no choice' experiments

When applied to leaf discs of Brussels sprout all insecticides, to varying extents, led to a reduction in the overall time alate *M. persicae* spent probing/feeding in 'no choice' experiments, compared to the untreated control experiment (68.5%) (Fig. 5.2).

The least time spent probing/feeding was observed on leaf discs treated with lambda-cyhalothrin (44.6%: Fig. 5.2A) followed by Plant Extract 2 (47.4%: Fig. 5.2D). In both cases, *M. persicae* spent a considerable amount of the time observed walking in the 'arenas' (45% and 37.1%, respectively) compared to the untreated control (18.8%). Both Systemic Insecticides 1 and 2 (Figs. 5.2E and F), led to a reduction in the time spent probing/feeding (to 51.5% and 48.7%, respectively) relative to the control due to the effects of convulsion/tremors, rendering intoxicated aphids immobile. Alate *M. persicae* displayed symptoms of intoxication for 18.4% and 21.8% of the time observed in 'no choice' 'arenas' containing leaf discs treated with Systemic Insecticide 1 and Systemic Insecticide 2, respectively. The only other insecticide to induce intoxication was lambda-cyhalothrin, which caused aphids to walk erratically for 11.7% of the time observed (Fig. 5.2A).

In 'arenas' with leaf discs treated with spirotetramat (Fig. 5.2B) and Plant Extract 1, (Fig. 5.2D) alate *M. persicae* displayed similar behaviour to aphids in the untreated control experiments, with only slight differences in the total amount of time aphids spent probing/feeding (< 7% reductions) and walking (< 10% increases).

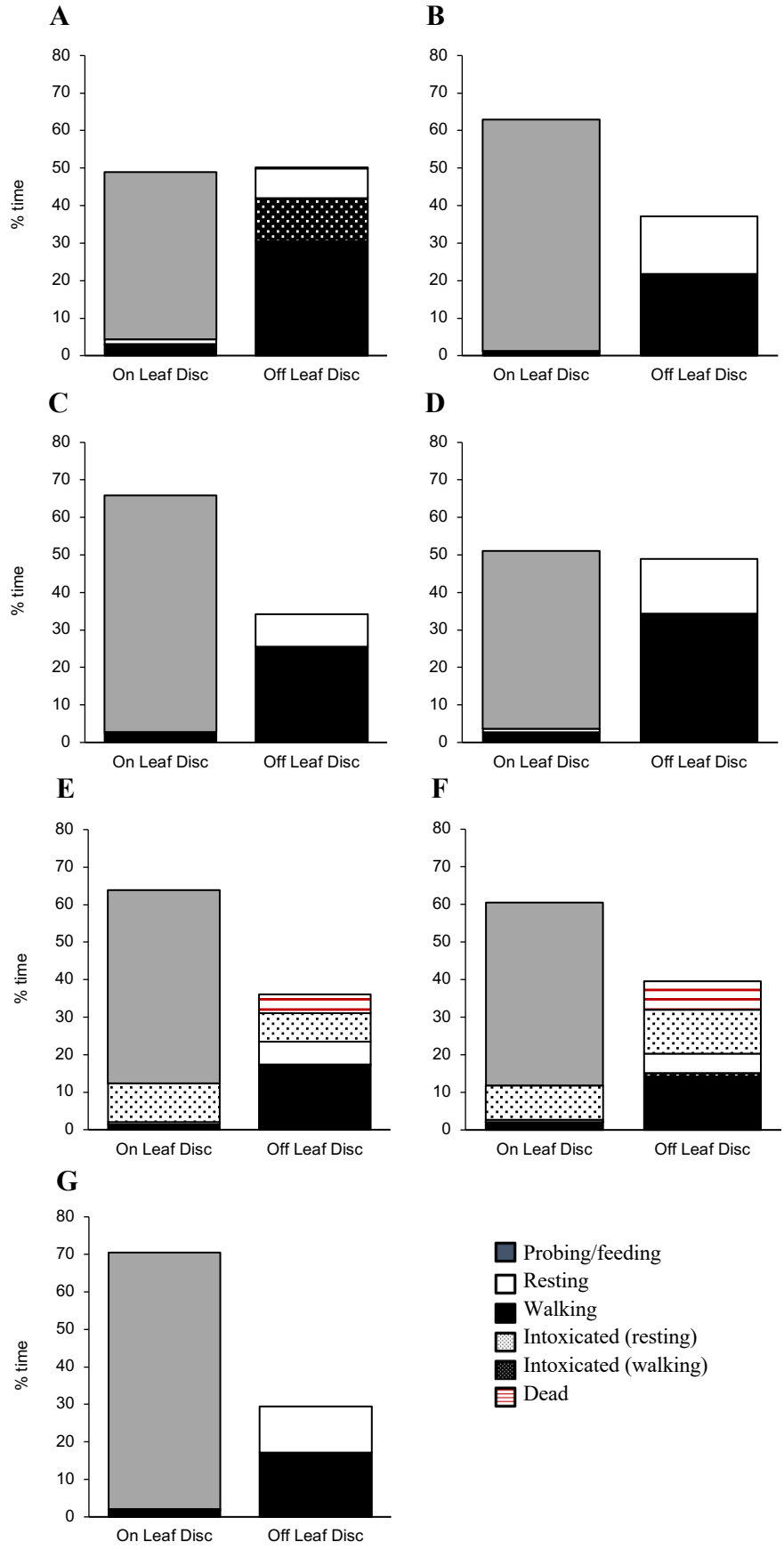

Figure 5.2 – Behaviour of alate *Myzus persicae* during the 'no choice' experiments with leaf discs treated with **(A)** lambda-cyhalothrin, **(B)** spirotetramat, **(C)** Plant Extract 1, **(D)** Plant Extract 2, **(E)** Systemic Insecticide 1, **(F)** Systemic Insecticide 2 and **(G)** the untreated control.

For each insecticide, the proportion of time *M. persicae* spent probing/feeding or walking during each 'no choice' experiment was compared to the untreated control with a binomial GLM (logit link function) (Table 5.3).

In the 'no choice' 'arenas' *M. persicae* spent significantly less time probing/feeding on Brussels sprout leaf discs treated with lambda-cyhalothrin, Plant Extract 2 and Systemic Insecticides 1 and 2. Spirotetramat and Plant Extract 1, however, did not significantly affect the time that aphids spent probing/feeding relative to the untreated control. There were also statistically significant differences in the time spent walking in 'no choice' 'arenas' for lambda-cyhalothrin (45%) and Plant Extracts 1 (27.9%) and 2 (37.1%) compared to the untreated control (18.8%).

Table 5.3 – Analysis (binomial GLM: logit link function) of the time alate *Myzus persicae* spent probing/feeding or walking in the 'no choice' experiments with insecticide-treated leaf discs of Brussels sprout compared with untreated control 'no choice' experiment.

Treatment	Behaviour	Coefficient	Std. error	95% Wald Confidence Interval		Wald chi-square	d.f.	P
				Lower	Upper			
Lambda-cyhalothrin	Probing/feeding	-0.996	0.231	-1.448	-0.543	18.572	1	**<0.001**
Spirotetramat	Probing/feeding	-0.301	0.2334	-0.759	0.156	1.668	1	0.197
Plant Extract 1	Probing/feeding	-0.242	0.2343	-0.701	0.217	1.064	1	0.302
Plant Extract 2	Probing/feeding	-0.881	0.2305	-1.333	-0.43	14.621	1	**<0.001**
Systemic Insecticide 1	Probing/feeding	-0.717	0.2304	-1.169	-0.265	9.68	1	**0.002**
Systemic Insecticide 2	Probing/feeding	-0.831	0.2304	-1.282	-0.379	13.002	1	**<0.001**
Lambda-cyhalothrin	Walking	1.259	0.2078	0.851	1.666	36.693	1	**<0.001**
Spirotetramat	Walking	0.25	0.223	-0.187	0.687	1.259	1	0.262
Plant Extract 1	Walking	0.512	0.2167	0.088	0.937	5.589	1	**0.018**
Plant Extract 2	Walking	0.932	0.2102	0.52	1.344	19.678	1	**<0.001**
Systemic Insecticide 1	Walking	-0.002	0.2311	-0.455	0.451	0	1	0.994
Systemic Insecticide 2	Walking	-0.107	0.2351	-0.568	0.354	0.208	1	0.649

Leaf disc 'no choice' experiments with carrot and lettuce plants

For several insecticides, similar effects on the behaviour of *M. persicae* were observed in 'arenas' containing leaf discs of carrot or lettuce (Tables 5.4 and 5.5). In the 'no choice' 'arenas' containing carrot or lettuce leaf discs treated with lambda-cyhalothrin, aphids again spent significantly more time walking (50.8% and 54.1%, respectively) and less time probing/feeding (19.9% and 12.7%, respectively) compared to the respective control experiments (carrot: probing/feeding: 31.8%; walking: 32.8% and lettuce: probing/feeding: 24.3%; walking: 40%).

As in the experiments with Brussels sprout, spirotetramat and Plant Extract 1 did not significantly affect the time aphids spent probing/feeding (carrot: 32.5% and 28.4%, respectively; lettuce: 25.7% and 22.5%) relative to the respective untreated control experiments. Unlike the experiments with Brussels sprout leaf discs; however, Plant Extract 1 did not significantly increase the time aphids spent walking when applied to carrot (42.8%) or lettuce (42.1%).

When applied to leaf discs of carrot and lettuce, Plant Extract 2 led to a statistically significant increase in the time aphids spent walking in 'no choice' 'arenas' (carrot 48.4% and lettuce: 54.5%), but only significantly reduced the time aphids spent probing/feeding on carrot leaf discs (carrot: 19.8%; lettuce: 15.1%) relative to the respective untreated control experiments.

Systemic Insecticides 1 and 2 did not affect the time *M. persicae* spent probing/feeding on leaf discs of carrot (24.9% and 25.5%, respectively) or lettuce (18.7% and 14.4%, respectively). However, both insecticides significantly reduced the time aphids spent walking when applied to carrot (21.5% and 21.4% respectively) and lettuce (25.4% and 26.1%, respectively) compared to the respective untreated control experiments. This was likely due to the induction of intoxication, which rendered some aphids immobile after probing/feeding.

Table 5.4 – Analysis (binomial GLM: logit link function) of the time alate *Myzus persicae* spent probing/feeding or walking in the 'no choice' experiments with insecticide-treated leaf discs of carrot compared with untreated control 'no choice' experiment.

Treatment	Behaviour	Coefficient	Std. error	95% Wald Confidence Interval		Wald chi-square	d.f.	P
				Lower	Upper			
Lambda-cyhalothrin	Probing/feeding	-0.627	0.2815	-1.178	-0.075	4.954	1	**0.026**
Spirotetramat	Probing/feeding	0.032	0.2585	-0.475	0.539	0.015	1	0.902
Plant Extract 1	Probing/feeding	-0.161	0.2635	-0.677	0.356	0.371	1	0.542
Plant Extract 2	Probing/feeding	-0.636	0.282	-1.189	-0.084	5.09	1	**0.024**
Systemic Insecticide 1	Probing/feeding	-0.341	0.2694	-0.869	0.187	1.605	1	0.205
Systemic Insecticide 2	Probing/feeding	-0.307	0.2682	-0.832	0.219	1.307	1	0.253
Lambda-cyhalothrin	Walking	0.753	0.2362	0.29	1.216	10.173	1	**0.001**
Spirotetramat	Walking	0.347	0.2381	-0.12	0.814	2.123	1	0.145
Plant Extract 1	Walking	0.431	0.2374	-0.034	0.896	3.295	1	0.069
Plant Extract 2	Walking	0.657	0.2363	0.194	1.12	7.727	1	**0.005**
Systemic Insecticide 1	Walking	-0.577	0.2822	-1.13	-0.024	4.184	1	**0.041**
Systemic Insecticide 2	Walking	-0.584	0.2618	-1.097	-0.071	4.972	1	**0.026**

Table 5.5 – Analysis (binomial GLM: logit link function) of the time alate *Myzus persicae* spent probing/feeding or walking in the 'no choice' experiments with insecticide-treated leaf discs of lettuce compared with untreated control 'no choice' experiment.

Treatment	Behaviour	Coefficient	Std. error	95% Wald Confidence Interval		Wald chi-square	d.f.	P
				Lower	Upper			
Lambda-cyhalothrin	Probing/feeding	-0.794	0.3939	-1.566	-0.021	4.058	1	**0.044**
Spirotetramat	Probing/feeding	0.076	0.3383	-0.587	0.739	0.051	1	0.822
Plant Extract 1	Probing/feeding	-0.1	0.3461	-0.778	0.578	0.083	1	0.773
Plant Extract 2	Probing/feeding	-0.594	0.3771	-1.333	0.145	2.484	1	0.115
Systemic Insecticide 1	Probing/feeding	-0.337	0.3592	-1.041	0.367	0.879	1	0.348
Systemic Insecticide 2	Probing/feeding	-0.643	0.3809	-1.389	0.104	2.848	1	0.091
Lambda-cyhalothrin	Walking	0.567	0.2233	0.13	1.005	6.458	1	**0.011**
Spirotetramat	Walking	0.18	0.2236	-0.258	0.618	0.648	1	0.421
Plant Extract 1	Walking	0.083	0.2243	-0.356	0.523	0.138	1	0.711
Plant Extract 2	Walking	0.585	0.2234	0.147	1.023	6.854	1	**0.009**
Systemic Insecticide 1	Walking	-0.671	0.2396	-1.141	-0.202	7.847	1	**0.005**
Systemic Insecticide 2	Walking	-0.636	0.2385	-1.103	-0.168	7.105	1	**0.008**

Analysis of time until 'lock-in'

To assess the settling behaviour of alate *M. persicae* during the leaf disc 'no choice' experiments, the time until 'lock-in' on insecticide-treated Brussels sprout leaf discs was compared to the untreated control experiment using Kaplan-Meier estimators with log-rank tests (Fig. 5.3). Statistically significant differences in the time alate *M. persicae* took to 'lock-in' were identified for all treatments except for Spirotetramat (log-rank test: $P = 0.075$) and Plant Extract 1 (log-rank test: $P = 0.162$). All insecticides, however, reduced the initial settling behaviour of alate *M. persicae*, with < 45% aphids 'locked-in' 1 h after placing alate adults into the leaf disc 'arenas' compared to the untreated control (66.7%). Two hours after infestation more aphids had 'locked-in' to feed on leaf discs treated with spirotetramat (66.7%) and Plant Extract 1 (70.8%) and the untreated control (79.1%). On leaf discs treated with lambda-cyhalothrin, only 58.3% aphids had 'locked-in' to feed 5 h after infestation compared to the untreated control (95.8%), whereas on leaf discs treated with Plants Extract 2, a considerable proportion of aphids 'locked-in' 3 h after infestation (62.5%) and by 5 h after infestation 83.3% had 'locked-in'.

Alate *M. persicae* were relatively quick to settle on leaf discs treated with Systemic Insecticides 1 and 2, however, these treatments induced high rates of intoxication and in several cases death within 5 h. For this reason, fewer aphids had 'locked-in' to feed on leaf discs at the end of 5 h study period compared to the untreated control. For both treatments, ≤ 20% alate *M. persicae* were 'locked-in' 5 h after being placed into leaf disc 'arenas'.

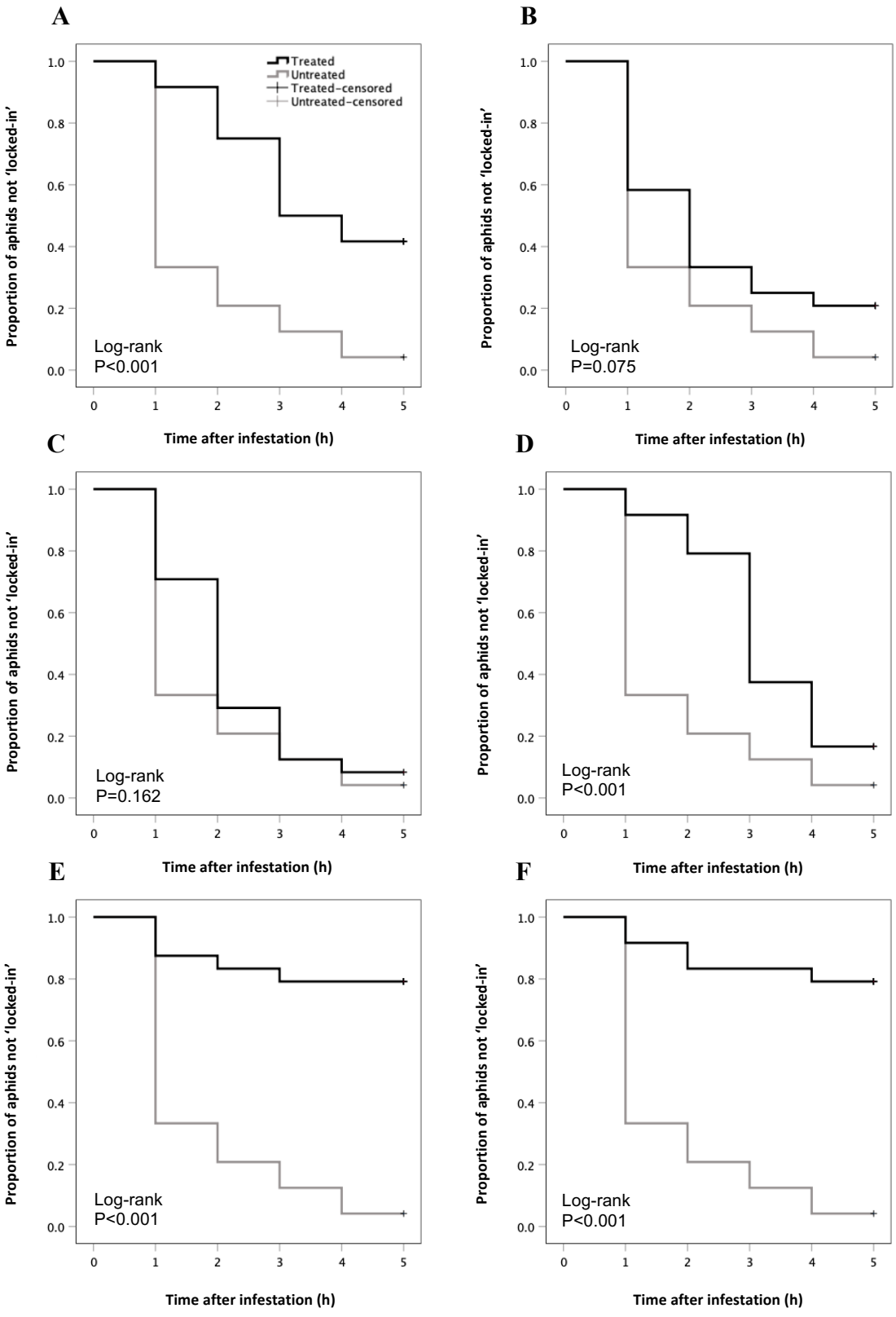

Figure 5.3 – Kaplan-Meier plots showing the effect of insecticides on the time until 'lock-in' on Brussels sprout leaf discs during the 'no choice' experiments with alate *Myzus persicae* for **(A)** lambda-cyhalothrin, **(B)** spirotetramat, **(C)** Plant Extract 1, **(D)** Plant Extract 2, **(E)** Systemic Insecticide 1 and **(F)** Systemic Insecticide 2.

Analysis of time until 'lock-in' on alternative host plants

The time alate *M. persicae* took to 'lock-in' on insecticide-treated leaf discs of carrot and lettuce during the various 'no choice' experiments were compared to the respective untreated control experiments using Kaplan-Meier estimators with log-rank tests (Table 5.6). Statistically significant differences in the time alate *M. persicae* took to 'lock-in' on leaf discs were found only when Systemic Insecticides 1 and 2 were applied to carrot (log-rank test: P = 0.001 and P = 0.013, respectively) and when Systemic Insecticide 1 was applied to lettuce (P = 0.016).

Table 5.6 – Log-rank test comparisons of the 'lock-in' curves for alate *Myzus persicae* during the 'no choice' experiments with insecticide-treated leaf discs and the respective untreated (control) leaf disc 'no choice' experiments for carrot and lettuce.

Host	Insecticide	d.f.	Chi-square	P
Carrot	Lambda-cyhalothrin	1	1.732	0.188
	Spirotetramat	1	0.105	0.746
	Plant Extract 1	1	0.027	0.87
	Plant Extract 2	1	0.081	0.776
	Systemic Insecticide 1	1	10.598	**0.001**
	Systemic Insecticide 2	1	6.12	**0.013**
Lettuce	Lambda-cyhalothrin	1	1.684	0.194
	Spirotetramat	1	0.071	0.79
	Plant Extract 1	1	0.255	0.614
	Plant Extract 2	1	0.057	0.812
	Systemic Insecticide 1	1	5.807	**0.016**
	Systemic Insecticide 2	1	2.8	0.094

The probing behaviour of Myzus persicae during leaf disc 'no choice' experiments

The mean numbers of short duration probes (< 1 min) performed by alate *M. persicae* during each leaf disc 'no choice' experiment are shown in Table 5.7. For each insecticide, the number of short duration probes on treated leaf discs was compared to the untreated control experiment using a Mann-Whitney U test. In 'no choice' 'arenas' with Brussels sprout and carrot leaf discs that were treated with lambda-cyhalothrin, there was a statistically significant increase in the number of short duration probes performed by aphids compared to the untreated control experiment. For all other treatment/host plant combinations a similar number of short duration probes were performed on leaf discs compared to the respective control experiments ($P > 0.05$).

Table 5.7 – Probing behaviour of alate *Myzus persicae* during leaf disc 'no choice' experiments with the different insecticides. All leaf disc 'no choice' experiments were compared to the untreated control experiment with a Mann-Whitney U test.

Host	Leaf disc 'no choice' experiment	Mean no. short duration probes	S.E.	U	P
Brussels sprout	Untreated control	2.5	0.83		
	Lambda-cyhalothrin	8.3	1.01	98.5	**<0.001**
	Spirotetramat	2.5	0.53	271.5	0.706
	Plant Extract 1	1.8	0.47	273	0.729
	Plant Extract 2	4.1	1.04	241	0.272
	Systemic Insecticide 1	2.6	0.47	260.5	0.543
	Systemic Insecticide 2	2.4	0.42	270	0.691
Carrot	Untreated control	4.4	0.98		
	Lambda-cyhalothrin	8.2	1.51	199	**0.05**
	Spirotetramat	4.8	0.93	256	0.495
	Plant Extract 1	4.1	0.64	274	0.775
	Plant Extract 2	4.7	0.88	256	0.493
	Systemic Insecticide 1	3.7	0.71	284	0.932
	Systemic Insecticide 2	4.2	0.70	271	0.724
Lettuce	Untreated control	7.5	2.27		
	Lambda-cyhalothrin	9.1	1.63	248	0.373
	Spirotetramat	7.5	0.93	221	0.151
	Plant Extract 1	5.7	1.32	281	0.872
	Plant Extract 2	6.6	0.87	239	0.292
	Systemic Insecticide 1	5.6	1.05	262	0.570
	Systemic Insecticide 2	6.9	0.8	231.5	0.223

Leaf disc 'choice' experiments

During the leaf disc 'choice' experiments alate *M. persicae* preferentially probed/fed on untreated Brussels sprout leaf discs compared to leaf discs treated with several of the test products (Fig. 5.4). Less time was spent probing/feeding on the leaf discs treated with lambda-cyhalothrin and Plant Extract 2 (15.2% and 16.6%, respectively) compared to on the untreated leaf discs (37.8% and 39.5%, respectively). Additionally, aphids spent a considerable amount of time (~30%) walking in these leaf disc 'arenas'.

Relative to the 'no choice' experiment with two untreated leaf discs, Systemic Insecticides 1 and 2, led to 16.7% and 22.1% reductions, respectively, in the total time spent probing/feeding in 'choice' 'arenas' most likely through the induction of intoxication after probing/feeding on treated leaf discs. However, there was little difference between the total number of aphids that chose to probe/feed on treated and untreated leaf discs in the 'choice' experiments.

Aphids spent a similar amount of time probing/feeding on untreated leaf discs and those treated with either spirotetramat or Plant Extract 1 during the 'choice' experiment. Unlike, in the 'no choice' 'arenas' there was not a statistically significant difference in the time *M. persicae* spent walking in the 'choice' 'arena' containing leaf discs treated with Plant Extract 1 relative to the untreated control experiment.

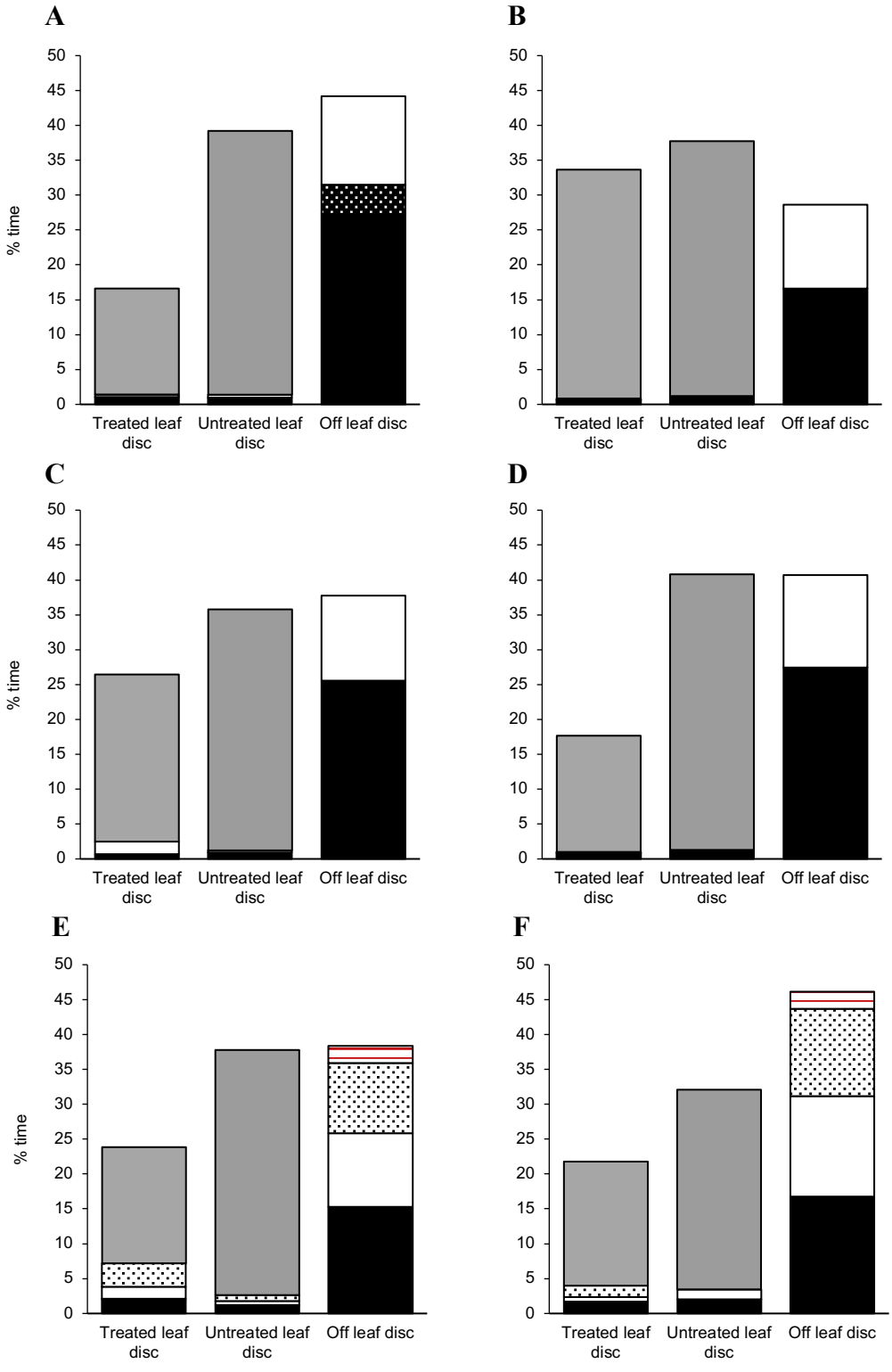

Figure 5.4 – Behaviour of alate *Myzus persicae* during the 'choice' experiments with leaf discs treated with **(A)** lambda-cyhalothrin, **(B)** spirotetramat, **(C)** Plant Extract 1, **(D)** Plant Extract 2, **(E)** Systemic Insecticide 1 and **(F)** Systemic Insecticide 2.

For each insecticide, the proportion of time *M. persicae* spent probing/feeding or walking during each 'choice' experiment was compared to the untreated 'no choice' control with a binomial GLM (logit link function).

In the 'choice' 'arenas' *M. persicae* spent significantly less time probing/feeding in 'arenas' that contained a leaf disc treated with lambda-cyhalothrin and Systemic Insecticides 1 and 2 (Table 5.8). Spirotetramat and Plant Extracts 1 and 2, however, did not significantly affect the time that aphids spent probing/feeding relative to the untreated 'no choice' control experiment.

As with the 'no choice' 'arenas', lambda-cyhalothrin and Plant Extract 2, led to a statistically significant increase in the time spent walking in 'choice' 'arenas' compared to the untreated 'no choice' control experiment.

Table 5.8 – Analysis (binomial GLM: logit link function) of the time alate *Myzus persicae* spent probing/feeding or walking in the 'choice' experiments with insecticide-treated Brussels sprout leaf discs compared with untreated control 'no choice' experiment.

Treatment	Behaviour	Coefficient	Std. error	95% Wald Confidence Interval		Wald chi-square	d.f.	P
				Lower	Upper			
Lambda-cyhalothrin	Probing/feeding	-0.657	0.3131	-1.271	-0.043	4.399	1	**0.036**
Spirotetramat	Probing/feeding	0.039	0.3254	-0.599	0.677	0.014	1	0.905
Plant Extract 1	Probing/feeding	-0.434	0.315	-1.051	0.184	1.894	1	0.169
Plant Extract 2	Probing/feeding	-0.529	0.314	-1.144	0.086	2.838	1	0.092
Systemic Insecticide 1	Probing/feeding	-0.706	0.313	-1.319	-0.092	5.086	1	**0.024**
Systemic Insecticide 2	Probing/feeding	-0.921	0.3133	-1.535	-0.307	8.646	1	**0.003**
Lambda-cyhalothrin	Walking	0.778	0.2562	0.276	1.28	9.229	1	**0.002**
Spirotetramat	Walking	-0.042	0.281	-0.593	0.508	0.023	1	0.88
Plant Extract 1	Walking	0.475	0.2627	-0.04	0.99	3.274	1	0.07
Plant Extract 2	Walking	0.58	0.2602	0.07	1.09	4.963	1	**0.026**
Systemic Insecticide 1	Walking	-0.01	0.2795	-0.558	0.538	0.001	1	0.971
Systemic Insecticide 2	Walking	0.102	0.2749	-0.437	0.641	0.138	1	0.71

5.3.2 Cage 'choice' and 'no choice' experiments

In the cage 'choice' experiments with Brussels sprout plants, alate *M. persicae* accumulated in greater numbers on the untreated plants compared to the treated plants for the six insecticides tested (Fig. 5.5). After 5 h, the numbers of alate adults on the treated and untreated plants differed the most for Plant Extract 2 (treated: 20 ±4.1%, untreated: 45 ±2.9%, off plant: 32.5 ±4.8%) and Systemic Insecticide 2 (treated: 22.5 ±2.5%, untreated: 42.5 ±2.5%, off plant: 22.5 ±2.5%). For the other insecticides, there was a difference of < 20% in the numbers of aphids accumulated on the treated and untreated plants after 5 h (lambda-cyhalothrin: treated: 20%, untreated: 37.5 ±2.5%, off plant: 40 ±4.1%; spirotetramat: treated: 37.5 ±4.8%, untreated: 42.5 ±2.5%, off plant: 20 ±4.1%; Plant Extract 1: treated: 30 ±7.1%, untreated: 42.5 ±4.8%, off plant: 27.5 ±11.1% and Systemic Insecticide 1: treated: 27.5 ±2.5%, untreated: 40 ±4.1%, off plant: 22.5 ±2.5%).

For alate *M. persicae* that accumulated on plants, Chi-square (χ^2) goodness of fit tests were used to compare the numbers on either the treated or untreated plants after 5 h. A statistically significant difference was found for Plant Extract 2 (χ^2 = 3.846, d.f. = 1, P = 0.05). For all other insecticides, there was not a statistically significant difference: lambda-cyhalothrin (χ^2 = 2.13, d.f. = 1, P = 0.144), spirotetramat (χ^2 = 0.125 d.f. = 1, P = 0.724), Plant Extract 1 (χ^2 = 0.862, d.f. = 1, P = 0.353) and Systemic Insecticides 1 (χ^2 = 0.926, d.f. = 1, P = 0.336) and 2 (χ^2 = 2.462, d.f. = 1, P = 0.117).

However, compared to the 'no choice' experiment with only untreated (control) plants, fewer *M. persicae* (< 80%) settled on plants after 5 h in all 'choice' cages containing plants treated with the different insecticides. Statistically significant differences were found using independent samples t-tests for lambda-cyhalothrin (57.5 ±2.5%; t = 4.7, d.f. = 6, P = 0.003), Plant Extract 2 (65 ±2.89%; t = 3, d.f. = 6, P = 0.024), Systemic Insecticide 1 (67.5 ±2.5%; t = 2.611, d.f. = 6, P = 0.04) and Systemic Insecticide 2 (65 ±2.89%; t = 3, d.f. = 6, P = 0.024).

Aphid mortality was observed in 'choice' cages containing plants treated with lambda-cyhalothrin (2.5 ±2.5%), Plant Extract 2 (2.5 ±2.5%) and Systemic Insecticides 1 (10 ±4.1%) and 2 (12.5 ±2.5%).

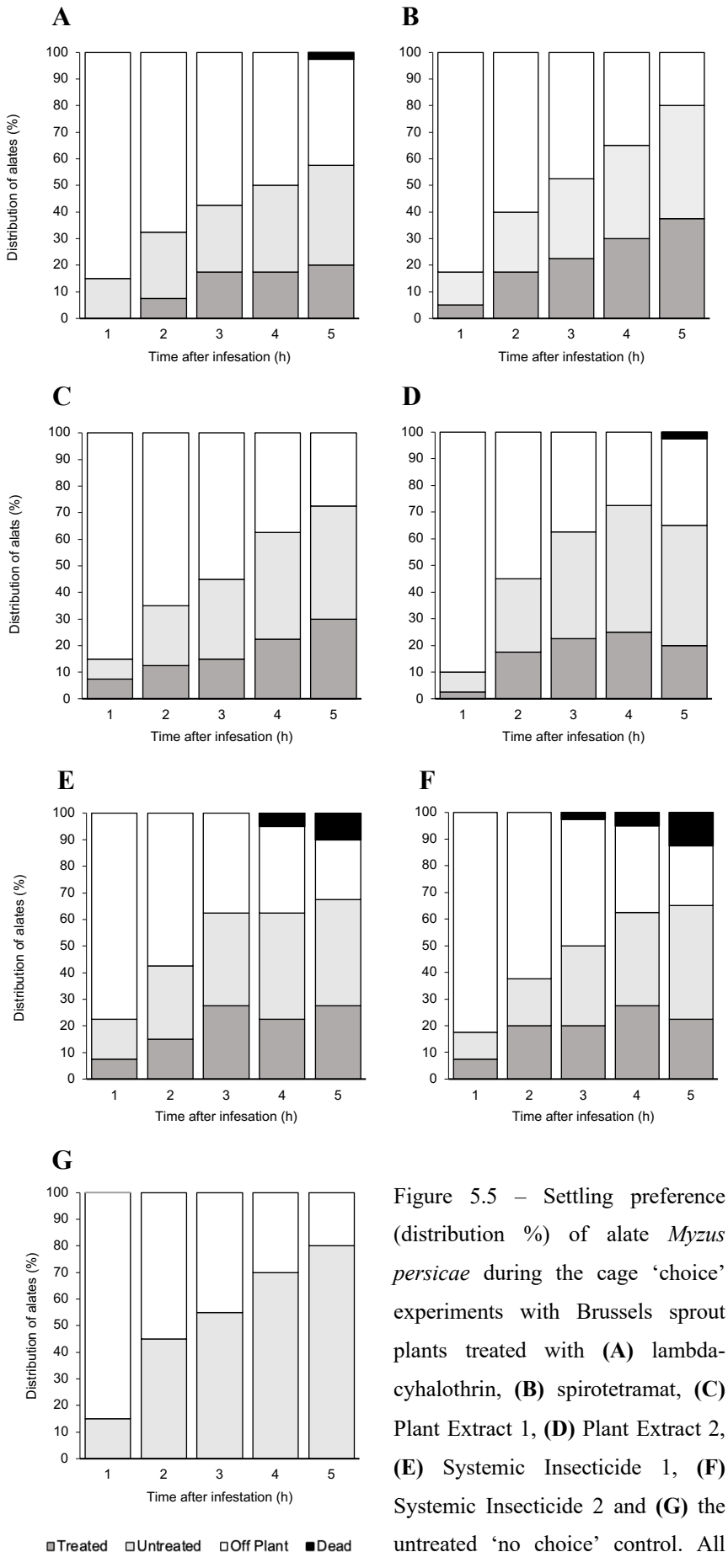

Figure 5.5 – Settling preference (distribution %) of alate *Myzus persicae* during the cage 'choice' experiments with Brussels sprout plants treated with **(A)** lambda-cyhalothrin, **(B)** spirotetramat, **(C)** Plant Extract 1, **(D)** Plant Extract 2, **(E)** Systemic Insecticide 1, **(F)** Systemic Insecticide 2 and **(G)** the untreated 'no choice' control. All aphids were reared on Brussels sprout cv. Doric F1.

Cage 'choice' experiments with carrot and lettuce plants

As with the cage 'choice' experiments with Brussels sprout plants, alate *M. persicae* accumulated in greater numbers on the untreated carrot and lettuce plants compared to the those treated with the six test products (Table 5.9). However, for both host plants, statistically significant differences were found only between the number of alate *M. persicae* accumulated on the untreated plants and plants treated with lambda-cyhalothrin (carrot: $\chi^2 = 4.571$, d.f. = 1, P = 0.033; lettuce: $\chi^2 = 4$, d.f. = 1, P = 0.046) and Plant Extract 2 (carrot: $\chi^2 = 3.769$, d.f. = 1, P = 0.05; lettuce: $\chi^2 = 4.263$, d.f. = 1, P = 0.039). Compared to the respective 'no choice' experiments with only untreated (control) plants, after 5 h fewer alate *M. persicae* settled in the 'choice' cages with carrot plants treated with Plant Extract 2 (55 ±6.5% and 32.5 ±6.3%, respectively) (independent samples t-test: carrot: $t = 2.496$, d.f. = 6, P = 0.047). No statistically significant differences were found for any of the other treatment and host plant combinations (P > 0.05).

Table 5.9 – Settling preference (distribution %) of alate *Myzus persicae* on carrot and lettuce plants treated with the different insecticides during the cage 'choice' experiments. The respective untreated control ('no choice') experiments are shown for comparison.

Host	Treatment	Mean % aphids on treated plants after 5h	Mean % aphids on untreated plants after 5h	Mean % aphids 'off plants' after 5h	% mortality
Carrot	Untreated control	-	55 ±6.5%	45 ±6.5%	0
	Lambda-cyhalothrin	7.5 ±2.5%	27.5 ±4.8%	62.5 ±4.8%	2.5 ±2.5
	Spirotetramat	27.5 ±2.5%	30 ±4.1%	42.5 ±4.8%	0
	Plant Extract 1	25 ±2.9%	30 ±4.1%	45 ±6.5%	0
	Plant Extract 2	7.5 ±4.8%	25 ±2.9%	65 ±5%	2.5 ±2.5%
	Systemic Insecticide 1	20%	22.5 ±4.8%	50 ±4.1%	7.5 ±2.5%
	Systemic Insecticide 2	15 ±6.5%	25 ±2.9%	52.5 ±2.5%	7.5 ±2.5%
Lettuce	Untreated control	-	50 ±4.1%	50 ±4.1%	0
	Lambda-cyhalothrin	10 ±4.1%	30 ±4.1%	57.5 ±2.5%	2.5 ±2.5%
	Spirotetramat	22.5 ±4.8%	25 ±2.9%	52.5 ±6.3%	0
	Plant Extract 1	17.5 ±4.8%	30 ±4.1%	52.5 ±4.8%	0
	Plant Extract 2	12.5 ±4.8%	35 ±5%	50 ±4.1%	2.5 ±2.5%
	Systemic Insecticide 1	20 ±4.1%	25 ±6.5%	50 ±4.1%	5 ±2.9%
	Systemic Insecticide 2	17.5 ±4.8%	20 ±4.1%	55 ±6.5%	7.5 ±2.5%

Cage 'no choice' experiments

For each insecticide, the mean numbers of alate *M. persicae* which had accumulated on treated Brussels sprout plants 5h after infestation in the cage 'no choice' experiments were compared to the untreated control using an independent samples t-test.

On untreated (control) plants a mean of > 50% aphids had accumulated 3 h after infestation and a mean of 80 ±4.1% aphids had accumulated on plants 5 h after infestation (Fig. 5.6G). With the exception of spirotetramat (Fig. 5.6B; 70 ±4.1% aphids accumulated on plants after 5h; $t = 1.732$, d.f. = 6, P = 0.134), all insecticides led to a statistically significant reduction in the settling behaviour of alate *M. persicae* on Brussels sprout plants compared to the untreated control experiment (Fig 5.6). A mean of 40–50% of the alate adults had accumulated on treated plants 5 h after infestation: lambda-cyhalothrin (42.5 ±4.8%; $t = 5.960$, d.f. = 6, P = 0.001), Plant Extract 1 (50 ±4.1%; $t = 5.196$, d.f. = 6, P = 0.002), Plant Extract 2 (45 ±6.5%; $t = 4.583$, d.f. = 6, P = 0.004), Systemic Insecticide 1 (40 ±4.1%; $t = 6.928$, d.f. = 6, P < 0.001) and Systemic Insecticide 2 (42.5 ±6.3%; $t = 5$, d.f. = 6, P = 0.002).

Compared to the untreated control experiments, the statistically significant differences obtained for Systemic Insecticides 1 and 2 are likely to result from an increase in the mortality of *M. persicae* when exposed to these treatments (Systemic Insecticide 1 = 32.5 ±2.5% mortality and Systemic Insecticide 2 = 27.5 ±2.5% mortality).

Considerably lower mortality, however, was observed in cages containing plants treated with lambda-cyhalothrin (2.5 ±2.5% mortality), Plant Extract 1 (2.5 ±2.5% mortality) and Plant Extract 2 (5% ±2.9% mortality) and no mortality was observed in cages containing plants treated with spirotetramat or in the untreated control.

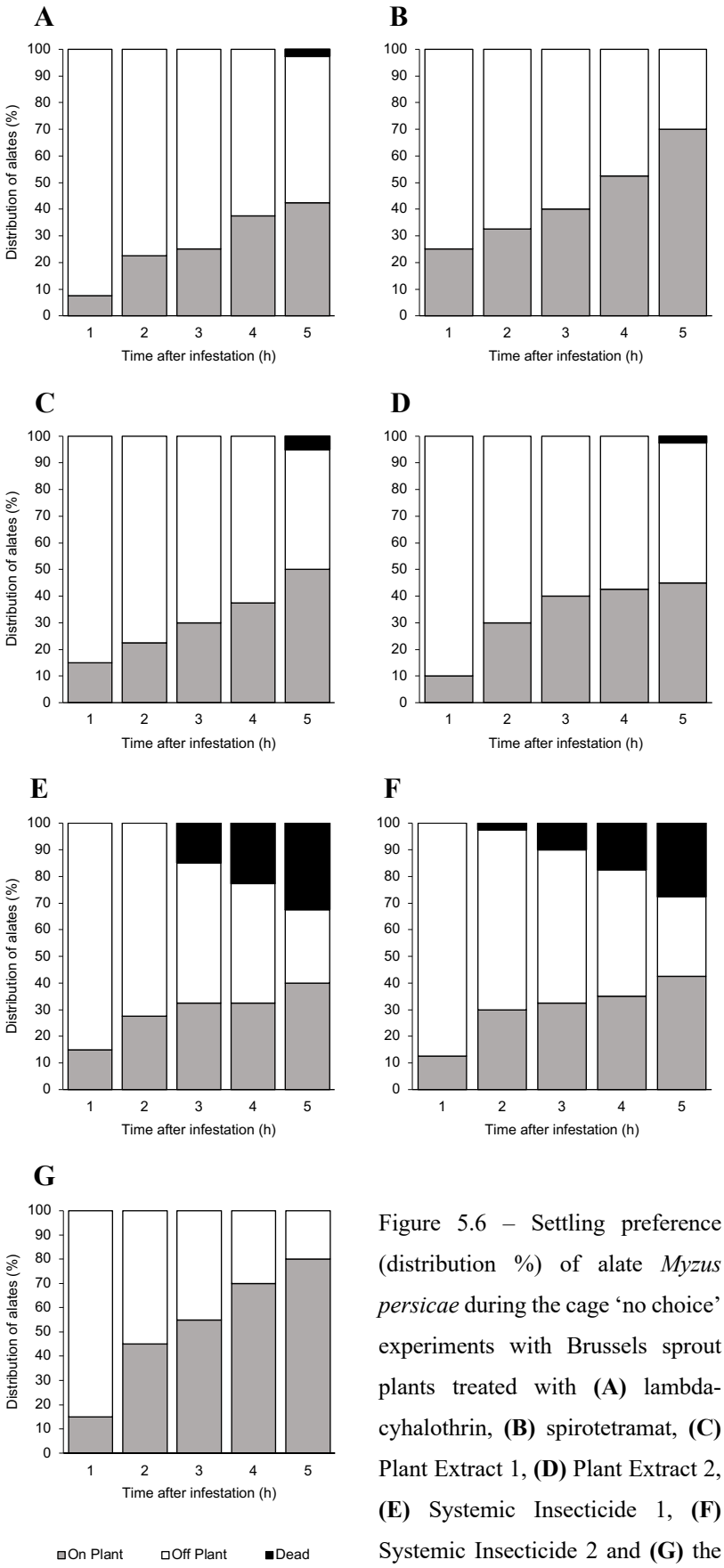

Figure 5.6 – Settling preference (distribution %) of alate *Myzus persicae* during the cage 'no choice' experiments with Brussels sprout plants treated with **(A)** lambda-cyhalothrin, **(B)** spirotetramat, **(C)** Plant Extract 1, **(D)** Plant Extract 2, **(E)** Systemic Insecticide 1, **(F)** Systemic Insecticide 2 and **(G)** the untreated control. All aphids were reared on Brussels sprout cv. Doric F1.

Cage 'no choice' experiments with carrot and lettuce plants

Similar effects were observed on carrot and lettuce plants treated with lambda-cyhalothrin during cage 'no choice' experiments (Table 5.10). Compared to the respective untreated control experiments, there was a > 20% reduction in the mean number of alate *M. persicae* accumulated on treated plants after 5 h (independent samples t-tests; carrot: P = 0.047, lettuce P = 0.017) (Table 5.10). While three insecticides (spirotetramat, Plant Extracts 1 and 2) led to slight reductions in the mean number of alate *M. persicae* accumulated on carrot and lettuce plants after 5 h, these numbers did not significantly differ to those on untreated control plants (P > 0.05). On carrot plants treated with Systemic Insecticides 1 and 2, there were statistically significant reductions (> 20%) in the mean number of aphids accumulated on plants after 5 h compared to the untreated control. However, no statistically significant differences were found when these treatments were applied to lettuce plants compared to untreated (control) 'no choice' experiments.

Table 5.10 – Settling preference (distribution %) of alate *Myzus persicae* on carrot and lettuce plants treated with the different insecticides during the cage 'no choice' experiments. All cage 'no choice' experiments were compared to the respective untreated control experiment with independent samples t-tests.

Host	Insecticide	Mean % aphids on plants after 5h	Std. Error	*t*	d.f.	P
Carrot	Untreated (control)	55%	6.45			
	Lambda-cyhalothrin	32.5%	6.29	2.496	6	**0.047**
	Spirotetramat	50%	4.08	0.655	6	0.537
	Plant Extract 1	47.5%	6.29	0.832	6	0.437
	Plant Extract 2	40%	10.41	1.633	6	0.154
	Systemic Insecticide 1	30%	4.08	3.273	6	**0.017**
	Systemic Insecticide 2	32.5%	4.79	2.800	6	**0.031**
Lettuce	Untreated (control)	50%	4.08			
	Lambda-cyhalothrin	25%	6.45	3.273	6	**0.017**
	Spirotetramat	47.5%	4.79	0.397	6	0.705
	Plant Extract 1	45%	6.45	0.655	6	0.537
	Plant Extract 2	37.5%	8.54	1.321	6	0.235
	Systemic Insecticide 1	32.5%	7.50	2.049	6	0.086
	Systemic Insecticide 2	37.5%	6.29	1.667	6	0.147

Settling behaviour of Myzus persicae on Brussels sprout plants treated with insecticides

The settling behaviour of alate *M. persicae* on treated plants during the cage 'no choice' experiments was assessed using Kaplan-Meier estimators. Each insecticide and host plant combination was compared to the respective untreated (control) experiment with a log-rank test (Table 5.9).

With the exception of spirotetramat, the settling time of *M. persicae* significantly differed in 'no choice' cages containing insecticide-treated Brussels sprout plants compared to the untreated control (Fig. 5.7). For these insecticides, fewer alate *M. persicae* accumulated on the treated plants and, in some cases, were considerably slower to settle.

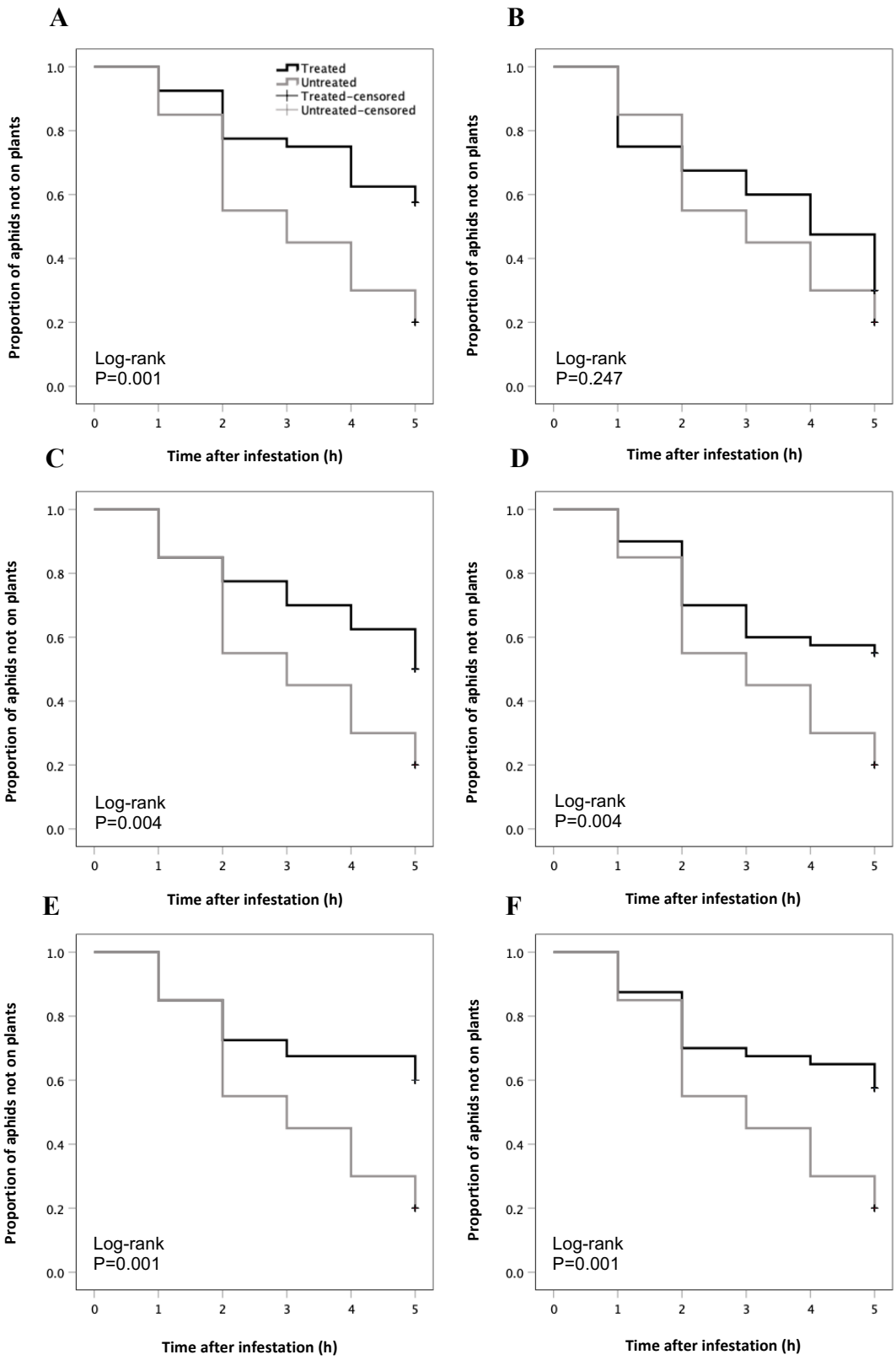

Figure 5.7 – Kaplan-Meier plots showing the effect of insecticide treatment relative to the untreated (control) on the settling time of alate *Myzus persicae* on Brussels sprout plants treated with **(A)** lambda-cyhalothrin, **(B)** spirotetramat, **(C)** Plant Extract 1, **(D)** Plant Extract 2, **(E)** Systemic Insecticide 1 and **(F)** Systemic Insecticide 2 during the cage 'no choice' experiments.

Settling behaviour of Myzus persicae on carrot and lettuce plants treated with insecticides

Compared to the respective control 'no choice' experiments with only untreated carrot or lettuce plants, a statistically significant difference was found only for the settling time of alate *M. persicae* on lettuce plants treated with lambda-cyhalothrin (Table 5.11; P = 0.036).

Table 5.11 – Log-rank test comparisons of the settling curves for alate *Myzus persicae* during the cage 'no choice' experiments with insecticide-treated host plants (Brussels sprout, carrot and lettuce) and the respective untreated (control) experiments.

Host	Insecticide	d.f.	Chi-square	P
Brussels sprout	Lambda-cyhalothrin	1	11.871	**0.001**
	Spirotetramat	1	1.341	0.247
	Plant Extract 1	1	8.097	**0.004**
	Plant Extract 2	1	8.207	**0.004**
	Systemic Insecticide 1	1	11.09	**0.001**
	Systemic Insecticide 2	1	10.114	**0.001**
Carrot	Lambda-cyhalothrin	1	3.322	0.068
	Spirotetramat	1	0.063	0.802
	Plant Extract 1	1	0.18	0.672
	Plant Extract 2	1	2.674	0.102
	Systemic Insecticide 1	1	3.467	0.063
	Systemic Insecticide 2	1	2.821	0.093
Lettuce	Lambda-cyhalothrin	1	4.408	**0.036**
	Spirotetramat	1	0.002	0.962
	Plant Extract 1	1	0.07	0.792
	Plant Extract 2	1	0.812	0.367
	Systemic Insecticide 1	1	1.396	0.237
	Systemic Insecticide 2	1	0.685	0.408

5.4 The effects of insecticides on the behaviour of *Myzus persicae*: Discussion

5.4.1 The effects of insecticides on the behaviour of alate *Myzus persicae*

The probing/feeding and settling behaviour of alate *M. persicae* was affected by the application of several test products that were investigated during 'choice' and 'no choice' experiments. While Systemic Insecticides 1 and 2 affected the behaviour of alate *M. persicae* through neurotoxicity, there was some evidence to suggest that several of the test products had an effect on aphid behaviour due to the sensory perception of the insecticides.

Lambda-cyhalothrin

Lambda-cyhalothrin reduced the time *M. persicae* spent probing/feeding on the three host plants (Brussels sprout, carrot and lettuce) compared to the respective untreated control experiments. In leaf disc 'arenas' and cages containing plant material treated with lambda-cyhalothrin aphids displayed 'restless' behaviour and spent a considerable amount of time walking in 'arenas' or on the walls/ceiling in cages. This was observed both in 'choice' and 'no choice' experiments. This behaviour may relate to the reported repellent properties of lambda-cyhalothrin. Nevertheless, for other species of insect, there have been varying reports regarding the repellency of lambda-cyhalothrin. For example, repellent effects could not be demonstrated consistently for lambda-cyhalothrin against the German cockroach, *Blattella germanica* L. (Blattodea: Ectobiidae) and the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Wege *et al*., 1999). Whereas, the repellent effects against the biting midge, *Culicoides imicola* (Kieffer, 1931) (Diptera: Ceratopogonidae) were reported to last only up to 1 h (Braverman *et al*., 2004). For aphids, several studies have reported that pyrethroid insecticides, like lambda-cyhalothrin, affect the behaviour largely through intoxication. For example, upon the exposure to pyrethroids, many apparent cases of repellency can be explained instead by the rapid induction of excitatory symptoms (Gibson *et al*., 1982b; Rice *et al*., 1983; Lowery and Boiteau, 1988).

The results from the cage experiments in this study did not provide any evidence for repellent properties as similar numbers of alate *M. persicae* accumulated on the treated plants and the untreated plants after 5 h. However, there was an increase in the time

alate *M. persicae* took to accumulate on Brussels sprout and lettuce plants that were treated with lambda-cyhalothrin. It is possible that this behaviour could simply relate to a reduction in settling behaviour, potentially through the induction of excitatory symptoms, after the alate adults made contact with the insecticide.

Lambda-cyhalothrin induced a moderate level of intoxication during 'choice' and 'no choice' experiments, notably in the leaf disc 'arenas' where aphids were observed walking erratically. On leaf discs treated with lambda-cyhalothrin, intoxication led to the arrest of sustained feeding behaviour before mortality was induced. Previously, an inhibition in feeding has been reported as an initial symptom of intoxication though the contact action of lambda-cyhalothrin (Perrin, 1985). In the experiments in this study, intoxicated aphids were still capable of walking and probing. Sustained feeding, however, was reduced in intoxicated individuals. This may explain why alate adults performed more short duration probes (< 1 min) when lambda-cyhalothrin was applied to leaf discs of Brussels sprout and carrot compared to the respective untreated control experiments. On leaf discs of lettuce treated with lambda-cyhalothrin, the number of short duration probes performed did not significantly differ from the untreated control. This may be explained by the fact that aphids spent less time probing/feeding on treated leaf discs of lettuce, giving rise to a lower total number of intoxicated aphids (12.5%) compared to in Brussels sprout 'arenas' (41.7%).

To clarify whether the observed changes in the behaviour of *M. persicae* were due to intoxication (*i.e.* excitatory symptoms) rather than repellency, behavioural assays could be repeated with the pyrethroid-resistant clone of *M. persicae* (2050A). In the absence of intoxication, aphids would be expected to behave in a similar manner to those in the untreated control experiment.

It is possible that the 'restless' behaviour induced by the application of lambda-cyhalothrin may have implications in terms of the transmission of plant viruses. For example, it has been reported that insecticides that reduce settling behaviour and/or increase aphid dispersal may limit the success of the management of plant viruses by increasing the contact made between viruliferous aphids and susceptible host plants (Roberts *et al*., 1993). An increase in probing behaviour could also prove problematic, particularly for the management of non-persistent viruses.

Spirotetramat

Of the six insecticides investigated, spirotetramat had the least effect on the behaviour of alate *M. persicae* relative to the untreated control experiments. While there were some reductions in the time spent probing/feeding in the leaf disc experiments and the numbers of aphids accumulated on plants during cage experiments, these did not significantly differ from the untreated control experiments with the three species of host plants. Kaplan-Meier plots produced for spirotetramat displayed similar settling curves to the untreated controls in both the leaf disc (time until 'lock-in') and the cage experiments (time until aphids settled on plants).

Additionally, when applied to the three host plants spirotetramat did not significantly affect the time aphids spent walking relative to the untreated control experiments. These findings suggest that spirotetramat has little/no repellent or anti-feedant properties. It is likely that *M. persicae* was either unable to detect this insecticide on a plant surface or it was undeterred by its presence.

Spirotetramat did not cause any intoxication or death within 5 h. This is likely to relate to the slower mode action (ACC inhibitor/interruption of lipid biosynthesis) of the insecticide (Bretschneider *et al*., 2007; Nauen *et al*., 2008). The insecticide also requires time for conversion to the active enol-form and the subsequent translocation throughout the plant (Nauen *et al*., 2008). For these reasons, after exposure to the treatment there is often a lag period before considerable insecticidal effects are observed.

These properties would make spirotetramat unsuitable for the management of non-persistent plant viruses. However, as the application of spirotetramat did not appear to reduce the settling behaviour of *M. persicae,* the use of spirotetramat may be beneficial once aphid populations are already established in crops to prevent a disruption to settling behaviour and/or the redistribution of aphids within crops.

Plant Extract 1

Plant Extract 1 displayed considerable variation with regard to its effects on the behaviour of alate *M. persicae* during the 'choice' and 'no choice' experiments. When applied to the three host plants, Plant Extract 1 did not significantly affect the time *M.*

persicae spent probing/feeding on leaf discs relative to the untreated control experiments. However, when applied to Brussels sprout, but not to carrot and lettuce, Plant Extract l considerably increased the time aphids spent walking in the leaf disc 'no choice' 'arenas' relative to the untreated control.

The difference between plant species may relate to the host preference of *M. persicae* and/or the volatility of Plant Extract 1. For example, it was previously found that *M. persicae* spent considerably more time walking during the first few hours (exhibiting 'restless' behaviour) when presented with 'less-preferred' host plants (*i.e*. carrot and lettuce) compared to Brussels sprout. It is possible that by the time aphids decide to feed on leaf discs of carrot or lettuce they have become habituated to any volatiles present in the leaf disc 'arenas'. This would explain why Plant Extract 1 had no overall effect on the time aphids spent walking in carrot and lettuce 'arenas'. This may also explain why 1 h after placing the aphids into leaf disc 'arenas' with Brussels sprout, only 29.2% of aphids had 'locked-in' to leaf discs compared to 66.7% during the untreated control experiment. Two hours after placing aphids into Brussels sprout 'arenas' a similar number of aphids had 'locked-in' to feed on leaf discs in both the treated and untreated 'no choice' experiments (70.8% and 79.2%, respectively).

In the 'choice' cages, after 5 h a similar number of aphids accumulated on untreated plants and those treated with Plant Extract 1 for the three different host species. However, in 'no choice' experiments significantly fewer aphids accumulated on Brussels sprout plants treated with Plant Extract 1 relative to the 'no choice' experiment with only untreated plants. There was also a statistically significant difference in the time *M. persicae* took to settle on Brussels sprout plants when Plant Extract 1 was applied compared to the untreated control experiment. If volatiles are responsible for the disruption in the settling behaviour of *M. persicae* by Plant Extract 1, their concentration would be notably higher (approximately two-fold) in 'no choice' cages that contained four treated plants compared to only two treated plants in 'choice' cages. In the cage 'no choice' experiments with carrot and lettuce plants, however, no statistically significant differences were found in the number of aphids accumulated on treated plants 5 h after infestation compared with the respective untreated control experiments. The reason for this is unclear, but could again relate to the relative settling time of *M. persicae* on these host plants compared to on Brussels sprout.

Overall, there is evidence to suggest that Plant Extract 1 leads to an initial reduction in the settling behaviour of *M. persicae*. These effects were most noticeable when *M. persicae* was first placed into leaf disc 'arenas' (0–1 h) or released into cages (1–2 h).

Plant Extract 2

With regard to the leaf disc 'no choice' experiments, the application of Plant Extract 2 led to a reduction in the time aphids spent probing/feeding on leaf discs of Brussels sprout and carrot, but not on lettuce. This may relate to the relative feeding preference of *M. persicae* for these host plants. 'Restless' behaviour (*e.g.* more time spent walking) and reduced probing/feeding was previously observed on leaf discs of lettuce compared to the other plant species in host preference studies. It is possible that if the duration of the behaviour assays were extended, a notable difference in the time aphids spend probing/feeding on treated leaf discs of lettuce may have been detected.

Additionally, the time *M. persicae* took to 'lock-in' to feed on treated leaf discs, only significantly differed from the untreated control when Plant Extract 2 was applied to Brussels sprout. As aphids were slower to 'lock-in' on leaf discs of carrot and lettuce, it is possible that like Plant Extract 1, this difference could relate to habituation of alate adults to volatiles from the insecticide over time.

On leaf discs of all host plants, however, Plant Extract 2 increased the time aphids spent walking in leaf disc 'choice' and 'no choice' 'arenas'. This suggests that the compound may have initial repellent and/or anti-feedant properties, which disrupted the settling behaviour of *M. persicae.*

Results from the cage 'choice' experiments provide further evidence of an initial anti-feedant or repellent effect of Plant Extract 2, as aphids accumulated in greater numbers on untreated plants opposed to those treated with Plant Extract 2 for all three species of host plant. However, in cage 'no choice' experiments a statistically significant difference between the number of *M. persicae* accumulated on treated plants relative to the untreated control experiment was only found for Brussels sprout.

Both Plant Extracts 1 and 2 caused low levels of mortality in *M. persicae* (≤ 5%) during the 'choice' and 'no choice' experiments. However, these levels were unlikely

to have a major effect on the overall probing/feeding and settling behaviour of *M. persicae.*

Systemic Insecticides 1 and 2

During the leaf disc and cage experiments, Systemic Insecticides 1 and 2 induced considerable levels of intoxication and mortality in alate *M. persicae* that probed/fed on treated plant material. Aphids exhibited little selectivity between treated and untreated plant material suggesting that Systemic Insecticides 1 and 2 had little/no repellent or anti-feedant properties.

In both 'choice' and 'no choice' experiments, however, the application of Systemic Insecticides 1 and 2 reduced the total amount of time alate *M. persicae* spent probing/feeding due to the induction of convulsion/tremors and in some cases death. When applied to carrot or lettuce, while reductions in probing/feeding behaviour were still observed, they were not significantly different from the respective control experiments. This is likely to be related to the host preference behaviour of *M. persicae*. For example, in the 'no choice' experiments with Systemic Insecticides 1 and 2, *M. persicae* generally made faster decisions to feed on leaf discs of Brussels sprout compared to carrot and lettuce. For this reason, in Brussels sprout 'arenas' intoxication often occurred earlier (within 0–2 hours) compared with in 'arenas' containing treated carrot and lettuce leaf discs (3–4 hours).

However, there were statistically significant reductions in the time aphids spent walking in 'arenas' containing leaf discs of carrot and lettuce relative to the untreated control experiments. Again, this was likely due to intoxication which rendered some aphids immobile after periods of probing/feeding. During the control experiments with untreated leaf discs of lettuce and carrot, aphids exhibited 'restless' behaviour; spending a considerable amount of time walking after making initial exploratory probes on leaf discs. No statistically significant differences were found, however, between the time aphids spent walking in 'arenas' containing Brussels sprout leaf discs and the respective control experiments. This relates to the settling time of aphids in the control 'arenas' containing leaf discs of Brussels sprout. Here, aphids made faster decisions to feed and spent considerably less time walking.

The Kaplan-Meier estimators with log-rank tests found statistically significant differences between the 'lock-in' curves in 'no choice' experiments when these insecticides were applied to leaf discs of the three host species, with the exception of when Systemic Insecticide 2 was applied to lettuce. Again, this is likely to be due to the rapid induction of intoxication/death which considerably reduced the number of aphids that had 'locked-in' to feed by the end of the 'no choice' experiments. In several cases, aphids ceased probing/feeding and moved away from the leaf discs where convulsion/tremors occurred.

In the cage 'choice' experiments with Systemic Insecticides 1 and 2, after 5 h fewer aphids had accumulated on treated Brussels sprout plants compared to untreated plants. This difference was likely to be due to an increase in mortality when *M. persicae* selected plants treated with Systemic Insecticides 1 and 2 to settle on (10% and 12.5% mortality, respectively). However, this difference was not observed between treated and untreated carrot and lettuce plants. This may be explained by the relative time aphids took to settle and feed on these hosts. As aphids were quicker to settle on Brussels sprout plants, they were exposed to the treatments for longer durations, leading to the higher rate of mortality observed during the 5 h study period. In cages containing carrot and lettuce plants, it is possible that statistically significant differences between the number of aphids accumulated on treated and untreated plants may have been found if the study period was extended for longer than 5 h due to an increase in intoxication and mortality over time.

In the cage 'no choice' experiments when Systemic Insecticides 1 and 2 were applied to Brussels sprout and carrot plants there were statistically significant reductions (> 20%) in the number of aphids accumulated on plants after 5 h compared to the respective untreated control experiments. However, when applied to lettuce plants similar numbers of aphids accumulated on the treated and the untreated (control) plants. Once more, this may relate to the host preference of *M. persicae* for these crops. As *M. persicae* generally takes longer to settle on lettuce plants compared to the other two hosts; this may explain the lower rate of mortality on lettuce plants observed during these experiments.

It is possible that the rapid induction of intoxication/mortality by Systemic Insecticides 1 and 2 could aid in the management of plant viruses by reducing probing/feeding behaviour. Previously several studies have highlighted how the neurotoxic effects of insecticides (*e.g*. paralysis) or rapid knock-down have contributed to a reduction in the transmission of plant viruses through a reduction in probing/feeding behaviour (Collar *et al*., 1997; Zhu *et al*., 2010).

5.4.2 Limitations and further research

The leaf disc and cage systems used in this chapter provided a reliable way to collect information on the effects of insecticides on the behaviour of alate *M. persicae*. To gain further clarification of these effects, time-lapse video filming (Nauen, 1995) or EPG motoring techniques could be employed to supplement the information that was collected during these 'no choice' and no choice' experiments. Additionally, as all 'choice' and 'no choice' experiments were based on closed systems (*e.g*. Petri dishes or insect cages) aphids were limited in their dispersal response. It is possible that in the field or a semi-field environment, after the detection of certain insecticides on plant surfaces, aphids may disperse rapidly away from plants. There is potential to investigate the effects of insecticides on the behaviour of *M. persicae* in more open systems (*e.g.* polytunnels or field trials) with whole plants.

Furthermore, during these experiments, the effects of insecticides on aphid behaviour were only examined on the day the test products were applied. In the field, the retention of repellent, anti-feedant and/or neurotoxic effects of insecticides are crucial for the successful control of pest populations and the management of plant viruses. In these experiments the sub-lethal effects of insecticides on aphid behaviour over time were not investigated. It is possible that the test products may elicit different effects on aphid behaviour as the active ingredient degrades over time, compared to when they are freshly applied.

It is also important to extend these behavioural assays to other species of aphid to test the hypothesis that the test products would have similar effects on different species of aphid.

5.4.3 Conclusions

The probing/feeding and settling behaviour of alate *M. persicae* was affected by the application of several test products investigated during the 'choice' and 'no choice' behaviour experiments.

- Lambda-cyhalothrin led to a considerable reduction in settling behaviour. There was evidence to suggest that this reduction occurred through neurotoxicity and, to some extent, the sensory perception of the insecticide.

- Spirotetramat had very little effect on probing/feeding and settling behaviour.

- Plant Extracts 1 and 2 displayed considerable variability in terms of their effects on probing/feeding and settling behaviour. Results suggest that Plant Extracts 1 and 2 disrupt the initial settling of alate *M. persicae* on treated plant surfaces.

- Systemic Insecticides 1 and 2 affected the behaviour of alate *M. persicae* largely through the rapid induction of neurotoxicity and subsequent mortality.

Chapter 6 — The Efficacy of Insecticides for the Management of Non-persistent Plant Virus Transmission by *Myzus persicae*

6.1.1 The transmission of non-persistent plant viruses by aphids

Many plant viruses rely on insect vectors for their transmission and survival (Day and Irzykiewicz, 1954; Nault, 1997). Of these insects, aphids are thought to account for the transmission of at least 50% of plant viruses (Nault, 1997). Aphids are highly efficient virus vectors due to their ability to undertake parthenogenic reproduction, the polyphagous feeding behaviour of several species and the possession of stylet mouthparts that are ideal for virus transmission (Ng and Perry, 2004). Insect-borne plant viruses can be separated into two main groups, known as 'persistent' and 'non-persistent' viruses (Watson and Roberts, 1939). However, several viruses have been described as 'semi-persistent' which relates to the duration of time that a vector remains viruliferous after probing/feeding on an infected plant (Sylvester, 1956a, 1956b; Pirone and Harris, 1977).

Persistent plant viruses

The distinction between these main groups of viruses relates to the mode of their transmission and whether virions (virus particles) are circulative or non-circulative within the vector (Fig. 6.1) (Ng and Perry, 2004). Persistent viruses are transmitted in a circulative manner, in which they move into cells and are transported in the haemolymph of insects (Fig. 6.1) (Kennedy *et al*., 1962; Power, 2000). Several persistent viruses, known as circulative propagative viruses, may replicate in insect vectors (*e.g.* lettuce necrotic yellows virus, LNYV) (Stubbs and Grogan, 1963; Sylvester, 1980).

The viruses are then transmitted in the saliva of insects when they feed (Ng and Perry, 2004). The vectors of persistent viruses usually remain viruliferous for a considerable amount of time (days to months) (Day and Irzykiewicz, 1954). However, there is often a latent period (lasting hours to weeks) after the acquisition of the virus by the vector before it can be transmitted successfully to susceptible plants (Fereres and Collar, 2001).

Non-persistent plant viruses

Non-circulative viruses are only associated with an insect's stylet and/or foregut and are referred to as non-persistent or in some cases semi-persistent viruses (Fig. 6.1) (Ng and Perry, 2004).

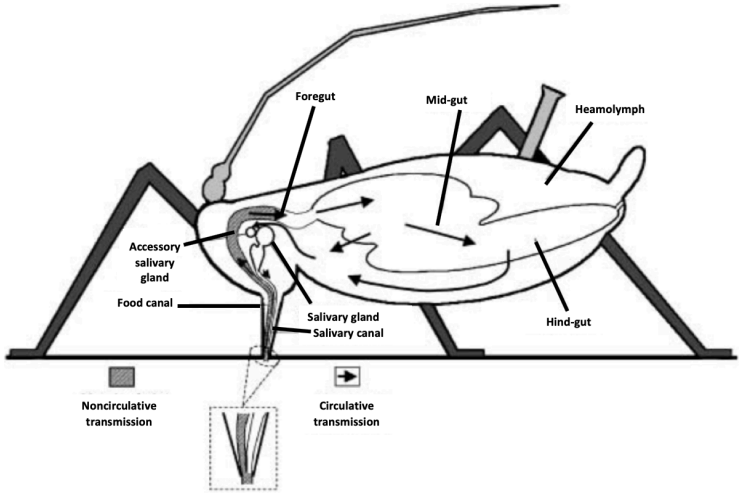

Figure 6.1 – Schematic diagram of the pathways taken by circulative and non-circulative viruses in an aphid after their acquisition. Plant fluids are taken up in the food canal of the stylet bundle and the virions of non-circulative viruses bind in the insect stylet or foregut. These virions are then released during the inoculation process through saliva. Circulative viruses move through an aphid's body via the midgut and hindgut. These viruses are often transported through the haemolymph to the salivary glands and then exit through the salivary canal. Circulative propagative viruses can enter through the midgut and may replicate inside the vector. Aphid organs are not shown to scale. Adapted from: Ng, J. C. and Perry, K. L. (2004). Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology*, *5*(5), pp.505-511.

Non-persistent virus transmission is almost exclusively carried out by aphids and approximately 75% of the plant viruses with aphid vectors are transmitted in a non-persistent manner (Nault, 1997). Many of these viruses infect crops of high economic importance including cereals, potatoes and many other vegetable and salad crops (Katis and Gibson, 1985; Tomlinson, 1987; Plumb, 2002). Non-persistent viruses are typically distributed throughout the tissues of plants, including the epidermal layer (Loebenstein and Raccah, 1980; Stevens and Lacomme, 2017). In host plants, the symptoms of infection with a non-persistent virus can include leaf distortion, stunted

plant growth, vein clearing and necrosis, leaf mosaic and mottling and flower breaking (*e.g.* turnip mosaic virus, TuMV, in certain hosts) (Stevens and Lacomme, 2017). In certain cases, infection may also be asymptomatic (*e.g*. Alfalfa mosaic virus, AMV) (Balasubramaniam *et al*., 2006).

The transmission of non-persistent plant viruses differs to that of persistent plant viruses as they may be transmitted immediately after their acquisition (Pirone and Perry, 2002). The acquisition of non-persistent plant viruses occurs optimally through very brief periods of probing (< 1 min) (Stevens and Lacomme, 2017). During these stylet penetrations, aphids reach the epidermal layer, where the concentration of a virus can be high (Loebenstein and Raccah, 1980). Prior to acquiring a virus, the fasting can increase the efficacy of transmission by aphids. This is likely to relate to behavioural changes that occur in starved aphids, which tend to make more short duration probes (< 1 min). This behaviour facilitates the rapid acquisition and transmission of non-persistent plant viruses (Bradley, 1952). The rate of virus acquisition decreases with prolonged periods of probing/feeding (> 1 min) on virus source plants as this often represents stylet penetration past the epidermis (Ng and Perry, 2004). Unlike the vectors of persistent plant viruses, aphids carrying a non-persistent plant virus generally remain viruliferous for only short durations of time once they have left a virus source plant (Ng and Perry, 2004). Non-persistent transmission is often carried out by alate adults during host selection, whereas persistent plant viruses are spread only by colonising aphids (Stevens and Lacomme, 2017).

In many cases, aphids lose their ability to transmit non-persistent viruses after moulting, as any retained virions are lost with the shedding of the stylets and/or circular lining of the foregut (Ng and Perry, 2004). For this reason, aphids may quickly lose their vectoring ability when they come into contact with a plant (Watson and Roberts, 1940; Bradley, 1959). If aphids are not actively feeding, however, they may remain viruliferous for extended periods of time, for example, at least 18 h in the case of maize dwarf mosaic virus (MDMV) for several species of aphid (Berger *et al*., 1987).

In the field, the spread of plant viruses by aphid vectors has two stages; primary and secondary infection (Stevens and Lacomme, 2017). Primary infection arises after the

arrival of viruliferous alate adults on plants and/or through infected seed (Johansen *et al*., 1994). Secondary infection, however, results from the movement and redistribution of both alate and apterous aphids on plants (Swenson, 1968; Stevens and Lacomme, 2017). There are a range of factors that can affect the spread of plant viruses within plants. These include; the source of the virus and its relative levels, the nature of its transmission (*i.e*. persistent, non-persistent or semi-persistent), the timing of aphid migration, the age of the plants upon the arrival of aphids and also environmental conditions, such as the weather, which can affect both the phenology of aphids and plant development (Stevens and Lacomme, 2017). Furthermore, it has been shown in several studies that infected host plants may lead to an increase in the production of alate adults (Blua and Perring, 1992; Gildow, 1980), which has implications in terms of the spread of virus (Montllor and Gildow, 1986; Hodge and Powell, 2010).

6.1.2 Efficacy of insecticides for managing the transmission of non-persistent plant viruses

The transmission of persistent plant viruses can be managed effectively through the use of insecticides (Broadbent, 1957; Mowry, 2005). This is because viruliferous aphids may be killed during the period after the acquisition of virus and prior to its transmission to susceptible plants, which can be hours to weeks (Perring *et al.,* 1999; Mowry, 2005; Stevens and Lacomme, 2017). The application of insecticides, however, often has limited success in the management of non-persistent plant viruses (Loebenstein and Raccah, 1980). This is mainly due to the transitory nature of aphid vectors and the rapid acquisition and inoculation characteristics of non-persistent viruses. In many cases, insecticides act too slowly on aphids when they first arrive in crops to prevent primary infection (Loebenstein and Raccah, 1980). Often, this is the case when aphid vectors land on plants they subsequently do not colonise; as aphids remain in contact with an insecticide for only a short duration of time (Margaritopoulos *et al*., 2010).

Additionally, some insecticides may instead enhance the activity of aphid vectors by inducing ‘restless’ behaviour (Collar *et al*., 1997; Stevens and Lacomme, 2017). In certain cases, this may contribute to a rise in virus incidence by increasing the contact

made between viruliferous aphids and susceptible plants (Ferro *et al*., 1980; Irwin and Thresh, 1990).

Nevertheless, insecticides can be used to manage the populations of vectors by reducing the number of aphids that can acquire and then transmit viruses. To some extent, this may contribute to a reduction in disease incidence (Castle *et al*., 2009). In particular, insecticides with a fast knock-down effect on aphids (*e.g.* pyrethroids and neonicotinoids) may reduce or prevent the secondary spread of non-persistent plant viruses in crops (Broadbent *et al*., 1956; Gibson *et al*., 1982b; Gibson and Cayley, 1984; Perring and Farrar 1993). However, successfully managing the primary spread of a non-persistent virus with these insecticides is difficult (Jayasena and Randles, 1985; Stevens and Lacomme, 2017).

Additional properties of insecticides, other than lethal toxicity, can be important for the management of non-persistent viruses, particularly anti-feeding or repellent mechanisms (Castle *et al*., 2009). Pyrethroids (3A), for example, are regularly reported to have repellent properties (Ruscoe, 1977; Dewar and Denholm, 2017). Insecticides with repellent effects are potentially valuable tools for managing the transmission of non-persistent viruses by alate adults. However, the repellent effects of pyrethroids have not been demonstrated consistently in the literature (Gibson *et al*., 1982; Lowery and Boiteau, 1988).

Margaritopoulos *et al.* (2010) investigated the efficacy of pymetrozine (9B) in reducing the non-persistent transmission of potato virus Y (PYV) by apterous *M. persicae nicotianae*. They found that when applied to tobacco plants, pymetrozine inhibited the transmission of PVY by affecting the acquisition and/or inoculation phase of the virus through an inhibition of feeding. The effects of insecticides on the acquisition and inoculation phases have different benefits. For example, treatments that affect acquisition are important for managing the secondary spread of virus within crops, whereas those that impact inoculation may reduce the primary spread of a virus in crops by immigrant aphid vectors (Margaritopoulos *et al.,* 2010).

It is also reported that some mineral oils may inhibit the transmission of certain non-persistent viruses to plants (*e.g.* PVY in seed potato crops) (Bradley *et al*., 1962b; Simons and Zitter, 1980; Powell, 1992). This is thought to occur through interference

with the virions in an aphid's stylet (Gibson and Rice, 1986). However, changes in aphid behaviour (*e.g*. reduced feeding) due to the presence of certain mineral oils may also be important (Simons *et al.,* 1977). These mineral oils can be combined with insecticides to target the transmission of viruses (*e.g.* MDMV and BtMV) (Ferro *et al*., 1980; Gibson and Rice, 1986). Walkey and Dance (1979) observed a reduction of 86% in the transmission of turnip mosaic virus (TuMV) by *M. persicae* to mustard with the application of 1% mineral oil, whereas as the insecticide, pirimicarb (carbamate, 1A), did not reduce transmission.

In similar laboratory studies, Lowery *et al*. (1990) investigated the transmission of TuMV in swede (*Brassica napus* var. *napobrassica* L.) by apterous and alate *M. persicae*. They found that applications of 1% mineral oil (Sunspray 6E, Sunoco Inc.) in combination with the insecticide cypermethrin (pyrethroid, 3A) (15.2 ppm) reduced virus infection by > 80% relative to the untreated control. A reduction in virus infection was also observed in field trials when insecticides were applied in combination with the mineral oil.

However, the use of mineral oils for virus management is not widespread, often due to low efficacy, high volatility or viscosity, low persistence due to rainfall and/or irrigation and in some cases phytotoxicity (de Wijs, 1980; Stevens and Lacomme, 2017).

6.1.3 Turnip mosaic virus (TuMV)

Turnip mosaic virus (TuMV) is a member of the Potyvirus genus, which is part of the family Potyviridae. The virus has a wide host range of over 300 plant species from more than 43 plant families. These include members of the Asteraceae, Brassicaceae, Chenopodiaceae and Fabaceae families (Tomlinson, 1987; Walsh and Jenner, 2002). Infection with TuMV can often lead to considerable economic losses through a reduction in both the yield and quality of crops (Walsh *et al*., 2002). The typical symptoms of TuMV-infection in plants include systemic mosaic patterning in the leaves, leaf curling and mottling, chlorotic and necrotic lesions, vein clearing and stunted plant growth. The severity of these symptoms, however, is determined by the virulence of the virus pathotypes and host susceptibility (Jenner and Walsh, 1996).

The transmission of TuMV is thought to be carried out by at least 89 aphid species, including *M. persicae,* in a non-persistent stylet-borne manner (Edwardson and Christie, 1986). Like other potyviruses, a helper component protein acts as a 'bridge' between the viral coat protein and the stylet of an aphid. This permits virions to be retained in the mouthparts of aphids prior to the inoculation of susceptible plants (Govier and Kassanis, 1974). Due to the nature of its transmission, the application of insecticides often proves inadequate for the management of TuMV in plants (Walsh and Jenner, 2002). Instead, cultural approaches are regularly employed, including the removal of infected plant material and timing planting dates to avoid periods of peak aphid migration into crops. This can allow crops to develop sufficiently before exposure to vectors (Shattuck, 1992). Additionally, the use of immune or resistant cultivars is important for protecting plants against TuMV in the field (Shattuck, 1992).

In this chapter, turnip mosaic virus (TuMV) was used as a 'model' to test the efficacy of insecticides for managing the transmission of a non-persistent virus by alate *M. persicae. Myzus persicae* has been used previously in several studies to inoculate plants with TuMV due to the species being an efficient vector of the virus (Walkey and Dance, 1979; Lowery *et al*., 1990; Jenner *et al*., 2010).

6.1.4 The transmission of non-persistent plant viruses by aphids: aims

The aims of this chapter were to:

- Investigate the efficacy of insecticides for managing the transmission of a non-persistent virus (TuMV) by alate *M. persicae*. In particular, focusing on primary infection when aphids initially land on plants during the processes of host selection and colonisation.
- Examine whether certain insecticides contribute to a reduction or an increase in the transmission of TuMV by alate *M. persicae* and relate this information to what was found previously in terms of the effects of insecticides on aphid behaviour (*i.e*. test the hypothesis that insecticide-induced 'restless behaviour' increases the transmission of TuMV by alate *M. persicae*).
- Infer the effects of other insecticides with similar MoAs and/or properties on the transmission of a non-persistent virus, based on the findings of this chapter.

6.2 The efficacy of insecticides for the management of non-persistent plant viruses transmitted by *Myzus persicae*: Materials and Methods

6.2.1 The development and settling behaviour of *Myzus persicae* on *Brassica rapa* ssp. *perviridis* cv. Tendergreen

Plant material

Tendergreen mustard plants (*Brassica rapa* ssp. *perviridis* cv. Tendergreen) were grown initially at 20°C, under a photoperiod of 16L:8D in pots (9 x 9 x 8 cm) containing M2 compost (Levington® medium grade sphagnum moss peat: Everris Limited, Ipswich, UK: pH 5.3–6.0; N = 192, P = 98, K = 319 mg/litre) in the Insect Rearing Unit, Warwick Crop Centre, School of Life Sciences, Wellesbourne. Tendergreen mustard plants (TGM) were used due to their susceptibility to TuMV and the clear phenotypic symptoms they display when infected.

Insect material

Myzus persicae 2050A were maintained at 18°C, under a photoperiod of 16L:8D on *Brassica rapa* ssp. *perviridis* cv. Tendergreen (Tendergreen mustard; TGM) in rearing cages (47.5 x 47.5 x 47.5 cm) with very fine (150 x 150) mesh nylon sides (Bugdorm-4F4545; Watkins and Doncaster Ltd., Herefordshire, UK) in the Insect Rearing Unit, Warwick Crop Centre, School of Life Sciences, Wellesbourne. Aphids were reared on TGM plants for at least two generations prior to use in the bioassays.

Development of Myzus persicae on Brassica rapa* ssp. *perviridis cv. Tendergreen

The development of *M. persicae* clones MP1S and 2050A was first investigated on *Brassica rapa* ssp. *perviridis* cv. Tendergreen (Tendergreen mustard; TGM) (3–4 weeks after sowing). The method employed was the same as Chapter 3.2.2 (pp.87–88).

Settling behaviour of alate Myzus persicae on Brassica rapa ssp. perviridis cv. Tendergreen

Cage experiments were carried to examine aphid settling behaviour by focusing on the accumulation of alate *M. persicae* 2050A (0–3 days old) on TGM plants over time (0–5 h). The method used was similar to the *Cage system*: Chapter 3.2.3 (pp.91–93)

with only the cage 'no choice' experiment performed with four untreated TGM plants per cage. The experiment was replicated in time, giving a total of two cages (or replicates) per treatment. Each cage contained four TGM plants and was infested with ten alate *M. persicae*.

6.2.2 Mechanical transmission of turnip mosaic virus (TuMV)

The mechanical transmission of TuMV in Tendergreen mustard (TGM) plants was carried out in a 'sterile' controlled-environment room (18°C) in the Insect Rearing Unit (IRU), Warwick Crop Centre, School of Life Sciences, Wellesbourne using the method described by Walsh (1989). Leaf samples infected with TuMV (source inoculum) were ground with a pestle and mortar and mixed with an inoculation buffer of 1 g K_2HPO_4; 0.1 g $Na_2SO_3.7H_2O$; 100 ml diluted water. Using muslin cloth, the inoculum was then rubbed into the leaves of healthy TGM plants that were dusted with abrasive carborundum (silicon carbide). Mock-inoculations of TGM plants were carried out at the same time using the sap from healthy (uninfected) leaves. The last leaf of each plant to be inoculated was pierced with a small hole to allow the detection of the systemic spread of the virus into any new leaves that developed after inoculation. The inoculated TGM plants were tested for the presence of TuMV with plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA) (see Chapter 6.2.4: pp.199–201) prior to using them as virus source plants in the bioassays.

6.2.3 Transmission of turnip mosaic virus (TuMV) by alate *Myzus persicae*

A system was developed to investigate the efficacy of insecticides in managing the transmission of TuMV by alate *Myzus persicae* clone 2050A (0–3 days old). Alate *M. persicae* were initially starved for 1 h in empty clip cages (2.5 cm diameter) (approximately ten aphids per clip cage). Clip cages were then attached to the leaves of TGM plants that had been mechanically inoculated with TuMV (source plants). Aphids were left to probe/feed for a 10 min acquisition period. Alate *M. persicae* that were observed probing/feeding were then collected by mechanically stimulating the aphid's dorsum with a fine paintbrush to encourage aphids to remove their stylet from the substrate. Aphids were then starved again for 1 h in empty Petri dishes (SARSTEDT, Germany).

Tendergreen mustard plants (3–4 true leaf stage) were dipped in an emulsion (300 ml) of the test products, prepared at the field recommended dose for *Brassica* species as indicated on the insecticide labels (*e.g.* Movento®, spirotetramat, at 0.5 L/ha, concentration of 1.7ml/L) in the Pesticide Handling Unit (PHU) at Warwick Crop Centre, School of Life Sciences, Wellesbourne.

Cages (Bugdorm-4F4545, UK) containing four healthy TGM plants that had been treated with one of the test products (one cage/test product) were each infested with ten alate adults by placing Petri dishes into the centre of each cage and then removing the lids. Three insecticides were tested: spirotetramat, Plant Extract 2 and Systemic Insecticide 1. The treatments were selected based on the different effects they had on the settling behaviour of alate *M. persicae* (see Chapter 5.3: pp.160–180). Control experiments with untreated TGM plants (dipped in an equal amount of water) were carried out at the same time. Positive control cages were infested with viruliferous aphids and negative control cages were infested with non-viruliferous aphids that had previously been left to feed on healthy TGM plants.

Alate adults were left in the cages for 2 h and were then removed manually with a fine paintbrush. All plants were then treated with pymetrozine (Plenum®, Syngenta®, Basel, Switzerland) at the recommended dose for *Brassica* species (0.4 kg/ha) in order to kill any alate adults that were not found and/or any nymphs that may have been deposited. Plants were then placed into micro-perforated polypropylene bags (200 x 500 mm) (Cryovac®, New Jersey, USA) and kept in cages (Bugdorm-4F4545, UK) at 18°C, under a photoperiod of 16L:8D. Plants were examined the following day and then weekly for any signs of aphid infestation to avoid the potential for secondary virus infection.

6.2.4 Detection of turnip mosaic virus (TuMV)

Phenotypic assessments of Tendergreen mustard plants

Visual assessments of the TGM plants were carried out each week for four weeks after their exposure to alate *M. persicae*. The classification system that was used was adapted from Jenner and Walsh (1996); 0: no detectable symptoms, +: systemic mosaic infection, +N: systemic infection with necrosis. Tendergreen mustard plants

with systemic mosaic infection (+) displayed clear symptoms of infection including; systemic mosaic patterning in the leaves, leaf curling and stunted growth (Fig. 6.2).

Figure 6.2 – *Brassica rapa* ssp. *perviridis* cv. Tendergreen (Tendergreen mustard; TGM) infected with turnip mosaic virus (TuMV) displaying symptoms of systemic mosaic patterning in the leaves, leaf mottling, and stunted growth.

Enzyme-linked Immunosorbent Assay (ELISA)

Single leaves were removed from TGM plants 4–5 weeks after exposure to alate *M. persicae*. Samples were ground through electric steel rollers (Meku-Pollahne, Wennigsen, Germany) with the sap collected into separate 2 ml microcentrifuge tubes (Eppendorf Safe-Lock Tubes, Eppendorf Quality™, Hamburg, Germany).

Plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA) was then carried out to test for the presence of TuMV in microtitre plates (Nunc-Immuno™ MicroWell™ 96 well solid plates, MaxiSorp™, Gibco Ltd., Uxbridge, UK). The method employed was described by Walsh *et al.* (1999). Firstly, 100µl of coating buffer (0.05 M sodium carbonate) were pipetted into each well of the ELISA plate. The test samples (100µl of leaf sap) were then randomly assigned into paired wells. Positive and negative controls were included in the outside wells of the ELISA plate, surrounded by blank wells. Plates were left overnight at 4°C.

The following day a series of antibodies were used to detect the presence of viral protein (antigens) in the test samples. The first antibody added was a mouse monoclonal antibody EMA67 (Jenner *et al*., 1999) and the second antibody added was goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma-Aldrich Chemical Co., Poole, UK). The antibodies were first diluted (1/500 and 1/2000, respectively) in phosphate-buffered saline (pH 7.3) containing Tween 20 (PBS-T) and bovine serum albumin (0.2g/L) and incubated with the samples for 2 h at room temperature. The substrate p-nitrophenyl phosphate (1 mg/mL in 0.1 M diethanolamine, pH 9.8) was then added to the samples and the reaction was left to proceed at room temperature. Once the reaction had progressed sufficiently, and if viral protein was present, this led to a colour change (clear to yellow) in the substrate.

The optical absorbance of the samples was then measured at 405 nm using a Biochem Anthos Labtec HT2 microplate reader (TechGen International Ltd., London, UK). The colour strength provided an indication of the levels of viral protein present. Data were exported into Microsoft® Excel (Microsoft® Office 2016 for Mac, Version 15.0) and infection by TuMV was determined by subtracting the mean value for the two negative control wells from the mean values of the paired wells for each plant test sample. Positive values confirmed infection.

6.2.5 Statistical analysis

As the expected counts were fewer than five (due to a small sample size) Fisher's exact test was carried out in in R (Version 3.5.1) to test the null hypotheses that treatment had no statistically significant effect on the transmission of TuMV by alate *M. persicae*.

6.3 The efficacy of insecticides for the management of non-persistent plant viruses transmitted by *Myzus persicae*: Results

6.3.1 The development and settling behaviour of *Myzus persicae* on *Brassica rapa* ssp. *perviridis* cv. Tendergreen

Development of Myzus persicae on Brassica rapa* ssp. *perviridis cv. Tendergreen

On Tendergreen mustard plants, the mean development time and mean daily rate of reproduction (mean number of nymphs produced per day) for *M. persicae* were 7.08 ±0.3 days and 1.78 ±0.1, respectively. The intrinsic rate of increase (r_m) was calculated to be 0.27 ±0.01. This value was similar to the r_m of *M. persicae* on Brussels sprout cv. Doric F1 plants (Table 6.1).

Table 6.1 – Mean development time in days, mean daily rate of reproduction (number of nymphs per day) and the intrinsic rate of increase r_m (see Chapter 3.2.2: pp.87–88 for method of calculation) of *Myzus persicae* on Brussels sprout cv. Doric F1 and Tendergreen mustard (TGM) plants (*Brassica rapa* ssp. *perviridis* cv. Tendergreen). Data are shown ± standard error of the mean. All experiments were carried out at 20°C.

Measure of aphid development	Host plant	
	Brussels sprout	TGM
Mean development time	7.42 ±0.4	7.08 ±0.3
Mean daily rate of reproduction	1.73 ±0.1	1.78 ±0.1
r_m	0.26 ±0.01	0.27 ±0.01

Settling behaviour of alate Myzus persicae on Brassica rapa ssp. perviridis cv. Tendergreen

Cage system: 'no choice' experiment

The settling behaviour of alate *M. persicae* (2050A) was investigated in 'no choice' cages each containing four untreated TGM plants. In these cages, 52.5 ±2.2% of aphids had settled on TGM plants 2 h after infestation and by 5 h 80 ±3.5% aphids had accumulated on the plants (Fig. 6.3).

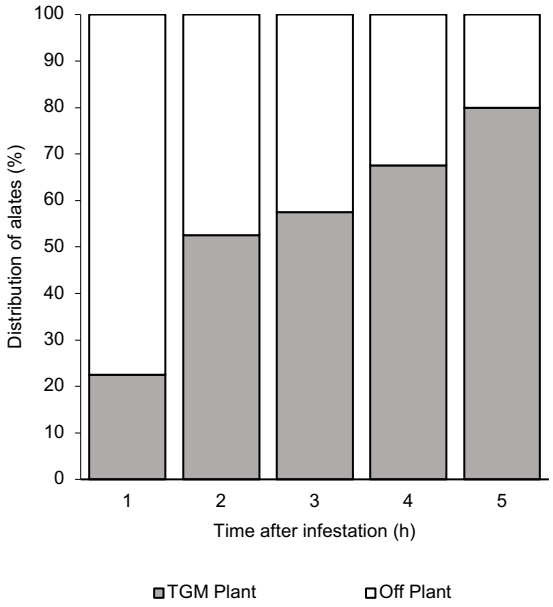

Figure 6.3 – Settling preference (distribution %) of alate *Myzus persicae* 2050A on Tendergreen mustard (TGM) plants (*Brassica rapa* ssp. *perviridis* cv. Tendergreen) during cage 'no choice' experiments. All aphids were reared on TGM plants at 18°C.

6.3.2 The efficacy of insecticides in the management of the transmission of turnip mosaic virus (TuMV) by alate *Myzus persicae*

After 2 h, the mean percentage of alate *M. persicae* 'on plants', 'off plants' and dead (% mortality) are shown in Table 6.2 during the virus (TuMV) transmission experiments. For all treatments 30–50% of *M. persicae* settled on plants 2 h after infestation. Mortality was only observed in cages containing TGM plants treated with Plant Extract 2 (5 ±3.5%) and Systemic Insecticide 1 (25 ±3.5%).

Table 6.2 – Settling preference (distribution %) after 2 h of alate *Myzus persicae* 2050A in 'no choice' cages containing either treated or untreated (control) Tendergreen mustard (TGM) plants (*Brassica rapa* ssp. *perviridis* cv. Tendergreen). Data are shown ± standard error of the mean.

Treatment	**% *M. persicae* accumulated 'on plants'**	**% *M. persicae* accumulated 'off plants'**	**% *M. persicae* mortality**
Negative control	50 ±7.1	50 ±3.5	0
Positive control	55 ±3.5	45 ±3.5	0
Spirotetramat	45 ±3.5	55 ±7.1	0
Plant Extract 2	40 ±7.1	55 ±3.5	5 ±3.5
Systemic Insecticide 1	30 ±0.0	45 ±3.5	25 ±3.5

The total % of TGM plants infected with TuMV (as confirmed by ELISA testing) is shown in Table 6.3. As expected, none of the negative control plants were infected with the virus. Infection was highest in the positive control plants (87.5%) followed by in TGM plants that had been treated with spirotetramat (75%). Compared to the positive control plants, the application of Plant Extract 2 and Systemic Insecticide 1, led to reductions in the presence of TuMV in plants (62.5% infected in both treatments).

Table 6.3 – Infection of Tendergreen mustard (TGM) plants (*Brassica rapa* ssp. *perviridis* cv. Tendergreen), *n* = 8, with turnip mosaic virus (TuMV) (%) four–five weeks after exposure to viruliferous and non-viruliferous (negative control) alate *Myzus persicae* 2050A. Phenotypes; 0: no detectable symptoms, +: systemic mosaic infection, $+_N$: systemic infection with necrosis.

Treatment	Main phenotype(s)	% TGM plants infected with TuMV
Negative control	0	0.0
Positive control	+/ $+_N$/ 0	87.5
Spirotetramat	+/ $+_N$/ 0	75.0
Plant Extract 2	+/ 0	62.5
Systemic Insecticide 1	+/ 0	62.5

Excluding the negative control plants, for the remaining treatments Fisher's exact test was carried out to test the null hypotheses that treatment had no effect on the transmission of TuMV by alate *M. persicae*. No statistically significant difference was found (Fisher's exact test: $P > 0.05$).

6.4 The efficacy of insecticides for the management of non-persistent plant viruses transmitted by *Myzus persicae*: Discussion

6.4.1 Efficacy of insecticides in the management of a non-persistent virus

The main aim of this chapter was to investigate the efficacy of insecticides in managing the transmission (primary infection) of a non-persistent plant virus (TuMV) by simulating when alate *M. persicae* first arrive in crops during host selection/host colonisation.

None of the insecticides investigated prevented the transmission of TuMV entirely. Additionally, for the three treatments there was no statistically significant difference in terms of the percentage of TGM plants infected with the virus. This may relate to the lack of replicates (two replicates/treatment) that were carried out. However, both the applications of Plant Extract 2 and Systemic Insecticide 1 led to a modest reduction in virus transmission. This could be due to repellent effects in the case of Plant Extract 2 and increased mortality of alate adults in the case of Systemic Insecticide 1. In both cases, this would have limited the contact made between viruliferous *M. persicae* and susceptible TGM plants. Due to the similar effects Systemic Insecticides 1 and 2 had on the behaviour of alate *M. persicae* (see Chapter 5.3: pp.160–180), it is likely that if Systemic Insecticide 2 had also been tested in the virus transmission experiments, it would have performed in a similar manner to Systemic Insecticide 1.

Compared to the positive control, spirotetramat had little effect on the transmission of TuMV. This was not unexpected, as it was determined previously that spirotetramat did not affect the initial settling behaviour of alate *M. persicae* and did not cause a notable increase in mortality until 3–4 days after its application, relative to the untreated control experiments.

Unfortunately, during the experiments in was not possible to obtain a sufficient number of alate *M. persicae* from the pyrethroid-susceptible clone (MP1S) to test the effects of lambda-cyhalothrin on the transmission of TuMV. It was determined that the application of lambda-cyhalothrin led to a reduction in the settling behaviour of alate *M. persicae* and increased the number of short duration probes (< 1 min)

performed on treated plant material (see Chapter 5.3: pp.160–180). It is possible that if virus transmission experiments had been carried out with lambda-cyhalothrin, any 'restless' behaviour may have increased the transmission of TuMV. In previous field trials, Lowery *et al*. (1990) found that the application of lambda-cyhalothrin (15 g AI/ha), on some occasions led to a modest reduction in the number of swede plants infected with TuMV, but overall the insecticide did not provide consistent management of the transmission of the virus by aphids. Perrin (1985) suggested that lambda-cyhalothrin has more of an effect on reducing the secondary spread of non-persistent viruses in crops compared to combating the initial introduction of viruses to crops (primary infection) by alate adults.

6.4.2 Developing a system to investigate the efficacy of insecticides in the management of a non-persistent plant virus transmitted by *Myzus persicae*

The method developed provided a way of investigating the efficacy of insecticides for the management of a non-persistent plant virus (TuMV) in a controlled environment. Due to time constraints, the level of replication was low, with a limited number of plants tested per treatment (n = 8) across two replicates. Ideally, a much large number of replicates would have been carried out. This would allow for more meaningful conclusions to be drawn with regard to the overall effects, if any, of the different insecticides on the transmission of TuMV.

To refine the system, single test plants, as opposed to four, could have been placed into the individual cages. This would increase the likelihood of contact being made between aphid vectors and susceptible TGM plants. This could reduce the number of 'false positives' in which, the treatment appears to reduce virus transmission (resulting in no infection) when in reality it is possible that no contact (*e.g.* probing/feeding) was made between a viruliferous aphid and a plant during the 2 h of 'exposure' time. It is possible that no contact may be the result of chance rather than due to any repellent effects.

Going forward, a system that approaches biological reality could be employed. For example, a set amount of viruliferous alate *M. persicae* could be released into glasshouse compartments containing a number of treated and untreated TGM plants.

This would considerably increase the amount of replication with regard to the number of test plants. After a specified duration of time, plants could be treated with an insecticide to kill alate adults and any deposited nymphs to exclude the possibility of secondary infection.

6.4.3 Conclusions

- None of the test products (spirotetramat, Plant Extract 2 and Systemic Insecticide 1) prevented the transmission of TuMV by alate *M. persicae* to TGM plants.

- There is some initial evidence to suggest that insecticides with fast knock-down (*e.g.* Systemic Insecticide 1) may have some impact on the transmission of TuMV by alate *M. persicae*, however, more replication is needed.

Chapter 7 — General Discussion

7.1 The process of crop colonisation by aphids

Aphids are some of the most damaging pests of horticultural crops worldwide. The reasons for managing aphid populations are often two-fold; to prevent damage through direct feeding and to reduce/prevent the transmission of plant viruses to crops (Dewar and Denholm, 2017). Aphids are managed largely through the application of synthetic chemical insecticides. In the UK, the widespread distribution of aphids and their importance as pests, has led to considerable applications of insecticides made to crops each year (Garthwaite *et al*., 2017).

The sustainability of crop protection depends greatly on optimising the use of insecticides and the discovery and development of novel insecticide classes, including biopesticides (Chandler *et al*., 2011). In the UK, these factors are particularly important due to recent declines in the availability of synthetic chemical insecticides. Notably, the loss of the outdoor use of several neonicotinoid insecticides (European Food Safety Authority, 2018a, 2018b, 2018c) has major implications for the UK's crop production industry. The development of insecticide resistance by aphids is also a growing concern, which in part may result from a reduction in the number of chemical options that are available to growers (Bass *et al*., 2015; IRAG, 2019).

This project focused on the process of crop colonisation by aphids. Forecasting models were developed to predict aphid phenology to warn of crop infestation and periods with potential for virus transmission. However, once in crops, host plant selection by aphids and their responses to the presence of insecticides can have implications for the severity of infestations and virus transmission. Several synthetic chemical insecticides and compounds based on plant extracts were investigated in terms of their effects on aphid survival, behaviour, and virus transmission when applied prophylactically to commercially-important host plants.

The information gathered in the project can be used to inform and improve the approaches that are applied for the control of aphids in the UK. In particular, the findings offer the potential to refine components within Integrated Pest Management strategies, which could (i) help to target the use of insecticides and (ii) inform on the

selection of plant protection products based on an improved understanding of their effects on aphid survival and behaviour.

The project focused on several important pest aphids of horticultural crops; *M. persicae* (Chapters 2–6), *C. aegopodii*, *C. pastinaceae* and *C. theobaldi* (Chapter 2) and was divided into five areas of research that each addressed a central aim. The discussion will be structured in accordance to these aims:

1) Investigate methods of predicting aphid phenology (**Chapter 2**).

2) Examine development and colonisation behaviour of *M. persicae* on several commercially-important host plants (**Chapter 3**).

3) Examine the efficacy and persistence of a number of synthetic chemical insecticides and compounds based on plant extracts for the control of *M. persicae* (**Chapter 4**).

4) Determine the effects of a number of synthetic chemical insecticides and compounds based on plant extracts on the behaviour of *M. persicae* (**Chapter 5**).

5) Investigate the efficacy of synthetic chemical insecticides and compounds based on plant extracts in managing the transmission of a non-persistent plant virus by alate *M. persicae* (**Chapter 6**).

7.2 Methods of predicting aphid phenology

The validity of using different types of forecasts to predict aphid phenology was investigated. For *C. aegopodii*, there were strong relationships between accumulated day-degrees and the timing of its migration. In terms of their accuracy, the models improved on the current day-degree forecast that is used in the UK to the predict the onset of migration (360D° accumulated above 4.4°C from 1st February), often by several days. There is still uncertainty, however, surrounding the exact base temperature for the development of *C. aegopodii*. This could be clarified by conducting laboratory studies using diapausing eggs that could be collected from the field or produced in the laboratory.

Similar relationships with accumulated day-degree sums were not found for *C. pastinaceae* and *C. theobaldi*. This was unexpected as the species share a similar life cycle (*e.g.* holocyclic and host alternating) with *C. aegopodii*. Overall, there is relatively little information on the biology and ecology of *C. pastinaceae* and *C. theobaldi* in the surrounding literature. Predictive models may benefit from a more detailed understanding of these factors. However, in recent years (2013–2018) these species were considerably less abundant at suction trap sites compared to *C. aegopodii* and *M. persicae*. This raises questions about their relative importance as virus vectors in the UK. In several studies, *C. pastinaceae* and *C. theobaldi* have been shown to transmit anthriscus yellows virus (AYV), parsnip yellow fleck virus (PYFV) and carrot yellow leaf virus (CYLV) to carrot with low efficiency (Van Dijk and Bos, 1989; Fox *et al*., 2015). In the field, these aphids do not colonise carrot and for this reason, *C. pastinaceae* and *C.* are not regarded as important vectors of viruses affecting carrot (Van Dijk and Bos, 1985). However, it is possible that *C. pastinaceae* and *C. theobaldi* may be important in terms of transmitting viruses to other apiaceous crops, such as parsnip. For carrot, the relative importance of different vectors and the timing of transmission of the key viruses, *e.g.* carrot yellow leaf virus (CYLV) and carrot red leaf virus (CtRLV) by aphids, are being investigated by FERA and Warwick Crop Centre in an ongoing AHDB project (FV 460).

For *C. pastinaceae* and *C. theobaldi*, novel forecasts were instead developed using measures of mean air temperature averaged over defined periods in the winter/early spring. There was considerable variability of the accuracy of these models, which predicted capture dates to within seven to twenty days. For both species, forecasts of the mid-point of the spring/summer migration (capture of 50% of the spring/summer migrants) yielded the most reliable predictions. The inconsistencies in model accuracy may be explained by the limited data sets that were used to develop and validate the forecasts. For example, there were a considerable number of years with very low numbers of aphids (< 50) caught by the suction traps that were not included in the analysis. Additionally, access to reliable weather records for recent years was limited. Going forward, stronger relationships between mean air temperature and the phenology of *Cavariella* spp. and may be established if additional years of capture data and weather records are incorporated into the models.

Forecasts based on mean air temperature were also able to predict the phenology of *C. aegopodii*, however, with less accuracy than the day-degree forecasts. For *M. persicae*, it was not possible to develop a day-degree forecast but strong relationships were identified with mean air temperature in the winter/spring. Similar relationships with mean air temperature in the winter/spring have been observed previously for *M. persicae,* with an earlier onset of migration following warmer winters (Zhou *et al*., 1995). In part, this is likely to be explained by the predominantly anholocyclic life cycle of *M. persicae* in the UK and higher survival at warmer temperatures (van Emden *et al.,* 1969). While a forecast based on mean air temperature during January–February (inclusive) is used currently to predict the onset of migration by *M. persicae* (RIS, 2019), in this project forecasts were also developed to predict the capture of 10%, 25%, 50% and 90% of the spring/summer migrants and the week of the summer peak in aphid numbers. These can provide useful monitoring points of the migration of *M. persicae* over the course of the growing season.

Additionally, the dates of the first and 90% capture of *C. aegopodii* and *M. persicae* at individual sites during 2013–2018, gave an indication of the potential 'risk periods' for virus transmission. The mean duration of the 'risk period' at sites ranged from 44–54 days (from the end of April to mid-June) for *C. aegopodii*, and 35–67 days (from the beginning of May to mid-July) for *M. persicae*. In terms of managing the plant viruses transmitted by these species, this information could inform growers on the length of time control approaches may be required during the spring/summer.

The decision to apply a treatment based on the output of forecasting models, however, should always be supported by the findings of local monitoring programmes. During the project, the numbers of alate *C. aegopodii* were monitored in carrot plots using yellow water traps (YWTs). Aphids were always caught first in YWTs during 2016–2018 at Wellesbourne, UK (one–two weeks before the first capture by the suction trap). Generally, the predicted dates from the day-degree models were closer to when aphids were caught in the suction trap, compared to in YWTs. It is possible that the early captures in YWTs were anholocyclic clones that had overwintered on carrot. However, the presence and relative numbers of aphids caught in YWTs can provide an early indication of the onset of migration and/or presence of a species in crops. For managing the transmission of non-persistent viruses this early warning may be

important when the numbers of aphid migrants are not sufficiently large enough to exceed the threshold for detection by suction traps.

Importance of forecasting models and their uptake by growers

Overall, the employment of robust forecasting systems has the potential to enhance the use of crop monitoring resources, target applications of insecticides and combat the development of insecticide resistance by reducing selection pressure. These outcomes are likely to provide financial benefits for growers, prove favourable with consumers, reduce environmental damage and non-target effects and help to preserve the efficacy of insecticide treatments. Forecasting models, however, should always be used in conjunction with regular crop monitoring (*e.g.* crop walking/in-crop trapping methods) at sites and grower experience.

At present growers receive information on aphid activity from 'Pest Bulletins' and forecasts in 'AHDB News' It is not clear how growers use this information or what value forecasts have in terms of improving aphid control and/or informing insecticide use.

Results from a recent survey (AHDB, 2019b) showed that of 123 growers and 79 agronomists of horticultural crops, 46.3% and 73.4% used the pest monitoring services provided by AHDB, respectively. The provision of early warning forecasts was one of the most common answers when participants were asked how pest monitoring services could be improved. Interestingly, 'parsnip aphids' (*C. pastinaceae* and *C. theobaldi*) were species that growers and agronomists wanted to see these services extended to.

In addition, growers and agronomists expressed interest in the increased promotion of pest monitoring services and different methods for disseminating information. There are several potential ways to disseminate aphid forecasts. For example, growers and agronomists could be supplied with the appropriate forecasting equations to be used with temperature data from their own sites. Additionally, in recent years there has been considerable innovation in the decision support tools that are available to growers and agronomists. In particular, the use of statistical or simulation models for disease outbreaks have been made available as commercial and free access solutions, for example, through mobile applications, such as BYDV ASSIST (Syngenta®). This

model employs a day-degree forecast (170D° accumulated above 3°C from the date of crop emergence) to predict the production of the second generation of apterous aphids (progeny of alate colonisers). This information can be used to inform the timing of insecticide applications to target to the secondary spread of barley yellow dwarf virus, BYDV by *R. padi* and the grain aphid, *Sitobion avenae* (Fabricius, 1775) (Hemiptera: Aphididae) in cereal crops. There is opportunity to extend the use of this technology to provide specific forecasts for a variety of pest aphids. The applications could be developed to permit growers to input their site details and to use local weather data. This would overcome one limitation associated with current forecasts and weekly aphid bulletins, which are restricted in their geographical range. It is possible that if growers were to use forecasting models with information from their own site (*e.g.* local temperature data) the accuracy of predictions may be improved.

Going forward, it will also be important to address whether sufficient numbers of aphids are likely to enter a crop to warrant the application of insecticides, particularly when virus transmission is not a major concern. Forecasts of aphid abundance have so far been limited in terms of both their accuracy and uptake (Kindlmann and Dixon, 2010). For *M. persicae*, each year the RIS predicts the expected numbers of aphids captured by 17th June (AHDB, 2019a) at several suction trap locations in the UK. However, beyond this, there are no other forecasts of abundance currently available for *M. persicae* in the UK.

Losses in crop yields due to pest insects are predicted to increase considerably as a result of global warming (Deutsch *et al.*, 2018). In the UK, with recent trends in higher winter and spring temperatures (Met Office, 2018) aphids, like *M. persicae* and *C. aegopodii*, are arriving in crops earlier when plants may be more susceptible to damage (Harrington and Clark, 2010; Bell *et al*., 2014). Again, this highlights the need for robust forecasting models. However, forecasting infestations by aphids in the long term, particularly in relation to climate change, carries a risk. For example, temperature variables in the future may lie outside of the range of those used to construct current models (Harrington and Clark, 2010). For this reason, it will be important to continue to model trends in aphid migration in the coming years.

7.3 The development and colonisation behaviour of *Myzus persicae*

Before investigating the effects of insecticides on the survival and behaviour of *M. persicae* it was important to gather information on the relative development and settling preference of aphids on the different host plants. At 20°C the development of *M. persicae* varied on the three species of host plant that were tested. The intrinsic rates of increase (r_m) were calculated to be: 0.26 ±0.01 (Brussels sprout), 0.16 ±0.01 (carrot) and 0.06 ±0.01 (lettuce). In terms of host preference, *M. persicae* settled and fed more readily on Brussels sprout than on carrot and lettuce. On the 'less-preferred' host plants (carrot and lettuce) there was a tendency for *M. persicae* to exhibit 'restless' behaviour, with aphids taking a considerable amount of time to settle. This could have implications in terms of virus transmission to these crops. While the relative preference of *M. persicae* for *Brassica* species is reported regularly by growers, there are few recent studies examining aphid preference in relation to other host plants.

In this project, previous host (rearing) experience did not significantly affect the preference behaviour of alate *M. persicae* in the 'choice' and 'no choice' bioassays. This may be related to the number of generations (minimum of two) that *M. persicae* was reared on new host for prior to examining host preference behaviour. There is also a possibility that aphid clones will always perform better on hosts from which they originated. In this case, the 2050A clone originated on Brussels sprout (field-collected in 1996) (S. Foster, personal communication). Going forward, it would be worth investigating the effects of rearing generation on the host preference of *M. persicae*.

7.4 Efficacy and persistence of insecticides applied for the control of *Myzus persicae*

Understanding the effects of insecticides on the survival, reproduction and behaviour of aphids is crucial for developing sustainable crop protection programmes. This knowledge is important for *M. persicae*, which is not only a prolific crop pest and an important virus vector, but also has the ability to rapidly evolve mechanisms of resistance to insecticides (Bass *et al*., 2014). For these reasons, information on the efficacy and persistence of insecticides can be useful for informing the timing of their applications for the control of *M. persicae*.

Over the last ten years, lambda-cyhalothrin and spirotetramat have been applied widely for the control of aphids in the UK (Garthwaite *et al*., 2017). However, due to the decreasing number of modes of action available to growers, there are concerns about an overreliance on these products and the subsequent development of resistance. Some field populations of aphids (*e.g.* clone 2050A *M. persicae* and a sample of *C. aegopodii* collected from North Yorkshire) have already developed resistance to lambda-cyhalothrin and other pyrethroid insecticides. In the UK, around 70% of the samples of *M. persicae* analysed at Rothamsted Research are reported to have super-kdr resistance (IRAG, 2019). However, as of yet, there are no reported cases of resistance to spirotetramat in the UK (IRAG, 2019). In the project, the efficacy, persistence and behaviour-modifying effects of lambda-cyhalothrin and spirotetramat were investigated, alongside four novel treatments that are not authorised currently for outdoor use in the UK.

On Brussels sprout plants infested on the day of spraying, lambda-cyhalothrin, spirotetramat and Systemic Insecticides 1 and 2 led to statistically significant reductions in the number of live alate adults over time, compared to the untreated controls. The insecticides, however, differed in terms of their levels of efficacy, persistence and speed of knock-down. Of these treatments, lambda-cyhalothrin displayed the lowest persistence, with reduced levels of mortality on plants infested seven days after spraying. In part, this could be due to the contact action of lambda-cyhalothrin. For example, insecticidal effects would not be observed if alate adults only made contact with/fed on new leaves that developed after treatment. For optimal use, the insecticide should be sprayed directly onto infested crops to target aphids, rather than used as a prophylactic measure, as in the experiments carried out here.

Both Systemic Insecticides 1 and 2 led to the rapid knock-down of *M. persicae*, inducing up to 80% mortality after one day and 100% mortality within four to five days. Strong insecticidal effects persisted on plants infested seven days after treatment. The properties of Systemic Insecticides 1 and 2 make them ideal for the use against migrating aphids. Their use could also help to prevent/reduce the transmission of plant viruses, particularly those spread in a persistent manner or the secondary spread of non-persistent viruses. It is possible that these treatments will be authorised for outdoor use in the UK in the coming years.

For spirotetramat, there was a lag period of three to four days before notable insecticidal effects were seen. This was observed on plants infested zero, three and seven days after treatment. This suggests that the lag period is due to the MoA of spirotetramat (ACC inhibitor/interruption of lipid biosynthesis) rather than due solely to the translocation of the active ingredient throughout the plant. However, after this point spirotetramat provided good levels of control, inducing considerable levels of mortality (~60% after four days) and notably affecting reproduction and/or nymph survival of *M. persicae*. However, the application of spirotetramat is unlikely to provide successful management of non-persistent plant viruses due to its slow speed of action.

While there were slight reductions in the survival of alate *M. persicae* after the application of Plant Extracts 1 and 2, the overall levels of mortality on treated plants did not differ significantly to the untreated controls, even on plants infested 1 h after spraying. Variable effects of biopesticides are reported regularly. However, this is often related to their performance and persistence in the field (Copping and Menn, 2000; Chandler *et al*., 2011). Due to the withdrawal and restriction of the use of many synthetic chemical insecticides, there has been a resurgence in the interest of biopesticides (IBMA, 2016). While their sustainability makes them good candidates for use in IPM programmes, sufficient levels of efficacy and persistence need to be demonstrated consistently in both laboratory and field studies for pest control. From the findings of this project, it is unlikely that the applications of Plant Extracts 1 and 2 in the field would provide the adequate levels of control required for aphid populations in crop production systems.

The experiments in the project were carried out in a controlled laboratory environment (18°C, under a photoperiod of 16L:8D). Nevertheless, in the field, insecticides are subject to changes in environmental conditions. These include variation in air temperature, exposure to UV light and adverse weather conditions, such as heavy rainfall (Soliman, 2012). Due to these factors, there may be differences between how the insecticides perform in terms of their efficacy and persistence in the laboratory compared to in the field. To investigate this, field trials would be the next logical stage for this research.

7.5 Effects of insecticides on the behaviour of alate *Myzus persicae*

In terms of their effects on aphid behaviour, the six test products showed considerable variation. Lambda-cyhalothrin led to reductions in settling behaviour and increased the number of short duration probes (< 1 min) performed by alate *M. persicae*. This raises questions about the impact that lambda-cyhalothrin may have in terms of the spread of non-persistent plant viruses, as periods of short duration probing often facilitate virus transmission (Stevens and Lacomme, 2017). There was evidence to suggest that this reduction in aphid settling occurred through neurotoxicity, and to some extent, the sensory perception of the insecticide. Conversely, spirotetramat had very little effect on probing/feeding and settling behaviour, whereas Plant Extracts 1 and 2 displayed considerable variability in terms of their effects. It is possible that Plant Extract 2 may influence the settling behaviour of alate *M. persicae* through repellent and/or anti-feedant properties; but these effects were not demonstrated consistently in this study. Systemic Insecticides 1 and 2 affected the behaviour of alate *M. persicae* largely through the rapid induction of neurotoxicity (convulsion/tremors) and in several cases led to death within 5 h.

7.6 Efficacy of insecticides for the management of the transmission of a non-persistent plant virus by alate *Myzus persicae*

Host plant selection and the subsequent probing/feeding behaviour by aphids can considerably affect their potential as vectors of plant viruses. These factors can reduce or increase the transmission of a particular plant virus (Powell *et al*., 2006). The extent to which virus transmission is affected, however, depends on the specific virus, the nature of its transmission and its vectors (Ng and Perry, 2004). For this reason, knowledge of the behaviour of key virus vectors, like *M. persicae,* is crucial for understanding the spread and management of economically-important viruses in crops.

Here a system was developed to investigate the efficacy of three insecticides (spirotetramat, Plant Extract 2 and Systemic Insecticide 1) in reducing the transmission of an important non-persistent virus of *Brassica* spp. (turnip mosaic virus, TuMV) by alate *M. persicae*. While there was some initial evidence to suggest that insecticides with fast knock-down (*e.g.* Systemic Insecticide 1) or potential

repellent/anti-feedant properties (*e.g.* Plant Extract 2) may have some impact on virus transmission, the findings were limited by the amount of replication. However, now that a system has been developed there is the opportunity to increase replication as well as to potentially extend these studies to different host plants, aphid vectors, insecticides and viruses.

The information gathered in Chapters 4–6 could aid in the timing the application of the test products. For example, in terms of aphid vectors of non-persistent plant viruses, it may be beneficial to apply treatments with rapid knock-down (*e.g*. Systemic Insecticides 1 and 2) and/or those with anti-feedant properties when aphids first arrive in crops. Forecasting systems can aid greatly in timing these initial applications to correspond with the onset of aphid migration. Contact treatments (*e.g*. lambda-cyhalothrin), applied reactively, may then be useful for aphids that are established in crops. However, as resistance to pyrethroid insecticides has been reported for several species of aphid (Mota-Sanchez and Wise, 2019), careful consideration is required prior to their application in the field. Going forward the use of pyrethroids for aphid control may not provide a sustainable option. In addition, selecting treatments that help to reduce reproduction to keep pest populations under control (*e.g.* spirotetramat) would be beneficial when aphids are already present in a crop. If and when aphid populations begin to increase (*e.g.* greater pest pressure arising later in the season) treatments with high efficacy and fast knock-down may be required again (*e.g*. Systemic Insecticides 1 and 2).

However, in cases where plant viruses are not a major issue or persistent plant viruses are the primary concern for crop growers, the application sequence of insecticides may be different. For example, there is more opportunity to employ treatments with slower modes of action and reserve the use of those with rapid knock-down for periods of high abundance.

7.7 Summary

In this project, the forecasting models developed to predict aphid phenology improve and/or expand on what is available currently to growers (*e.g.* a day-degree model with improved accuracy for *C. aegopodii,* novel forecasts based on mean air temperature for *C. pastinaceae* and *C. theobaldi* and forecasts based on mean air temperature to

predict key events in the spring/summer migration of *M. persicae*). However, there are still several important gaps in the knowledge of the species that were investigated. For example, the base temperature for the development of *C. aegopodii* requires clarification. This may help to refine day-degree models. For *C. pastinaceae* and *C. theobaldi*, it is unclear why their phenology could not be described in terms of accumulated day-degree sums. This calls for an improved understanding of their biology and ecology. In several cases, forecast development was limited by access to recent years of reliable weather records during this project. Going forward, linear regression analyses could be rerun with these data, in order to account for recent trends in winter/spring temperature and aphid phenology.

Additionally, the findings of the project can be used to inform the selection, timing and sequence of application of several insecticides for the control of *M. persicae*. This is based on an improved understanding of their efficacy, persistence and effects on aphid behaviour. Notably, Systemic Insecticides 1 and 2 had high levels of efficacy and persistence and led to the rapid knock-down of alate *M. persicae*. In the field, it is possible that these treatments could contribute to a reduction in the transmission of plant viruses. In terms of managing the secondary spread of non-persistent plant viruses, the application of Systemic Insecticides 1 and 2 are likely to be important prior to aphids migrating to crops during their host selection flights.

However, the prophylactic use of novel chemistries can be expensive or these treatments may be restricted in terms of their number of authorised applications. For these reasons, the identification of key virus vectors (in addition to *M. persicae*) and determining their phenology is crucial for successfully targeting the use of insecticides.

The applications of Plant Extracts 1 and 2, varied in terms of their effects on the behaviour of alate *M. persicae*. The properties of Plant Extract 2 still require further investigation to determine any potential repellent and/or anti-feedant effects. However, due to their low levels of efficacy and persistence, these treatments are unlikely to provide effective control of aphid populations in the field when used as stand-alone treatments.

Interestingly, when applied prophylactically, lambda-cyhalothrin reduced aphid settling behaviour and significantly increased the number of short duration probes (< 1 min) performed by alate *M. persicae,* relative to the untreated controls. In the field, this behaviour could have implication for the transmission of non-persistent plant viruses. While the effects of lambda-cyhalothrin on the transmission of TuMV were not investigated in the project, it will be important to investigate these effects to test this hypothesis. However, due to the increasing number of cases of insecticide resistance, there is considerable uncertainty surrounding the sustainability of the use of pyrethroids for aphid control.

Going forward, it will be important to extend aphid behavioural studies and virus transmission experiments to different species of aphid and host plants. It is possible that the use of some insecticides may be more suited to certain crops. This is particularly relevant for the control of *M. persicae* due to its polyphagous nature and wide host range. Currently, the efficacy and persistence of a number of insecticides are being tested for the control of several species of apterous aphids in trials (SCEPTREplus) at Warwick Crop Centre, School of Life Sciences, Wellesbourne.

7.8 New findings from the project

The findings of this project improve the understanding of methods for predicting the phenology of key pest aphids (*M. persicae*, *C. aegopodii*, *C. pastinaceae* and *C. theobaldi*). The project also characterised the responses of alate *M. persicae* to commercially-important host plants and several insecticides.

- A refined day-degree model was developed to predict the onset of migration by *C. aegopodii*. The model offers improved accuracy over the day-degree model that is employed currently in the UK.

- Novel forecasts based on mean air temperature, averaged over defined periods in the winter/early spring, were developed to predict the timing of the migrations of *C. pastinaceae* and *C. theobaldi* and when the summer peak in numbers of *M. persicae* will occur.

- The behavioural responses of alate *M. persicae* on three host plants (Brussels sprout, carrot and lettuce) and to six insecticides (including four novel treatments) were characterised. On Brussels sprout, alate *M. persicae* initiated probing behaviour more quickly, settled and fed more readily. However, *M. persicae* exhibited 'restless' behaviour on carrot and lettuce. This behaviour could have implications for the transmission of non-persistent plant viruses.

- The effects of Systemic Insecticides 1 and 2 on alate *M. persicae* were promising in terms of their efficacy, persistence and speed of knockdown. Both insecticides led to a rapid induction of neurotoxicity, rendering aphids immobile. In the field, the application of Systemic Insecticides 1 and 2 may help to reduce the transmission of plant viruses by alate *M. persicae*.

References

Agelopoulos, N., Birkett, M. A., Hick, A. J., Hooper, A. M., Pickett, J. A., Pow, E. M., Smart, L. E., Smiley, D. W., Wadhams, L. J. and Woodcock, C. M. (1999). Exploiting semiochemicals in insect control. *Pesticide Science*, *55*(3), pp.225-235.

AHDB. (2013). Grown in Britain: Guide to British Fresh Produce. Available online: https://horticulture.ahdb.org.uk/download/7106/file [Accessed: July 2019].

AHDB. (2018). Pyrethroid resistance in willow-carrot aphid. Available online: https://horticulture.ahdb.org.uk/news-item/pyrethroid-resistance-willow-carrot-aphid [Accessed: February 2019].

AHDB. (2019a). Agriculture Horticultural Development Board Aphid News – Potato and Brassica Aphids forecast 2019. Available online: https://ahdb.org.uk/aphid-news [Accessed: July 2019]

AHDB. (2019b). Agriculture Horticultural Development Board Pest Monitoring Survey Report of levy payers and industry professionals (November 2018–January 2019).

Ahmed, N., Darshanee, H. L. C., Khan, I. A., Zhang, Z. F. and Liu, T. X. (2019). Host Selection behaviour of the green peach aphid, *Myzus persicae*, in response to volatile organic compounds and nitrogen contents of cabbage cultivars. *Frontiers in Plant Science*, *10*, p.79.

Alford, L., Blackburn, T. M. and Bale, J. S. (2012). Effects of acclimation and latitude on the activity thresholds of the aphid *Myzus persicae* in Europe. *Journal of Applied Entomology*, *136*(5), pp.332-346.

Annis, B., Berry, R. E. and Tamaki, G. (1982). Host preferences of the green peach aphid, *Myzus persicae* (Hemiptera: Aphididae). *Environmental Entomology*, *11*(4), pp.824-827.

Anthon, E. W. (1955). Evidence for green peach aphid resistance to organo-phosphorous insecticides. *Journal of Economic Entomology*, *48*(1), pp.56-57.

Auclair, J. L. (1966). Dissimilarities in the biology of the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphidae) on alfalfa and peas in New Mexico. *Annals of the Entomological Society of America*, *59*(4), pp.780-786.

Awmack, C., Harrington, R. and Leather, S. (1997). Host plant effects on the performance of the aphid *Aulacorthum solani* (Kalt.) (Homoptera: Aphididae) at ambient and elevated CO_2. *Global Change Biology*, *3*(6), pp.545-549.

Ayalew, G., Sciarretta, A., Baumgartner, J., Ogol, C. and Lohr, B. (2008). Spatial distribution of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), at the field and the regional level in Ethiopia. *International Journal of Pest Management 54*(1), pp.31-38.

Bailey, A. S., Bertaglia, M., Fraser, I. M., Sharma, A. and Douarin, E. (2009). Integrated pest management portfolios in UK arable farming: results of a farmer survey. *Pest Management Science, 65*(9), pp.1030-1039.

Balasubramaniam, M., Ibrahim, A., Kim, B. S. and Loesch-Fries, L. S. (2006). *Arabidopsis thaliana* is an asymptomatic host of Alfalfa mosaic virus. *Virus Research*, *121*(2), pp.215-219.

Bale J. S., Masters G. J., Hodkinson I. D., Awmack C., Bezemer T. M., Brown V. K., Butterfield J., Buse A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., Whittaker, J. B. (2002). Herbivory in global climate change research: direct effects of rising temperatures on insect herbivores. *Global Change Biology, 8*(1)*,* pp.1-16.

Bale, J. S. (1991). Insects at low temperature: a predictable relationship? *Functional Ecology*, *5*(2), pp.291-298.

Barlow, C. A. (1962). The influence of temperature on the growth of experimental populations of *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Aphididae). *Canadian Journal of Zoology*, *40*(2), pp.145-156.

Barzman, M., Bàrberi, P., Birch, A. N. E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J. E., Kiss, J., Kudsk, P. and Lamichhane, J. R. (2015). Eight principles of integrated pest management. *Agronomy for Sustainable Development*, *35*(4), pp.1199-1215.

Baskerville, G. L. and Emin, P. (1969). Rapid estimation of heat accumulation from maximum and minimum temperatures. *Ecology*, *50*(3), pp.514-517.

Bass, C., Denholm, I., Williamson, M. S. and Nauen, R. (2015). The global status of insect resistance to neonicotinoid insecticides. *Pesticide Biochemistry and Physiology*, *121*, pp.78-87.

Bass, C., Puinean, A. M., Andrews, M., Cutler, P., Daniels, M., Elias, J., Paul, V. L., Crossthwaite, A. J., Denholm, I., Field, L. M. and Foster, S. P. (2011). Mutation of a nicotinic acetylcholine receptor β subunit is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *BMC Neuroscience*, *12*(1), p.51.

Bass, C., Puinean, A. M., Zimmer, C. T., Denholm, I., Field, L.M., Foster, S. P., Gutbrod, O., Nauen, R., Slater, R. and Williamson, M. S. (2014). The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochemistry and Molecular Biology*, *51*, pp.41-51.

Bass, C., Zimmer, C. T., Riveron, J. M., Wilding, C. S., Wondji, C. S., Kaussmann, M., Field, L. M., Williamson, M. S. and Nauen, R. (2013). Gene amplification and microsatellite polymorphism underlie a recent insect host shift. *Proceedings of the National Academy of Sciences*, *110*(48), pp.19460-19465.

Bate, R. (2007). The rise, fall, rise, and imminent fall of DDT. *American Enterprise Institute for Public Policy Research 14*(4), pp.1-9.

Baulcombe, D., Crute, I., Davies, B., Dunwell, J., Gale, M., Jones, J., Pretty, J., Sutherland, W. and Toulmin, C. (2009).*Reaping the benefits: science and the sustainable intensification of global agriculture*. The Royal Society.

Bedford, I. D., Kelly, A., Banks, G. K., Fuog, D. and Markham, P. G. (1998). The effect of pymetrozine, a feeding inhibitor of Homoptera, in preventing transmission of cauliflower mosaic caulimovirus by the aphid species *Myzus persicae* (Sulzer). *Annals of Applied Biology* 132, pp.453–462.

Bell, J. R., Alderson, L., Izera, D., Kruger, T., Parker, S., Pickup, J., Shortall, C. R., Taylor, M. S., Verrier, P. and Harrington, R. (2014). Long-term phenological trends, species accumulation rates, aphid traits and climate: five decades of change in migrating aphids. *Journal of Animal Ecology*, *84*(1), pp.21-34.

Bell, J. R., Taylor, M. S., Shortall, C. R., Welham, S. J. and Harrington, R. (2012). The trait and host plant ecology of aphids and their distribution and abundance in the United Kingdom. *Global Ecology and Biogeography*, *21*(4), pp.405-415.

Berger, P. H., Zeyen, R. J. and Groth, J. V. (1987). Aphid retention of maize dwarf mosaic virus (potyvirus): epidemiological implications. *Annals of Applied Biology*, *111*(2), pp.337-344.

Bernays, E. A. and Chapman, R. F. (1994). Behaviour: the process of host finding. In: E. A. Bernays, R. F. Chapman (eds.). *Host Plant Selection by Phytophagous Insects*, Chapman and Hall, New York, USA, pp.95-165.

Bernays, E. A. and Funk, D. J. (1999). Specialists make faster decisions than generalists: experiments with aphids. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1415), pp.151-156.

Birch, A. N. E., Begg, G. S. and Squire, G. R. (2011). How agroecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. *Journal of Experimental Botany*, *62*(10), pp.3251-3261.

Blackman, R. L. (1971). Variation in the photoperiodic response within natural populations of *Myzus persicae* (Sulz.). *Bulletin of Entomological Research*, *60*(4), pp.533-546.

Blackman, R. L. (1974). Life-cycle variation of *Myzus persicae* (Sulz.) (Hom., Aphididae) in different parts of the world, in relation to genotype and environment. *Bulletin of Entomological Research*, *63*(4), pp.595-607.

Blackman, R. L. (1987). Morphological discrimination of a tobacco-feeding form from *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (*Nectarosiphon*) species. *Bulletin of Entomological Research*, *77*(4), pp.713-730.

Blackman, R. L. (1990). Specificity in aphid/plant genetic interactions, with particular attention to the role of the alate colonizer. In: R. K. Campbell and R. D. Eikenbary (eds.). Aphid–Plant Genotype Interactions, Elsevier, New York, USA, pp.251–274.

Blackman, R. L. and Eastop, V. F. (2017). Taxonomic issues. In: H. F. van Emden, R. Harrington (eds.). *Aphids as Crop Pests,* 2nd edition, CABI, Wallingford, UK, pp.1-36.

Blackman, R. L. and Eastop, V. F. (2018). Aphids on the World's Plants: an online identification and information guide. Available online: http://www.aphidsonworldsplants.info/ [Accessed: January 2019].

Blua, M. J. and Perring, T.M. (1992). Alatae production and population increase of aphid vectors on virus-infected host plants. *Oecologia*, *92*(1), pp.65-67.

Boiteau, G. (1990). Effect of trap colour and size on relative efficiency of water-pan traps for sampling alate aphids (Homoptera: Aphididae) on potato. *Journal of Economic Entomology*, *83*(3), pp.937-942.

Boiteau, G., King, R. R. and Levesque, D. (1985). Lethal and Sublethal effects of aldicarb on two potato aphids (Homoptera: aphidae): *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas). *Journal of Economic Entomology*, *78*(1), pp.41-44.

Bonferroni, C. E. (1936). Statistical theory of classification and calculation of the probability. *Publication of the R Institute Superiore of the Science of Economy and Commerce of Florence*, *8*, pp.3-62.

Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E. A. and Noome, D. A. (2015). Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research*, *22*(1), pp.35-67.

Boquel, S., Zhang, J., Goyer, C., Giguère, M.A., Clark, C. and Pelletier, Y. (2015). Effect of insecticide-treated potato plants on aphid behaviour and potato virus Y acquisition. *Pest Management Science*, *71*(8), pp.1106-1112.

Bradley, R. H. E. (1952). Studies on the aphid transmission of a strain of henbane mosaic virus. *Annals of Applied Biology*, *39*(1), pp.78-97.

Bradley, R. H. E. (1959). Loss of virus from the stylets of aphids. *Virology*, *8*(3), pp.308-318.

Bradley, R. H. E., Sylvester, E. S. and Wade, C. V. (1962a). Note on the movements of the mandibular and maxillary stylets of the aphid vector *Myzus persicae* (Sulzer). *The Canadian Entomologist, 94*(6) pp.653-654.

Bradley, R. H. E., Wade, C. V. and Wood, F. A. (1962b). Aphid transmission of potato virus Y inhibited by oils. *Virology*, *18*(2), pp.327-329.

Braverman, Y., Chizov-Ginzburg, A., Pener, H. and Wilamowski, A. (2004). Susceptibility and repellency of *Culicoides imicola* and *Culex pipiens* to lambda-cyhalothrin. *Veterinaria Italiana, 40*(3), pp.336-339.

Bretschneider, T., Fischer, R., Nauen, R. (2007). Inhibitors of lipid synthesis (acetyl-CoA-carboxylase inhibitors). In: W. Krämer and U. Schirmer (eds.). *Modern Crop Protection Compounds*, *1*, Wiley-VCH, Weinheim, Germany, pp. 909-925.

Broadbent, L. (1957). Insecticidal control of the spread of plant viruses. *Annual Review of Entomology*, *2*(1), pp.339-354.

Broadbent, L. and Hollings, M. (1951). The influence of heat on some aphids. *Annals of Applied Biology*, *38*(3), pp.577-581.

Bruce, T. J., Wadhams, L J. and Woodcock, C. M. (2005). Insect host location: a volatile situation. *Trends in Plant Science*, *10*(6), pp.269-274.

Brück, E., Elbert, A., Fischer, R., Krueger, S., Kühnhold, J., Klueken, A. M., Nauen, R., Niebes, J. F., Reckmann, U., Schnorbach, H. J. and Steffens, R. (2009). Movento®, an innovative ambimobile insecticide for sucking insect pest control in agriculture: biological profile and field performance. *Crop Protection*, *28*(10), pp.838-844.

Bryant, S. R., Bale, J. S. and Thomas, C. D. (1998). Modification of the triangle method of degree-day accumulation to allow for behavioural thermoregulation in insects. *Journal of Applied Ecology*, *35*(6), pp.921-927.

C-IPM. (2016). Coordinated Integrated Pest Management in Europe: Strategic research agenda for IPM in Europe. Available online: http://projects.au.dk/fileadmin/75813_36sider_brochure_C-IPMnovember_2016.pdf [Accessed: December 2018]

CABI. (2019). *Myzus persicae* (green peach aphid). Available online: https://www.cabi.org/isc/datasheet/35642 [Accessed: June 2019].

Calabrese, E. J. and Edwards, L. J. (1976). Light and gravity in leaf-side selection by the green peach aphid, *Myzus persicae*. *Annals of the Entomological Society of America*, *69*(6), pp.1145-1146.

Campbell, A., Frazer, B. D., Gilbert, N. G. A. P., Gutierrez, A. P. and Mackauer, M. (1974). Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology*, *11*(2), pp.431-438.

Casida, J. E. (1963). Mode of action of carbamates. *Annual Review of Entomology*, *8*(1), pp.39-58.

Castle, S., Palumbo, J. and Prabhaker, N. (2009). Newer insecticides for plant virus disease management. *Virus Research*, *141*(2), pp.131-139.

Chandler, D., Bailey, A. S., Tatchell, G. M., Davidson, G., Greaves, J. and Grant, W. P. (2011). The development, regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*(1573), pp.1987-1998.

Chapman, R. F., Bernays, E. A. and Simpson, S. J. (1981). Attraction and repulsion of the aphid, *Cavariella aegopodii*, by plant odours. *Journal of Chemical Ecology, 7*(5), pp.881-888.

Cho, S. R., Koo, H. N., Yoon, C. and Kim, G. H. (2011). Sublethal effects of flonicamid and thiamethoxam on green peach aphid, *Myzus persicae* and feeding behaviour analysis. *Journal of the Korean Society for Applied Biological Chemistry*, *54*(6), pp.889-898.

Ciss, M., Parisey, N., Moreau, F., Dedryver, C.A. and Pierre, J. S. (2014). A spatiotemporal model for predicting grain aphid population dynamics and optimising insecticide sprays at the scale of continental France. *Environmental Science and Pollution Research*, *21*(7), pp.4819-4827.

Clarke, J. H., Wynn, S. C., and Twining, S. E. (2011). Impact of changing pesticide availability. *Aspects of Applied Biology*, *106*, pp.263-267.

Cloyd, R. A., Bethke, J. A. and Cowles, R. S. (2011). Systemic insecticides and their use in ornamental plant systems. *Floriculture Ornamental Biotech*, *5*(1), pp.1-9.

Coats, J. R. (1994). Risks from natural versus synthetic insecticides. *Annual Review of Entomology*, *39*(1), pp.489-515.

Cocu, N., Harrington, R., Rounsevell, M. D. A., Worner, S. P., Hulle, M. and Examine Project Participants (2005). Geographical location, climate and land use influences on the phenology and numbers of the aphid, *Myzus persicae*, in Europe. *Journal of Biogeography*, *32*(4), pp.615-632.

Cole, R. A. (1997). The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. *Entomologia Experimentalis et Applicata*, *85*(2), pp.121-133.

Collar, J. L., Avilla, C., Duque, M. and Fereres, A. (1997). Behavioural response and virus vector ability of *Myzus persicae* (Homoptera: Aphididae) probing on pepper plants treated with aphicides. *Journal of Economic Entomology*, *90*(6), pp.1628-1634.

Collier, R. H. (2014). Spinach: Further studies on forecasting migrations of *Aphis fabae* into spinach crops. HDC Project FV 407a. Agriculture and Horticulture Development Board (AHDB) Report.

Collier, R. H. and Finch, S. (2001). Forecasting attacks by pest insects of cruciferous crops. *Proceedings of the 4th International Workshop: The management of diamondback moth and other crucifer pests*. Melbourne, Australia, 26-29th November 2001, pp.163-168.

Collier, R. H., Davies, J. A., Roberts, M., Leatherland, M., Runham, S. R. and Blood Smyth, J. A. (1994). Monitoring and forecasting the times of attack of the lettuce root aphid, *Pemphigus bursarius* L. *Bulletin IOBC/WPRS, 22*(5), pp.25-35.

Collier, R. H., Wilson, D. J., Jukes, A., Elliott, M. S., Warmington, R. and Clarkson, J. (2017). Research on integrated pest and disease management in carrot crops. *Acta Horticulturae. 1153*, pp.225-230.

Cook, P. P. and Sylvester, E. S. (1961). Influence of caging and transferring techniques on aphid mortality and virus transmission. *Journal of Economic Entomology*, *54*(1), pp.101-103.

Copping, L.G. and Menn, J. J. (2000). Biopesticides: a review of their action, applications and efficacy. *Pest Management Science*, *56*(8), pp.651-676.

Crawley, J. M. (2009). Population dynamics of natural enemies and their prey: In: M. J. Crawley (ed.). *Natural enemies: the population biology of predators, parasites and diseases*, *6,* Blackwell Scientific Publications Ltd., Oxford, UK, pp.40-60.

Criniti, A., Mazzoni, E., Cassanelli, S., Cravedi, P., Tondelli, A., Bizzaro, D. and Manicardi, G. C. (2008). Biochemical and molecular diagnosis of insecticide resistance conferred by esterase, MACE, kdr and super-kdr based mechanisms in Italian strains of the peach potato aphid, *Myzus persicae* (Sulzer). *Pesticide Biochemistry and Physiology*, *90*(3), pp.168-174.

Crist, E., Mora, C. and Engelman, R. (2017). The interaction of human population, food production, and biodiversity protection. *Science*, *356*(6335), pp.260-264.

Culliney, T. W. (2014). Crop losses to arthropods. D. Pimental, R. Peshin (eds.). *Integrated Pest Management*, Publ. Springer, New York, USA, pp.201-225.

Davis, G. K. (2012). Cyclical parthenogenesis and viviparity in aphids as evolutionary novelties. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *318*(6), pp.448-459.

Day, M. F. and Irzykiewicz, H. (1954). On the mechanism of transmission of non-persistent phytopathogenic viruses by aphids. *Australian Journal of Biological Sciences*, *7*(3), pp.251-273.

de Wijs, J. J. (1980). The characteristics of mineral oils in relation to their inhibitory activity on the aphid transmission of potato virus Y. *Netherlands Journal of Plant Pathology*, *86*(6), pp.291-300.

Dean, G. J. (1974). Effect of temperature on the cereal aphids *Metopolophium dirhodum* (Wlk.). *Bulletin of Entomological Research*, *63*(401), p.109.

Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B. and Naylor, R.L. (2018). Increase in crop losses to insect pests in a warming climate. *Science*, *361*(6405), pp.916-919.

Devonshire, A. L. and Moores, G. D. (1982). A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae). Pesticide Biochemistry and Physiology*, *18*(2), pp.235-246.

Dewar, A. M. and Denholm, I. (2017). Chemical control. In: H. F. van Emden, R. Harrington (eds.). *Aphids as Crop Pests,* 2nd edition, CABI, Wallingford, UK, pp.398-425.

Dinant, S., Bonnemain, J. L., Girousse, C. and Kehr, J. (2010). Phloem sap intricacy and interplay with aphid feeding. *Comptes Rendus Biologies*, *333*(6-7), pp.504-515.

Dixon, A. F. G. (1992). Constraints on the rate of parthenogenetic reproduction and pest status of aphids. *Invertebrate Reproduction and Development*, *22*(1-3), pp.159-163.

Dixon, A. F. G. (2012). Resource tracking in space. In: A. F. G. Dixon (ed.). *Aphid Ecology an Optimization Approach*, Springer, Dordrecht, Netherlands, pp.171-188.

Dixon, A. F. G. and Hopkins, G. W. (2010). Temperature, Seasonal Development and Distribution of Insects with Particular Reference to Aphids. In: P. Kindlmann, K. Houdkova, A. F. G. Dixon (eds.) *Aphid Biodiversity under Environmental Change*. Springer, Netherlands, pp.129-147.

Döring, T. F. (2014). How aphids find their host plants, and how they don't. *Annals of Applied Biology*, *165*(1), pp.3-26.

Dunn, J. A. (1965). Studies on the aphid, *Cavariella aegopodii* Scop: I. On willow and carrot. *Annals of Applied Biology*, *56*(3), pp.429-438.

Dunn, J. A. (1969). The colonization by *Cavariella aegopodii* Scop, of carrot plants of different sizes. *Annals of Applied Biology*, *63*(2), pp.318-324.

Dunn, J. A. (1970). The susceptibility of varieties of carrot to attack by the aphid, *Cavariella aegopodii* (Scop.). *Annals of Applied Biology*, *66*(2), pp.301-31.

Dunn, J. A. and Kempton, D. P. H. (1967). Effect of the timing of insecticide applications on infestation by *Cavariella aegopodii* Scop, on carrots. *Annals of Applied Biology*, *60*(1), pp.33-42.

Dunn, J. A. and Kirkley, J. (1966). Studies on the aphid, *Cavariella aegopodii* Scop: On secondary hosts other than carrot. *Annals of Applied Biology*, *58*(2), pp.213-217.

Dunn, O. J. (1961). The Dunn Multiple Comparison Test. *Journal of the American Statistical Association*, *56*, pp.52-64.

Eastop, V. F. (1955). Selection of aphid species by different kinds of insect traps. *Nature*, *176*(4489), p.936.

Edwardson J. R. and Christie R. G. (1986). Turnip mosaic virus. In: J. R. Edwardson (ed.). *Viruses Infecting Forage Legumes, 2*. Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, USA, pp. 438-453.

Elbert, A., Becker, B., Hartwig, J. and Erdelen, C. (1991). Imidacloprid – a new systemic insecticide. *Pflanzenschutz-Nachrichten Bayer (Germany, FR)*, *44*(2), pp.113-136.

Elbert, A., Haas, M., Springer, B., Thielert, W. and Nauen, R. (2008). Applied aspects of neonicotinoid uses in crop protection. *Pest Management Science, 64*(11), pp.1099-1105.

Elnagar, S. and Murant, A. F. (1976). The role of the helper virus, anthriscus yellows, in the transmission of parsnip yellow fleck virus by the aphid *Cavariella aegopodii*. *Annals of Applied Biology*, *84*(2), pp.169-181.

European Food Safety Authority (EFSA). (2018a). Peer review of the pesticide risk assessment for bees for the active substance clothianidin considering the uses as seed treatments and granules. *EFSA Journal*, *16*(2), p.e05177.

European Food Safety Authority (EFSA). (2018b). Peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering the uses as seed treatments and granules. *EFSA Journal*, *16*(2), p.e05178.

European Food Safety Authority (EFSA). (2018c). Peer review of the pesticide risk assessment for bees for the active substance thiamethoxam considering the uses as seed treatments and granules. *EFSA Journal*, *16*(2), p.e05179.

European Union. (2009). Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. *Official Journal of the European Union*, *309*, pp.71-86.

FERA. (2016). *PUS STAT: Pesticide Usage Surveys.* Available online: https://secure.fera.defra.gov.uk/pusstats/myindex.cfm [Accessed: January 2019].

FERA. (2020). LIAISON database. FERA Science Ltd. Available online: https://secure.fera.defra.gov.uk/liaison/ [Accessed: March 2020]

Fereres, A. and Collar, J. L. (2001). Analysis of noncirculative transmission by electrical penetration graphs. In: K. F. Harris, O. P. Smith, J. E. Duffus (eds.). *Virus-insect-plant Interactions*, Elsevier, Amsterdam, Netherlands, pp.87-109.

Fereres, A. and Moreno, A. (2009). Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus Research*, *141*(2), pp.158-168.

Ferro, D. N., Mackenzie, J. D. and Margolies, D. C. (1980). Effect of mineral oil and a systemic insecticide on field spread of aphid-borne maize dwarf mosaic virus in sweet corn. *Journal of Economic Entomology*, *73*(5), pp.730-735.

ffrench-Constant, R. H., Anthony, N., Aronstein, K., Rocheleau, T. and Stilwell, G. (2000). Cyclodiene insecticide resistance: from molecular to population genetics. *Annual Review of Entomology*, *45*(1), pp.449-466.

Fidelis, E. G., Farias, E. S., Lopes, M.C., Sousa, F. F., Zanuncio, J. C. and Picanço, M. C. (2019). Contributions of climate, plant phenology and natural enemies to the seasonal variation of aphids on cabbage. *Journal of Applied Entomology*, *143*(4), pp.365-370.

Finch, S. (1980). Chemical attraction of plant-feeding insects to plants. In: T. H. Coaker (ed.). *Applied Biology V*, Academic Press, London and New York, pp.67-143.

Finch, S. and Collier, R. H. (2000). Host-plant selection by insects–a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. *Entomologia Experimentalis et Applicata*, *96*(2), pp.91-102.

Finch, S. and Collier, R. H. (2003). Host-plant Selection by Insects-the" Missing Link". *IOBC WPRS Bulletin*, *26*(3), pp.103-108.

Flint, M. L. and van den Bosch, R. (2012). *Introduction to Integrated Pest Management*. Springer Science and Business Media, Berlin, Germany.

Fontaine, S., Caddoux, L., Brazier, C., Bertho, C., Bertolla, P., Micoud, A. and Roy, L. (2011). Uncommon associations in target resistance among French populations of *Myzus persicae* from oilseed rape crops. *Pest Management Science*, *67*(8), pp.881-885.

Foster, S. P. and Denholm, I. (2008). Stewardship of neonicotinoid insecticides. Agriculture and Horticulture Development Board (AHDB) Report.

Foster, S. P., Denholm, I. and Devonshire, A. L. (2000). The ups and downs of insecticide resistance in peach-potato aphids (*Myzus persicae*) in the UK. *Crop Protection*, *19*(8-10), pp.873-879.

Foster, S. P., Denholm, I. and Thompson, R. (2002). Bioassay and field-simulator studies of the efficacy of pymetrozine against peach-potato aphids, *Myzus persicae* (Hemiptera: Aphididae), possessing different mechanisms of insecticide resistance. *Pest Management Science, 58*(8), pp.805-810.

Foster, S. P., Denholm, I., Rison, J. L., Portillo, H. E., Margaritopoulos, J. and Slater, R. (2011). Susceptibility of standard clones and European field populations of the green peach aphid, *Myzus persicae,* and the cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae), to the novel anthranilic diamide insecticide cyantraniliprole. *Pest Management Science*, *68*(4), pp.629-633.

Foster, S. P., Devine, G. and Devonshire, A. L. (2017). Insecticide resistance. In: H. F. van Emden, R. Harrington (eds.). *Aphids as Crop Pests,* 2nd edition*,* CABI, Wallingford, UK, pp.426-447.

Fox, A., Skelton, A., Collins, L., Blackburn, L., Jackson, L. and Hinds, H. (2015). Carrots: The Epidemiology of Carrot yellow leaf virus (CYLV)-the development of a decision support system for the management of carrot viruses in the UK. HDC Project FV 382b. Agriculture and Horticulture Development Board (AHDB) Report.

Francis, F., Gerkens, P., Harmel, N., Mazzucchelli, G., De Pauw, E. and Haubruge, E. (2006). Proteomics in *Myzus persicae*: effect of aphid host plant switch. *Insect Biochemistry and Molecular Biology*, *36*(3), pp.219-227.

Fray, L. M., Leather, S. R., Powell, G., Slater, R., McIndoe, E. and Lind, R. J. (2014). Behavioural avoidance and enhanced dispersal in neonicotinoid-resistant *Myzus persicae* (Sulzer). *Pest Management Science*, *70*(1), pp.88-96.

Funk, D. J. and Bernays, E. A. (2001). Geographic variation in host specificity reveals host range evolution in *Uroleucon ambrosiae* aphids. *Ecology*, *82*(3), pp.726-739.

Gabrys, B., Tjallingii, W. F. and van Beek, T. A. (1997). Analysis of EPG recorded probing by cabbage aphid on host plant parts with different glucosinolate contents. *Journal of Chemical Ecology*, *23*(7), pp.1661-1673.

Garthwaite, G., Barker, I., Mace, A., Parrish, G., Ridley., L and Macarthur, R. (2017). Pesticide usage survey report: outdoor vegetable crops in Great Britain 2017. DEFRA, York, UK.

Gerwick, B. C. and Sparks, T. C. (2014). Natural products for pest control: an analysis of their role, value and future. *Pest Management Science*, *70*(8), pp.1169-1185.

Gibson, R. W. and Cayley, G. R. (1984). Improved control of potato virus Y by mineral oil plus the pyrethroid cypermethrin applied electrostatically. *Crop Protection*, *3*(4), pp.469-478.

Gibson, R. W. and Rice, A. D. (1986). The combined use of mineral oils and pyrethroids to control plant viruses transmitted non-and semi-persistently by *Myzus persicae*. *Annals of applied Biology*, *109*(3), pp.465-472.

Gibson, R. W., Rice, A. D., Pickett, J. A., Smith, M. C. and Sawicki, A. M. (1982a). The effects of the repellents dodecanoic acid and polygodial on the acquisition of non-, semi- and persistent plant viruses by the aphid *Myzus persicae*. *Annals of Applied Biology*, *100*(1), pp.55-59.

Gibson, R. W., Rice, A. D. and Sawicki, R. M. (1982b). Effects of the pyrethroid deltamethrin on the acquisition and inoculation of viruses by *Myzus persicae*. *Annals of Applied Biology*, *100*(1), pp.49-54.

Gildow, F. E. (1980). Increased production of alatae by aphids reared on oats infected with barley yellow dwarf virus. *Annals of the Entomological Society of America*, *73*(3), pp.343-347.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F. and Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *Science*, *327*(5967), pp.812-818.

Goggin, F. L., Quisenberry, S. S. and Ni, X. (2017). Feeding injury. In: H. F. van Emden, R. Harrington (eds.). *Aphids as Crop Pests,* 2nd edition, CABI, Wallingford, UK, pp.303-322.

Gong, Y., Shi, X., Desneux, N. and Gao, X. (2016). Effects of spirotetramat treatments on fecundity and carboxylesterase expression of *Aphis gossypii* Glover. *Ecotoxicology*, *25*(4), pp.655-663.

Goulson, D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology, 50*(4), pp.977-987.

Goulson, D. (2018). Call to restrict neonicotinoids. *Science*, *360*(6392), pp.973-973.

Govier, D. A. and Kassanis, B. (1974). Evidence that a component other than the virus particle is needed for aphid transmission of potato virus Y. *Virology*, *57*(1), pp.285-286.

Gratwick, M. (1992). Willow-carrot aphid (Leaflet 603). In: M. Gratwick (ed.). *Crop Pests in the UK: Collected edition of MAFF leaflets*. Springer Science and Business Media, Berlin, Germany, pp.73-77.

Grue, C. E., Gibert, P. L. and Seeley, M. E. (1997). Neurophysiological and behavioural changes in non-target wildlife exposed to organophosphate and carbamate pesticides: thermoregulation, food consumption, and reproduction. *American Zoologist*, *37*(4), pp.369-388.

Hardie, J. and Powell, G. (2000). Close-up video combined with electronic monitoring of plant penetration and behavioural effects of an aphid antifeedant. In: G. P. Walker, E. A. Backus (eds.). *Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behaviour*, Thomas Say Publ./ESA, Lanham, Maryland, USA pp.201-211.

Hardie, J., Isaacs, R., Pickett, J. A., Wadhams, L. J. and Woodcock, C. M. (1994b). Methyl salicylate and (−)-(1R, 5S)-myrtenal are plant-derived repellents for black bean aphid, *Aphis fabae* Scop.(Homoptera: Aphididae). *Journal of Chemical Ecology*, *20*(11), pp.2847-2855.

Hardie, J., Visser, J. H. and Piron, P. G. M. (1994a). Perception of volatiles associated with sex and food by different adult forms of the black-bean aphid, *Aphis fabae*. *Physiological Entomology, 19*(4), pp.278-284.

Hardin, M. R., Benrey, B., Coll, M., Lamp, W. O., Roderick, G. K. and Barbosa, P. (1995). Arthropod pest resurgence: an overview of potential mechanisms. *Crop Protection*, *14*(1), pp.3-18.

Harrewijn, P. and Kayser, H. (1997). Pymetrozine, a fast-acting and selective inhibitor of aphid feeding. In-situ studies with electronic monitoring of feeding behaviour. *Pesticide Science*, *49*(2), pp.130-140.

Harrington, R. and Clark, S. (2010). Trends in the timings of the start and end of annual flight periods. In: P. Kindlmann, K. Houdkova, A. F. Dixon (eds.). *Aphid Biodiversity under Environmental Change,* Springer, Dordrecht, Netherlands, pp.41-54.

Harrington, R. and Hullé, M. (2017). Monitoring and forecasting. In: H. F. van Emden, R. Harrington (eds.). *Aphids as Crop Pests,* 2nd edition, CABI, Wallingford, UK, pp.362-381.

Harrington, R., Bale, J. S. and Tatchell, G. M. (1995). Aphids in a changing climate. In: R. Harrington and N. E. Stork (eds.). *Insects in a Changing Environment*, Academic Press, London, UK, pp. 125-155.

Harrington, R., Clark, S. J., Welham, S. J., Verrier, P. J., Denholm, C. H., Hulle, M., Maurice, D., Rounsevellz, M. D. and Cocu, N. (2007). Environmental change and the phenology of European aphids. *Global Change Biology*, *13*(8), pp.1550-1564.

Harrington, R., Dewar, A. M. and George, B. (1989). Forecasting the incidence of virus yellows in sugar beet in England. *Annals of Applied Biology*, *114*(3), pp.459-469.

Harrington, R., Howling, G. G., Bale, J. S. and Clark, S. (1991). A new approach to the use of meteorological and suction trap data in predicting aphid problems. *EPPO Bulletin*, *21*(3), pp.499-505.

Harrington, R., Tatchell, G. M. and Bale, J. S. (1990). Weather, life cycle strategy and spring populations of aphids. *Acta Phytopathologica et Entomologica Hungarica, 25*, pp.423-432.

Haynes, K. F. (1988). Sublethal effects of neurotoxic insecticides on insect behaviour. *Annual Review of Entomology*, *33*(1), pp.149-168.

Heathcote, G. D. (1962). The suitability of some plant hosts for the development of the peach-potato aphid, *Myzus persicae* (Sulzer). *Entomologia Experimentalis et Applicata*, *5*(2), pp.114-118.

Heathcote, G. D., Palmer, J. M. P. and Taylor, L. R. (1969). Sampling for aphids by traps and by crop inspection. *Annals of Applied Biology*, *63*(1), pp.155-166.

Higley, L. G., Pedigo, L. P. and Ostlie, K. R. (1986). DEGDAY: a program for calculating degree-days, and assumptions behind the degree-day approach. *Environmental Entomology*, *15*(5), pp.999-1016.

Hille Ris Lambers, D. (1966). Polymorphism in Aphididae. *Annual Review of Entomology*, *11*(1), pp.47-78.

Hillocks, R. J. (2012). Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection*, *31*(1), pp.85-93.

Hodge, S. and Powell, G. (2010). Conditional facilitation of an aphid vector, *Acyrthosiphon pisum*, by the plant pathogen, pea enation mosaic virus. *Journal of Insect Science*, *10*(1), p.155.

Holman, J. (2009a). Introduction. In: J. Holman (ed.) *Host Plant Catalog of Aphids*, Springer, New York, USA, pp.1-7.

Holman, J. (2009b). The aphids and their host plants. In: J. Holman (ed.) *Host Plant Catalog of Aphids*, Springer, New York, USA. Pp.232–241

Horsfall, J. L. (1924). Life history studies of *Myzus persicae* Sulzer. Pennsylvania State College Agricultural Experiment Station.

Howard, M. T. and Dixon, A. F. G. (1990). Forecasting of peak population density of the rose grain aphid *Metopolophium dirhodum* on wheat. *Annals of Applied Biology*, *117*(1), pp.9-19.

Howling, G. G., Harrington, R., Clark, S. J. and Bale, J. S. (1993). The use of multiple regression via principal components in forecasting early season aphid (Homoptera: Aphididae) flight. *Bulletin of Entomological Research*, *83*(3), pp.377-381.

Hurej, M. and van der Werf, W. (1993). The influence of black bean aphid, *Aphis fabae* Scop., and its honey-dew on leaf growth and dry matter production of sugar beet. *Annals of Applied Biology 122*(2), pp.201-214.

Ibbotson, A. and Kennedy, J. S. (1959). Interaction between walking and probing in *Aphis fabae* Scop. *Journal of Experimental Biology*, *36*(2), pp.377-390.

IBMA. (2016). International Biocontrol Manufacturer's Association Biocontrol Survey Report. Available online: http://www.ibma-global.org/en/news/ibma-biocontrol-survey-report. [Accessed: June 2018].

Inglesfield, C. (1989). Pyrethroids and terrestrial non-target organisms. *Pesticide Science*, *27*(4), pp.387-428.

IRAC. (2009). Susceptibility Test Methods Series method No: 001 *Myzus persicae* (Version 3: June 2009) Available online:
https://www.irac-online.org/content/uploads/Method_001_v3_june09.pdf
[Accessed: April 2019].

IRAC. (2019). IRAC Mode of Action Classification Scheme: Version 9.1. Available online: https://www.irac-online.org/documents/moa-classification/ [Accessed: June 2019]

IRAG. (2019). Insecticide Resistance Action Group minutes of the 42nd meeting held at ADAS, Boxworth (24th April 2019).

Irwin, M. E. and Thresh, J. M. (1990). Epidemiology of barley yellow dwarf: a study in ecological complexity. *Annual Review of Phytopathology*, *28*(1), pp.393-424.

Jayasena, K. W. and Randles, J. W. (1985). The effect of insecticides and a plant barrier row on aphid populations and the spread of bean yellow mosaic potyvirus and subterranean clover red leaf luteovirus in *Vicia faba* in South Australia. *Annals of Applied Biology*, *107*(3), pp.355-364.

Jenner, C. E. and Walsh, J. A. (1996). Pathotypic variation in turnip mosaic virus with special reference to European isolates. *Plant Pathology*, *45*(5), pp.848-856.

Jenner, C. E., Nellist, C. F., Barker, G. C. and Walsh, J. A. (2010). Turnip mosaic virus (TuMV) is able to use alleles of both eIF4E and eIF (iso) 4E from multiple loci of the diploid *Brassica rapa*. *Molecular Plant-microbe Interactions*, *23*(11), pp.1498-1505.

Jenner, C., Keane, G., Jones, J. and Walsh, J. (1999). Serotypic variation in turnip mosaic virus. *Plant Pathology*, *48*(1), pp.101-108.

Jeschke, P., Nauen, R., Schindler, M. and Elbert, A. (2010). Overview of the status and global strategy for neonicotinoids. *Journal of Agricultural and Food Chemistry, 59*(7), pp.2897-2908.

Jess, S., Kildea, S., Moody, A., Rennick, G., Murchie, A. K. and Cooke, L. R. (2014). European Union policy on pesticides: implications for agriculture in Ireland. *Pest Management Science*, *70*(11), pp.1646-1654.

Johansen, E., Edwards, M. C. and Hampton, R. O. (1994). Seed transmission of viruses: current perspectives. *Annual Review of Phytopathology*, *32*(1), pp.363-386.

Johnson, C. G. (1954). Aphid migration in relation to weather. *Biological Reviews*, *29*(1), pp.87-118.

Johnson, C. G. and Taylor, L.R. (1957). Periodism and energy summation with special reference to flight rhythms in aphids. *Journal of Experimental Biology*, *34*(2), pp.209-221.

Johnson, C. G., Taylor, L. R. and Haine, E. (1957). The analysis and reconstruction of diurnal flight curves in alienicolae of *Aphis fabae* Scop. *Annals of Applied Biology*, *45*(4), pp.682-701.

Karley, A. J., Parker, W. E., Pitchford, J. W. and Douglas, A. E. (2004). The mid-season crash in aphid populations: why and how does it occur? *Ecological Entomology*, *29*(4), pp.383-388.

Katis, N. and Gibson, R. W. (1985). Transmission of potato virus Y by cereal aphids. *Potato Research*, *28*(1), pp.65-70.

Kennedy, J. S. (1958). Physiological condition of the host-plant and susceptibility to aphid attack. *Entomologia Experimentalis et Applicata, 1*(1), pp.50-65.

Kennedy, J. S. (1976). Host-plant finding by flying aphids. In: T. Jermy (eds.). *The Host-Plant in Relation to Insect Behaviour and Reproduction*, Springer, Boston, USA, pp.121-123.

Kennedy, J. S., Day, M. F. and Eastop, V. F. A. (1962). *A Conspectus of Aphids as Vectors of Plant Viruses*, Commonwealth Institute of Entomology, London, p.114.

Kindlmann, P. and Dixon, A. F. (2010). Modelling population dynamics of aphids and their natural enemies. In: P. Kindlmann, K. Houdkova, A. F. Dixon (eds.). *Aphid Biodiversity under Environmental Change,* Springer, Dordrecht, Netherlands, pp.1-20.

Kleier, D. A. (1994). Phloem mobility of xenobiotics. V. Structural requirements for phloem-systemic pesticides. *Pesticide Science*, *42*(1), pp.1-11.

Kleinbaum, D. G. and Klein, M. (2012). Kaplan-Meier survival curves and the log-rank test. In: D. G. Kleinbaum (ed.). *Survival Analysis,* Springer, New York, USA, pp.55-96.

Knisel, W. G. (1993). *GLEAMS: Groundwater Loading Effects of Agricultural Management Systems: Version 2.10* (No. 5). University of Georgia Coastal Plain Experiment Station, Biological and Agricultural Engineering.

Knowles, A. (2008). Recent developments of safer formulations of agrochemicals. *The Environmentalist*, *28*(1), pp.35-44.

Knutson, A. E. and Muegge, M. A. (2010). A degree-day model initiated by pheromone trap captures for managing pecan nut casebearer (Lepidoptera: Pyralidae) in pecans. *Journal of Economic Entomology*, *103*(3), pp.735-743.

Kobayashi, M. and Ishikawa, H. (1993). Breakdown of indirect flight muscles of alate aphids (*Acyrthosiphon pisum*) in relation to their flight, feeding and reproductive behaviour. *Journal of Insect Physiology, 39*(7), pp.549-554.

Kogan, M. (1998). Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology*, *43*(1), pp.243-270.

Kolbezen, M. J., Metcalf, R. L. and Fukuto, T. R. (1954). Insecticide structure and activity, insecticidal activity of carbamate cholinesterase inhibitors. *Journal of Agricultural and Food Chemistry*, *2*(17), pp.864-870.

Kundu, R. and Dixon, A. F. G. (1995). Evolution of complex life cycles in aphids. *Journal of Animal Ecology*, 64(2), pp.245-255.

Kurppa, S. (1989) Predicting outbreaks of *Rhopalosiphum padi* in Finland. *Annales Agriculturae Fenniae,* 28, pp.333-347.

Lahm, G. P., Selby, T. P., Freudenberger, J. H., Stevenson, T. M., Myers, B. J., Seburyamo, G., Smith, B. K., Flexner, L., Clark, C. E. and Cordova, D. (2005). Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators. *Bioorganic and Medicinal Chemistry Letters*, *15*(22), pp.4898-4906.

Lal, R. (1950). Biology and control of *Myzus persicae* (Sulzer) as a pest of potato at Delhi. *Indian Journal of Agricultural Science*, *20*, pp.87-100.

Leather, S. R., Walters, K. F. and Bale, J. S. (1995). *The Ecology of Insect Overwintering*. Cambridge University Press, Cambridge, UK.

Lees, A. D. (1966). The control of polymorphism in aphids. *Advances in Insect Physiology*, *3*, pp.207-277.

Lefebvre, M., Langrell, S. R. and Gomez-y-Paloma, S. (2015). Incentives and policies for integrated pest management in Europe: a review. *Agronomy for Sustainable Development*, *35*(1), pp.27-45.

Lewis, T. and Taylor, L. R. (1965). Diurnal periodicity of flight by insects. *Transactions of the Royal Entomological Society of London*, *116*(15), pp.393-435.

Liu, S. S. and Meng, X. D. (1999). Modelling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures. *Bulletin of Entomological Research*, *89*(1), pp.53-63.

Loebenstein, G. and Raccah, B. (1980). Control of non-persistently transmitted aphid-borne viruses. *Phytoparasitica*, *8*(3), pp.221-235.

Lowe, H. J. B. (1973). Variation in *Myzus persicae* (Sulz.) (Hemiptera, Aphididae) reared on different host-plants. *Bulletin of Entomological Research*, *62*(4), pp.549-556.

Lowery, D. T. and Boiteau, G. (1988). Effects of five insecticides on the probing, walking, and settling behavior of the green peach aphid and the buckthorn aphid (Homoptera: Aphididae) on potato. *Journal of Economic Entomology, 81*(1), pp.208-214.

Lowery, D. T., Sears, M. K. and Harmer, C. S. (1990). Control of turnip mosaic virus of rutabaga with applications of oil, whitewash, and insecticides. *Journal of Economic Entomology*, *83*(6), pp.2352-2356.

Lushai, G., Sherratt, T. N., David, O., De Barro, P. J. and Maclean, N. (1997). Host selection by winged summer females of the aphid *Sitobion avenae*. *Entomologia Experimentalis et Applicata*, *85*(3), pp.199-209.

Macaulay, E. D. M., Tatchell, G. M. and Taylor, L. R. (1988). The Rothamsted Insect Survey '12-metre'suction trap. *Bulletin of Entomological Research*, *78*(1), pp.121-128.

Macfadyen, S., Davies, A. P. and Zalucki, M. P. (2015). Assessing the impact of arthropod natural enemies on crop pests at the field scale. *Insect Science*, *22*(1), pp.20-34.

MacGillivray, M. E. and Anderson, G. B. (1958). Development of four species of aphids (Homoptera) on potato. *The Canadian Entomologist*, *90*(3), pp.148-155.

Margaritopoulos, J. T., Tsamandani, K., Kanavaki, O. M., Katis, N. I. and Tsitsipis, J. A. (2010). Efficacy of pymetrozine against *Myzus persicae* and in reducing potato virus Y transmission on tobacco plants. *Journal of Applied Entomology*, *134*(4), pp.323-332.

Margaritopoulos, J. T., Tsourapas, C., Tzortzi, M., Kanavaki, O. M. and Tsitsipis, J. A. (2005). Host selection by winged colonisers within the *Myzus persicae* group: a contribution towards understanding host specialisation. *Ecological Entomology*, *30*(4), pp.406-418.

Markkula, M. and Roukka, K. (1970). Resistance of plants to the pea aphid *Acyrthosiphon pisum* Harris (Homoptera: Aphididae). I. Fecundity of the biotypes on different host plants. *Annales Agriculturae Fenniae*, *9*, pp.127-132.

Martinez-Torres, D., Devonshire, A. L. and Williamson, M. S. (1997). Molecular studies of knockdown resistance to pyrethroids: cloning of domain II sodium channel gene sequences from insects. *Pesticide Science*, *51*(3), pp.265-270.

Martinez-Torres, D., Foster, S. P., Field, L. M., Devonshire, A. L. and Williamson, M. S. (1999). A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Insect Molecular Biology*, *8*(3), pp.339-346.

McCaffery, A. and Nauen, R. (2006). The insecticide resistance action committee (IRAC): public responsibility and enlightened industrial self-interest. *Outlooks on Pest Management*, *17*(1), pp.11-14.

Met Office. (1928). Tables for the evaluation of daily values of accumulated temperature above and below 42' F from daily values of maximum and minimum. *Air Ministry Form 3300.*

Met Office. (2012). Met Office Integrated Data Archive System Land and Marine Surface Stations Data (1853-current). NCAS British Atmospheric Data Centre. Available online: http://catalogue.ceda.ac.uk/uuid/220a65615218d5c9cc9e4785a3234bd0 [Accessed: 2015–2019].

Met Office. (2018). UKCP18 science overview report. Version 2. Available online: https://www.metoffice.gov.uk/pub/data/weather/uk/ukcp18/science-reports/UKCP18-Overview-report.pdf [Accessed: August 2019].

Metcalf, R. L. (1989). Insect resistance to insecticides. *Pesticide Science*, *26*(4), pp.333-358.

Miles, P. W. (1999). Aphid saliva. *Biological Reviews*, *74*(1), pp.41-85.

Montllor, C. B. and Gildow, F. E. (1986). Feeding responses of two grain aphids to barley yellow dwarf virus-infected oats. *Entomologia Experimentalis et Applicata*, *42*(1), pp.63-69.

Moores, G. D., Devine, G. J. and Devonshire, A. L. (1994). Insecticide-insensitive acetylcholinesterase can enhance esterase-based resistance in *Myzus persicae* and *Myzus nicotianae*. *Pesticide Biochemistry and Physiology*, *49*(2), pp.114-120.

Morales, M. (2017). *sciplot: Scientific Graphing Functions for Factorial Designs. R package version 1.1-1*. Available online: https://cran.r-project.org/web/packages/sciplot/index.html [Accessed: April 2019].

Morgan, D. (2004). Parsnip Yellow Fleck Virus: development of a disease management strategy. HDC Project FV 228a. Agriculture and Horticulture Development Board (AHDB) Report.

Moriarty, F. (1969). The sublethal effects of synthetic insecticides on insects. *Biological Reviews*, *44*(3), pp.321-356

Morita, M., Ueda, T., Yoneda, T., Koyanagi, T. and Haga, T. (2007). Flonicamid, a novel insecticide with a rapid inhibitory effect on aphid feeding. *Pest Management Science*, *63*(10), pp.969-973.

Mota-Sanchez, D. and Wise, J, C. (2019). The Arthropod Pesticide Resistance Database. Michigan State University. Available online: http://www.pesticideresistance.org [Accessed: August 2019].

Mowry, T. M. (2005). Insecticidal reduction of Potato leafroll virus transmission by *Myzus persicae*. *Annals of Applied Biology*, *146*(1), pp.81-88.

Namba, R. and Sylvester, E. S. (1981). Transmission of cauliflower mosaic virus by the green peach, turnip, cabbage, and pea aphids. *Journal of Economic Entomology*, *74*(5), pp.546-551.

National Biodiversity Network. (2019). National Biodiversity Network Atlas: UK distribution of *Salix* L. Available online:
https://species.nbnatlas.org/species/NHMSYS0000463078#tab_mapView
[Accessed: August 2019].

Nauen, R. (1995). Behaviour modifying effects of low systemic concentrations of imidacloprid on *Myzus persicae* with special reference to an antifeeding response. *Pesticide Science*, *44*(2), pp.145-153.

Nauen, R. (2006). Insecticide mode of action: return of the ryanodine receptor. *Pest Management Science, 62*(8), pp.690-692.

Nauen, R. and Denholm, I. (2005). Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. *Archives of Insect Biochemistry and Physiology, 58*(4), pp.200-215.

Nauen, R. and Elbert, A. (1997). Apparent tolerance of a field-collected strain of *Myzus nicotianae* to imidacloprid due to strong antifeeding responses. *Pesticide Science*, *49*(3), pp.252-258.

Nauen, R., Ebbinghaus-Kintscher, U., Elbert, A., Jeschke, P. and Tietjen, K. (2001). Acetylcholine receptors as sites for developing neonicotinoid insecticides. In: I. Ishaaya (ed.). *Biochemical Sites of Insecticide Action and Resistance*, Springer, Berlin, Heidelberg, Germany, pp.77-105.

Nauen, R., Reckmann, U., Thomzik, J. and Thielert, W. (2008). Biological profile of spirotetramat (Movento®)–a new two-way systemic (ambimobile) insecticide against sucking pest species. *Bayer CropScience Journal*, *61*(2), pp.245-278.

Nault, L. R. (1997). Arthropod transmission of plant viruses: a new synthesis. *Annals of the Entomological Society of America*, *90*(5), pp.521-541.

Neal, J. J., Tingey, W. M. and Steffens, J. C. (1990). Sucrose esters of carboxylic-acids in glandular trichomes of *Solanum berthaultii* deter settling and probing by green peach aphid. *Journal of Chemical Ecology, 16*(2), pp.487-497.

Newton, J. H., Palmer, M. A. and List, G. M. (1953). Fall migration of aphids with special reference to the green peach aphid. *Journal of Economic Entomology*, *46*(4), pp.667-670.

Ng, J. C. and Perry, K. L. (2004). Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology*, *5*(5), pp.505-511.

Niemeyer, H. M. (1990). The role of secondary plant compounds in aphid-host interactions. In: R. K. Campbell, R. D. Eikenbary (eds.). *Aphid-plant Genotype Interactions.* Elsevier, Amsterdam, Netherlands, pp. 787-205.

Nikolakakis, N. N., Margaritopoulos, J. T. and Tsitsipis, J. A. (2003). Performance of *Myzus persicae* (Hemiptera: Aphididae) clones on different host-plants and their host preference. *Bulletin of Entomological Research, 93*(3), pp.235-242.

Nottingham, S. F. and Hardie, J. (1993). Flight behavior of the black bean aphid, *Aphis fabae*, and the cabbage aphid, *Brevicoryne brassicae*, in host and nonhost plant odour. *Physiological Entomology, 18*(4), pp.389-394

Nottingham, S. F., Hardie, J., Dawson, G. W., Hick, A. J., Pickett, J. A., Wadhams, L. J. and Woodcock, C. M. (1991). Behavioural and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology*, *17*(6), pp.1231-1242.

Oberemok, V. V., Laikova, K. V., Gninenko, Y. I., Zaitsev, A. S., Nyadar, P. M. and Adeyemi, T. A. (2015). A short history of insecticides. *Journal of Plant Protection Research*, *55*(3), pp.221-226.

Panini, M., Dradi, D., Marani, G., Butturini, A. and Mazzoni, E. (2014). Detecting the presence of target-site resistance to neonicotinoids and pyrethroids in Italian populations of *Myzus persicae. Pest Management Science*, *70*(6), pp.931-938.

Parker, W. E. (1998). Forecasting the timing and size of aphid populations (*Myzus persicae* and *Macrosiphum euphorbiae*) on potato. *Aspects of Applied Biology*, *52*, pp.31-38.

Pelletier, Y. (1990). The role of the colour of the substratum on the initiation of the probing behaviour in *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Homoptera, Aphididae). *Canadian Journal of Zoology, 68*(4), pp.694-698.

Perrin, R. M. (1985). Karate (PP 321) control of plant virus vectors. Section Report 2, ICI Plant Protection Division. Bracknell, Berkshire, UK, ICI Agrochemicals, Jealott's Hill Research Station, pp.26.

Perring, T. M., Gruenhagen, N. M. and Farrar, C. A. (1999). Management of plant viral diseases through chemical control of insect vectors. *Annual Review of Entomology*, *44*(1), pp.457-481.

Phelps, K., Collier, R. H., Reader, R. J. and Finch, S. (1993). Monte Carlo simulation method for forecasting the timing of pest insect attacks. *Crop Protection*, *12*(5), pp.335-342.

Philippou, D., Field, L. and Moores, G. (2010). Metabolic enzyme (s) confer imidacloprid resistance in a clone of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) from Greece. *Pest Management Science*, *66*(4), pp.390-395.

Pilkington, L. J., Messelink, G., van Lenteren, J. C., and Le Mottee, K. (2010). "Protected Biological Control"–Biological pest management in the greenhouse industry. *Biological Control*, *52*(3), pp.216-220.

Pirone, T. P. and Harris, K. F. (1977). Nonpersistent transmission of plant viruses by aphids. *Annual Review of Phytopathology*, *15*(1), pp.55-73.

Pirone, T. P. and Perry, K. L. (2002). Aphids: non-persistent transmission. *Advances in Botanical Research*, *36*, pp.1-19.

Pisa, L. W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J. M., Downs, C. A., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., McField, M. and Morrissey, C. A. (2015). Effects of neonicotinoids and fipronil on non-target invertebrates. *Environmental Science and Pollution Research, 22*(1), pp.68-102.

Plumb, R. T. (2002). Viruses of Poaceae: a case history in plant pathology. *Plant pathology*, *51*(6), pp.673-682.

Potter, C. (1952). An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids. *Annals of Applied Biology, 39*(1), pp.1-28.

Powell, G. (1992). The effect of mineral oil on stylet activities and potato virus Y transmission by aphids. *Entomologia Experimentalis et Applicata*, *63*(3), pp.237-242.

Powell, G. and Hardie, J. (2000). Host-selection behaviour by genetically identical aphids with different plant preferences. *Physiological Entomology, 25*(1), pp.54-62.

Powell, G., Hardie, J. and Pickett, J. A. (1995). Behavioural evidence for detection of the repellent polygodial by aphid antennal tip sensilla. *Physiological Entomology, 20*(2), pp.141-146.

Powell, G., Maniar, S. P., Pickett, J. A. and Hardie, J. (1999). Aphid responses to non-host epicuticular lipids. *Entomologia Experimentalis et Applicata*, *91,* pp.115-123.

Powell, G., Tosh, C. and Hardie, J. (2004). Parturition by colonising aphids: no correlation with phloem ingestion. In: J. C. Simon, C. A. Dedryver, C. Rispe, M. Hulle (eds.). *Aphids in a New Millennium*, INRA, Paris, France, pp.485-489.

Powell, G., Tosh, C. R. and Hardie, J. (2006). Host plant selection by aphids: behavioural, evolutionary, and applied perspectives. *Annual Review of Entomology, 51*, pp.309-330.

Power, A. G. (2000). Insect transmission of plant viruses: a constraint on virus variability. *Current Opinion in Plant Biology*, *3*(4), pp.336-340.

Prabhaker, N., Castle, S. J., Naranjo, S. E., Toscano, N. C. and Morse, J. G. (2011). Compatibility of two systemic neonicotinoids, imidacloprid and thiamethoxam, with various natural enemies of agricultural pests. *Journal of Economic Entomology*, *104*(3), pp.773-781.

Prado, E. and Tjallingii, W. F. (1994). Aphid activities during sieve element punctures. *Entomologia Experimentalis et Applicata*, *72*(2), pp.157-165.

Prasad, Y. and Prabhakar, M. (2012). Pest monitoring and forecasting. In: D. P. Abrol, U. Shankar (eds.). *Integrated Pest Management: Principles and Practice.* CABI, Oxford, UK, pp.41-57.

Pretty, J. (2008). Agricultural sustainability: concepts, principles and evidence. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *363*(1491), pp.447-465.

Pretty, J. and Bharucha, Z. P. (2014). Sustainable intensification in agricultural systems. *Annals of Botany, 114*(8), pp.1571-1596.

Pruess, K. P. (1983). Day-degree methods for pest management. *Environmental Entomology*, *12*(3), pp.613-619.

Puinean, A. M., Foster, S. P., Oliphant, L., Denholm, I., Field, L. M., Millar, N. S., Williamson, M. S. and Bass, C. (2010). Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae. PLOS Genetics*, *6*(6), p.e1000999.

Remaudière, G. and Remaudière, M. (1997). *Catalogue of the world's Aphididae: Homoptera Aphidoidea*. Institut National de la Recherche Agronomique (INRA), Paris, France, p.473.

Rice, A. D., Gibson, R. W. and Stribley, M. F. (1983). Effects of deltamethrin on walking, flight and potato virus Y-transmission by pyrethroid-resistant *Myzus persicae*. *Annals of Applied Biology*, *102*(2), pp.229-236.

Ro, T. H., Long, G. E. and Toba, H. H. (1998). Predicting phenology of green peach aphid (Homoptera: Aphididae) using degree-days. *Environmental Entomology*, *27*(2), pp.337-343.

Roberts, J. M. F., Hodgson, C. J., Jackai, L. E. N., Thottappilly, G., Singh, S. (1993). Interaction between two synthetic pyrethroids and the spread of two non-persistent viruses in cowpea. *Annals of Applied Biology, 122,* pp. 57-67.

Rochow, W. F. (1972). The role of mixed infections in the transmission of plant viruses by aphids. *Annual Review of Phytopathology*, *10*(1), pp.101-124.

Rundlöf, M., Andersson, G. K., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., Jonsson, O., Klatt, B. K., Pedersen, T. R., Yourstone, J. and Smith, H. G. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature*, *521*(7550), p.77.

Ruscoe, C. N. E. (1977). The new NRDC pyrethroids as agricultural insecticides. *Pesticide Science*, *8*(3), pp.236-242.

Russell, G. E. (1966). Observations on the settling behaviour of aphids on sugar-beet plants in the glasshouse. *The Journal of Agricultural Science*, *67*(3), pp.405-410.

Sánchez-Bayo, F., Tennekes, H. A. and Goka, K. (2013). Impact of systemic insecticides on organisms and ecosystems. In: S. Trdan (ed.). *Insecticides – Development of Safer and More Effective Technologies,* IntechOpen, London, UK, pp.367-416.

Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), p.671.

Scott, C. and Bilsborrow, P. E. (2019). The impact of the EU neonicotinoid seed-dressing ban on oilseed rape production in England. *Pest Management Science*, *75*(1), pp.125-133.

Selby, T. P., Lahm, G.P., Stevenson, T. M., Hughes, K. A., Cordova, D., Annan, I. B., Barry, J. D., Benner, E. A., Currie, M. J. and Pahutski, T. F. (2013). Discovery of cyantraniliprole, a potent and selective anthranilic diamide ryanodine receptor activator with cross-spectrum insecticidal activity. *Bioorganic and Medicinal Chemistry Letters*, *23*(23), pp.6341-6345.

Shaffer, P. L. (1983). Prediction of variation in development period of insects and mites reared at constant temperatures. *Environmental Entomology*, *12*(4), pp.1012-1019.

Shaposhnikov, G. C. (1961). Specificity and appearance of adaptations to new hosts in aphids during the process of natural selection. *Entomologiceskoe Obozrenie*, *40*, pp.739-762.

Shattuck, V. I. (1992). The biology, epidemiology, and control of turnip mosaic virus. *Horticultural Reviews*, *14*, pp.199-238.

Shepherd, T., Robertson, G. W., Griffiths, D. W. and Birch, A. N. E. (1999). Epicuticular wax composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeu*s L.). *Phytochemistry*, *52*(7), pp.1239-1254.

Sicbaldi, F., Sacchi, G. A., Trevisan, M. and Del Re, A. A. (1997). Root uptake and xylem translocation of pesticides from different chemical classes. *Pesticide Science*, *50*(2), pp.111-119.

Sigvald, R. (1992). Progress in aphid forecasting systems. *Netherlands Journal of Plant Pathology*, *98*(2), pp.55-62.

Simons, J. N. and Zitter, T. A. (1980). Use of oils to control aphid-borne viruses. *Plant Disease*, *64*(6), p.543.

Simons, J. N., McLean, D. L. and Kinsey, M. G. (1977). Effects of mineral oil on probing behaviour and transmission of stylet-borne viruses by *Myzus persicae*. *Journal of Economic Entomology*, *70*(3), pp.309-315.

Singer, M. C. (2000). Reducing ambiguity in describing plant-insect interactions: "preference", " acceptability" and “electivity". *Ecology Letters*, *3*(3), pp.159-162.

Slater, R., Paul, V. L., Andrews, M., Garbay, M. and Camblin, P. (2011). Identifying the presence of neonicotinoid-resistant peach-potato aphid (*Myzus persicae*) in the peach-growing regions of southern France and northern Spain. *Pest Management Science*, *68*(4), pp.634-638.

Soderlund, D. M. and Bloomquist, J. R. (1989). Neurotoxic actions of pyrethroid insecticides. *Annual Review of Entomology*, *34*(1), pp.77-96.

Soliman, M. (2012). Effects of UV-light, temperature and storage on the stability and biological effectiveness of some insecticides. *Journal of Plant Protection Research*, *52*(2), pp.275-280.

Sparks, T. C. and Nauen, R. (2015). IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, *121*(1), pp.122-128.

Stern, D. L. (2008). Aphids. *Current Biology*, *18*(12), pp.R504-R505.

Stevens, M. and Lacomme, C. (2017). Transmission of plant viruses. In: H. F. van Emden, R. Harrington (eds.). *Aphids as Crop Pests,* 2nd edition, CABI, Wallingford, UK, pp.323-361.

Storer, J. R., Powell, G., Hardie, J. (1996). Settling responses of aphids in air permeated with non-host plant volatiles. *Entomologia Experimentalis et Applicata, 80*, pp.76-78.

Stroyan, H. L. G. (1969). Notes on some species of *Cavariella*. Del Guercio, 1911 (Homoptera: Aphidoidea). *Proceedings of the Royal Entomological Society of London. Series B, Taxonomy*, *38*(1-2), pp.7-19.

Stubbs, L. L. and Grogan, R. G. (1963). Necrotic yellows: a newly recognized virus disease of lettuce. *Australian Journal of Agricultural Research*, *14*(4), pp.439-459.

Swenson, K. G. (1968). Role of aphids in the ecology of plant viruses. *Annual Review of Phytopathology*, *6*(1), pp.351-374.

Sylvester, E. S. (1956a). Beet mosaic and beet yellows virus transmission by the green peach aphid. *Journal of the American Society of Sugar Beet Technologists*, *9*(1), pp.56-61.

Sylvester, E. S. (1956b). Beet yellows virus transmission by the green peach aphid. *Journal of Economic Entomology*, *49*, pp.789-800.

Sylvester, E. S. (1980). Circulative and propagative virus transmission by aphids. *Annual Review of Entomology*, *25*(1), pp.257-286.

Syngenta. (2019). AHDB Pest Bulletin (sponsored by Syngenta®) updated by R. Collier in association with the University of Warwick: willow-carrot aphid. Available online: https://www.syngenta.co.uk/ahdb-pest-bulletin [Accessed: May 2019].

Taylor, L. R. (1974). Insect migration, flight periodicity and the boundary layer. *The Journal of Animal Ecology*, pp.225-238.

Tenhumberg, B., Tyre, A. J. and Rebarber, R. (2009). Model complexity affects transient population dynamics following a dispersal event: a case study with pea aphids. *Ecology*, *90*(7), pp.1878-1890.

Thacker, J. I., Thieme, T. and Dixon A. F. G. (1997). Forecasting of periodic fluctuations in annual abundance of the bean aphid: the role of density dependence and weather. *Journal of Applied Entomology, 121*(1-5), pp.137-145.

Thackray, D. J., Diggle, A. J. and Jones, R. A. C. (2009). BYDV PREDICTOR: a simulation model to predict aphid arrival, epidemics of Barley yellow dwarf virus and yield losses in wheat crops in a Mediterranean-type environment. *Plant Pathology*, *58*(1), pp.186-202.

Tilman, D., Balzer, C., Hill, J. and Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences*, *108*(50), pp.20260-20264.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R. and Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature*, *418*(6898), p.671.

Tjallingii, W. F. (1994). Sieve element acceptance by aphids. *European Journal of Entomology, 91*, pp.47- 52.

Tomlinson, J. A. (1987). Epidemiology and control of virus diseases of vegetables. *Annals of Applied Biology*, *110*(3), pp.661-681.

Tosh, C. R., Powell, G. and Hardie, J. (2002). Maternal reproductive decisions are independent of feeding in the black bean aphid, *Aphis fabae*. *Journal of Insect Physiology, 48*(6), pp.619-629.

Tosh, C. R., Powell, G. and Hardie, J. (2003). Decision making by generalist and specialist aphids with the same genotype. *Journal of Insect Physiology, 49*(7), pp.659-669.

Troncoso, A. J., Vargas, R. R., Tapia, D. H., Olivares-Donoso, R. and Niemeyer, H. M. (2005). Host selection by the generalist aphid *Myzus persicae* (Hemiptera: Aphididae) and its subspecies specialized on tobacco, after being reared on the same host. *Bulletin of Entomological Research*, *95*(1), pp.23-28.

Tsitsipis, J. A. and Mittler, T. E. (1976). Development, growth, reproduction, and survival of apterous virginoparae of *Aphis fabae* at different temperatures. *Entomologia Experimentalis et Applicata*, *19*(1), pp.1-10.

UC IPM. (2003). Degree-Days. Agriculture and Natural Resources, University of California. Available online: http://www.ipm.ucdavis.edu/WEATHER/ddconcepts.html [Accessed: January 2019].

United Nations. (2015). World Population Prospects, the 2015 revision. Available online: https://population.un.org/wpp/ [Accessed: January 2019].

Van Dijk, P. and Bos, L. (1985). Viral dieback of carrot and other Umbelliferae caused by the Anthriscus strain of parsnip yellow fleck virus, and its distinction from carrot motley dwarf. *Netherlands Journal of Plant Pathology*, *91*(4), pp.169-187.

Van Dijk, P. and Bos, L. (1989). Survey and biological differentiation of viruses of wild and cultivated Umbelliferae in the Netherlands. *Netherlands Journal of Plant Pathology*, *95*(2), pp.1-34.

van Emden, H. F., Eastop, V. F., Hughes, R. D. and Way, M. J. (1969). The ecology of *Myzus persicae*. *Annual Review of Entomology*, *14*(1), pp.197-270.

van Lenteren, J. C. (2000). A greenhouse without pesticides: fact or fantasy? *Crop Protection*, *19*(6), pp.375-384.

van Lexmond, M. B., Bonmatin, J. M., Goulson, D. and Noome, D. A. (2015). Worldwide integrated assessment on systemic pesticides. *Environmental Science and Pollution Research. 22*(1), pp.1-4.

Vargas, R. R., Troncoso, A. J., Tapia, D. H., Olivares-Donoso, R. and Niemeyer, H. M. (2005). Behavioural differences during host selection between alate virginoparae of generalist and tobacco-specialist *Myzus persicae*. *Entomologia Experimentalis et Applicata*, *116*(1), pp.43-53.

Via, S. (1991). Specialized host plant performance of pea aphid clones is not altered by experience. *Ecology*, *72*(4), pp.1420-1427.

Visser J. H, Piron, P. G. M. and Hardie, J. (1996). The aphids' peripheral perception of plant volatiles. *Entomologia Experimentalis et Applicata, 80*(1), pp.35-38.

Visser, J. H. (1986). Host odor perception in phytophagous insects. *Annual Review of Entomology*, *31*(1), pp.121-144.

Von Keyserlingk. H. C. (1985). The significance of behaviour studies in insecticide research. *Pesticide Science, 1*(6), pp. 558-559.

Walkey, D. G. A. and Dance, M. C. (1979). The effect of oil sprays on aphid transmission of turnip mosaic, beet yellows, bean common mosaic, and bean yellow mosaic viruses. *Plant Disease Reporter*, *63*(10), pp.877-881.

Walsh, J. A. (1989). Genetic control of immunity to turnip mosaic virus in winter oilseed rape (*Brassica napus* ssp. *oleifera*) and the effect of foreign isolates of the virus. *Annals of Applied Biology*, *115*(1), pp.89-99.

Walsh, J. A. and Jenner, C. E. (2002). Turnip mosaic virus and the quest for durable resistance. *Molecular Plant Pathology*, *3*(5), pp.289-300.

Walsh, J. A., Rusholme, R. L., Hughes, S. L., Jenner, C. E., Bambridge, J. M., Lydiate, D. J. and Green, S. K. (2002). Different classes of resistance to turnip mosaic virus in *Brassica rapa*. *European Journal of Plant Pathology*, *108*(1), pp.15-20.

Walsh, J. A., Sharpe, A. G., Jenner, C. E. and Lydiate, D. J. (1999). Characterisation of resistance to turnip mosaic virus in oilseed rape (*Brassica napus*) and genetic mapping of TuRB01. *Theoretical and Applied Genetics*, *99*(7-8), pp.1149-1154.

Walters, K. F. A. and Dewar, A. M. (1986). Overwintering strategy and the timing of the spring migration of the cereal aphids *Sitobion avenae* and *Sitobion fragariae*. *Journal of Applied Ecology, 23*(3), pp.905-915.

Wang, Z. H., Gong, Y. J., Jin, G. H., Zhu, L. and Wei, S.J. (2016). Effects of spirotetramat on development and reproduction of *Myzus persicae* (Hemiptera: Aphididae). *Austral Entomology*, *55*(3), pp.235-241.

Waterfield, G. and Zilberman, D. (2012). Pest management in food systems: an economic perspective. *Annual Review of Environment and Resources*, *37*, pp.223-245.

Waterhouse, P. M. and Murant, A. F. (1983). Further evidence on the nature of the dependence of carrot mottle virus on carrot red leaf virus for transmission by aphids. *Annals of Applied Biology*, *103*(3), pp.455-464.

Watson, M. A. and Roberts, F. M. (1939). A comparative study of the transmission of Hyoscyamus virus 3, potato virus Y and cucumber virus 1 by the vectors *Myzus persicae* (Sulz), *M. circumflexus* (Buckton), and *Macrosiphum gei* (Koch). *Proceedings of the Royal Society of London. Series B-Biological Sciences*, *127*(849), pp.543-576.

Watson, M. A. and Roberts, F. M. (1940). Evidence against the hypothesis that certain plant viruses are transmitted mechanically by aphids. *Annals of Applied Biology*, *27*(2), pp.227-233.

Watson, M. A., Serjeant, E. P. and Lennon, E. A. (1964). Carrot motley dwarf and parsnip mottle viruses. *Annals of Applied Biology*, *54*(2), pp.153-166.

Way, M. J., Cammell, M. E., Taylor, L. R. and Woiwood, I. P. (1981). The use of egg counts and suction trap samples to forecast the infestation of spring-sown field beans, *Vicia faba,* by the black bean aphid, *Aphis fabae. Annals of Applied Biology 98*(1), pp.21-34.

Weber, G. (1985). Genetic variability in host plant adaptation of the green peach aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata*, *38*(1), pp.49-56.

Weber, G. (1986). Ecological genetics of host plant exploitation in the green peach aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata*, *40*(2), pp.161-168.

Webster, B. (2012). The role of olfaction in aphid host location. *Physiological Entomology*, *37*(1), pp.10-18.

Weed, A. (1927). Metamorphosis and reproduction in apterous forms of *Myzus persicae* Sulzer as influenced by temperature and humidity. *Journal of Economic Entomology, 20*(1), pp.150-157.

Wege, P. J., Hoppe, M. A., Bywater, A. F., Weeks, S. D. and Gallo, T. S. (1999). A microencapsulated formulation of lambda-cyhalothrin. *Proceedings of the 3rd International Conference on Urban Pests*, pp. 301-310.

Wensler, R. J. D. (1962). Mode of host selection by an aphid. *Nature, 195*(4843), p.830.

Wensler, R. J. D. (1977). The fine structure of distal receptors on the labium of the aphid, *Brevicoryne brassicae* L. (Homoptera) implications for current theories of sensory transduction. *Cell and Tissue Research, 181*(3), pp.409-422.

Wensler, R. J. D. and Filshie, B. K. (1969). Gustatory sense organs in the food canal of aphids. *Journal of Morphology, 129*(40), pp.473-492.

Whalon, M. E. and Smilowitz, Z. (1977). Determination of constant temperature developmental thresholds for *Myzus persicae* (Sulzer) (Homoptera: Aphididae) *Journal of the New York Entomological Society, 85*(8), p.206.

Whalon, M. E. and Smilowitz, Z. (1979). Temperature-dependent model for predicting field populations of green peach aphid, *Myzus persicae* (Homoptera: Aphididae). *The Canadian Entomologist*, *111*(9), pp.1025-1032.

Whitehorn, P. R., O'connor, S., Wackers, F. L. and Goulson, D. (2012). Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science*, *336*(6079), pp.351-352.

Wiktelius, S. (1982). Flight phenology of cereal aphids and possibilities of using suction trap catches as an aid in forecasting outbreaks. *Swedish Journal of Agricultural Research, 12*, pp.9-16.

Wood, T. J. and Goulson, D. (2017). The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environmental Science and Pollution Research*, *24*(21), pp.17285-17325.

Worner, S. P., Tatchell, G. M. and Woiwod, I. P. (1995). Predicting spring migration of the damson-hop aphid *Phorodon humuli* (Homoptera: Aphididae) from historical records of host-plant flowering phenology and weather. *Journal of Applied Ecology*, pp.17-28.

Wyatt, I. J. and White, P. F. (1977). Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *Journal of Applied Ecology*, *14*(3), pp.757-766.

Zhou, X., Harrington, R., Wowod, I. P., Perry, J. N., Bale, J. S., and Clark, S. J. (1995). Effects of temperature on aphid phenology. *Global Change Biology*, *1*(4), pp.303-313.

Zhu, Y., Loso, M. R., Watson, G. B., Sparks, T. C., Rogers, R. B., Huang, J. X., Gerwick, B. C., Babcock, J. M., Kelley, D., Hegde, V. B. and Nugent, B. M. (2010). Discovery and characterization of sulfoxaflor, a novel insecticide targeting sap-feeding pests. *Journal of Agricultural and Food Chemistry*, *59*(7), pp.2950-2957.

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Appendix A

Appendix A.1 – Data sets used for the development and validation of forecasts (both day-degree forecasts and forecasts based on mean air temperature) for the four species of aphid: *Myzus persicae*, *Cavariella aegopodii*, *Cavariella pastinaceae* and *Cavariella theobaldi*. Sites are abbreviated as Broom's Barn (BB), East Craigs (EC), Hereford (H), Kirton (K), Preston (P), Rothamsted (R), Starcross (S), Writtle (Wr) and Wye (W). Sites x years indicates the number of occasions with reliable (few days of suction trap inactivity) and sufficient (≥ 50 aphids captured before cut-off point) suction trap data and air temperature records available for a given site x year combination.

Species	Data set	Sites used	Years used	Sites x years
Myzus persicae	Forecast development (phenology)	BB, K, R	1965–1999	71
	Forecast validation (phenology)	BB, K, R	2000–2010	24
	Forecast development ('aphid peak')	BB, K, R	1965–1999	50
	Forecast validation ('aphid peak')	BB, K, R	2000–2010	18
Cavariella aegopodii	Forecast development (phenology)	BB, K, P, R, Wr, W	1996–2006	56
	Forecast validation (phenology)	BB, EC, H, K, P, R, S, Wr, W	1981–1988	55
Cavariella pastinaceae	Forecast development (phenology)	BB, K, P, R, Wr, W	1996–2006	45
	Forecast validation (phenology)	BB, K, R	2008–2015	18
Cavariella theobaldi	Forecast development (phenology)	BB, K, P, R, Wr, W	1996–2006	35
	Forecast validation (phenology)	BB, K, R	2008–2015	12

Appendix A.2 – Mean capture dates (2013–2018) of the first, 10% and 50% of spring/summer migrant(s) for each species at six suction trap locations in the UK at which the total annual number of aphids caught ≥ 50. Mean values are shown with standard error of the mean (SEM) and the sample size used (*n* = number of years).

		Date of first capture			**Date of 10% capture**			**Date of 50% capture**		
Species	**Site**	**Mean**	**SEM**	***n***	**Mean**	**SEM**	***n***	**Mean**	**SEM**	***n***
Cavariella aegopodii	Preston	127.5	4.039	6	146.67	3.694	6	154.5	3.538	6
	Kirton	115.17	9.148	6	144.67	4.951	6	157	6.919	6
	Broom's Barn	113.5	8.429	6	141.17	4.167	6	151.83	4.922	6
	Rothamsted	122.67	5.835	6	140.33	3.565	6	154.67	4.485	6
	Writtle	119	6.072	6	140	3.337	6	151.5	4.072	6
	Wye	125	6.107	5	139	3.821	5	151.8	4.913	5
Cavariella pastinaceae	Preston	150.2	3.569	5	156.4	4.202	5	178	10.237	5
	Kirton	154.25	6.074	4	185	6.311	4	192.5	7.411	4
	Broom's Barn	144.5	6.764	4	167.5	11.288	4	188.25	7.134	4
	Rothamsted	145.25	8.29	4	166.75	8.779	4	190.25	7.284	4
	Writtle	141.5	6.198	4	164.75	8.097	4	183.5	6.344	4
	Wye	133.5	13.5	2	146.5	7.5	2	166	17	2
Cavariella theobaldi	Preston	133.5	12.5	2	149	7	2	163.5	18.5	2
	Kirton	146.75	15.95	4	176	12.955	4	193	7.416	4
	Broom's Barn	134.67	11.348	3	163	13.796	3	182.33	18.55	3
	Rothamsted	128	–	1	144	–	1	170	–	1
	Writtle	124.67	6.386	3	140	4.041	3	171	14.742	3
	Wye	123	12	2	137.5	5.5	2	156.5	10.5	2
Myzus persicae	Preston	151	6.843	4	161.25	6.223	4	177.75	6.663	4
	Kirton	125.33	13.208	6	166.67	10.045	6	179.33	8.011	6
	Broom's Barn	131.17	9.666	6	157.5	7.375	6	172.5	8.613	6
	Rothamsted	130.67	8.192	6	160	9.448	6	171.33	7.279	6
	Writtle	125.83	10.035	6	155.67	7.504	6	173.83	6.21	6
	Wye	125	3.028	4	148.5	5.331	4	168.5	4.093	4

Appendix A.3 – Linear regression analyses of the weeks of first, 10%, 25%, 50% and 90% capture of spring/summer *Myzus persicae* migrants (x) and mean air temperature (y), $y = mx + c$ (71 sites x years).

Capture	Mean Air Temperature	Parameter		S.E.	R^2	P-value
First	January–February	c	27.34099	0.66633	0.663	<0.001
		m	-1.96213	0.16841		<0.001
First	January–March	c	29.34943	0.95133	0.59585	<0.001
		m	-2.09616	0.20783		<0.001
10%	January–February	c	30.96507	0.64692	0.57876	<0.001
		m	-1.59195	0.1635		<0.001
10%	January–March	c	32.86566	0.86314	0.5588	<0.001
		m	-1.76278	0.18856		<0.001
25%	January–February	c	31.28322	0.508	0.59203	<0.001
		m	-1.28475	0.12839		<0.001
25%	January–March	c	32.90671	0.66536	0.58824	<0.001
		m	-1.44315	0.14536		<0.001
50%	January–February	c	31.69637	0.48843	0.56067	<0.001
		m	-1.15839	0.12344		<0.001
50%	January–March	c	33.19554	0.63433	0.56403	<0.001
		m	-1.3093	0.13858		<0.001
50%	January–April	c	36.85483	0.8891	0.62654	<0.001
		m	-1.79306	0.16666		<0.001
90%	January–February	c	32.46113	0.49666	0.41654	<0.001
		m	-0.88101	0.12552		<0.001
90%	January–March	c	33.33633	0.67309	0.36952	<0.001
		m	-0.93509	0.14704		<0.001
90%	January–April	c	35.87761	0.99299	0.40167	<0.001
		m	-1.2668	0.18613		<0.001
90%	January–May	c	38.35004	1.22948	0.44829	<0.001
		m	-1.4147	0.18894		<0.001

Appendix B

Appendix B.1 – Settling preference (distribution %) of alate *Myzus persicae* 2050A on sugar maple (non-host) during cage 'no choice' experiment. Aphids were reared on Brussels sprout cv. Doric F1.