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The Biology and Ecology of *Aleyrodes proletella*, the
Cabbage Whitefly; a Pest of *Brassica* Crops

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the degree of Doctor of Philosophy

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Declaration

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

Summary

Whilst not a ‘new’ pest in the UK, *Aleyrodes proletella* has become an increasing problem for the *Brassica* industry in recent years, especially on Brussels sprout and kale. The reason for the increasing problem is unknown, but it is believed to be due to a combination of climate change, removal of certain active ingredients from use and later harvest times of crops. Relatively little research has focused on this species as, historically; it has been regarded as a minor pest. Knowledge about the biology of *A. proletella* is limited and some of what is currently understood about its ecology has been inferred from anecdotal evidence.

The overall aim of this project is to understand population trends of *A. proletella* in the most vulnerable crops, Brussels sprout and kale. This includes understanding the key times of population increase and colonisation of new crops. This information can then be used to inform the development of an integrated control strategy using insecticides and other tools, which might include biological control agents and methods of cultural or physical control.

Experiments to investigate the vertical and horizontal distribution of flights by *A. proletella* showed that *A. proletella* performs mainly low, short distance flights throughout most of the year and it is these flights that are mostly responsible for colonisation of new vulnerable crops, which can be achieved by overwintering females early in the season. Monitoring of field populations on kale has shown that populations of whitefly develop without regulation by predators or parasitoids, with increases in numbers mostly determined by the development of further generations from the initial immigrants to the crop. The size of a population of *A. proletella* that can be achieved within a crop seems to be governed by the number of generations that can develop before the onset of diapause in September, which prevents further reproduction. A new fungal pathogen, which causes epizootics within the field, has been observed. This killed up to >90% of adult *A. proletella*. Of all potential natural enemies this pathogen had the largest potential to reduce *A. proletella* infestations and offers potential for the development of a new method of biological control.

1 Introduction

The fresh vegetable farming industry is of considerable importance to the UK economy, estimated to be worth over £1 Billion in 2015. Of that, Brassicas (cabbage, cauliflower, broccoli, Brussels sprout and kale) are a large aspect of horticultural industry in the UK, with 72 thousand tonnes produced in 2015 alone and showing a trend of increasing in value (DEFRA, 2015). Due to the high value of brassica crops much research has focused on the biology of many of its insect pest species allowing the development of efficient control strategies. For example, the study of development of the cabbage root fly (*Delia radicum*) led to the creation of a forecast day-degree model predicting times of adult activity used to time pesticide applications (Collier and Finch, 1985).

Recently *Aleyrodes proletella* has become an increasing pest in Europe particularly of Brussels sprout and kale (Nebreda *et al.* 2005). The cause of this is not fully understood, but is believed to be due to a combination of climate change, removal of certain active ingredients from use and later harvest times of crops. Effective control of *A. proletella* with insecticides is difficult due to the positioning of the nymphs, and the leaf structure of the most susceptible crops (Brussels sprout and kale) further adds to the difficulty of achieving good coverage with insecticides. Currently, the most effective pesticide for the control of this species is the systemic insecticide Movento[®] (spirotetramat) (Richter, 2010; Collier, 2012). Resistance to pyrethroid insecticides has been documented in *A. proletella* (Springate and Colvin, 2011), indicating that resistance management is very important. Little research has focused on this species as, historically, it has been regarded as a minor pest. Knowledge about the biology of *A. proletella* is limited and most of what is currently understood about its ecology has been inferred from anecdotal evidence. In the scientific literature, whiteflies (Aleyroidae) are particularly under-represented taxa, with research focused mainly on two species: *Bemisia tabaci* and *Trialeurodes vaporariorum*, due to their significant economic importance, with very few other species studied to such an extent.

1.1 Whitefly

Whiteflies are a group of insects from the family Aleyrodidae within the order Hemiptera. It is a herbivorous family of insects feeding from the phloem sap of their plant hosts (Byrne and Bellows, 1991). The adults have two pairs of membranous wings and development involves incomplete metamorphosis. Whiteflies reproduce by arrhenotoky, males are haploid arising from unfertilized eggs and diploid females develop from fertilized eggs. Unmated females lay male eggs while mated females lay eggs with equal numbers of males and females (Butler, 1938a). A commonality to whiteflies is the production of wax from plates with rows of microtrichia on the ventro-lateral abdominal surface. The waxes are spread over the entire body surface by the hind tibia. The function of these waxes is unknown, however it is believed that they may offer protection against desiccation and fungal pathogens (Kanagaratnam *et al.*, 1982, Byrne and Hadley, 1988).

1.1.1 As Pests

Although traditionally more associated with the tropical regions, whiteflies have spread as pests throughout much of the world partially due to the extensive transportation of host plants by man. The transporting of whiteflies to new localities along with transforming the natural environment to favour the survival of whiteflies has meant large populations of whitefly can be supported on crops that would not have naturally occurred. Of the approximately 1,400 currently described species of whitefly only a handful are regarded as pests, *Bemisia tabaci* and *Trialeurodes vaporariorum* being the most well known (Mound and Martin, 2007). Whiteflies can act as pests in three potential ways. Firstly, a direct impact of feeding, removing nutrients from the plant host leading to reduced productivity and reduced yield returns of the crops. Secondly, whiteflies produce large volumes of sugary excretions from feeding from the phloem, honeydew. These collect immediately below the whiteflies often falling on leaves below. This can allow the growth of sooty moulds that can significantly reduce the photosynthetic efficiency of the plant. Direct contamination of harvestable products by honeydew is also often a problem.

Whiteflies have also been shown to be vectors of a number of plant viruses, most notably the African Cassava Mosaic Virus transmitted by *B. tabaci*.

1.1.2 Chemical control methods

Currently 15 known modes of action are active on whiteflies (IRAC, 2014), however effective control with contact insecticides is often difficult. Whiteflies are often late season pests (Gerling, 1990) when the crop canopy is fully developed at the time of application with a very low probability of sprays fully penetrating the canopy. Whiteflies are mainly on the underside of the leaves and droplets mostly impact upon the upper side of the leaf; deposition of pesticides can be as low as 5% on the underside of the leaf when compared with the upper (Uk and Courshee, 1982). Systemic insecticides are often the most effective method of control as the problem of reaching the insect is overcome due to the translocation of active ingredient throughout the entire plant. Whiteflies, specifically *Bemisia tabaci*, have developed resistance to some 54 insecticides in the field (Sparks and Nauen, 2015) further adding to the pest status of this family of insect.

1.1.3 *Aleyrodes proletella*, the Cabbage whitefly.

Aleyrodes proletella (L.) was previously known under the synonym *Aleyrodes brassicae* (Walk.).

1.1.3.1 Distribution

Aleyrodes proletella has an expansive distribution, being found in Africa, New Zealand, throughout Europe and, most recently, Australia. It has yet to establish fully within the USA (Evans, 2008); its presence in the eastern states has been recorded and introductions as a result of transportation of host plants has been documented in California (NPAG, 2001). It is frequently transported throughout the world as its host plants are traded worldwide, especially *Brassica oleracea* (Dale *et al.*, 1975; Evans, 2008). In addition, the cosmopolitan distribution of hosts that are common weed species, such as *Sonchus* spp, has helped it to spread and establish in new territories worldwide.

1.1.3.2 Host plants

Aleyrodes proletella is a polyphagous species limited mainly to the families Brassicaceae and Asteraceae. Members of the Brassicaceae are the main hosts of this species, which shows a particular high affinity for *Brassica oleracea*, probably due to its wide cultivation by man. Brussels sprout (var. *gemmifera*), kale (var. *acephala*), cauliflower (var. *botrytis*) and broccoli (var. *italica*), show particularly high infestations in the field (Butler, 1938a). Together with reproduction on other members of the *Brassicaceae* family, *A. proletella* has also been recorded reproducing on dandelion (*Taraxacum* spp.) and sow-thistle (*Sonchus oleracea*) (Butler, 1938a). These are common cosmopolitan weeds and new records of *A. proletella* in both Australia and New Zealand mention these species as host plants (Dale *et al.*, 1975, DeBarro and Carver, 1997). There has been a suggestion that there is a difference in host preferences between the summer and overwintering generations, and it is likely that the apparent host ‘preference’ in winter is based on structural features, which provide increased protection (Butler, 1938a). An extensive list of known host plants can be found in Mound and Halsey (1978).

1.1.3.3 Developmental stages

Like most whiteflies, *A. proletella* shows distinct stages of development, eggs first instar (crawler), second instar nymph, third instar nymph and fourth instar nymph/pupae, which are distinguishable from each other by size and slight morphological differences. The most notable differences are in the lingula, a small dorsal appendage used to propel honeydew away from the insect (Butler, 1938a).

The egg

The egg is elongate (~0.26 mm) and pyriform with a pedicel attached at the broader end and is laid on foliage. It is often covered with white wax, but it is pale yellow underneath, changing to brown and then dark brown prior to hatching. The first instar nymph emerges through a slit at the narrower end of the egg where it crawls onto the leaf (Butler, 1938a). Eggs are nearly always laid on the underside of leaves, a preference that is conserved regardless of orientation of the leaf (Al-Houty, 1979). The lower threshold temperature for development has been estimated as 10°C, with development taking 8 days at 20°C (Iheagwam, 1978).

The first instar nymph

On emergence from the egg, the nymph is approximately 0.34 mm long, an oval shape and slightly convex dorsally. The abdominal, but not the thoracic, segments are demarcated. The ligula consists of one pair of short and one pair of long bristles. Three pairs of five segmented legs are present and the nymph is capable of movement. After up to 3 days of movement, the nymph settles down and becomes attached to the leaf by its mouthparts, although it rarely moves far from where it hatched (Butler, 1938a). Powdery wax collects, primarily on the marginal bristles, soon after emergence. The lower temperature threshold for development has been estimated as 7.3°C and completion of this stage takes 3-4 days at 20°C (Iheagwam, 1978).

The second instar nymph

The second instar nymph is approximately 0.56 mm long, oval in shape and with broad marginal fringes of wax. Immediately after moulting, the nymph appears yellow, but then turns brown after a few days. Segmentation is not defined in the thorax but is clearly evident in the abdomen. The ligula is covered in small hairs and possesses one pair of long bristles at its apex (Butler, 1938a). The lower temperature threshold for development has been estimated as being 5°C, and completion of this stage takes 2-3 days at 20°C (Iheagwam, 1978).

The third instar nymph

The third instar is approximately 0.76 mm long, oval in shape and with a humped dorsum. The nymph is translucent yellow after moulting, turning brown in colour, with broad fringes of wax evident. The legs and antennae are greatly atrophied, the thoracic segments are not easily discernible and the abdominal segments are visible with the ligula shaped like a convex spoon (Butler, 1938a). The lower temperature threshold for development has been estimated as 6.5°C and the stage takes 3 days to complete at 20°C (Iheagwam, 1978).

The fourth instar pupae

The fourth instar is approximately 1.51 mm long, roughly oval in shape and with a markedly humped dorsum with white fringes of wax. It is yellow, brown or translucent yellow in colour. The legs and antennae are greatly atrophied and both the thoracic and abdominal segments are distinct. There are no hairs on the crenulate margin except for one pair of long bristles at the posterior end of body. Apolysis takes place during the second part of the fourth instar, where the cuticle of the adult is laid down and it is here when the red eyes become visible, lending the term 'red-eyed nymph' to this stage (Gerling, 1990). The lower temperature threshold for development has been estimated as 10.4°C and the stage takes 7-8 days to complete at 20°C (Iheagwam, 1978). Although not a true pupal stage (Gerling, 1990) the fourth instar in most whiteflies is commonly regarded as a pupae and this stage will be referred to as pupae throughout this thesis.

The adult

The average length is 1.4 mm. Immediately on emergence from the pupa, the insect is quite soft, delicate, shining and pale yellow in colour. A black colour begins to develop on the body after about 5 hours. It develops first on the thorax and then it appears on the ventral side of the abdomen and then on the dorsal side. In living specimens the whole of the thorax is a mixture of black and yellow. Each compound eye is completely divided into two parts by a strip-like projection of chitin. The whole eye and the chitinous projection are hairy (Deshpande, 1933).

The two pairs of wings appear white with the powdery wax which covers them, but immediately on hatching they are pale, translucent yellow and delicate. The veins in the wings are of the same colour. As soon as the dark colour develops on the body, the veins assume a dark tinge and also black spots begin to develop. Only the Rs and cubitus veins are visible on the forewing, with the cubitus only suggested by a faint line. The Rs is bent twice, once in the middle and again towards the end, with two dark patches that develop, one at the first bend and the second near the tip. In the hind-wing only one vein is present, the Rs, which is centrally situated. It has one dark area in the centre. (Figure 1.1, Deshpande, 1931; Deshpande, 1933)

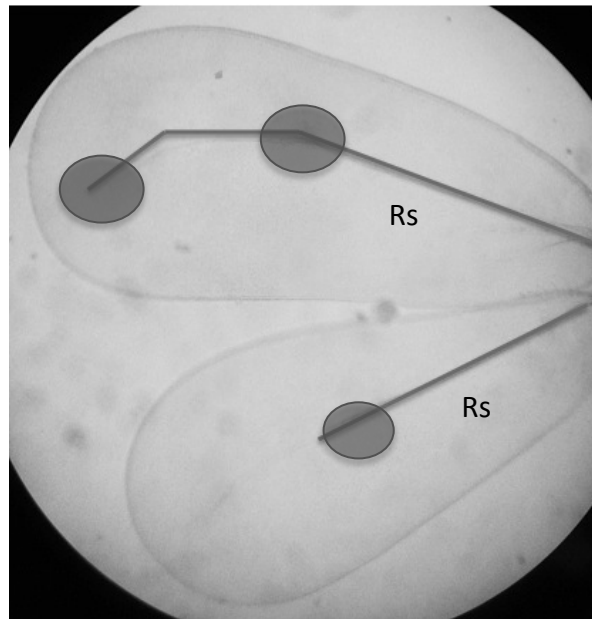


Figure 1.1. Fore- and hind-wings of *Aleyrodes proletella* with veins and darkened patches marked out. On the forewing only the 'Rs' persists, bent twice with two dark patches. The Cubitus is extremely faint if visible at all. On the hind wing only the 'Rs' vein is visible with one dark patch near the centre (from Deshpande, 1931; Deshpande, 1933)

Approximately 250 day degrees of development above a threshold temperature 10°C are required to complete development from egg through to eclosion (Iheagwam, 1978). The maximum development rate was observed at 28°C and the upper lethal temperature was estimated at ~38°C (Alonso *et al.*, 2009). No studies have focused on the development times at temperatures lower than 14°C, which may invalidate the assumption of a linear relationship between development rate and temperature, and alter estimates of the lower temperature thresholds for development. Development rate has also been shown to vary depending on the host plant. Development times at 22°C varied from ~19 days for Broccoli (cv Agripa) to ~27 days for early cauliflower (cv Nautilus) (Nebrada *et al.*, 2005). Development rate has also been shown to decrease with increasing densities of nymphs (Iheagwam, 1982).

1.1.4 Life cycle

Overwintered female *A. proletella* begin laying eggs in the spring and increasing temperatures are responsible for stimulating egg laying after a winter diapause (Iheagwam, 1978). *Aleyrodes proletella* is a multivoltine species and three to five generations occur per year in England. Ambient temperatures have the largest

influence on the number of generations in a season (Butler, 1938a; El Khidir, 1963, Al-Houty, 1979). It is thought that the first generation develops on the overwintering host the newly emergent adults then migrate to summer hosts (Butler, 1938a; El Khidir, 1963), because new infestations of *A. proletella* on crops usually occur when the adults of the first generation emerge (Butler, 1938a; Al-Houty, 1979; El Khidir, 1963). Evidence for this, however, is anecdotal and the timing of colonisation of newly planted crops has not been substantiated. Reproduction continues until late September after which females enter diapause and egg laying ceases.

1.1.5 Diapause

Female *A. proletella* overwinter in a state of ovarian diapause induced by the shortening day length late in the season. The second nymphal instar is the most responsive to changes in photoperiod. As daylength decreases to a photoperiod of 15¾ hours late in the season, second nymphal instars experiencing this critical reducing photoperiod will emerge as female adults in a state of ovarian diapause from September onwards. In Warwickshire, UK, this critical daylength is achieved on approximately 1st August and nymphs developing after this time will emerge as adult females in ovarian diapause. Females in ovarian diapause emerge without fully developed ovarioles and thus egg laying is not possible subsequently (El Khidir, 1963, Adams 1985a; 1985b). Overwintering is achieved primarily by these females which can tolerate temperatures as low as -18°C for short periods of time (Butler, 1938a). Adult males have been shown to have a considerably lower tolerance to cold, and after mating only have a life expectancy of ~10 days (Butler, 1938a; 1938b). Iheagwam (1977a) suggested that the later nymphal instars can also overwinter and this is supported by their ability to withstand sub-zero temperatures that would occur during the winter months (Butler, 1938b). The degree to which this occurs has not been substantiated and indeed most pupae are likely to perish when the plant sheds its older leaves late in the winter (El Khidir, 1963).

1.1.6 Longevity / fecundity.

Al-Houty (1979) noted that under glasshouse conditions female *A. proletella* laid an average of 225 eggs and that they continued laying for up to 50 days. Butler (1938b) discovered a linear relationship between the rate of oviposition and temperature but did not record whether there was a relationship between the duration of oviposition

and temperature. According to Butler's study, an increase of approximately 1°C leads to an increase in egg laying rate of 0.5 eggs per female over 10 days. Female longevity was found to be inversely related to ambient temperature, however the total number of eggs laid over a female's lifetime decreased with increasing temperature (Butler, 1938b). The host plant was also shown to have a large impact on longevity. At 20°C a range of female longevity from 16 to 48 days was observed when reared on different varieties of cabbage (Iheagwam, 1981).

1.1.7 Dispersal

The first instar nymph rarely moves more than a few centimeters away from where it hatched (Butler, 1938a). The subsequent instars are sessile and dispersal is achieved predominantly by adults. A more detailed review of literature regarding this subject is given in Chapter 2.

1.1.8 Control

Pesticidal control of *A. proletella* is used mainly in field brassicas, especially kale and Brussels sprout. Kale is greatly affected by *A. proletella* as the leaves where *A. proletella* spend the duration of its life as sessile nymphs are the harvestable crop. Nymphs, along with waxy deposits, contaminate the leaves that are harvested causing significant crop damage and rejections. Brussels sprout buttons are mainly impacted by the development of sooty moulds contaminating the buttons that develop directly beneath the feeding *A. proletella*.

Infestations of *A. proletella* can be hard to control. The most effective pesticide for the control of this species is the systemic insecticide Movento[®] (spirotetramat) (Richter, 2010; Collier, 2012). Resistance of *A. proletella* to pyrethroid insecticides has been documented (Springate and Colvin, 2011); therefore resistance management is of great importance.

Natural enemies of *A. proletella* are not common within the UK. A parasitoid *Encarsia tricolor* is resident, however populations are rarely large enough to control infestations (Springate and Colvin, 2013). The specialist ladybird *Clitostethus arcuatus* is also resident, but at low densities, and is often limited to woodland,

possibly feeding on the honeysuckle whitefly, *Aleyrodes lonicerae*. (Springate and Arnold, 2012) causing it to be rare within cultivated fields.

Whilst not a ‘new’ pest in the UK, *Aleyrodes proletella* has become an increasing challenge for the *Brassica* industry in recent years, especially on Brussels sprout and kale. The reason for the increasing problem is unknown, but it is believed to be due to a combination of climate change, removal of certain active ingredients from use and later harvest times of crops. Relatively little research has focused on this species as, historically, it has been regarded as a minor pest. Knowledge about the biology of *A. proletella* is limited and some of what is currently understood about its ecology has been inferred from anecdotal evidence.

1.2 Aims and Objectives

The overall aim of this project is to understand population trends of *A. proletella* in the most vulnerable crops, Brussels sprout and kale. This includes understanding the key times of population increase and colonisation of new crops. This information can then be used to inform the development of an integrated control strategy using insecticides and other tools, which might include biological control agents and methods of cultural or physical control.

The objectives of the work are to:

- Develop a phenological model for *Aleyrodes proletella* development within a crop.
- Understand the dispersal potential of *Aleyrodes proletella*
- Understand the natural pattern of crop colonisation.
- Identify factors limiting population growth.

2 Movement, Dispersal and Migration of *Aleyrodes proletella*.

2.1 Introduction

Understanding the movement of pest insects is important for understanding patterns of colonisation of vulnerable crops. Being able to understand the timing of increased movement in a species allows the prediction of periods of colonisation and the degree to which colonisation may occur. Such information is of considerable value in formulating management strategies for pest control. Southwood (1962) considered two distinct forms of movement in insects; trivial and migration. Trivial flights can be regarded as short duration flights, usually between hosts, where flight is directed to host plants. It is often the movement resulting from searching for food or oviposition sites. Migration has been extensively defined by entomologists as it has far-reaching physiological, behavioural and ecological importance. Migratory behaviour as defined by Kennedy (1961) is:

“Persistent and straightened-out movement effected by the animal’s own locomotory exertions or by its active embarkation on a vehicle. It depends on some temporary inhibition of station-keeping responses, but promotes their eventual disinhibition and recurrence.”

In many pest insect species migration is undistracted flight whereby the insect ignores normally attractive vegetative cues and focuses on others, such as skylight, causing the insect to fly upwards, out of the crop, and to move to a new habitat. Dingle (1996) proposed that migration is a specific behaviour that allows the long-range displacement from one habitat to a new one.

Among insects, aphids have probably been studied to the greatest extent with respect to the migratory phase of their life cycle. The holocyclic life cycle of many aphids means that migration is vital for moving between their two host plants within a year. Migration involves strong rejection of an old habitat with upward flight taking the aphid away. Usually a positive phototactic response is seen with an attraction to short

wavelengths (<400 nm, blue-ultraviolet) and then, the aphid performs what is termed 'distance flight' (Kring, 1972). The height of flight is such that the aphid leaves the boundary layer and often begins to travel with prevailing winds. Such a migration, aided by wind, leads to a dispersive migration with little directional control from the aphid over a potentially vast distance (Southwood 1962). Flight often continues for an extended time (~2 hours) after which an attraction to vegetative cues, yellow wavelengths ~600nm, causes the aphid to descend and alight onto potential host plants within a new habitat (Kring, 1972). In many Aphididae the migration from one host to another takes place during a defined season and, sometimes, it is the only time that alates are produced, while in others migration is also elicited when overcrowding occurs and a habitat becomes unfavourable.

The migration of pest aphids (e.g. *Nasonovia ribisnigri*, *Cavariella aegopodii*, *Aphis fabae*) can have a predictable pattern, with flight often occurring after one generation develops on the primary host, causing them to migrate onto the secondary host. The Rothamsted Insect Survey records the numbers of aphid species that are caught by suction traps in 15 locations around the UK. The traps are 12.2 m high, sampling a constant volume of air ($45\text{m}^3 \text{ min}^{-1}$) all year round. Aphids caught at this height are highly likely to be migratory individuals capable of long distance dispersal (Macaulay *et al.*, 1988). The timings of such migrations recorded by the suction traps inform farmers of potential migrants that are likely to colonise vulnerable crops in the region. This is important in forecasting and monitoring the migration of virus vectors such as *Myzus persicae* as new migrants are likely transmit virus into a crop. It has also been shown that these data sets are more informative for predicting the timing of initial immigration into crops than local crop inspections (Heathcote *et al.*, 1969).

Migration is not, however, the only method by which pest insects move to colonise vulnerable crops. Populations of insects that are at high densities in a locality often spread out into the surrounding area. Such dispersal is described as a function of a population in which the average spacing between individuals increases, so that insects, in effect, disperse outwards from an area of high infestation (Southwood, 1966). This is often due to the trivial movement of pest insects performing short flights searching for food and/or oviposition sites. Here, populations are likely to

diffuse outwards colonising host plants in the surrounding area. Taylor (1978) described the relationship between dispersal probabilities and distances as a power relationship whereby one can expect an exponential decline in dispersal rate with increasing distance from the source. The probability of an individual colonising a location at distance, x , from the source has been termed the dispersal probability kernel. Such probabilities rarely follow a normal distribution; they are often leptokurtic; that is they have a higher peak of dispersal near the source with a sharp drop in probability of dispersing at short distances, however, there are higher probabilities at extreme distances than what a normal distribution would predict. Colonisation rates on new vulnerable crops are therefore likely to be highly influenced by their distance from the source population. The parameters of a mathematical model of a dispersal kernel can describe dispersion of a pest insect from a source and also provide information on its aggregation or dispersal potential through the model's parameters (Southwood, 1966). The carrot fly (*Psila rosae*) was studied to try to understand its movement from a source through dispersal caused by trivial ('neighbourhood') movement. Here a power relationship was found between the distance from the source and the catch rate. Such a relationship described a $1/66$ decrease in numbers with a ten-fold increase in distance from the source. This is informative; showing that isolation of new crops from overwintering sites by relatively small distances may have large impacts on rates of colonisation by carrot fly. Such information on dispersal/movement potential of the carrot fly has provided the opportunity to develop management strategies based on the isolation of new crops from overwintering sites (Finch and Collier, 2004)

2.1.1 Movement, dispersal and migration by *Aleyrodes proletella*.

Due to the mostly sessile nature of the juvenile stages of *A. proletella*, dispersal/movement is achieved predominantly by the adults. Females show a considerably higher rate of dispersal than males. For example, very few males were caught in suction traps at heights of 9ft above the crop, while females were common (El Khidir, 1963). It is likely that males respond to olfactory or visual cues from females as they have been observed 'waiting' for females to emerge from their puparia (Butler, 1938a).

Distribution patterns of the whitefly *Bemisia tabaci* emanating from a source follow a bimodal distribution, supporting the notion that morphs display either trivial or migratory flight (Byrne *et al.*, 1996). Morphological differences were also found between individuals that performed short-range or long-range dispersion, supporting the hypothesis that there are morphs that undertake migration or trivial flights (Blackmer *et al.*, 1995). Iheagwam (1977b) identified two ‘seasonal morphs’ of *A. proletella* that differed in their dispersal behavior. The ‘summer morph’ was shown to be reluctant to fly and when it did fly this was for only short durations returning quickly to vegetation. This ‘morph’ can be regarded as performing mainly trivial dispersal. The winter diapausing morph showed increased flight behaviour, with flights of long duration that probably enable the whiteflies to reach up to 40 m in height (Iheagwam, 1976; Iheagwam, 1977b).

Summer morphs of *A. proletella* are attracted to yellow-green light (500-600nm), which is close to the wavelength of light reflected by vegetation, and a similar response has been documented for other species of whitefly (Butler, 1938a, Mound, 1962). This attraction to yellow-green light is likely to keep whiteflies close to their host plants. Such behaviour is unlikely to elicit migratory flights and these individuals are likely to perform trivial movements. In contrast, diapausing individuals of *A. proletella* demonstrated positive phototaxis in response to an overhead light source rather than landing on available host plants (Iheagwam, 1977b). Changes in phototactic behavior by migratory and non-migratory morphs have also been observed in *B. tabaci*, a change from attraction to yellow/green to ultraviolet light, which is believed to induce the whiteflies to fly upwards towards the sky, eliciting higher flights for migration (Mound, 1962).

The distance over which whitefly species migrate has not been quantified in detail. *Bemisia tabaci* has been shown to migrate distances of over 2 km, although this is likely to be a conservative estimate as prevailing winds are likely to aid their migration (Byrne *et al.*, 1996). Although whitefly migration is likely aided by prevailing winds it should be noted that it is not regarded as a passive migration, as dispersal does not follow a diffusive pattern that would be predicted if this were the case. Thus they are likely to be performing an active migratory process (Byrne *et al.* 1996).

The heights that can be achieved by migrating *A. proletella* have not been investigated. In laboratory studies, ascending flight has shown them to have the potential to reach heights of over 40 m and such heights could potentially allow for vast migratory distances (Iheagwam, 1977b). However, *A. proletella* have only been captured in suction traps up to 9ft in height in the field (El Khidir, 1963).

Understanding the dispersal potential of *A. proletella* both spatially and temporally can only provide increased understanding of the colonisation of new, vulnerable crops. Information which can be used to develop effective management practices to reduce the impact of this problematic pest will be beneficial to growers.

The aims of the experiments described in this chapter are to understand methods and timings of colonisation by *A. proletella* onto newly planted vulnerable crops. This involves understanding the role long-range migration or trivial dispersal is involved in the movement of individuals and the timings of such movements. Such information would be valuable to growers as timings of immigration may be predicted through temporal changes of migratory behaviour and the method of colonisation would be better understood.

2.2 Materials and methods

2.2.1 Experiment 2.1. Developing a method for trapping active adult *Aleyrodes proletella* in the field.

A small plot, 3 m x 10 m, of 30 Brussels sprout plants (cv. Trafalgar) with a heavy infestation of *A. proletella* was used to investigate any preferences for colour and height. Four replicate treatments were set out in a randomized-block design within the Brussels sprout plot. The sticky traps (22 cm x 10 cm) were either yellow or blue (BHGS Ltd, UK) in colour and placed on the ground or at a height of 1 m. Traps located close to the ground were placed upon a plastic base (9 cm diameter) to suspend them approximately 1 cm above soil level, preventing ground-active insects (e.g. carabid beetles) being caught on the traps, which quickly reduced their efficiency to catch other insects. Traps located 1 m above the ground were attached between two 1 m long bamboo canes to secure them horizontally. All sticky traps were placed perpendicular to the ground. Traps were set out on 26 October 2012 and collected on 29 October 2012. The numbers of *A. proletella* caught were recorded on the day of collection using a x 45 microscope (Euromex E series, Holland). The resulting data were analysed using Analysis of Variance (ANOVA) testing for significant effects of colour or height on catch rate.

2.2.2 Experiment 2.2. Monitoring *Aleyrodes proletella* activity using yellow sticky traps on the ground.

Yellow sticky traps, 22 cm x 10 cm (BHGS Ltd, UK) were placed horizontally, 1 cm above the ground on the north, south, east and west side of Brussel sprout-kale plots with a high infestation of *A. proletella*, the Plots (A-E, Figure 2.1) used in Experiment 4.5. Traps were deployed for a 7-day period prior to a sampling event within the plots, so that captures were unaffected by the disturbance caused by assessing the plants in detail. The whiteflies on the traps were counted using a x 45 microscope (Euromex E series, Holland). Weather data were available from an automatic Meteorological Office weather station located nearby (Figure 2.1). To

reduce the potential for dead whiteflies to fall onto the traps, they were placed 30 cm from the nearest plant, since the aim was to capture individuals flying actively.

2.2.3 Experiment 2.3. Estimating the abundance of adult *Aleyrodes proletella* at different distances from an infested crop.

Yellow sticky traps and ‘trap plants’ were deployed in ‘transects’ at a range of distances from plots infested with *A. proletella* (also used in Experiment 4.5). Individual sticky traps were placed horizontally on a plastic tray ~1 cm above the ground at distances of 0 m, 5 m, 10 m, 20 m and 30 m from each plot. For the approach using ‘trap plants’, groups of three potted (9 x 9 x 8 cm) cauliflower plants (cv Skywalker) at the 7th true leaf stage, were placed at the required distances (0, 5, 15, 25, 45 m) along the line of the hedgerow adjacent to the infested plot. The plants were placed in a 30 x 20 cm white plastic tray to hold water. All transects went in a southerly direction from the plot ranging in dates from April-June (Table 2.1). Both the sticky traps and trap plants were left for 7 days, after which all adults on the plants were counted by eye. The sticky traps were taken into the laboratory where the whiteflies were counted using a x 45 microscope (Euromex E series, Holland).

Table 2.1. Dates and location (Figure 2.1) of transects set up in Experiment 2.3. (plots were those used in Experiment 4.2 and described in Chapter 4)

Date	Plot locations	Transect
3 rd April 2014	B,D,E	Plants
24 th April 2014	B,D,E	Sticky traps
30 th April 2014	A,B,C,D,E	Plants
9 th June 2014	B,D,E	Sticky traps

Experimental Fields

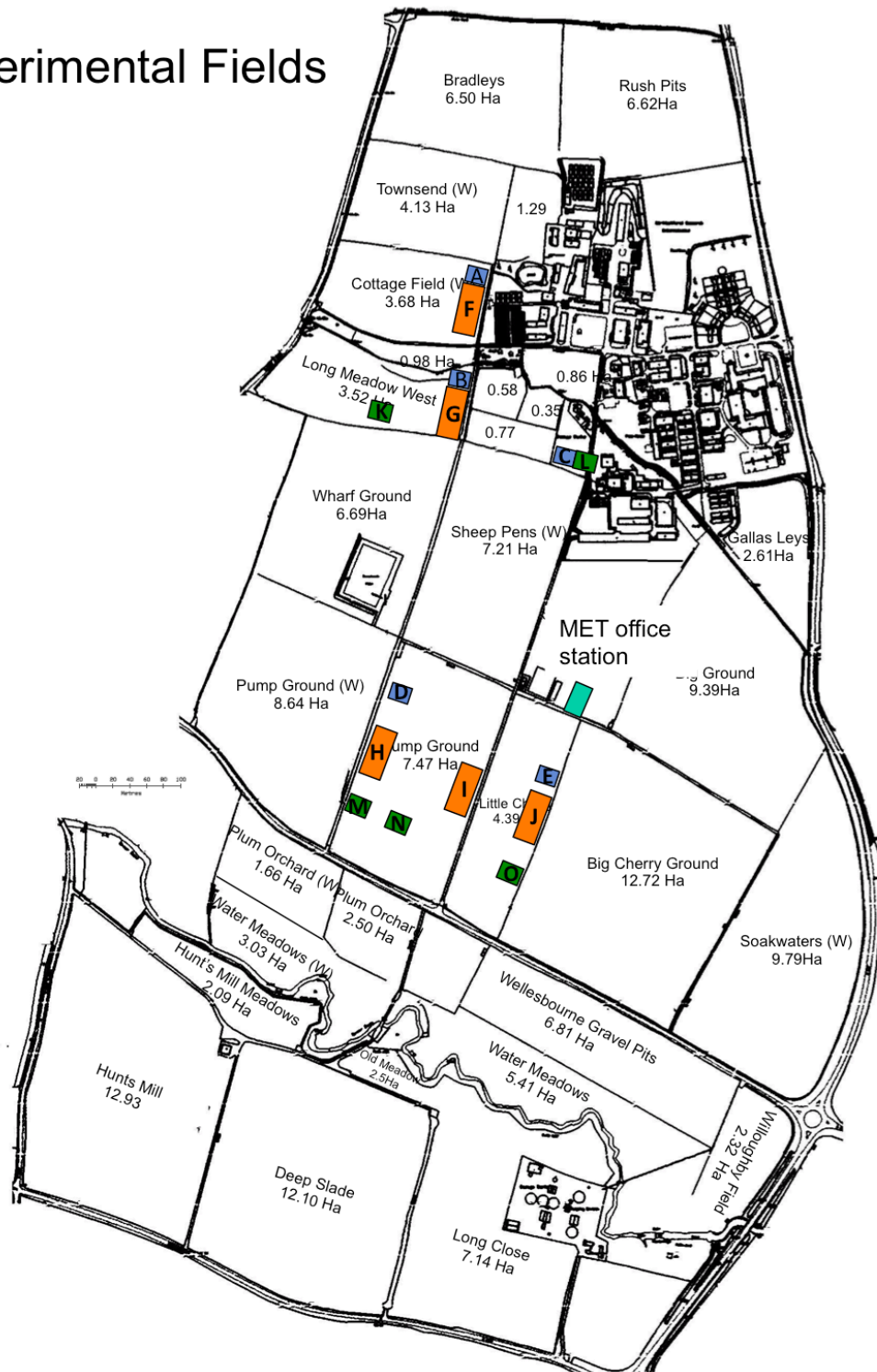


Figure 2.1. Locations of experimental plots for Experiment 4.5 (A–E), Experiment 4.6 (F–J) and Experiment 4.7 (K–O) at the Wellesbourne campus (SP27142 57772). The location of the meteorological office weather station is also shown. Experiment 4.5 (A–E), (F–J) and (K–O) at the Wellesbourne campus (SP27142 57772). The location of the meteorological office weather station is also shown.

2.2.4 Experiment 2.4. Determining the heights at which adult *Aleyrodes proletella* disperse.

Sticky traps deployed at various heights from the ground were used to monitor adult whiteflies to determine the vertical distribution of flight (Table 2.2). Telescopic ‘7 metre Flagpoles’ (Newquay Camping, UK) were erected within 5 m of plots infested with large numbers of *A. proletella* to increase the probability of capture; using the plots in Experiment 4.5, A-E (Figure 2.1). The sticky traps (22 cm x 10 cm) were either yellow or blue (BHGS Ltd, UK) and were rolled into a cylinder (10 cm tall, 8 cm diameter), to provide a coloured sticky surface that covered 360°. Replicate flagpoles (Figure 2.2) were set up on the same day and left for 7 days. The traps were then collected and the numbers of *A. proletella* were counted using a x 45 microscope (Euromex E series, Holland).

Table 2.2. Dates and locations (Figure 2.1) of flagpoles set up in Experiment 2.4

Date	Plot locations	Number of replicate flagpoles
22 nd October 2013	C	1
12 th December 2013	C	1
13 th April 2014	C	1
28 th April 2014	C	1
28 th May 2014	C,A,B,D	4
14 th June 2014	C,A,B,D	4
1 st August 2014	C,E,B,D	4
18 th September 2014	C,E,B	3
28 th October 2014	C,D,E,B	4
18 th November 2014	A,E,B,D	4

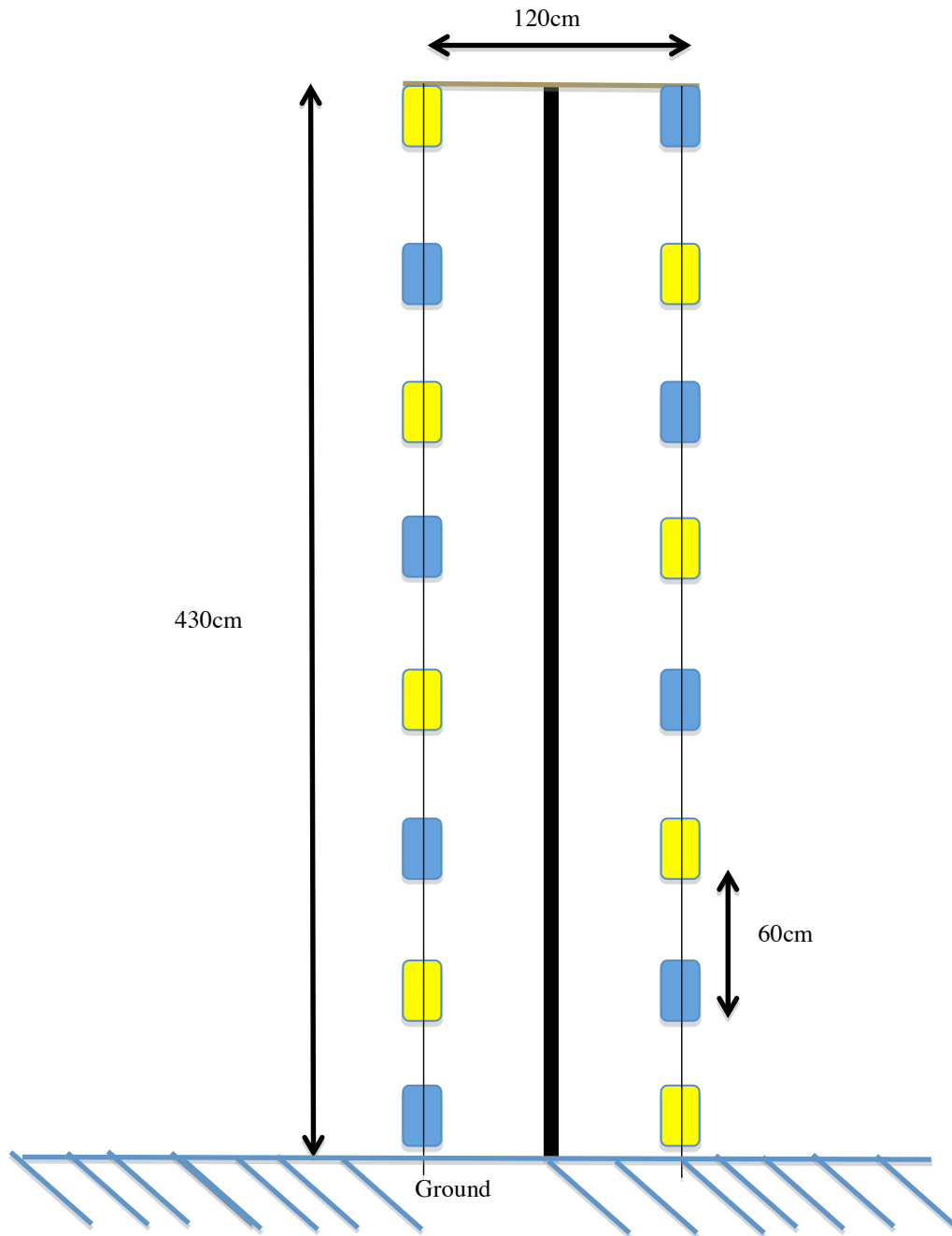


Figure 2.2. Diagram of a flagpole supporting yellow and blue sticky traps used in Experiment 2.4.

2.2.5 Experiment 2.5. Assessing Rothamsted suction trap samples for the presence of *Aleyrodes proletella*

Samples from suction traps run by the Rothamsted Insect Survey were sorted for *A. proletella*. The traps are 12.2 m tall and consist of a 224 mm diameter pipe, sampling

45 m³ of air per minute by means of an inlet fan (Macaulay *et al.*, 1988). All trapped animals are automatically collected in bottles containing 70% alcohol and located at the base of the trap. When the samples are removed from the trap they are transferred into 95% ethanol - 5% glycerol solutions for storage in small vials. Samples were viewed under x45 magnification (Euromex E series, Holland). Identification of whitefly individuals as *A. proletella* was confirmed using the characteristic wing venation (Figure 1.1). The samples assessed ranged January – December for Wellesbourne and Kirton suction traps from the years 2010 to 2015 (Table 2.3).

Table 2.3. Location, year and date of Rothamsted Insect Survey samples that were assessed for the presence of *Aleyrodes proletella*.

Trap location	Year	Dates	Total number of samples assessed.
Kirton, Lincolnshire, UK	2010	Aug-Dec	114
	2011	Aug-Dec	121
	2012	Aug-Dec	102
	2013	Aug-Dec	58*
	2014	Aug-Dec	84
Wellesbourne, Warwickshire, UK.	2012	Aug-Dec	106
	2013	Aug-Dec	116
	2014	Jan-Dec	222
	2015	March-Dec	230

*samples were missing due to short-term failure of suction trap.

2.3 Results

2.3.1 Experiment 2.1. Developing a method for trapping active adult *Aleyrodes proletella* in the field.

Yellow sticky traps caught over five times as many *A. proletella* as blue traps (ANOVA, $F_{(1,12)}=9.45$, $P<0.01$; Figure 2.3) and traps on the ground caught more *A. proletella* than those positioned 1 m above the ground (ANOVA, $F_{(1,12)}=11.09$, $P<0.01$, Figure 2.3). Figure 2.3) and traps on the ground caught more *A. proletella* than those positioned 1 m above the ground (ANOVA, $F_{(1,12)}=11.09$, $P<0.01$,). There was a statistically significant interaction between trap colour and height (ANOVA, $F_{(1,12)}=6.75$, $P<0.05$), with the effect of colour being less apparent at 1 m.

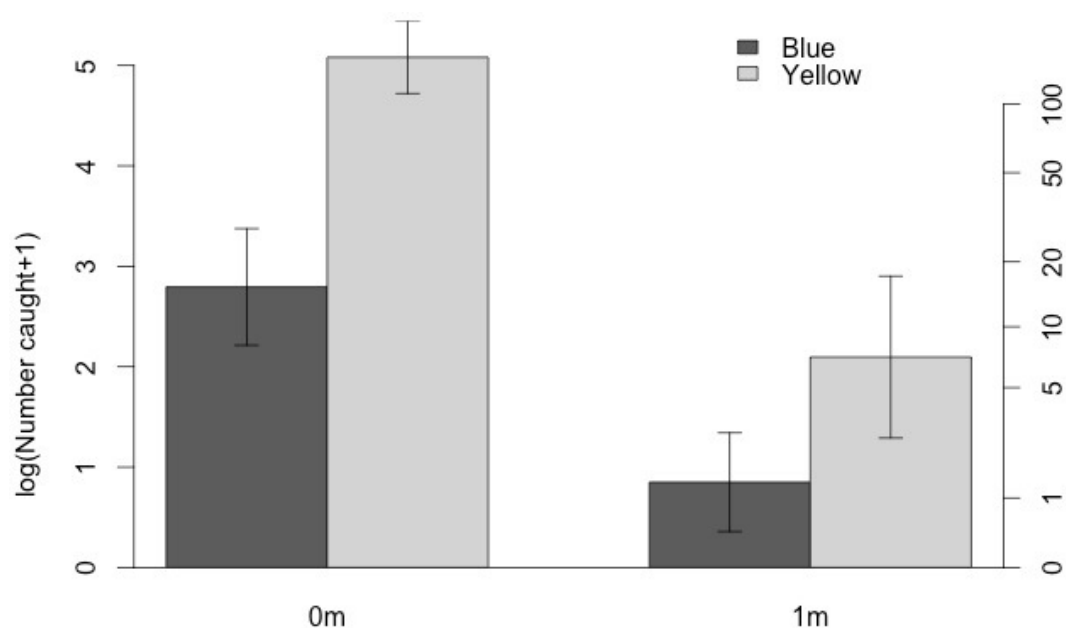


Figure 2.3. Log mean (\pm SE) number of *Aleyrodes proletella* caught by yellow or blue sticky traps either at ground level, 0 m, or 1 m above the ground. The right-hand axis shows the back-transformed counts, $n=4$.

2.3.2 Experiment 2.2. Monitoring *Aleyrodes proletella* activity using yellow sticky traps on the ground..

Trap location had a significant effect on the *A. proletella* catch rate (ANOVA, $F_{(3,276)}=24.9$, $P<0.01$, Figure 2.4). This difference was not evident for the majority of

the year, however, there was a period from December 2013 until March 2014 when traps to the north and the east caught higher numbers than those to the south and west (Figure 2.4).

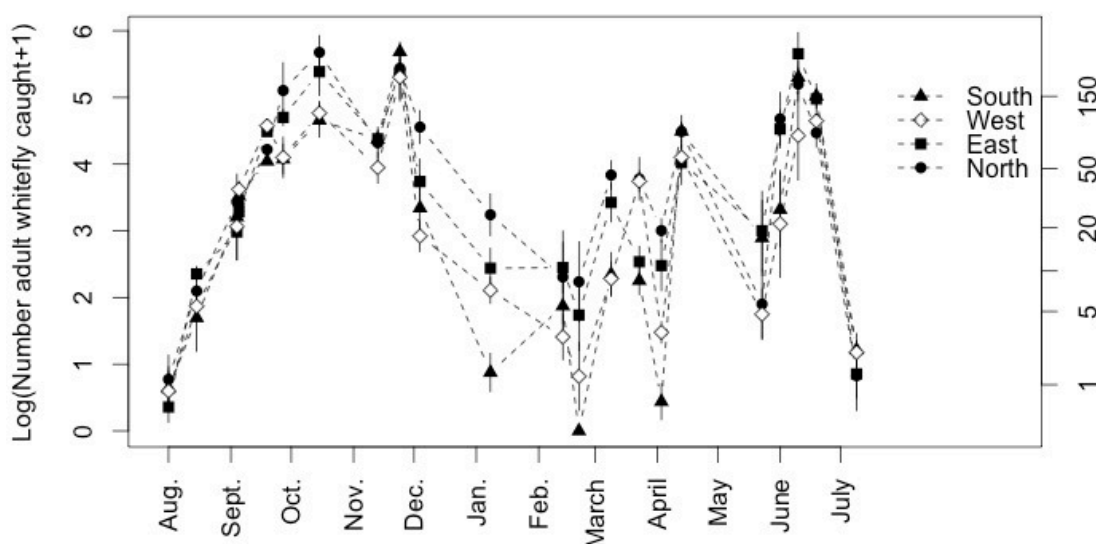


Figure 2.4. Mean (\pm SE) number of *Aleyrodes proletella* caught on traps placed in different directions from plots (north, south, east, west) from August 2013 until July 2014. The right axis shows the back transformed data values.

An ‘activity index’ was calculated for the dates between August 2013 and July 2015 - this was defined as

$$\text{Activity index} = \ln\left(\frac{\text{No. Whitefly caught on trap} + 0.1}{\text{Mean No. adult whitefly per plant in plot} + 0.1}\right)$$

Note that 0.1 was added to each value to avoid results = ∞ ; a value of 1 has a relatively large impact on small catches/populations therefore 0.1 was used.

The ‘activity index’ varied during the year with a similar pattern for 2013 and 2014 (Figure 2.5). An increase in activity occurred in the autumn, with a larger increase noted in 2014. This was followed by a decrease in activity over the winter months; it was at this time when activity was the lowest for the year. Activity began to increase

from March onwards in conjunction with an increase in temperature. Activity continued to increase until reaching its peak, in both years, around June.

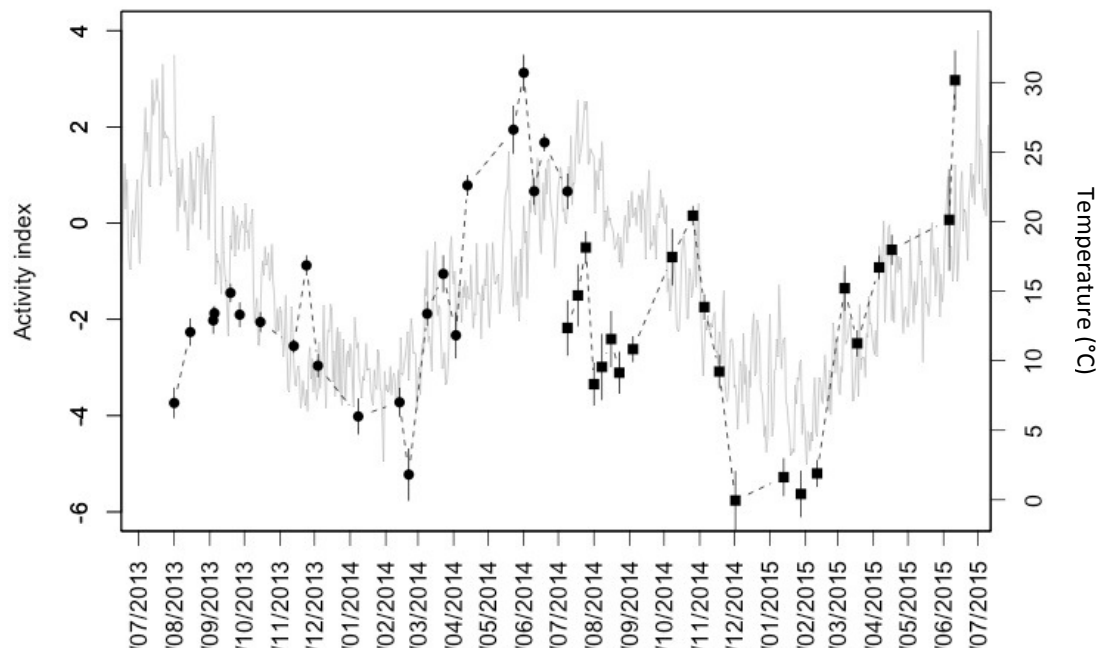


Figure 2.5. Mean (\pm SE) activity index [$\ln(\text{Number } A. \text{ proletella caught on traps} + 0.1 / \text{mean number per plant in plot} + 0.1)$] August 2013 – July 2015). The grey line represents daily maximum air temperatures ($^{\circ}\text{C}$) for Wellesbourne. Black circles and squares represent the activity index for 2013 plots and 2014 plots respectively.

It was possible to calculate activity indices separately for Brussels sprout and kale plots for 2013 (Figure 2.6), as traps (north, south, east, west) were adjacent to kale, Brussels sprouts or located intermediately between both. Data from sticky traps adjacent to Brussels sprout plots showed significantly higher activity indices than those that were adjacent to kale (ANOVA, $F_{(2,302)}=36.7$, $P<0.001$). The activity index for traps adjacent to Brussels sprout plots fell less dramatically, barely at all, over the winter period when compared to traps near kale. This difference in response to time of year was seen as a significant interaction of date and crop type (ANOVA, $F_{(42,302)}=1.604$, $P<0.05$).

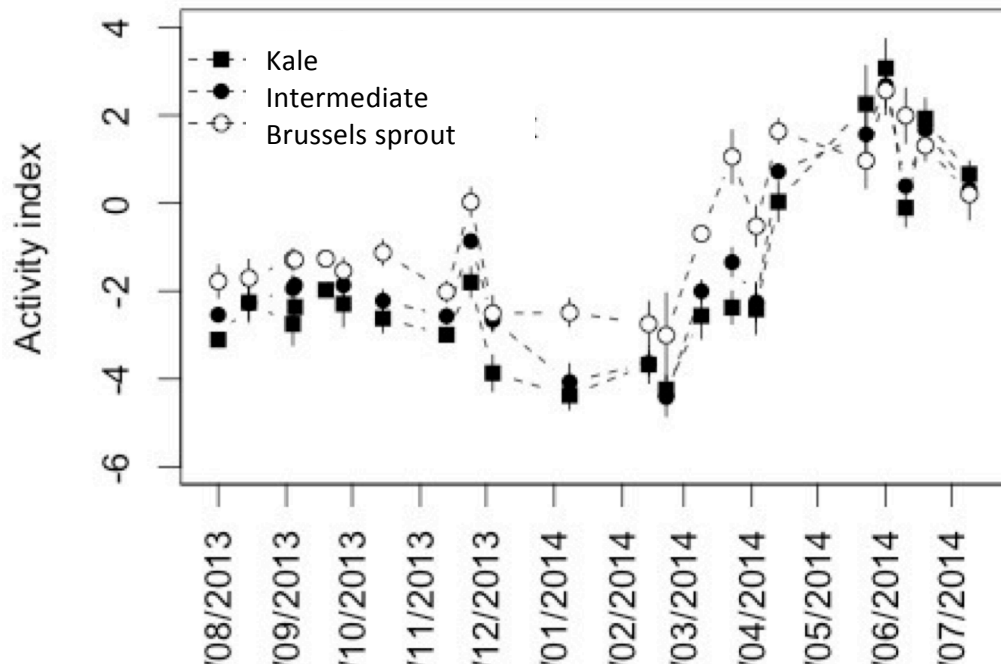


Figure 2.6. Mean (\pm SE) activity index for *Aleyrodes proletella* caught on sticky traps adjacent to different crops, kale, Brussels sprout and intermediate (trap between kale and Brussels sprout) for August 2013 until July 2014.

2.3.3 Experiment 2.3. Estimating the abundance of adult *Aleyrodes proletella* at different distances from an infested crop.

The proportions of *A. proletella* caught along a transect were modeled using Taylor's (1978) general model of insect dispersal.

$$y = e^{(a-b \cdot x^c)}$$

where y = frequency of capture at distance, x , from the source. a specifies the sample size and b is a scale factor depending on units distance is measured in. c is the dispersal parameter describing the rate of change of density with distance, whereby $c < 2$ suggests aggregation and $c > 2$ suggests active dispersal (Taylor, 1978). When the data were modeled allowing all parameters a , b and c to be variable the analysis returned the estimates shown in Table 2.4, however the estimate for b was not significant. Setting c as a constant, 1, which in effect becomes the dispersal equation as proposed by Gregory and Read (1949), allowed estimates of the parameters b and a that led to a significant description of the distribution of *A. proletella* catches.

Transects based on sticky traps or trap plants were modeled using the same parameters as there was no statistically significant effect of transect type (Figure 2.7.).

Table 2.4. Parameters for Taylor's (1978) dispersal model, $y = e^{(a-b.x^c)}$

Parameters.	Parameter	Estimate	Standard error	P-value
a,b,c -variable	a	-028784	0.03804	<0.01
	b	0.23246	0.15765	NS
	c	1.10521	0.38815	<0.01
a,b – variable c=1	a	-0.27439	0.04438	<0.01
	b	0.28736	0.03590	<0.01

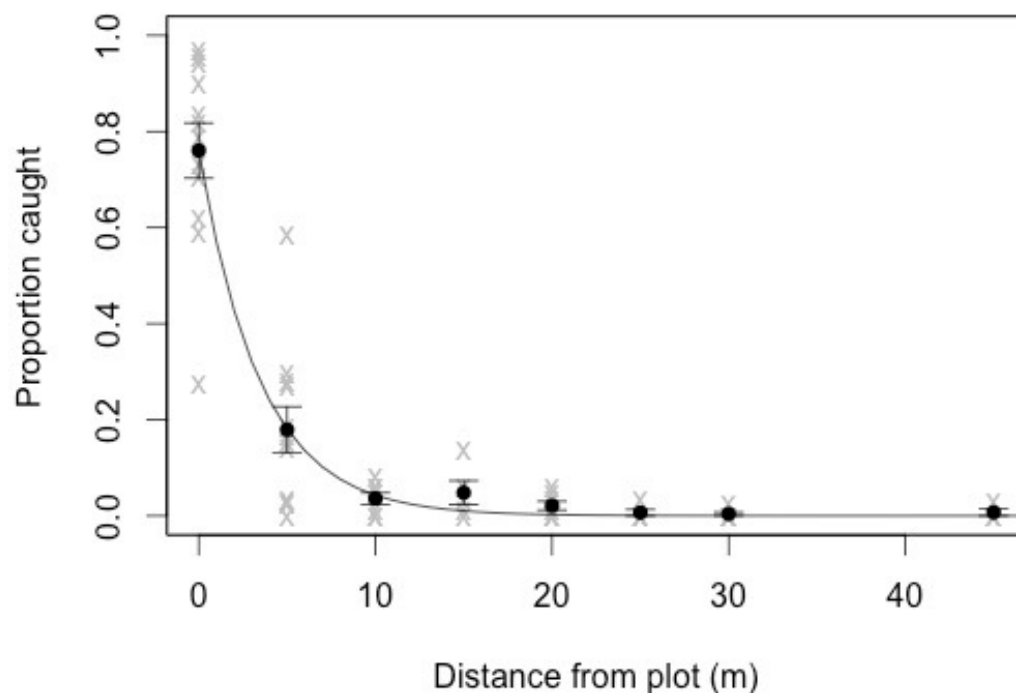


Figure 2.7. Mean (\pm SE) proportion of *Aleyrodes proletella* 'caught' at different distances from a plot of brassicas (Brussels sprout or kale) infested with *A. proletella*. The fitted line shows $y = e^{(-0.2439-(0.28736*x))}$, individual data points are denoted by an 'x'

2.3.4 Experiment 2.4. Determining the heights at which adult *Aleyrodes proletella* disperse.

Yellow sticky traps at ground level caught more *A. proletella* than all other traps combined, catching an average of ~50 individuals during the study period. The next highest rate of capture was by yellow traps at 60 cm and here the average rate of capture was only ~5 individuals. The vertical distribution of *A. proletella* captured on sticky traps differed between April-August and September-November ($\chi^2=176.9$, $df=6$, $P<0.01$) with larger numbers captured at greater heights during September-December. There was also a statistically significant increase in the proportion of *A. proletella* caught on blue sticky traps during September-December when compared with earlier in the year ($\chi^2=51.79$, $df=1$, $P<0.01$, Figure 2.8).Figure 2.8).

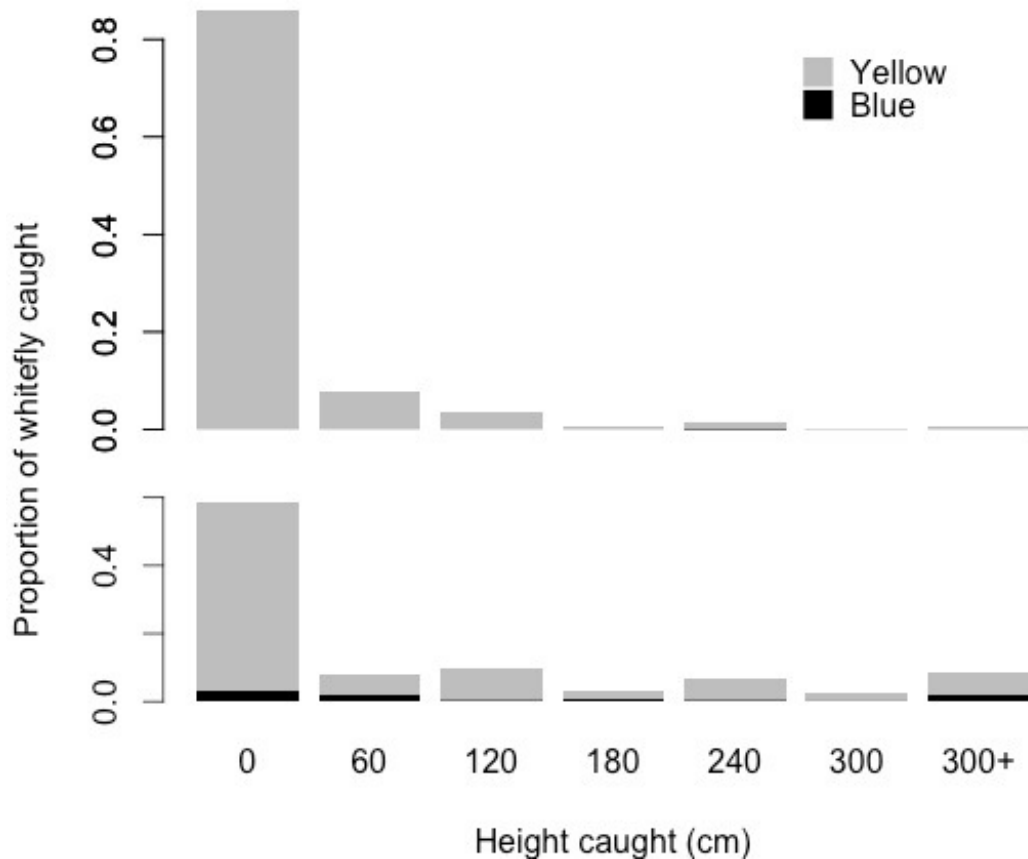


Figure 2.8. Proportion of *Aleyrodes proletella* caught on yellow or blue sticky traps at different heights from the ground (cm). Top: April-August (810*), Bottom: September-November (1810*). *Numbers in brackets denote total number of *A. proletella*.

The proportions of *A. proletella* caught at each height were modeled using Johnson's (1957) vertical density model.

$$y = a * (x + Ze)^b$$

Where y =density of insects at distance ' x ', from the ground a = scale factor dependent on size of population in the air, it is in fact the expected density when $(x + Ze)=1$, i.e. proportion caught at ground level, Ze =constant b = index of aerial diffusion process.

The parameters of the model were fitted separately for April-August and September-November and for captures by yellow or blue traps (Table 2.5). The model did not fit for *A. proletella* captures on blue traps during April-August as only two whiteflies were caught on one trap, on one occasion. Replicates where fewer than five whiteflies were captured were excluded from the model fitting, as at this level individual whiteflies have a large impact on the distribution. Model simplification showed that different parameters for April-August and September-November significantly improved the model's descriptive power (ANOVA, $df= 1$, $F=4.11$, $P<0.05$; Figures 2.9-2.11, Table 2.5)

Table 2.5. Parameters for Johnson's (1957) vertical density model, $y = a*(x + Ze)^b$ by fitting separate models for each trap colour x trapping period combination.

Model	Parameter*	Estimate	Standard error	P-value
Yellow April-August	a	0.82465	0.02688	<0.01
	b	-0.64567	0.06717	<0.01
Yellow September-December	a	0.66971	0.02781	<0.01
	b	-0.4976	0.04172	<0.01
Blue April-August	NA	NA	NA	NA
Blue September-December	a	0.53729	0.05827	<0.01
	b	-0.35623	0.05300	<0.01

*Ze was set at 1 for each model.

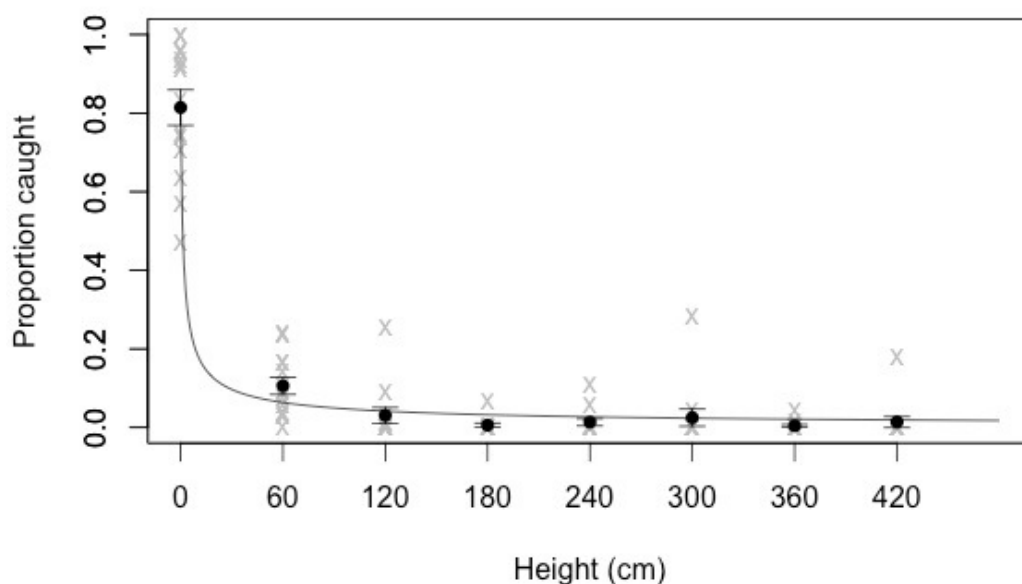


Figure 2.9. Mean (\pm SE) proportion of *Aleyrodes proletella* caught on yellow sticky traps at each height (April-August). Fitted line $y=0.82465*(x+1)^{-0.66971}$, $P<0.01$ (Table 2.5) is shown. Individual data points are denoted by 'x'.

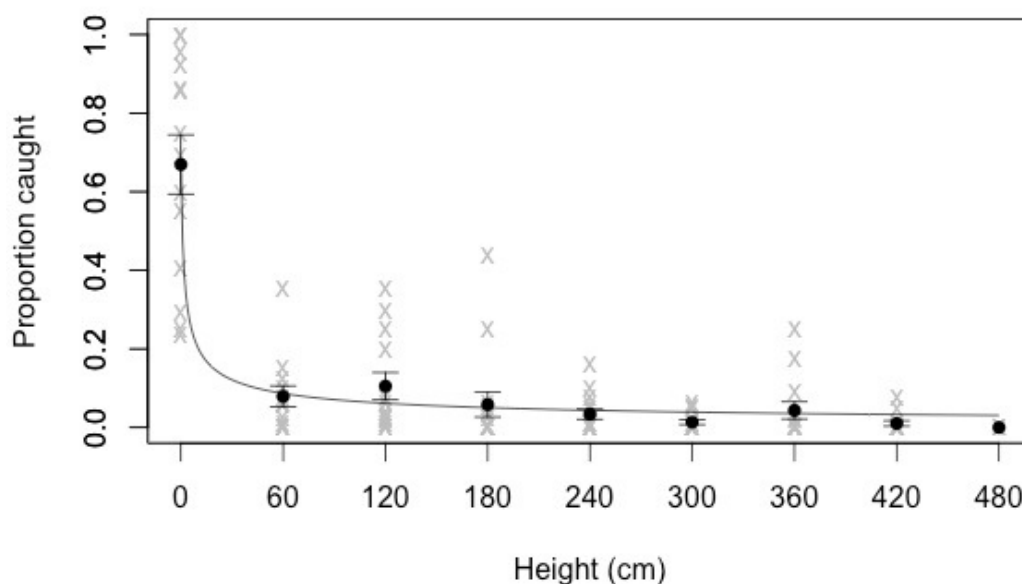


Figure 2.10. The mean (\pm SE) proportion of *Aleyrodes proletella* caught on yellow sticky traps at each height (September-December). The fitted line $y=0.6697*(x+1)^{-0.4976}$, $P<0.01$ (Table 2.5) is shown. Individual data points are denoted by 'x'.

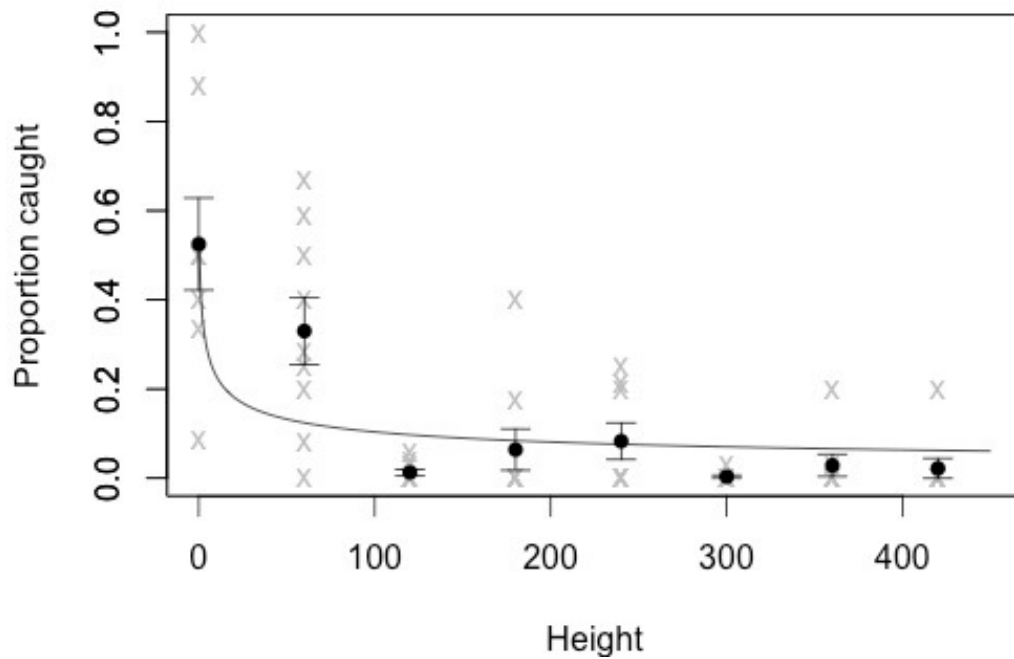


Figure 2.11. The mean (\pm SE) proportion of *Aleyrodes proletella* caught on blue sticky traps at each height (cm) (September-November). The fitted line $y=0.53729*(x+1)^{-0.35623}$, $P<0.01$ (Table 2.5) is shown. Individual data points are denoted by 'x'.

2.3.5 Experiment 2.5. Assessing Rothamsted suction trap samples for the presence of *Aleyrodes proletella*.

With the exception of 2010 at Kirton, the median date of capture of adult *A. proletella* by the suction traps was between 11th October and 12th November for the years sampled, *A. proletella* adults were caught about a month earlier at Kirton in 2010 (Figure 2.12). In the years where samples from both sites were assessed, the total numbers of *A. proletella* captured were very similar. No clear relationship was found between the median date of capture and accumulated day-degrees from 1 January or up to 1 August, which is the date after which nymphs go on to develop into adult females in ovarian diapause (Table 2.6).

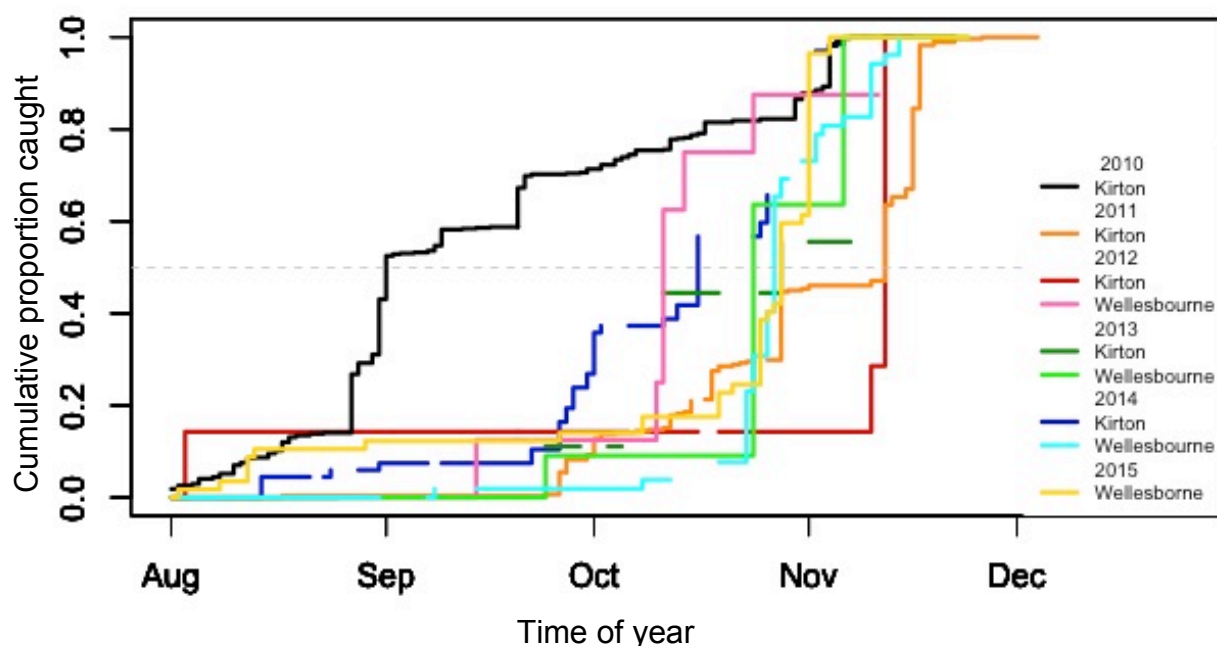


Figure 2.12. Cumulative captures from suction traps between August and December for Kirton (2010-2014) and Wellesbourne (2012-2015) shown as a proportion of the total number captured each year. Gaps in cumulative captures represent samples that were missing due to trap failure. The dashed line shows the point by which 50% of the total number of *Aleyrodes proletella* captured were caught each year (median date of capture).

During 2014 at Wellesbourne (one of two years when samples collected throughout the year were assessed), the majority of samples did not contain *A. proletella*. Most *A. proletella* were found in samples from October and November. Only three *A. proletella* were caught outside this period, one in September and two in June (Figure 2.13). The numbers of *A. proletella* captured were higher in 2015 and a larger number of captures occurred before August, 11 out of 68 whiteflies. Most of these were in late June/early July (Figure 2.14).

Table 2.6. Total captures of *Aleyrodes proletella* (Aug-Dec) for each trap location and date. The median date of capture is shown with the accumulated day degrees (T^t 6.3°C) from 1 January to that date, together with the accumulated day degrees to 1st August.

Year	Location	Total	Date of 50% catch	Acc. D° at 50% catch	Acc. D° at August 1st
2010	Kirton	541	1 st September	1266	965
2011	Kirton	291	12 th November	1886	1036
2012	Kirton	7	12 th November	1586	904
	Wellesbourne	8	11 th October	1506	919
2013	Kirton	9	28 th October	1615	862
	Wellesbourne	11	24 th October	1696	943
2014	Kirton	67	16 th October	1746	1108
	Wellesbourne	52 (54)*	27 th October	1844	1120
2015	Wellesbourne	57(68)*	28 th October	1601	945

*Numbers in brackets represent total catches for the year.

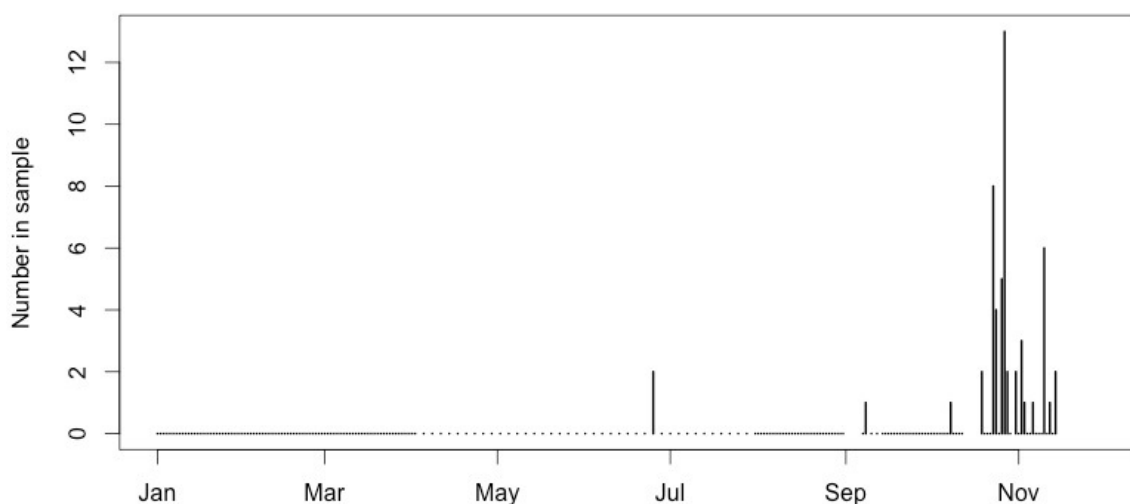


Figure 2.13. Daily number of *Aleyrodes proletella* caught in the Wellesbourne suction trap, April- December 2014.

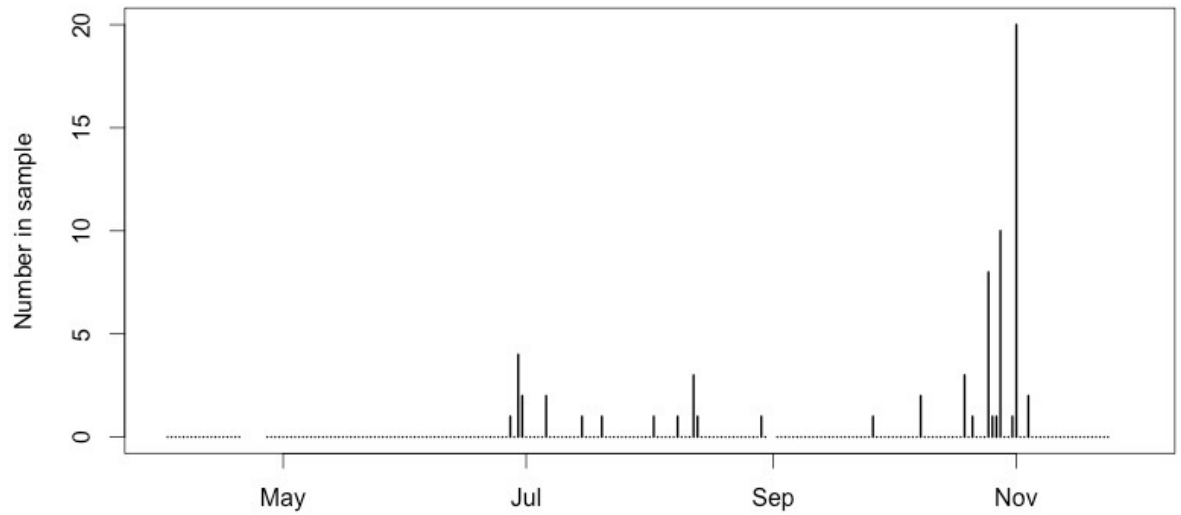


Figure 2.14. Daily number of *Aleyrodes proletella* caught in the Wellesbourne suction trap, April – December 2015.

2.4 Discussion

Although *A. proletella* appears to be ill-equipped to fly for long distances independently, it does show a considerable capacity to colonise new crops. The aim of the experimental work described in this chapter was to try and understand the dispersal behaviour of *A. proletella* in more detail in terms of the distances covered and the annual periodicity of activity, as both of these attributes are important for the colonisation of new crops. Information was gathered using data collected from different types of traps: sticky traps, trap plants and the network of suction traps run by the Rothamsted Insect Survey.

2.4.1 Yellow traps

Overall, close to infested crops, where it is likely that many individuals will be undertaking trivial movement, yellow sticky traps were more effective than blue sticky traps at capturing adult *A. proletella*. Most adult *A. proletella* were captured at ground level and capture of the largest numbers of individuals at ground level is consistent with the results of several studies on *Bemisia tabaci* (Gerling and Horowitz, 1984; Byrne *et al.*, 1996; Isaacs and Byrne, 1998; Atakan and Canhilal, 2004). Whilst undertaking trivial movement, adult *A. proletella* are likely to be flying close to the ground, and actively finding or re-finding a host plant. In particular, adult *A. proletella* are very easily disturbed when crop plants are moved and will need to relocate crop foliage once airborne.

Yellow traps can be interpreted as providing a super-normal stimulus to herbivorous insects seeking host plants since yellow light (~500 nm) is a major component of the reflected light from green leaves (Shull, 1929; Vaishampayan *et al.*, 1975). Overall, the majority of the data collected using yellow sticky traps suggest that, unsurprisingly, the aerial *A. proletella* captured on these traps are most abundant close to the crop in both the horizontal and vertical dimensions. The proportion captured on these traps decreased rapidly within a short distance from the plot.

Yellow traps and trap plants were used to study the dispersal rate of *A. proletella* from a known population source. The data obtained were modeled using the mathematical model described by Taylor (1978), although more replications would

be needed to establish statistically significant parameters for this model. The parameter 'c' in Taylor's model is important in describing the 'randomness' of dispersal. Only when $c \sim 2$ is dispersal considered random, following a diffusion-like process. Although not statistically significant, the value of 'c' estimated from fitting Taylor's model to the data for *A. proletella* was 1.1, indicating a tendency for aggregation. That is to say that the *A. proletella* are dispersing from the source rather poorly, leading to a high concentration in the immediate vicinity. The data were modeled more successfully using Gregory and Read's (1949) equation, setting c at 1, again indicating a propensity for *A. proletella* to stay within the locality and to aggregate. Thus, modeling the dispersal distribution of *A. proletella* confirms that there is a sharp drop in catch size with small increases in distance from the host crop and that the distribution of dispersion can be described as 'leptokurtic' (Southwood, 1966).

2.4.2 Blue versus yellow and long range dispersal

It is known that female *A. proletella* change their physiological status in late summer to early autumn as they enter ovarian diapause; and the occurrence of diapause was confirmed by monitoring data collected from the experimental plots at Wellesbourne (Experiment 4.4, Chapter 4). It has been suggested previously that this is the stage during which *A. proletella* undertakes long-range dispersal (El Khidir, 1963; Iheagwam, 1977b) and when females are likely to be more attracted to overhead sources of light rather than to host plants (Iheagwam, 1977b).

Attraction to short-wavelength light is believed to be important in eliciting long-range dispersal behaviour in other species of whitefly (Mound, 1962). Blue traps reflect short-wavelength light and it can be inferred that an increase in the proportion of *A. proletella* captured by blue traps compared with yellow traps later in the season denotes an increase in the number of *A. proletella* performing long-range dispersal. In contrast, almost no *A. proletella* were captured on blue sticky traps in the early part of the summer. In addition, the *A. proletella* found on blue traps were captured at a relatively greater height than those on yellow traps, particularly when comparing the proportion captured above a height of 3 m.

It was possible to model the vertical distribution of *A. proletella* caught on both yellow and blue traps using Johnson's (1957) vertical distribution model. The parameters of this model describe the aerial distribution of insects. Firstly, the parameter 'a' is the expected density (or 'catch rate') at ground level. When modeling the distribution obtained from yellow traps, 'a' was significantly lower in September-December than earlier in the year, indicating that a lower proportion of *A. proletella* were caught at ground level at that time. The parameter describing blue trap catches for September-December showed a marked 20% reduction in expected catch rates at ground level. There is a lower proportion of blue trap captures at ground level when compared with those caught on yellow traps, supporting the notion that the *A. proletella* attracted to blue traps are flying higher above the ground than those attracted to yellow traps.

The parameter 'b' describes the 'relative numbers at different heights; thus 'the more insects there are in the lower compared with the upper layers of air, the higher the value of b will be' (Johnson, 1957). Again, for the captures by yellow traps, 'b' was lower for data from September-December than from April-August, indicating that there was a shift in the distribution of captures towards greater heights. For data from the blue traps, 'b' was lower still, denoting an even larger shift in the distribution towards greater heights.

2.4.3 Long range dispersal and suction traps

The increased captures of *A. proletella* on blue sticky traps in September-December, and the reduced proportion captured on yellow (and blue) sticky traps at ground level, suggest that the proportion of *A. proletella* performing migratory flights had increased compared with earlier in the year. This was supported by the periodicity of the captures made by the Rothamsted suction traps at Wellesbourne, since relatively few *A. proletella* were captured between April and August in both 2014 and 2015, with the majority being captured from September onwards.

The suction traps capture a wide range of pest aphid species and much research has been focused on these. Taylor (1974) considered that captures of aphids by the suction traps indicate periods of long-range migration and are representative of the aerial population within a region rather than the abundance of aphids on crops in the

immediate locality. For example, the initial colonisation of crops by pest aphids, such as *Rhopalosiphum padi*, is related to the time at which the first aphid is captured in a nearby suction trap. Indeed the capture of the first aphid in the suction trap often provides an early warning within 10 days of their occurrence in the field (Heathcote *et al.*, 1969).

In contrast, in both 2014 and 2015, newly-planted plots of kale/Brussels sprout were first colonised by *A. proletella* within 2 weeks of planting, while the first capture in the suction trap was not until July. This supports the hypothesis that *A. proletella* are exhibiting trivial dispersal at this stage, such that new colonisers of crops are likely to be from the close vicinity and making short host-seeking flights. This behaviour is also seen in *B. tabaci*, where over 90% of individuals could be regarded as exhibiting trivial dispersal and the individuals that leave a host plant are likely to alight on another host as soon as it is encountered (Gerling and Horowitz, 1984).

Interestingly a small ‘peak’ in captures of *A. proletella* by suction traps occurred during June in both 2014 and 2015. El Khidir (1963) suggested that this limited amount of migratory activity was due to the senescence of some host plants such as oilseed rape. In the present study, the brassica plants used in the previous year’s experiments had begun to senesce in June, as had the oilseed rape crops in the surrounding area, and this may have led to the small increase in captures in the suction trap.

2.4.4 Activity

The numbers of insects captured on traps are the product of both population size and activity (Southwood, 1966). Thus the index calculated from catch size/population on host plants gives a relative estimate of activity over the year. The index varied over the course of the year with the lowest values occurring, as expected, during the winter months. At this time the temperatures were often below the estimated flight threshold of 9°C (Butler, 1938a) and it is unlikely that many *A. proletella* were moving around their hosts at this time. There was, however, some movement, and more *A. proletella* were captured on traps situated to the north and east of the plots. At this time the prevailing winds at Wellesbourne were in the north-easterly direction and it is certainly possible that some *A. proletella* were dislodged and blown onto the

traps. Activity increased early in the New Year, as temperatures increased, and this probably reflects an increase in trivial movement. At this time it is likely that *A. proletella* moving out from points of infestation may have been able to colonise nearby crops. The degree to which this might occur would be related to the dispersal probabilities described earlier. As the dispersal kernel is leptokurtic this suggests that there would be a sharp drop in colonisation with increasing distance.

Flight activity by *A. proletella* has been shown to increase with increasing temperature (Butler, 1938b). In the present study, however, peak periods of activity (in June 2014 and 2015) did not coincide with the periods when temperatures were at their highest (in July). In June-July, the activity indices were estimated from captures made close to plots of senescing plants, whereas in August they were estimated close to plots that had been recently transplanted. It is certainly possible that fewer young leaves would be available for colonisation in June, encouraging more *A. proletella* to leave their hosts. The senescence of host plants has been shown to increase the dispersal behaviour of *Trialeurodes vaporariorum* (Bonsignore, 2015) and it may be this behaviour that led to the small peak in activity in June. Unfortunately, in this study, June-August, time of year and quality of host plants are confounding variables.

There was a marked increase in the activity of *A. proletella* in the autumn (~October) in 2014, and to a lesser extent in 2013. This increase in activity was not related to an increase in temperature, and actually coincided with a slight decrease. Thus it is likely that this increase in activity is due to a behavioural change in *A. proletella* at this time. Iheagwam (1977b) showed that females in diapause, (i.e. those emerging after September), have stronger flight behaviour, leading to an increased likelihood of them leaving a host plant and flying for relatively long periods. It is likely that this increase in the activity index is indicative in a shift in the behaviour of *A. proletella* whereby they are becoming more active in flight from October.

2.4.5 Future work

The dispersal model (kernel) (Johnson, 1957) was fitted for trap captures made in April and July. It would be interesting to see if the dispersion parameters are different for captures made later in the year, in September-December, to test the

hypothesis that greater levels of dispersal would occur at this time. Similarly a comparison of dispersion parameters from trap captures made close to senescing or to young host plants would test the hypothesis that *A. proletella* disperse more readily from hosts that are senescing.

Johnson (1957)'s model does not fit the data from the blue traps as well as it does for the yellow traps, and a higher proportion of *A. proletella* were caught on the traps at 60 cm than the model predicts, although both fits are statistically significant. Johnson states that the equation no longer holds as aerial populations of insects are building. It could be that the *A. proletella* caught on blue traps are those that are caught in the first period of flight and therefore the vertical density profile has not been fully established. Possibly, the *A. proletella* caught at 60 cm were intercepted, preventing them from achieving greater heights, and this would lead to a higher catch rate at 60 cm.

The numbers of *A. proletella* caught by the Rothamsted suction traps varied greatly between years, but it was not possible to explain this variation. The year with the highest captures, 2010, was no warmer than the other years, and indeed it was a rather 'average' year. In 2010, the suction trap at Kirton also caught a relatively large number of *A. proletella* before the period when diapause would have occurred. It is possible that the increased captures earlier in the year may have been an artefact of the presence of a very large population of *A. proletella*, since *A. proletella* were very abundant in 2010 (Springate and Colvin, 2013). The highest daily catch for 2010 was 69, compared with the next highest year, 2011, when there were 15 *A. proletella*; a three-fold increase.

It is certainly possible that a small proportion of the *A. proletella* population exhibit migratory behaviour throughout the year, such has been observed in *B. tabaci* (Isaacs and Byrne, 1998). The higher numbers of *A. proletella* on crops in 2010 may have led to a, now measurable, small proportion of the population that performed migratory behaviour. Higher numbers of *A. proletella* on crops and wild hosts in 2010 may have also themselves led to an increased migratory response. Many species of insect respond to overcrowding by an increase in migratory behaviour, an approach that would allow colonisation of new, less crowded habitats (Kennedy,

1961). A number of species of aphid show this response, where overcrowding or poor host quality leads to the development of alates that perform migratory behaviour (Kring, 1972). Indeed, another species of whitefly, *Trialeurodes vaporariorum* shows greater flight activity at higher densities (Boinsogne, 2015).

Going through samples from the Rothamsted suction traps to identify and count *A. proletella* was very time-consuming and currently data have only been recorded for 2010-2014 at Kirton and 2012-2015 at Wellesbourne. Further samples would need to be investigated in order to determine relationships between the phenology and abundance of *A. proletella* and weather data, as has been achieved for some of the data on aphids (Heathcote *et al.*, 1969). Rothamsted Insect Survey samples hold a vast amount of information on seasonal activity and yearly relative abundance of *A. proletella* and much could be learnt from further work focusing on this resource in the future.

3 Effects of Temperature and Host Plant on *Aleyrodes proletella*.

3.1 Introduction.

Although pest insects are taxonomically diverse and infest a wide range of hosts, their success as pests often depends on a set of similar traits; in particular that they have high reproductive output (fecundity) and a short generation time, leading to rapid increases in population size within a season. Such species are considered to be 'r-selected' species and to have a high intrinsic rate of increase (Parry, 1981). Biotic and abiotic factors that influence the generation time and reproductive output of a species will impact on its intrinsic rate of increase and therefore its potential as a pest (Birch, 1948); environmental conditions that increase generation time and poor food quality reducing fecundity can reduce a pest's intrinsic rate of increase therefore reducing the potential for pest status.

Insects are poikilothermic (ectothermic), that is their body temperature varies with ambient temperature. The ambient temperature often determines the rate of development; increases in temperature leading to increased rates of development, due in the main to the increasing rate of enzymatic reactions (Higley *et al.*, 1986). Put more simply, generation time is often determined by the ambient temperature.

The rate of insect development has been shown to follow a sigmoidal relationship with temperature. Development rate increases with temperature until an optimum is reached, after which, increases in temperature have small or deleterious effects on development; until extreme temperatures cause death. In practice, the deleterious effects of high temperatures occur rarely in the field since ambient temperatures lie almost exclusively within the linear aspect of the relationship (Campbell *et al.*, 1974).

The linear relationship between development time and temperature can be described by Equation 1;

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

where, D = duration of development, T^o = temperature, T^t = lower temperature threshold for development, k = the gradient of the line. (Campbell *et al.*, 1974). In principle, the lower temperature threshold for development is the temperature below which development does not occur and can be estimated by extrapolation from the point where the fitted line intercepts the x-axis.

In effect, poikilotherms, such as insects (and also plants), require a certain amount of ‘heat’ to develop from one point in their life cycle to another (e.g. egg to larva). This measure of accumulated heat is known as ‘physiological time’. In general, for a given species, the amount of ‘heat’ required to complete a certain stage of development does not alter, and the combination of temperature (between upper and lower thresholds) and time is always the same. Physiological time is often expressed and approximated in units called day-degrees. A number of approaches are used to estimate day-degrees, the simplest being to subtract the value for the lower temperature threshold for development from the daily mean temperature (Higley *et al.*, 1986). The number of day-degrees that need to be accumulated to complete a particular stage of development can be calculated from Equation 1 and is equal to $\frac{1}{k}$.

The concept of using day-degrees to describe the rate of insect development is often applied to agricultural pests and has been shown to be useful in forecasting pest phenology within crops. For example, this approach has been used to predict when lettuce crops will be colonised by the lettuce root aphid (*Pemphigus bursarius*) (Collier *et al.*, 1994) and field populations of *Myzus persicae* can be predicted from a linear day-degree model suggesting timings for pesticide application (Whalon and Smilowitz, 1979). For species of whitefly in particular, development rates of the camellia spiny whitefly (*Aleurocanthus camelliae*) have been used to suggest timings for pesticide applications and inform growers of regions where increased numbers of generations are likely to lead to increased pest pressure (Kasai *et al.*, 2012). The phenology and population dynamics of *Bemisia tabaci* have been modeled in field crops using a day-degree forecasting system that allowed peaks of adult activity in

crops to be predicted (Awadalla *et al.*, 2014). It is likely that similar forecasts of the population development of *A. proletella* would, for example, support targeted pesticide applications and allow predictions of potential infestations within a crop.

Whilst ‘temperature’ is a major factor regulating the rate of insect development and thus generation time, it may also affect longevity, fecundity and, in cases of temperature extremes, survival.

Host plant quality can also influence both the rate of development and fecundity of phytophagous insects (Awmack and Leather, 2002). Hosts offering a food source of high nutritional content would be likely to support the highest fecundities and most rapid development; the converse would also be true. Poor host plants, either providing poor nutritional content or possessing one or more resistance mechanisms, would reduce fecundity and delay development, potentially reducing pest pressure overall. Host plants of *A. proletella* are mainly members of either the Brassicaceae, (e.g. *Brassica oleracea*) or Asteraceae, (e.g. *Sonchus oleracea*) (Mound and Hasley, 1978), and different hosts within these plant families may differ in quality and thus affect the intrinsic rate of increase differentially. For example, using different cultivars of *B. oleracea* as host plants, differential effects on the development and fecundity of *A. proletella* have been shown (Iheagwam, 1976; Alonso *et al.*, 2009). Such intra-specific effects on development between host plants may account for differences in the sizes of populations infesting certain field crops. This, for example, may be a reason why *A. proletella* infestations are often heavy on kale (Nebrada *et al.*, 2005).

An understanding of the relative effects of its host plant on the intrinsic rate of increase of *A. proletella* would be important for the development of an integrated pest management strategy. Using crop plants that support lower intrinsic rates of development would support slower rates of population growth reducing pest pressure. The aim of the experiments described in this chapter was to determine the effect of host and temperature on the population growth parameters, development rate and fecundity. The relationship temperature has on development rate will allow the development of a day-degree model allowing the forecasting of generations within the field.

3.2 Methods

3.2.1 Experiment 3.1. Longevity and fecundity of *Aleyrodes proletella* on three different crops of *Brassica oleracea*: Brussels sprout, kale and cauliflower.

Three different crop types of *Brassica oleracea* were evaluated to determine the fecundity of female *A. proletella* and the duration of oviposition. These were cauliflower (cv. Skywalker), Brussels sprout (cv. Revenge) and kale (cv. Reflex). All *A. proletella* used were reared in a controlled environment room at 20°C with a light regime of 16:8h (Light: Dark) on ~2 month old cauliflower (cv. Skywalker) plants. Newly-emerged *A. proletella* were collected by taking a sample of foliage infested with pupae from the laboratory culture and keeping it overnight in a closed Petri dish to prevent newly-emerged adults from escaping. The following day, any adult *A. proletella* that had emerged were sexed, males could be identified easily by the presence of two claspers on the abdomen. One adult of each sex was then placed onto a leaf of the appropriate host plant (grown in a 9 x 9 x 8 cm pot) using a fine paintbrush. This was performed over ice, providing chilled conditions to prevent individuals flying away. All the leaves that were infested were the second youngest leaf on the plant at the time of infestation. A total of five replicates were tested on each type of host plant. Plants were kept at 20°C with a photoperiod of 16:8h (L: D).

The *A. proletella* adults were enclosed within a ‘clip-cage’ to prevent them from escaping and to ensure feeding occurred on that particular leaf. The numbers of eggs laid were recorded every 2 days until the death of the female. Eggs were counted using a x10 magnification hand lens; care was taken to minimize disturbance to the female. Eggs were destroyed once counted. If the female whitefly was dislodged from the leaf, the insect was immediately placed back in the same location. Females were moved onto the next youngest leaf if the leaves showed signs of senescence.

3.2.2 Experiment 3.2. Development rate of *Aleyrodes proletella* held at a range of different constant temperatures.

The time required to complete development from egg to adult was monitored under controlled conditions for six 'constant' temperatures between 11°C and 26°C. Adults were allowed to lay eggs for a period of 24 hours at 20°C on the foliage of potted cauliflower plants (cv Skywalker) at the fifth true leaf stage. If more than 20 eggs were laid on a leaf, the excess were removed with a paintbrush, after which the plant was transferred to the constant temperature environment (incubator or rearing room). The developmental stage of each of the 20 eggs were monitored until adult emergence. The rearing temperature was monitored at plant height using ibutton® thermochrons (DS1921G) recorders.

3.2.3 Experiment 3.3. Field development of generations, validation of day-degree models.

Field data on the development of *A. proletella* generations were gathered from the overwintered plots used for Experiment 4.5 and Experiment 4.6. The date when the first empty pupal cases, exuviae, were present (evidence that adults had emerged from pupae) was taken as the observed date of emergence of the first generation. From this date a new cohort of insects were followed. Females were transferred to kale plants within a cage adjacent to Plot B (Figure 2.1) and females allowed to lay eggs. The eggs/nymphs were checked until either newly emerged females or empty pupal cases were seen.

When the first generation adults emerged, the development of the subsequent generation was determined by transferring adults onto a fresh kale plant (seventh true leaf stage) on the same day. The plant was positioned adjacent to Plot B (Figure 2.1). The adults were allowed to lay eggs and the occurrence of the next generation was recorded when these eggs developed and emerged as adults. The same method was used to time the third and fourth generations.

3.2.4 Experiment 3.4. Effects of host plant on fecundity and development rate of *Aleyrodes proletella*.

Host plant lines were received from the Genetic Resources Unit, Warwick Crop Centre, Wellesbourne Campus, University of Warwick and Elsoms Seeds Ltd, UK, wild host plants were grown from seeds collected on the Wellesbourne Campus in June 2014 (Table 3.1)

For each experimental block (replicate), four seeds of each plant line, (Table 3.1), were germinated in 135 ml modules in Hassey trays. Seedlings were allowed to grow until the second true leaf stage after which they were transferred to 9 x 9 x 8 cm plant pots. Plants were propagated in controlled conditions at 20°C, 16:8 (L:D). Each experimental block was set up when the study plants were in the fifth true leaf stage at least. A total of three experimental blocks were set up at different times. Two replicate plants of each seed line were tested in each block. Each plant was contained individually in a clear plastic 2 L bottle (30 cm x 10 cm diameter, polyethylene terephthalate, Figure 3.1) and infested with six newly-emerged adult females. To prevent females flying away, they were taken from a culture, identified over ice and placed within a 7 ml glass bijou vial using a fine paintbrush. The vial was inverted over the neck of the plastic bottle giving the females access to this container. The females were given 12 h to leave the vial and then the vial was replaced with a piece of plastic, perforated using a mounted needle, to allow air flow and prevent condensation forming.

The adults were left for 4 days and then the numbers of eggs laid were recorded along with the number of live females, which were removed afterwards. The plants were then removed from the bottles and transferred to cages and left there until pupae had developed. If no eggs were laid on a plant, six mobile first instar larvae (crawlers) were placed on the second youngest leaf with a paintbrush and left to develop. The dates of pupation and adult emergence were recorded. Plants were kept in controlled conditions at 20°C, 16:8 (L:D), for the entire study period.

Newly-emerged adults were transferred to 95% ethanol, 5% glycerol to kill and preserve them before morphometric measurements were taken. For each replicate

plant, the body length (head to end of abdomen), ventral width of the abdomen and tibia length of two females were measured under a microscope using a calibrated eye-piece graticule.



Figure 3.1. Individual host plant contained within plastic bottle allowing infestation with *Aleyrodes proletella* for Experiment 3.4.

Table 3.1. Host plants used in Experiment 3.4

Code	Seed book acc. No.*	Founder acc. No. *	Species	Cultivar
Bol01	GK040119	A12DHd ^a	<i>B. oleracea</i>	Chinese kale
Bol03	GT061881	CA25 ^b	<i>B. oleracea</i>	Autumn cauliflower
Bol06	GT130641	Early Big Broccoli ^c	<i>B. oleracea</i>	Broccoli
Bol07	GT080342	GDDH33 ^b	<i>B. oleracea</i>	Broccoli
Bol19	GT080484	HRIGRU005108 ^a	<i>B. oleracea</i>	Chinese kale
Bol30	GT070808	HRIGRU007799 ^a	<i>B. oleracea</i>	Trochuda Cabbage
Bol42	GT110245	Mar_34 ^c	<i>B. oleracea</i>	Broccoli
Bol48	GT081792	TO1000DH3 ^c	<i>B. oleracea</i>	Chinese white kale
CS04	C07081	HRIGRU012702 ^a	<i>B. montana</i>	Wild
CS08	C07020	HRIGRU012483B ^a	<i>B. hilarionis</i>	Wild
CS21	C07067	HRIGRU007343 ^a	<i>B. oleracea</i>	Wild
CS26	C07059	HRIGRU007319 ^a	<i>B. oleracea</i>	Wild
CS27	C07018	CA98004 ^a	<i>B. cretica</i>	Wild
CS39	C07022	HRIGRU007351 ^a	<i>B. incana</i>	Wild
CS40	C07096	HRIGRU007331 ^a	<i>B. villosa</i>	Wild
CS46	C07007	HRIGRU011452 ^a	<i>B. bourgaei</i>	Wild
CS48	C07121	LL25 ^a	<i>B. capitata</i>	Wild
CS49	C07046	HRIGRU013122 ^a	<i>B. macrocarpa</i>	Wild
CS51	C07029	HRIGRU007326 ^a	<i>B. insularis</i>	Wild
CS53	C07084	HRIGRU013131 ^a	<i>B. rupestris</i>	Wild
CS64	C07080	HRIGRU007337 ^a	<i>B. montana</i>	Wild

Code	Seed book acc. No.*	Founder acc. No. *	Species	Cultivar
CS65	C07017	CA02001 ^a	<i>B. cretica</i>	Wild
CS66	C07090	HRIGRU007345 ^a	<i>B. incana</i>	Wild
CS67	C07060	HRIGRU007320 ^a	<i>B. oleracea</i>	Wild
CS68	C07091	HRIGRU006691 ^a	<i>B. incana</i>	Wild
CDH01	C10001	DHSL150 ^d	<i>B. oleracea</i>	Cross
CDH04	C10063	HRIGRU006691 ^a	<i>B. incana x B. oleracea</i>	Cross
CDH05	C10064	HRIGRU006691 ^a	<i>B. incana x B. oleracea</i>	Cross
CDH08	C10140	HRIGRU007343 ^a	<i>B. oleracea x B. oleracea</i> <i>wild species</i>	Cross
CDH15	C10136	HRIGRU007320 ^a	<i>B. oleracea wild species x B.</i> <i>oleracea</i>	Cross
CDH16	C10118	HRIGRU007319 ^a	<i>B. oleracea wild species x B.</i> <i>oleracea</i>	Cross
CDH21	C10036	CA98004 ^a	<i>B. cretica x B. oleracea</i>	Cross
CDH22	C10034	CA98004 ^a	<i>B. cretica x B. oleracea</i>	Cross
CDH23	C10030	CA02001 ^a	<i>B. oleracea x B. cretica</i>	Cross
CDH27	C10027	HRIGRU011452 ^a	<i>B. bourgaei x B. oleracea</i>	Cross
CDH29	C10072	HRIGRU007345 ^a	<i>B. incana x B. oleracea</i>	Cross
CDH31	C10079	HRIGRU007345 ^a	<i>B. incana x B. oleracea</i>	Cross
CDH32	C10109	HRIGRU007337 ^a	<i>B. montana x B. oleracea</i>	Cross
CDH34	C10111	HRIGRU007337 ^a	<i>B. montana x B. oleracea</i>	Cross
Skywalker		Elsoms Seeds Ltd	<i>B. oleracea</i>	Cauliflower
Reflex		Elsoms Seeds Ltd	<i>B. oleracea</i>	Kale
Revenge		Elsoms Seeds Ltd	<i>B. oleracea</i>	Brussels sprout
Greater Celandine		Wild seeds ^f	<i>Chelidonium majus</i>	Wild

Code	Seed book acc. No.*	Founder acc. No. *	Species	Cultivar
Sowthistle		Wild seeds ^f	<i>Sonchus oleraceus</i>	Wild
Dandelion		Wild seeds ^f	<i>Taraxacum officinale</i>	Wild
Fat Hen		Wild seeds ^f	<i>Chenopodium album</i>	Wild
Species A		Wild seeds ^f	<i>Sonchus palustris</i>	Wild

^aNational Vegetable GeneBank, Genetic Resource Unit, Warwick Crop Centre, Wellesbourne Campus, University of Warwick.

^bSebastian *et al.*, 2000.

^cIniguez-Luy *et al.*, 2009.

^dHilton *et al.*, 2013.

^eWalley *et al.*, 2012.

^fWild seeds collected at Warwick Crop Centre, June 2014.

3.3 Results

3.3.1 Experiment 3.1. Longevity and fecundity of *Aleyrodes proletella* on three different crops of *Brassica oleracea*: Brussels sprout, kale and cauliflower.

The total number of eggs laid was highest on Brussels sprout plants and lowest on cauliflower plants. These differences were not, however, significantly different (ANOVA $F_{(2,19)}=3.31$, $P>0.05$, Figure 3.2). The mean longevity of females on the three host plants showed no significant differences (ANOVA $F_{(2,19)}=0.685$, $P>0.05$, Figure 3.3).

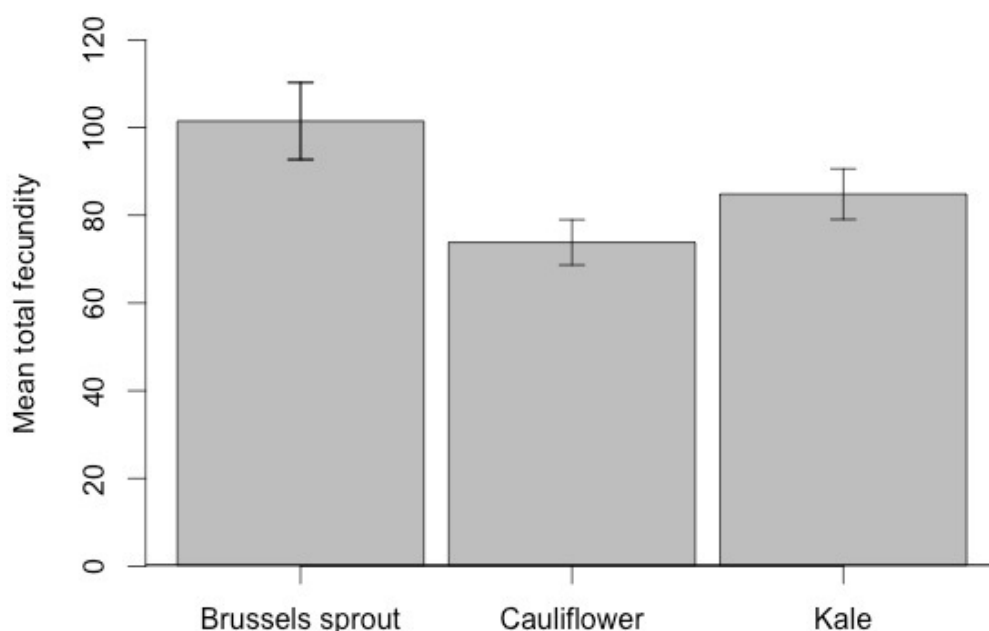


Figure 3.2. Mean (\pm SE) total fecundity, total eggs laid over lifetime, for female *Aleyrodes proletella* on Brussels sprout (cv. Revenge), cauliflower (cv. Skywalker) or kale (cv. Reflex) plants.

The mean rate of oviposition over 2 days was greatest on Brussels sprout plants, corresponding with the highest overall lifetime fecundity. The pattern of oviposition over the course of the life of a female was very similar on the three hosts; however the females on Brussels sprout plants showed a generally higher rate of oviposition, reaching a maximum rate of 8 eggs per 2 days within the first week, with a gradual decline in numbers until death (Figure 3.4).

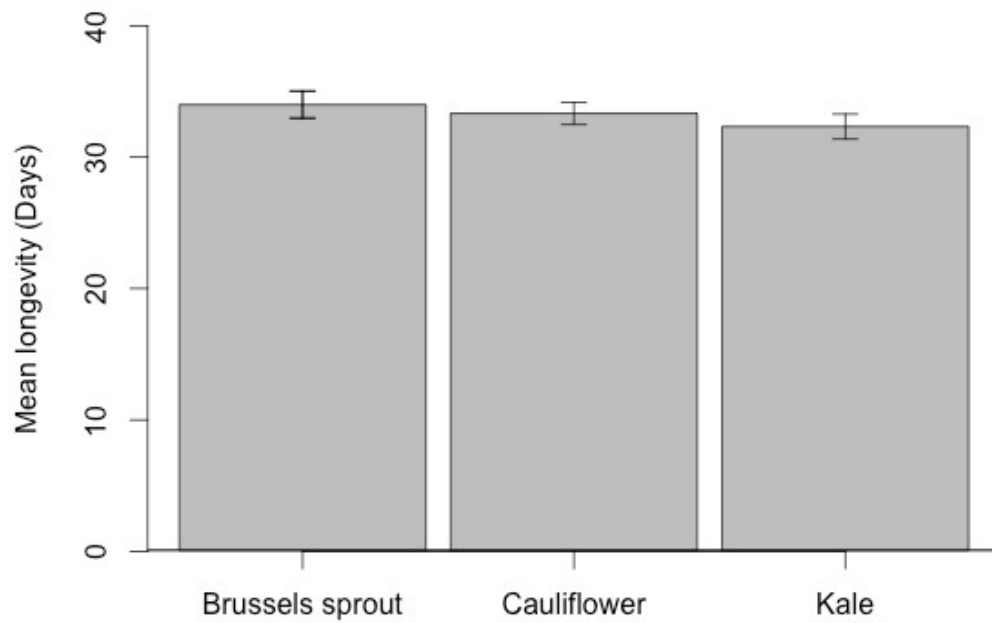


Figure 3.3. Mean (\pm SE) longevity, in days, of female *Aleyrodes proletella* on either Brussels sprout (cv. Revenge), Cauliflower (cv. Skywalker) or Kale (cv. Reflex) plants.

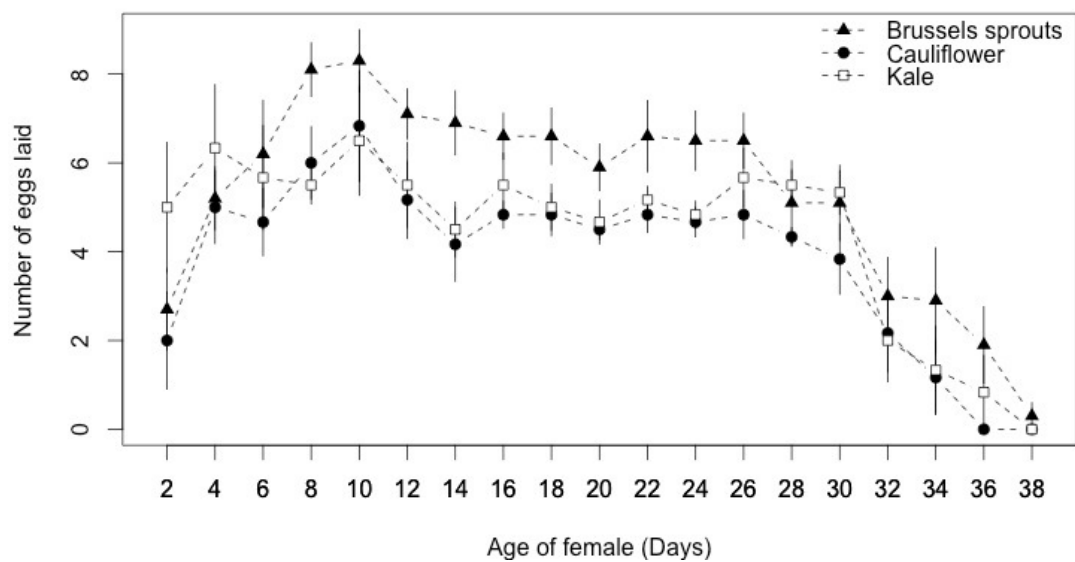


Figure 3.4. Mean (\pm SE) number of eggs laid over 2 day periods until death of the female.

3.3.2 Experiment 3.2. Development rate of *Aleyrodes proletella* held at a range of different constant temperatures..

The duration of development (days) of *A. proletella* held at each temperature ranged from 79 days at 11.9°C to 23 days at 25.5°C (Table 3.2). A straight line, $Y=0.0022*x-0.0138$, (ANOVA, $F_{(1,4)}=102.3$, $R^2=0.95$, $P<0.01$) was fitted to the data (Figure 3.5) and the estimated lower development threshold (T^t), the point where development is zero, determined as 6.3°C. The accumulated day-degrees above this threshold required to complete development were estimated to be 455 ($1/0.0022$). Table 3.2). A straight line, $Y=0.0022*x-0.0138$, (ANOVA, $F_{(1,4)}=102.3$, $R^2=0.95$, $P<0.01$) was fitted to the data () and the estimated lower development threshold (T^t), the point where development is zero, determined as 6.3°C. The accumulated day-degrees above this threshold required to complete development were estimated to be 455 ($1/0.0022$). Table 3.2).

Table 3.2 Mean temperature and mean duration of development in days for *Aleyrodes proletella* reared on cauliflower (cv Skywalker).

Mean (\pm SD) Temperature (°C)	Mean (\pm SE) length of development, in days (egg-adult)
11.9 \pm 0.46	78.6 \pm 2.26
14.8 \pm 0.23	48.2 \pm 4.93
18.5 \pm 0.45	43.6 \pm 6.72
21.5 \pm 0.31	29.1 \pm 2.78
22.7 \pm 1.1	27.7 \pm 3.42
25.5 \pm 0.76	23.1 \pm 3.10

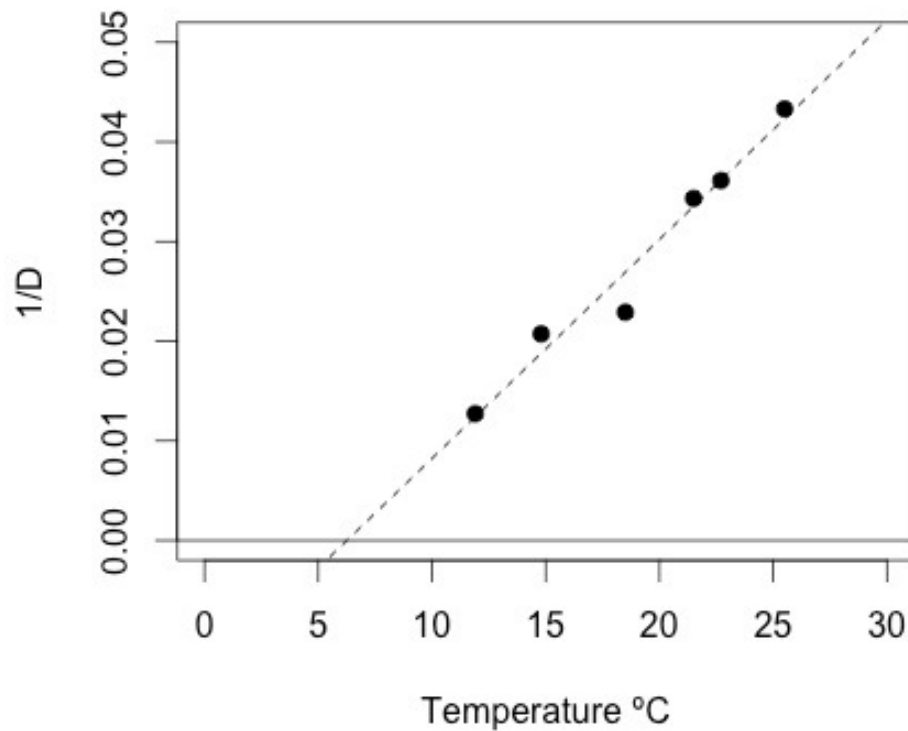


Figure 3.5 Mean development rate, 1/D (1/time to complete development in days, egg-adult) against temperature for *Aleyrodes proletella* in controlled conditions. Dashed line shows significant ($P < 0.01$) linear regression analysis ($Y = 0.0022 * x - 0.0138$).

3.3.3 Experiment 3.3. Field development of generations, validation of day-degree models.

Using this day-degree sum with Meteorological Office data collected at Warwick Crop Centre predicted emergences of generations were calculated (Table 3.3). Each generation was predicted at 455 day-degree above the lower threshold of 6.3°C (Table 3.3). A simple sine estimation of mean day temperature was used (MET Office, 1928; Lindsey and Newman, 1956).

Table 3.3. Start date of field validation of day degree model ($T^t=6.3^{\circ}\text{C}$). The date adults emerged after the egg-laying date along with the Accumulated day-degrees that occurred. The difference from the theoretical day-degrees to complete development, 455, is given.

Date at start	Adults emerged	Accumulated D°	Difference from 455.
10/02/2013	28/06/2013	483.505	28.505
11/01/2014	29/05/2014	436.4475	-18.5525
30/05/2014	09/07/2014	375.3725	-79.6275
10/07/2014	07/08/2014	358.325	-96.675
08/08/2014	12/09/2014	318.4725	-136.5275
09/01/2015	15/05/2015	274.4825	-180.5175
16/05/2015	26/06/2015	288.93	-166.07
27/06/2015	09/08/2015	466.48	11.48
10/08/2015	15/09/2015	331.33	-123.68

The largest difference between observed and predicted accumulated day-degree sums occurred when egg laying occurred on 9th January 2015 (Table 3.3). This showed a difference of 180 day-degrees between the predicted and observed values. The mean number of day-degrees needed to complete development for the range of dates monitored was 370.37, with the mean difference between the predicted (455) and observed day-degrees being -84.63. No relationship was found between the number of accumulated day-degrees to complete development and the mean temperature during the development period (Figure 3.6). The same was true for the relationship between accumulated day-degrees and the date when eggs were laid (Figure 3.7). Predictions for 2015 were less accurate, three of the four field development validations being overestimated by over 100 day-degrees (Table 3.3).

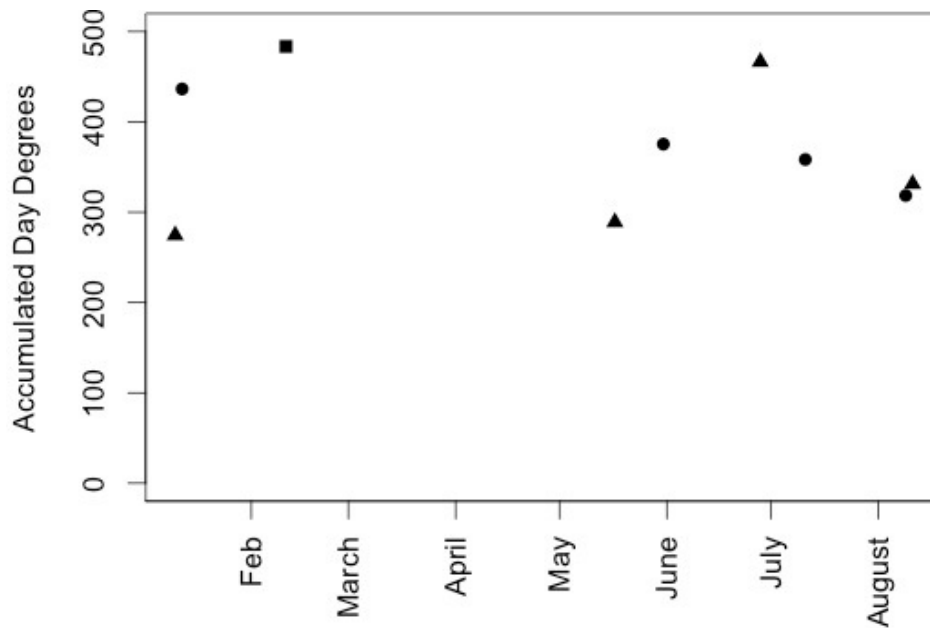


Figure 3.6. Number of accumulated day-degrees required to complete development (egg-adult) plotted against the date when eggs were first laid. Shape of symbol denotes year, squares-2013, circles-2014, triangles-2015

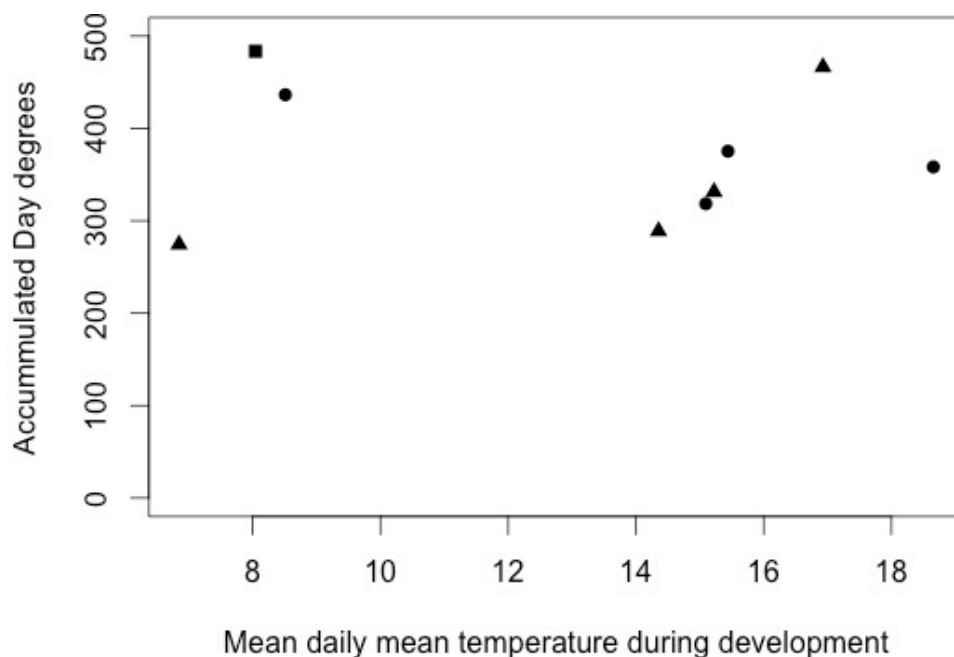


Figure 3.7. Number of accumulated day degrees to complete development (egg-adult) plotted against mean daily temperature during the development period. Shape of symbol denotes year, squares-2013, circles-2014, and triangles-2015

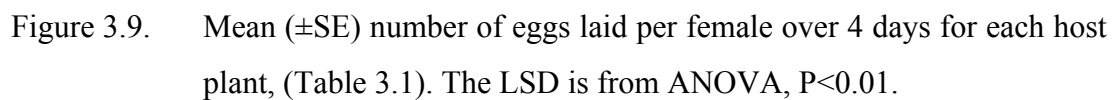
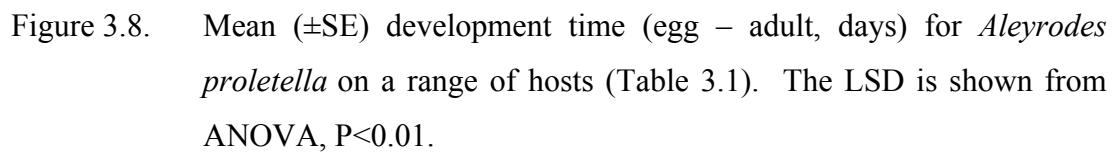
3.3.4 Experiment 3.4. Effects of host plant on fecundity and development rate of *Aleyrodes proletella*.

On the host plants tested, the mean development time (egg to adult) ranged from 22 to 36 days. This was a statistically significant difference (ANOVA, $F_{(48,98)}=1.79$, $P<0.01$; Figure 3.8). In particular, there was a marked difference in development time (14 days), between Bol48, a rapidly cycling Chinese white kale, and CDH32, a *B. oleracea* x *B. montana* cross.

The numbers of eggs laid over the four days per female were significantly different (ANOVA, $F_{(48,256)}=1.779$), $P<0.001$, Figure 3.9). Apart from the complete lack of egg laying on the host Fat Hen, *Chenopodium album*, females kept on Greater celandine, *Chelidonium majus*, plants showed the lowest oviposition rates with females laying eggs in only one of the replicates. The highest rate of oviposition occurred on Bol48, which also supported relatively rapid development.

There was a weak but statistically significant negative relationship between the duration of development and the number of eggs laid. ($y=-1.348*x+32.7$, $R^2=0.18$, $P<0.05$; Figure 3.10). Although there was considerable variation, there was a tendency for rapid development to be associated with the production of larger numbers of eggs (Figure 3.10).

Chenopodium album was the only host that did not support egg laying or development of *A. proletella* through to adults. Of the wild hosts tested (*Taraxacum officinal*, *Sonchus oleraceus*, *Chelidonium majus*, and *Lactuca* spp.) all had low rankings for the number of eggs laid and were in the top ten for the duration of development, suggesting a generally lower quality of host than the *Brassica* hosts.



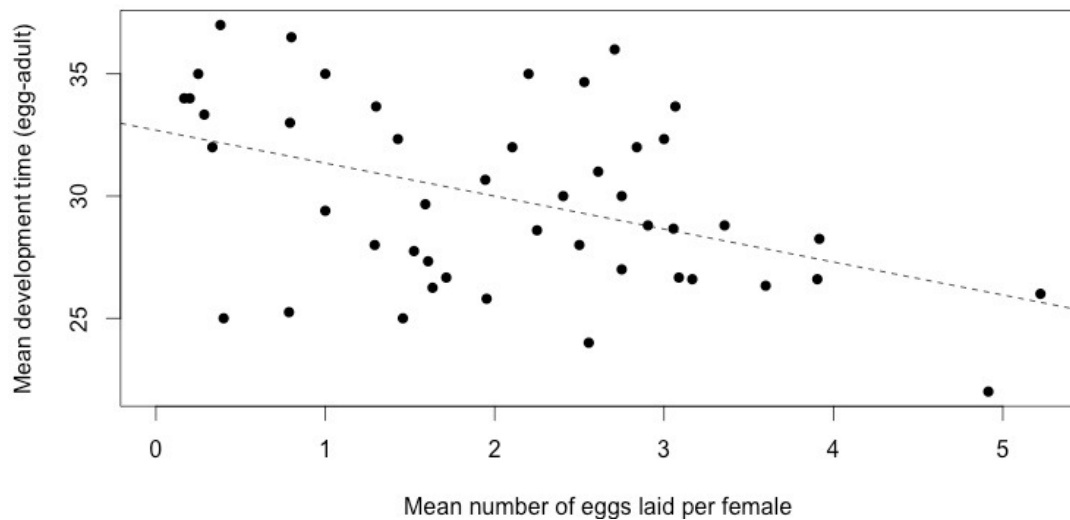


Figure 3.10 Mean development time, egg-adult (Days), plotted against the mean number of eggs laid per female over 4 days. The dashed line shows the fitted linear regression ($y = -1.3484x + 32.6960$, $R^2 = 0.18$, $P < 0.05$).

The tibia length of female *A. prolella* varied from 40-48 EPU (1 mm=140EPU), but there were no statistically-significant differences (ANOVA $F_{(40,98)} = 1.471$, $P = 0.0639$, Figure 3.11).

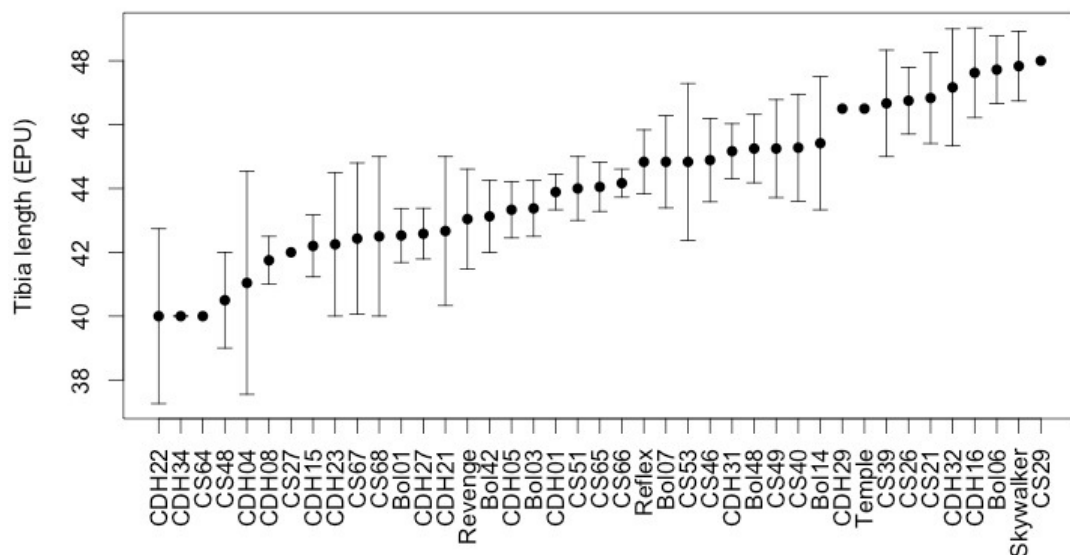


Figure 3.11. Mean (\pm SE) length of tibia (EPU) of adults that developed on each host plant. 1mm=140EPU.

There were also no statistically-significant differences in body width (ANOVA, $F_{(40,98)} = 0.629$, $P=0.95$; Figure 3.12) or length (ANOVA, $F_{(40,98)} = 0.988$, $P=0.503$; Figure 3.13) of newly-emerged adults taken from the different host plants..

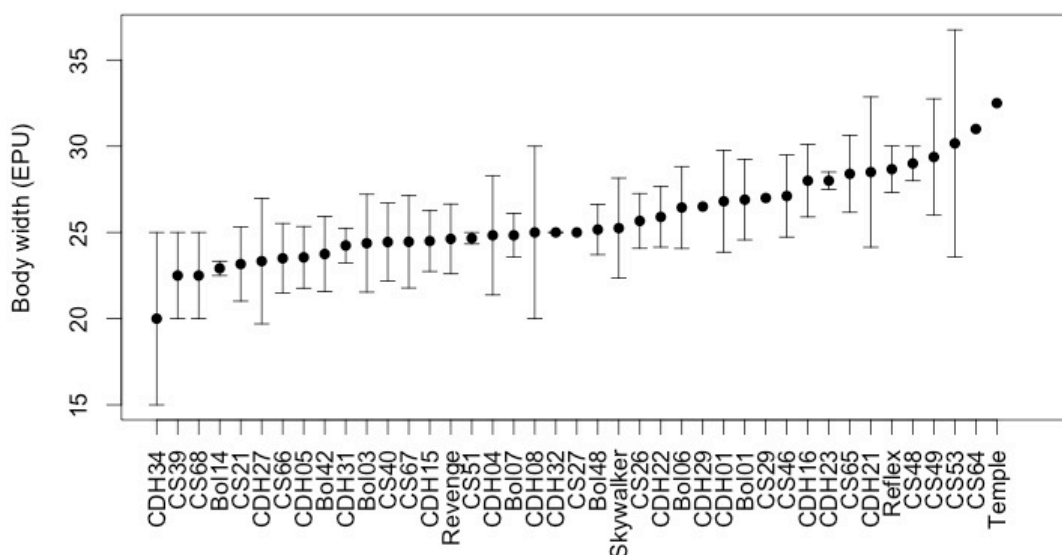


Figure 3.12. Mean (\pm SE) body, ventral abdomen, width (EPU) of adults that developed on each host plant. 1mm =74 EPU.

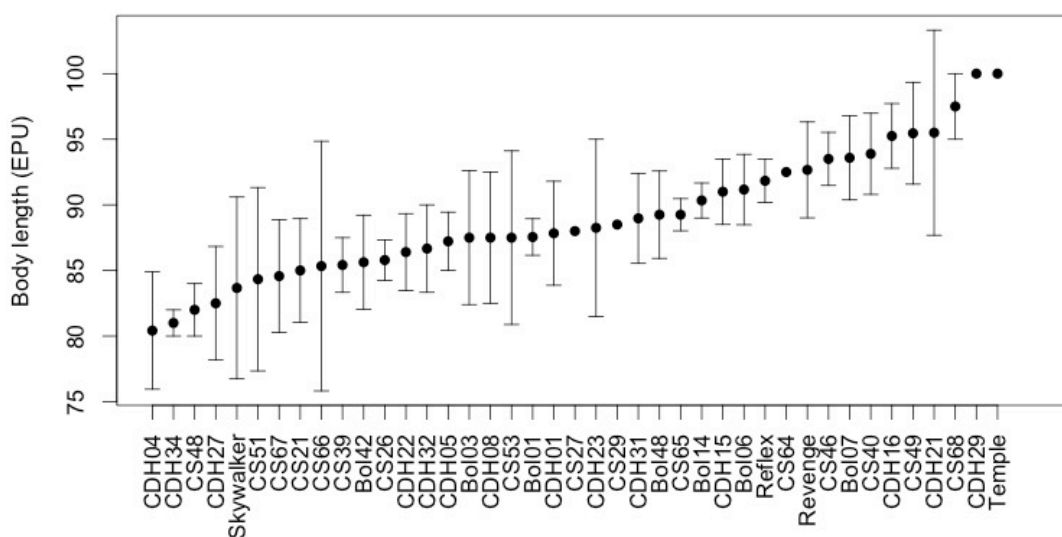


Figure 3.13. Mean (\pm SE) body length (EPU) of adults (head-abdomen) that developed on each host plant. 1 mm = 74 EPU.

Table 3.4. Mean values of development time, eggs laid per female, tibia length, body length and body width for *Aleyrodes proletella* on the host plants tested. The Least significant difference (LSD) from the subsequent Analysis of Variance of the variable is also shown.

Host	Development duration	Eggs laid per female	Tibia length	Body length	Body width
Bol01	32.00	0.33	42.53	87.55	26.90
Bol03	28.00	1.29	43.38	87.50	24.38
Bol06	26.67	3.09	47.72	91.17	26.44
Bol07	34.67	2.53	44.83	93.58	24.83
Bol14	26.00	5.22	45.42	90.33	22.92
Bol19	36.00	2.71	NA	NA	NA
Bol30	32.00	2.10	NA	NA	NA
Bol42	28.00	2.50	43.13	85.63	23.75
Bol48	22.00	4.91	45.25	89.25	25.17
CS04	29.00	NA	NA	NA	NA
CS08	35.00	1.00	NA	NA	NA
CS21	26.60	3.90	46.83	85.00	23.17
CS26	28.80	2.90	46.75	85.79	25.67
CS27	28.80	3.36	42.00	88.00	25.00
CS39	25.00	1.46	46.67	85.42	22.50
CS40	27.75	1.52	45.28	93.89	24.44
CS46	28.67	3.06	44.89	93.50	27.11
CS48	25.80	1.95	40.50	82.00	29.00
CS49	31.00	2.61	45.25	95.46	29.38
CS51	30.00	2.40	44.00	84.33	24.67
CS53	33.67	3.07	44.83	87.50	30.17
CS64	30.00	2.75	40.00	92.50	31.00
CS65	28.25	3.92	44.05	89.25	28.40
CS66	25.00	0.40	44.17	85.33	23.50
CS67	25.25	0.79	42.43	84.57	24.46
CS68	37.00	0.38	42.50	97.50	22.50
CDH01	26.67	1.71	43.89	87.83	26.81

CDH04	26.60	3.17	41.04	80.42	24.83
CDH05	35.00	2.20	43.33	87.22	23.56
CDH08	33.33	0.29	41.75	87.50	25.00
CDH15	27.33	1.61	42.20	91.00	24.50
CDH16	27.00	2.75	47.63	95.25	28.00
CDH21	26.25	1.63	42.67	95.50	28.50
CDH22	29.40	1.00	40.00	86.40	25.90
CDH23	33.67	1.30	42.25	88.25	28.00
CDH27	26.33	3.60	42.58	82.50	23.33
CDH29	29.67	1.59	46.50	100.00	26.50
CDH31	24.00	2.56	45.17	88.97	24.24
CDH32	NA	3.33	47.17	86.67	25.00
CDH34	32.33	1.43	40.00	81.00	20.00
Skywalker	30.67	1.94	47.83	83.67	25.25
Revenge	28.60	2.25	43.04	92.67	24.63
Reflex	32.33	3.00	44.83	91.83	28.67
Greater Celendine	34.00	0.17	NA	NA	NA
Species A	34.00	0.20	NA	NA	NA
Sow Thistle	33.00	0.79	NA	NA	NA
Temple	32.00	2.84	46.50	100.00	32.50
Dandelion	36.50	0.80	NA	NA	NA
Lactuca spp.	35.00	0.25	NA	NA	NA
Fat Hen	NA	0.00	NA	NA	NA
LSD	10.20	3.31	NS	NS	NS

No correlations were found between any of the morphometric measurements: tibia length, body length or body width. The mean tibia length of adults and the mean number of eggs laid were found to have a weak, yet significantly positive relationship ($y=0.6976 * x + 42.4053$, $R^2=0.1347$, $P<0.05$; Figure 3.14)

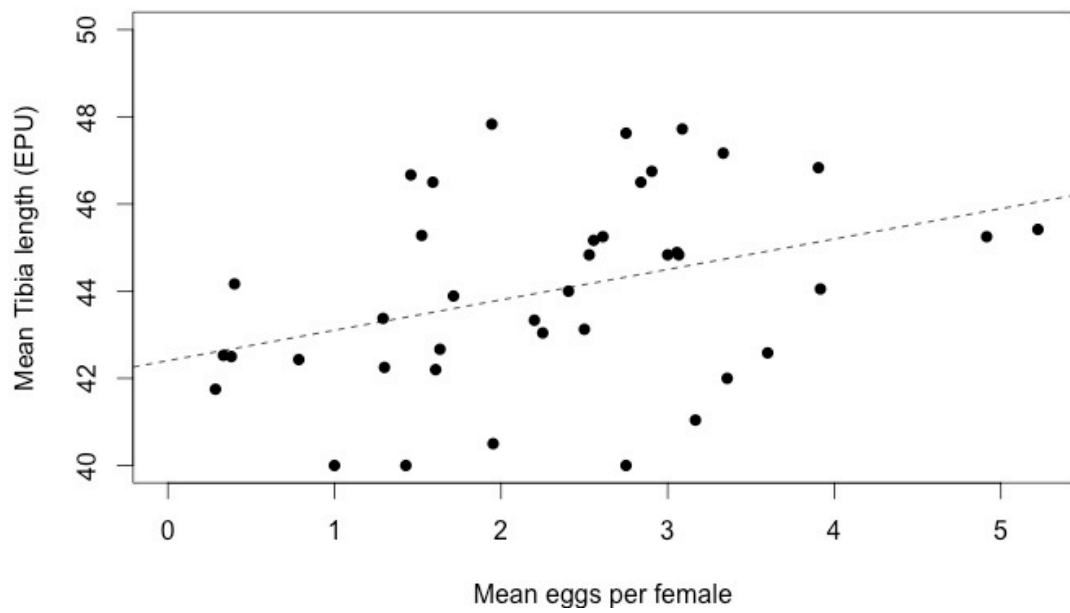


Figure 3.14. Mean tibia length plotted against the mean number of eggs laid per female over 4 days for each of the host plants investigated. The fitted line represents $y = 0.6976 * x + 42.4053$, $P < 0.05$.

3.4 Discussion

3.4.1 Host plants

For the majority of the host plants tested, the measures of fecundity, longevity and development rate did not differ significantly from one another. The most notably favourable host was Bol48, a white Chinese kale, which supported rapid development and a high oviposition rate. This host plant is known to be a rapid-cycling brassica and flowers more rapidly than all other the other plants used in the tests. The common weed species *Chenopodium album* did not support any egg laying or development of *A. proletella*. This is not surprising since there has been no record of *C. album* acting as a host for *A. proletella*. The oilseed rape cultivar ‘Temple’ was a relatively average host with a ranking falling close to the median value for both the rates of development and oviposition.

In terms of commercial brassica crops, kale is considered to be particularly vulnerable to *A. proletella* and supports large numbers of *A. proletella* in the field (Nebrada *et al.*, 2005). The results of the experiments described here suggest that the commercial kale cultivar, Reflex, is not significantly different as a host from other *Brassica* crop plants with regard to the rate of oviposition, development time and adult longevity. When compared to adult *A. proletella* fed on Brussels sprout or cauliflower plants, individuals fed on kale did not exhibit greater longevity or higher fecundity overall. Thus these results suggest that kale does not support a higher intrinsic rate of increase than the other crop plants tested.

There was also no separation between wild *Brassica* hosts, (plants beginning ‘CS’, Table 3.1) and those of cultivated varieties. The screen did not appear to detect any naturally occurring resistance mechanism impairing the development of *A. proletella*, which have been highlighted on occasions in other phenotypic screens of genetically diverse plant material (Wink, 1988).

Size has been shown to be related to fecundity in a range of insect taxa where size can be measured as dry weight (Honêk, 1993). Other substitute measures of insect size have been related to fecundity, with tibia length showing a positive relationship (Awmack and Leather, 2002). Tibia length has also been shown to be correlated with

the duration of the overall period of oviposition and with lifetime egg production (Thorne *et al.*, 2006), suggesting that tibia length can be used as a surrogate measure for potential fecundity. A correlation of tibia length with the weight of adult *A. proletella* (Iheagwam, 1981) has been found, adding to the validity of using tibia length as an index of weight in this study, and therefore potential fecundity.

Studies investigating the effects of host plant quality on other whitefly species have shown that size is often increased by feeding on good quality hosts. Adult *T. vaporariorum* have been shown to develop longer tibia when reared on hosts that have received an application of nitrogen fertilizer. The fertilizer led to increased nitrogen content within the plant, producing larger adult whitefly, measured by the length of their tibia (Jauset *et al.*, 2000). In the present study, tibia length was unaffected by host plant. The lack of any significant differences in the length of the tibia of *A. proletella* reared on the different hosts suggests that the hosts are not of differing quality, however, the mean EPU value ranges from 40-48. It could be that the considerable within-treatment variation has reduced the statistical power of the experiment and although the differences between host plants are not statistically significant, the ranking of each host may still suggest an order in host quality. It is possible that repeating this study with more replication would produce statistically significant results. The within-treatment variation in body length and width was greater than for tibia length and this may have led to the lack of significant differences in these measures.

There was, however, a tendency for adults reared on hosts that supported higher oviposition rates to have longer tibia. This implies that rearing *A. proletella* on good quality hosts supporting a higher oviposition rate may also lead to the increased fecundity of females in the next generation. Further studies evaluating the fecundity of females reared on different hosts could test this hypothesis. Since it appears that *A. proletella* are relatively immobile and may remain within the crop in which they emerged, the benefits of good quality hosts would be multiplicative through developing generations.

Interestingly *Chelidonium majus* was not a good host for *A. proletella* in this study, supporting no development to adulthood and with oviposition occurring in only one

of the replicates. *Chelidonium majus* has been recorded as a host of *A. proletella* on a number of occasions (Mound and Hasley, 1978; Klasa, 2011; Richter and Hirthe, 2014), and is believed to be one of the most important wild hosts for this species with large populations being recorded in the wild (Klasa, 2011). Why this screen did not confirm the apparent good status of *C. majus* as a host is interesting. The selection of biotypes has occurred in some field populations of whitefly, most importantly *Bemisia tabaci*, which has recently evolved biotypes that differ in their affinity for different host plants. One biotype of *B. tabaci*, for example, shows a clear ‘preference’ for Poinsettia compared with cotton, which is regarded as a preferred host for another biotype (Enkegaard, 1993)

The host plant preferences of different biotypes have been studied to a great extent in *B. tabaci* and alterations to specific host preferences have been shown to occur over the last 100 years, whereby ‘races’ have evolved different host preferences but are still morphologically indistinguishable (Brown *et al.*, 1995). In the present study the majority of cultivated and wild *Brassica* lines are grouped together, with the non-*Brassica* hosts supporting some of the lowest egg laying rates and the longest development times. This suggests that the population of *A. proletella* screened in this study have a preference for *Brassica* hosts.

In this study all host plant testing has been conducted on individuals collected from *Brassica* crops and these individuals may be from a ‘*Brassica* biotype’, which does not have an affinity, for example, for *C. majus*. A simple experiment, where one would collect populations from both *Brassica* crops and wild hosts, specifically *C. majus*, would help to determine whether there are biotypes which show differential host plant preferences.

Previous work on screening for resistance in host plants of *A. proletella* found that trichomes were very important in preventing females from attaching to leaves providing a structural method of resistance (Pelgrom *et al.*, 2015). This is likely to be the case for a few of the lines screened in this experiment. *Brassica incana*, CS68, has relatively more trichomes, especially when compared to kale plants, and this host plant supported one of the lowest oviposition rates and longest development times. Such physical properties of a host are likely to prevent adult females from attaching

to feed and from undertaking the normal circular movement exhibited when egg-laying (Butler, 1938a). Such a resistance mechanism would not be appropriate for introduction into kale varieties, as the structure of the leaf determines the palatability of the foliage, which is the marketed product. Kale leaves must be smooth and tender, probably providing a perfect leaf structure for *A. proletella*. Leaf structure, however, is less important in Brussels sprout where the foliage is usually not marketed and the introduction of trichomes might reduce the size of infestations, reducing contamination on the buttons, which are the harvested product.

Leaf structure is also unimportant in determining the quality of oilseed rape as the seed is the marketable product. Increasing the density of trichomes in oilseed rape through breeding might decrease its suitability as a host for *A. proletella*. Although *A. proletella* is not a problematic pest in oilseed rape it would reduce the potential for it to act as an overwintering host, offering a 'green-bridge' and allowing large populations to overwinter prior to migration onto more vulnerable crops, such as kale.

The majority of lines tested did not differ significantly from one another and therefore few further conclusions can be drawn from the data. The insect-insect variation was such that the power of the statistical analysis was low. The study was time-consuming and to a certain extent this determined the amount of replication. On a number of occasions, plants failed to germinate, reducing the amount of replication for that line. The experiment could be repeated with more replication to increase the statistical power. The lines at extreme ends of the spectrum such as *C. majus* and Bol48, the rapid-cycling Chinese kale, can be regarded as poor hosts and good hosts respectively. As Bol48 is known to be a rapid-cycling line, it would be interesting to ascertain if this was the reason for its status as a good host. It would be feasible to screen kale lines with differing cycling periods to see if this quality has a predictable impact on the development and oviposition rates of *A. proletella*. If this hypothesis was correct, a kale line with a long cycling period would be a host supporting a low intrinsic rate of increase by *A. proletella*.

3.4.2 Day-degree model.

The day degree model generated from laboratory studies led to rather accurate predictions of the timing of the emergence of first generation adults in 2013 and 2014, which was to within a week on both occasions. The emergence of first generation adults was not predicted accurately in 2015; there was discrepancy of 30 days.

There was no relationship between the estimated accumulated day-degrees to complete development in the field ($T^l=6.3$) and the mean temperature under which the insects developed or the time of year (Figure 3.7). This suggests that the discrepancies in the day-degree sums estimated from observing *A. proletella* in the field and the sum determined from the laboratory studies are unlikely to be due to an incorrect lower threshold temperature (Collier and Finch, 1985). The discrepancy between observations and predictions may not be due to a fundamental error in the day degree model but rather in the method of estimating the timing of emergence of each generation.

Whilst day-degree sums can be very useful for predicting the timing of key events in field populations of pest insects they do have several limitations. One of these regards the inherent intra-specific variation in development times shown by insects, with the development rates of individual insects varying around a mean with an approximate normal distribution (Shaffer, 1983; Phelps *et al.*, 1993). Accumulated day-degree requirements estimated from laboratory experiments generally predict the ‘mean’ or ‘median’ development times of a population and give no indication of the variation. Thus when using these estimates in comparison with field data, it is appropriate to compare them with the ‘mean’ or ‘median’ development times (e.g. when 50% of the population has emerged). In the present study, emergence in the field was recorded by surveying each plot until exuviae were present. Thus comparisons were made between the day-degree requirement established for the individuals close to the mean development time for the population with the ‘earliest’ individuals in the field population. The ‘position’ of these early individuals in the overall distribution of development times would depend on sample size (e.g. the larger the sample size, the earlier emergence would be detected). In 2015, more

plots (25) of kale were surveyed than in 2013 and 2014 (both five), leading to a larger sample size. Thus it is likely that emergence was detected relatively earlier in 2015 than in 2013 and 2014 simply because of sample size, although obviously it would also depend on the variation in population size from year to year.

To avoid at least one of the limitations of day-degree models, in relation to the inherent intra-specific variation in development times shown by insects, more sophisticated forecasting models have been developed for some species (e.g. Phelps *et al.*, 1993). Using a Monte-Carlo simulation approach, the development of any number ‘virtual insects’ can be described, allocating ‘individual development rates’ taken from a normal distribution about the mean. In this instance it is possible to predict the times when, for example, 1, 10 or 50% of insects will complete a particular development stage.

To remove the bias that may have been responsible for the discrepancies in estimation of day-degree sums in the field, a different approach to sampling could be used. For example, a set number of *A. proletella* could be followed throughout the season, recording emergence etc., for these individuals. Such information could be used to further validate the day-degree model for *A. proletella*. Another approach would be to develop a more sophisticated forecasting model for *A. proletella*, akin to a Monte-Carlo model, which would be likely to increase the reliability of predictions of the timing of generations in the field.

4 Monitoring Field Populations of *Aleyrodes proletella*

4.1 Introduction

The crops that are particularly susceptible to *A. proletella*, kale and Brussels sprout, are ‘long season’ crops in that they are planted usually in early summer and may not be harvested until the following winter or early spring (Elsoms, 2015), so there is a long period during which infestations can develop. Thus the sources of infestations and the pattern of their development subsequently are crucial information for implementation of an integrated control strategy. Growers have a limited number of tools available to them (mainly insecticides) to manage developing infestations to ensure that the harvested produce is free from contamination. No research has been undertaken to determine the distribution of *A. proletella* within or between plants or at the larger scale within fields. Such distributions have been investigated in detail for other pests of *Brassica* crops within the UK such as *Brevicoryne brassicae* (van Emden, 1965), and cabbage root fly (*Delia radicum*) (Finch *et al.*, 1975). Elucidation of the pattern of the within-field distribution of pests has been used to inform sampling procedures for estimating the size of field populations (e.g. Collier *et al.*, 2003). Similar information for *A. proletella* would inform potential survey methods and enable growers and advisors to estimate the size of infestations with a good level of accuracy.

Many studies have been undertaken to investigate population trends of pest whitefly species in vulnerable crops, including *Dialeurodes citri* in citrus groves (Bellows and Meisenbacher, 2007), *Bemisia tabaci* on cotton (Horowitz, 1986) and *Trialeurodes vaporariorum* in glasshouse conditions (Bi *et al.*, 2002). Very few studies have focused on *A. proletella* in an attempt to understand when colonisation of vulnerable crops occurs and describe seasonal trends in numbers. Generalisations drawn from studies on other species of whitefly should be avoided because their host plants exhibit different life-cycle strategies and also because of the different environmental conditions experienced by *A. proletella*, which is a temperate species (Butler, 1938a), compared with other whitefly pests which are adapted to tropical or

subtropical regions, with only a relatively recent expansion in their range towards the polar latitudes (Byrne and Bellows, 1991).

It may, however, be informative to compare the ‘infestation’ biology of *A. proletella* with other pests of *Brassica* crops. Initial colonisation by other pest species can be predicted with differing degrees of certainty. The first arrival of the pest aphids *B. brassicae* and *M. persicae* is often timed in accordance with the development of alates on overwintering hosts (Hafez, 1961) and day-degree models predicting the development of overwintering stages and migration into new crops can be used to forecast first occurrence with differing levels of accuracy (Collier and Finch, 1992; Cividanes *et al.*, 2012; Nematollahi *et al.*, 2016). Similarly, the first colonisation of crops by *D. radicum* is expected after the emergence of the first adults of the season from overwintering pupae and can be predicted with a degree of accuracy by a models based on accumulated temperatures (e.g. Phelps *et al.*, 1993).

A similar understanding of colonisation by *A. proletella* is lacking and further specific sampling work needs to be completed in order to gain this knowledge, with studies directed to ascertaining the times of first colonisation and population development within vulnerable crops. It has been suggested that *A. proletella* will colonise new crops following development into adults of the first generation of eggs laid in the spring. Little data supports this claim, with very little evidence that this occurs in field crops (Butler, 1938a, Al-Houty, 1979, Richter and Hirthe 2014). If the colonisation of crops occurs at the same time as emergence of the first generation of *A. proletella*, a model predicting emergence dates of the first generation would be useful in forecasting dates of colonisation.

Trends in the development of pest infestations have been studied for some of the other pest insect species infesting field *Brassica* crops. For example, infestations by *B. brassicae* generally show a rapid increase in numbers after colonisation followed by a mid season crash, the exact date of which cannot be predicted accurately at present (Hughes, 1963; Raworth *et al.*, 1984; Collier and Finch, 1992). The development of infestations of *D. radicum* can be predicted with some accuracy through forecasting models describing their life-cycle and indicating, for example,

times when peak numbers of female flies will be laying eggs (e.g. Phelps *et al.*, 1993).

There is currently no way of predicting accurately when infestations by *A. prolella* will occur and how they will develop over time and any indications are currently based on anecdotal evidence at best (Butler, 1938a, 1938b, Al-Houty, 1979). The aims of the studies described in the chapter were to determine the spatial and temporal population patterns of *A. prolella* on field crops and wild host species. Population growth of *A. prolella* on vulnerable crops is studied to see if predictable trends in population growth occur in the field.

4.2 Methods

4.2.1 Experiment 4.1. Distribution of *Aleyrodes proletella* on Brussels sprout plants.

To inform future sampling strategies a complete census was made of *A. proletella* living on five randomly selected Brussels sprout plants from a plot of 9 x 12 plants, 50 cm spacing, in Big Cherry Ground at Wellesbourne on 12th October 2012. *Aleyrodes proletella* of all life stages were recorded on each leaf, noting the location of the leaf in terms of its position relative to the terminal leaf.

4.2.2 Experiment 4.2. Distribution of *Aleyrodes proletella* in commercial crops of kale.

Adult *A. proletella* were counted on individual plants within three commercially grown crops (7-17 hectares) of kale, cv. Reflex, in Lincolnshire (Table 4.1).. The crops were sampled on 18th August 2014. Transects were taken from the middle of each side of the four field edges (north, south, east and west). Adult *A. proletella* were counted on four plants at distances of 0 (edge plants), 5, 15, 35, 75 and 155 plants into the field where the plant spacing was ~60 cm.

As the numbers of *A. proletella* in each transect seemed to differ, each field edge was sampled in more detail. The field edges of Field A and Field B were divided into potential sampling sections of 2 m and four sections were selected at random to become replicates for each field edge. The '0' point of the original transect for that edge constituted a fifth replicate. As an 'edge effect' was evident from the transect samples, none of the samples along an edge was taken within 50 m of an adjacent field edge (Figure 4.1). The crop had not been treated with insecticide beforehand. The resulting data were analysed using Analysis of Variance (ANOVA).

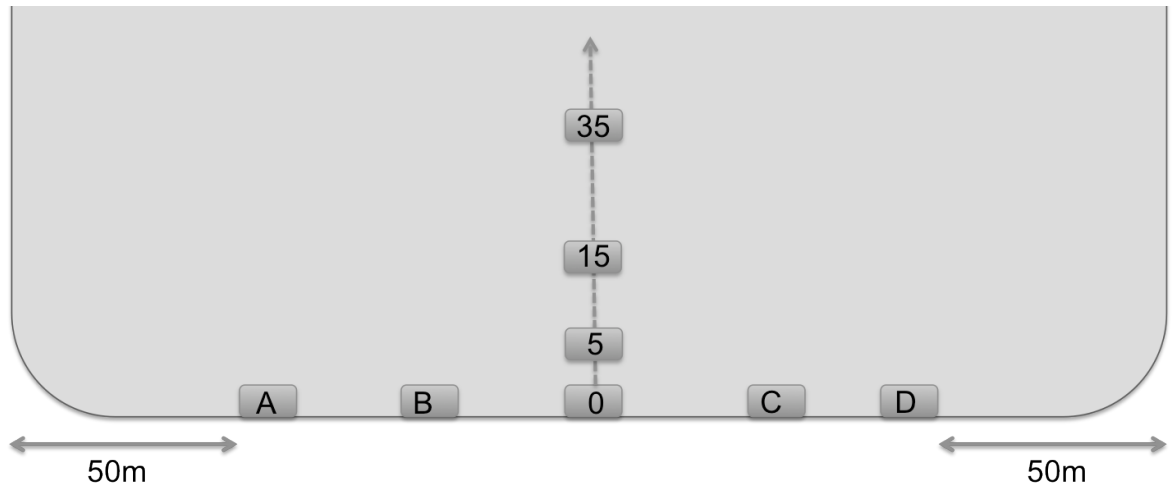


Figure 4.1. Sampling schematic for Experiment 4.2. Transects into the field are represented by numbers 0, 15, 35 etc. A, B, C and D represent the randomly selected sampling points on each field edge. No sampling occurred within 50 m of an adjacent field edge.

Table 4.1. Fields of kale sampled in Experiment 4.2: planting date, approximate size and UK grid reference.

Field	Approx. plant date	Size (hectares)	Grid reference
A	April	17	TF 33530 21723
B	June	7	TF 42318 28534
C	July	10	TF 42318 28534

4.2.3 Experiment 4.3. Survey of wild host plants for the presence of *Aleyrodes proletella* in uncultivated fields in 2014 and 2015.

Potential wild hosts, located within a single field, were sampled on 20th May 2013 and 22nd April 2014 for the presence of *A. proletella*. The field surveyed in 2013 was Cottage Field West at Warwick Crop Centre, Wellesbourne (approx. 1ha, Figure 2.1). The field had been removed from an arable crop rotation since 2008 when it entered management for the Entry Level Stewardship scheme for field margins, EF1 (DEFRA, 2005). Long Meadow West (approx. 1ha, Figure 2.1) was surveyed in

2014. The field had been left fallow for 2 years previously and was mowed when the vegetation grew taller than 40 cm.

Forty randomly-generated co-ordinates were used to identify sampling locations, where all known wild host plants (Mound and Hasley, 1978) within 0.25 m² quadrats were examined. These plants were checked thoroughly for the presence of *A. proletella*. The percentage ground cover in each quadrat and the numbers of each host plant present were also recorded.

4.2.3.1 *Ad hoc* Wild host surveys

Ad hoc sampling of wild hosts was conducted on a number of occasions in 2014. The host species, location, height of plant, habitat and number of *A. proletella* present were all recorded.

4.2.4 Experiment 4.4. Survey of commercial oilseed rape fields for *Aleyrodes proletella*.

4.2.4.1 2013

A commercial crop of Oilseed rape (OSR) *Brassica napus*, cv. Temple, approximately 10 ha in size and located close to the Wellesbourne campus (Grid reference SP27142 57772) was surveyed for the presence of *A. proletella* on three occasions during 2013: 20th April, 5th June and 17th July. A sampling grid 40 m x 40 m was used within the field (Figure 4.2). At each sampling point all plants within a 0.25 m² quadrat were investigated for the presence of all life stages of *A. proletella*. An estimate of the percentage ground cover by OSR plants and their distance from the field margin were also recorded.

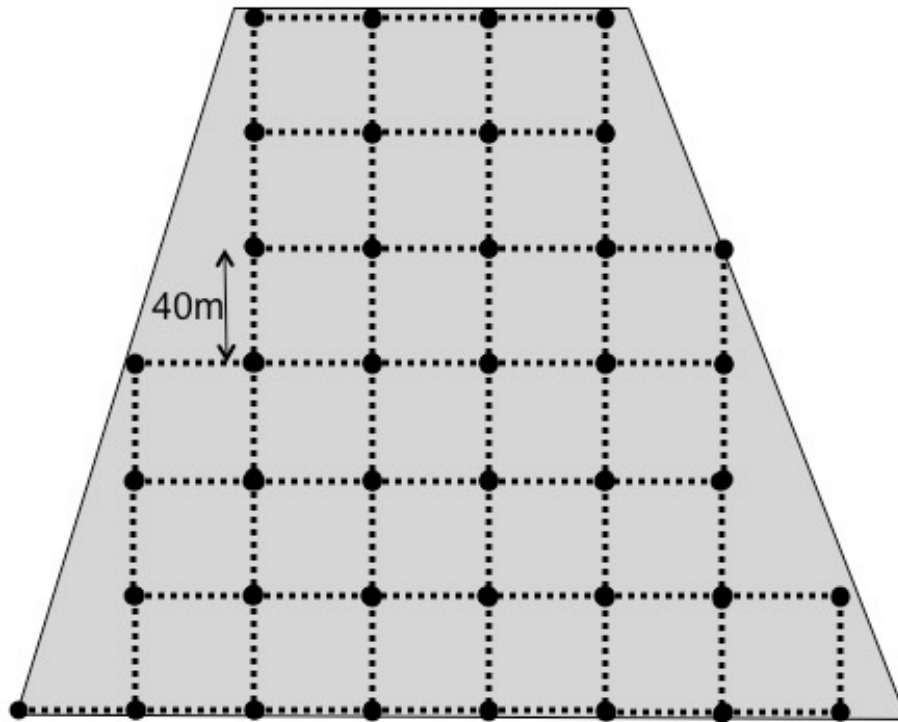


Figure 4.2. Sampling grid (40 m x 40 m) for oilseed rape crop in 2013. The black dots denote the location of a 0.25 m² area surveyed for *Aleyrodes proletella*.

4.2.4.2 2015

Adult *A. proletella* were counted on oilseed rape plants within 0.25 m² quadrats from four commercially grown OSR Fields (OSR Fields A-E, Figure 4.3) within the Wellesborne campus. The crops were sampled on 11th January 2015. Transects were taken from the middle of each side of the four edges (north, south, east and west) of each field. All adult *A. proletella* were counted within a 0.25 m² quadrat at distances of 0 (edge plants), 5, 15, 35, 75 and 155 m into the field. Each field edge was divided into potential sampling sections of 1 m and four sections were selected at random to become replicates for each field edge. The '0' point of the original transect for that edge constituted a fifth replicate. The eastern edges of OSR Fields A, B and C were surveyed for a second time on April 12th 2015.

Experimental Fields

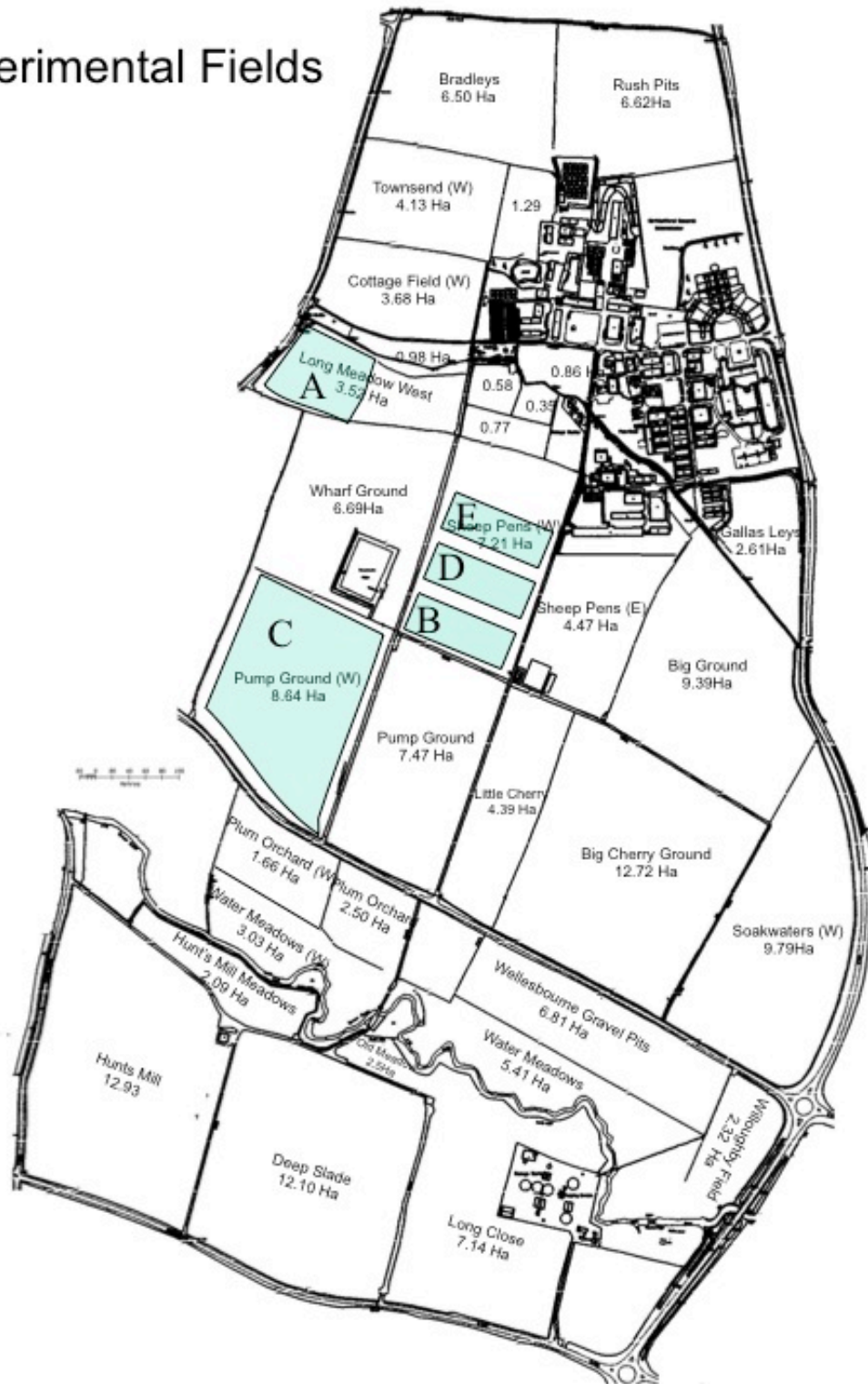


Figure 4.3 Locations of OSR Fields A-E surveyed for plots for Experiment 4.4 at the Wellesbourne campus (SP27142 57772), The University of Warwick.

4.2.5 Experiment 4.5. Monitoring *Aleyrodes proletella* on vulnerable field crops (Brussels sprout and kale) throughout the season.

Plots of Brussels sprout and kale were planted on 2nd May 2013 to investigate natural colonisation and population increase of *A. proletella* over a season. Plants were sown in modules and raised in a glasshouse for seven weeks prior to transplanting in the field. Five replicate Plots were planted in different locations on the Wellesbourne campus (Figure 2.1, Plots A-E). Plots consisted of 24 plants (3 x 8, 50 cm spacing) of each of kale (cv Reflex) and Brussels sprout (cv Revenge) (Figure 4.4). A drench of Dursban[®] (Chlorpyrifos) was applied to modules prior to planting in the field, after which no pesticides were applied. Plots were covered in netting to prevent damage by pigeons.



Figure 4.4. Study plot of Brussels sprout and kale covered in netting to prevent damage by pigeons.

To determine the date of first colonisation by *A. proletella* to within a week, all leaves of all plants were surveyed weekly for a month after planting. All *A. proletella* were surveyed using the naked eye. As the sampling effort increased significantly later in the season, due to increased numbers of leaves per plant and numbers of *A. proletella*, a method was developed to optimise sampling. A sampling approach adapted from Schultz *et al.*, (2010) was used to assess plants (Figure 4.5). Analysis

of initial data showed that the variance between plants was higher than that within plants, indicating that replication at a plant level would provide better estimations of the population (Southwood, 1966).

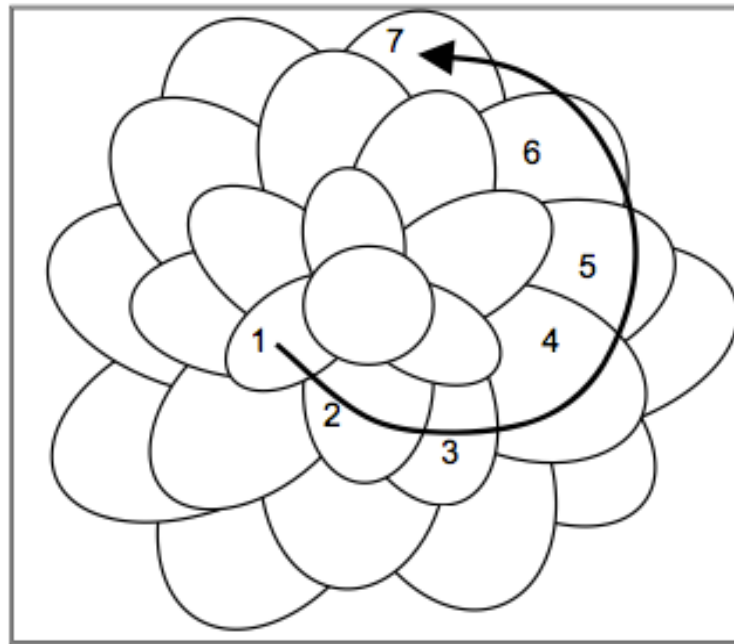


Figure 4.5. Schematic of sampling plan of plant to incorporate leaf-age distribution when sampling *Aleyrodes proletella* (adapted from Schultz *et al.*, 2010).

4.2.6 Experiment 4.6. Monitoring immigration and establishment of *Aleyrodes proletella* on spatially- and temporally-separated plantings of kale.

In 2014, plots of kale (cv. Reflex) were planted in five locations at Wellesbourne campus (Figure 2.1, F-J). Each plot consisted of five sub-plots of 6 x 6 kale plants separated by ~18 m (Figure 4.6). A single sub-plot was planted at each location on 19th May, 17th June, 19th July, 15th August and 16th September 2014, the positions of these plots at each location were allocated at random. Plants were sown in modules and raised in a glasshouse for 5 weeks prior to transplanting in the field. Before transplanting the plants were treated with Dursban[®] (chlorpyrifos) to reduce the risk of damage due to *D. radicum*; no other pesticides were applied. For the first month, all leaves of all plants were surveyed for the presence of *A. proletella* using the naked eye. When plant size increased, together with the size of the infestation, ten

randomly-selected plants were sampled completely. When the plants consisted of nine or more leaves, the sampling method shown in Figure 4.5, was adopted and random replication fell to three plants per subplot.

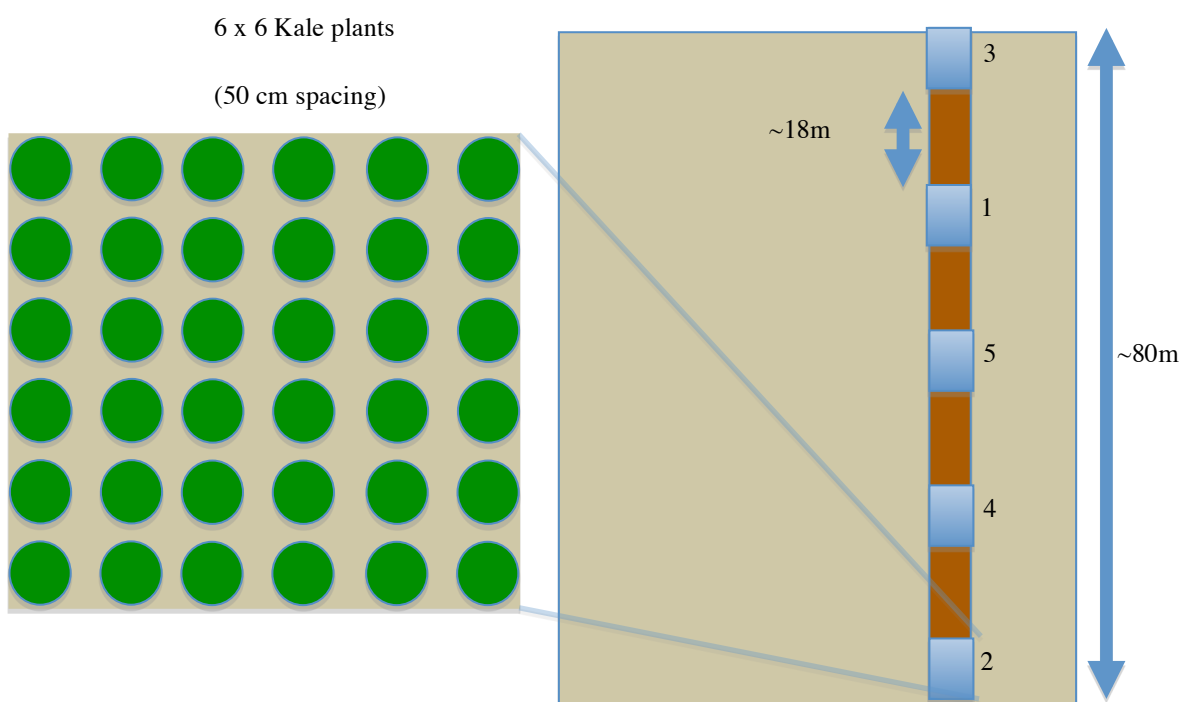


Figure 4.6. Schematic showing sequential plantings of plots of kale at each of the five locations at the Wellesbourne campus. The numbers 1-5 represent the randomized positions of the sub-plots (planted May-September).

4.2.7 Experiment 4.7. Monitoring *Aleyrodes proletella* populations on kale, 2015.

In 2015, plots of kale (cv. Reflex) were planted in 5 locations at Warwick Crop Centre, Wellesbourne (Figure 2.1, K-O). Each plot consisted of 6 x 6 kale plants (50 cm spacing). Plants were sown in modules and raised in a glasshouse for five weeks prior to transplanting in the field on 15th April. Before transplanting the plants were treated with Dursban[®] (chlorpyrifos) to reduce the risk of damage due to *D. radicum*; no other pesticides were applied. For the first month, all leaves of all plants were surveyed for the presence of *A. proletella* using the naked eye. When plant size increased, together with the size of the infestation, ten randomly-selected plants were sampled completely. When the plants consisted of nine or more leaves, the sampling method shown in Figure 4.5 was adopted and random replication fell to three plants per plot.

4.3 Results

4.3.1 Experiment 4.1. Distribution of *Aleyrodes proletella* on Brussels sprout plants.

The different life stages of *A. proletella* showed different patterns of distribution on the Brussels sprout plants. Adults showed the greatest variation in distribution with individuals being found on leaves of all ages, albeit in differing proportions (Figure 4.7). Overall, the proportion of adult *A. proletella* increased with leaf age. Very few leaves were infested with *A. proletella* eggs and, of those eggs, nearly 80% were found on Leaf 4 (Figure 4.8). The proportion of nymphs was also highest on Leaf 4 (Figure 4.9). As expected, pupae were absent from the young leaves and were only present on Leaf 5 and above (Figure 4.10).

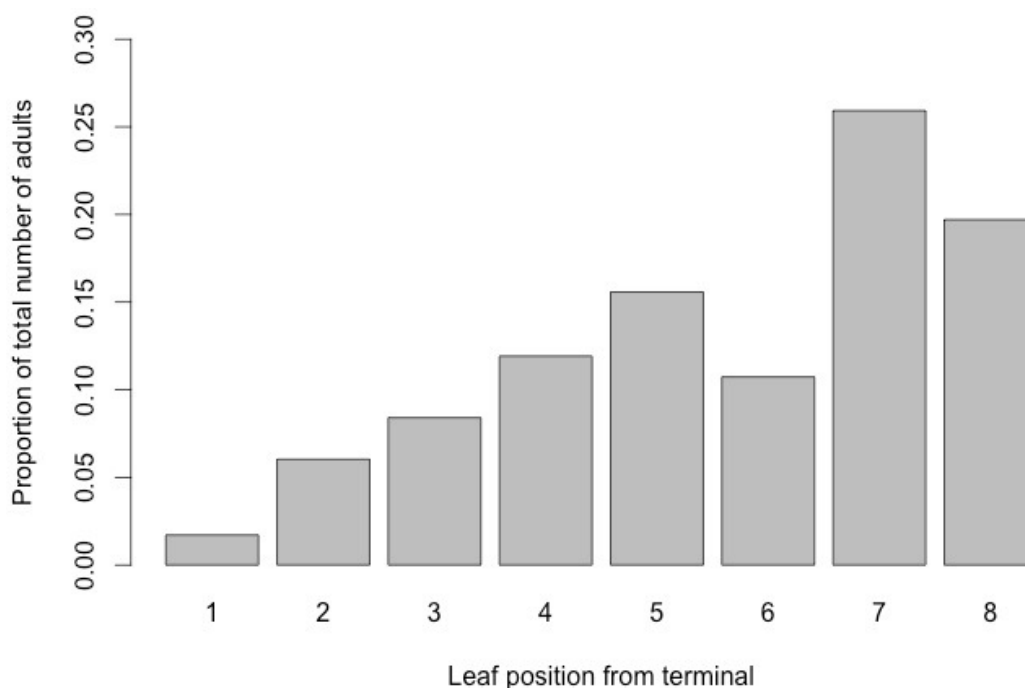


Figure 4.7. Proportions of total adult *Aleyrodes proletella*, total = 2106, on each leaf on Brussels sprout plants. Number 1 denotes terminal leaf.

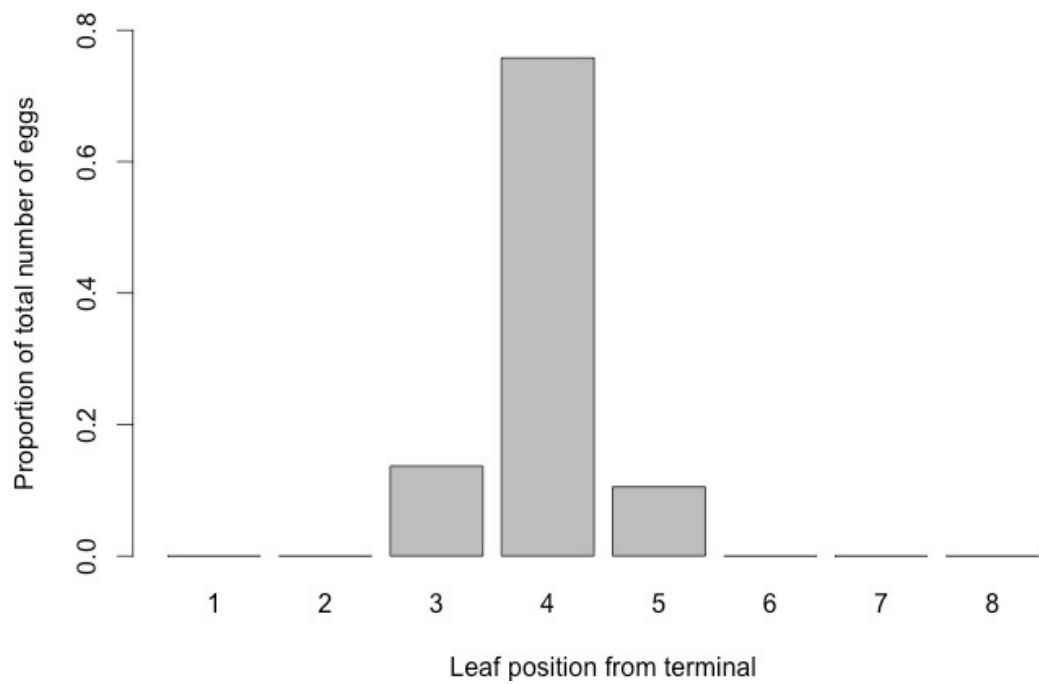


Figure 4.8. Proportions of total *Aleyrodes proletella* eggs, total = 95, on each leaf on Brussels sprout plants. Number 1 denotes terminal leaf.

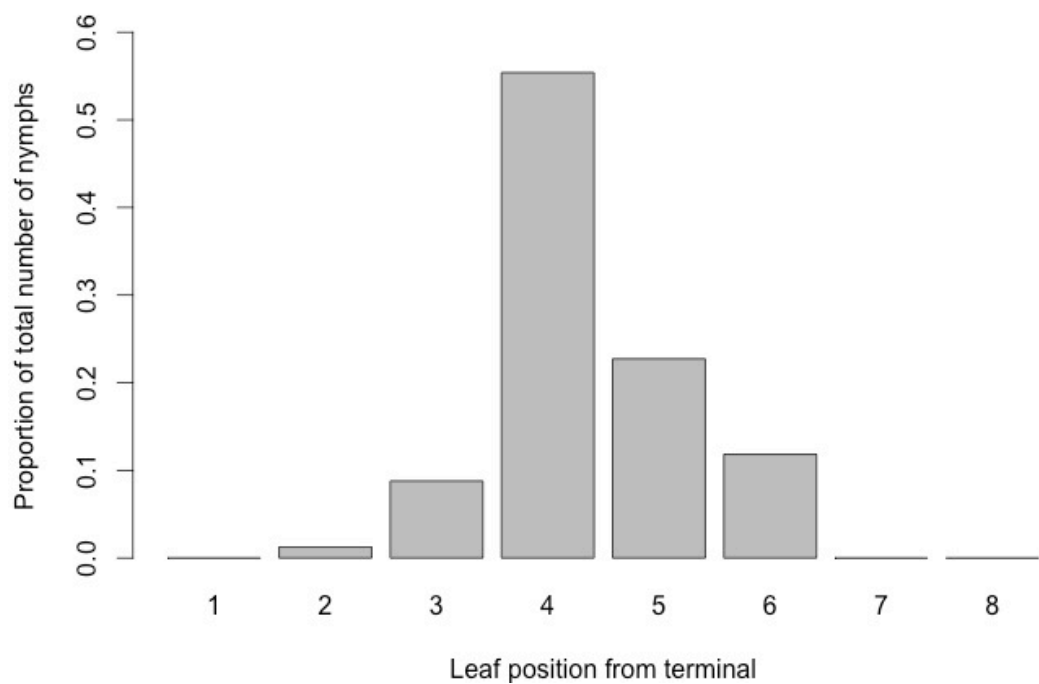


Figure 4.9. Proportions of total *Aleyrodes proletella* nymphs, total = 6374, on each leaf on Brussels sprout plants. Number 1 denotes terminal leaf.

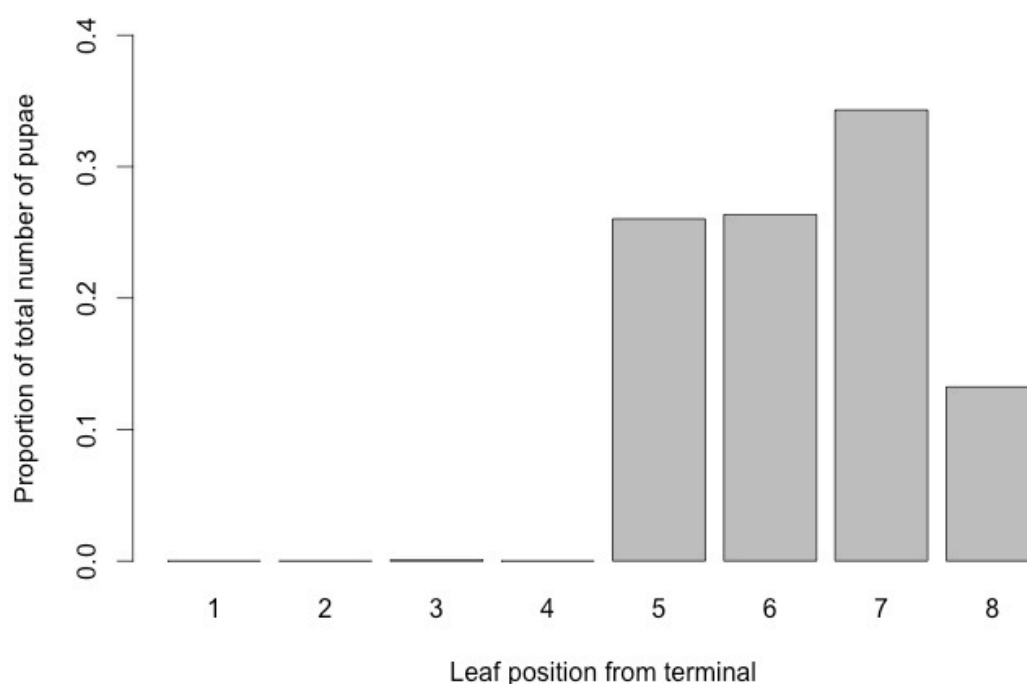


Figure 4.10. Proportions of total *Aleyrodes proletella* pupae, total = 9012, on each leaf on Brussels sprout plants. Number 1 denotes terminal leaf.

The variance (s^2) of all life stages increased more rapidly than the mean (\bar{x}), denoted by the value of the parameter $b > 1$ (Taylor, 1961). All values of b were larger than 1.5, with eggs, nymphs and pupae differing only slightly. Adults, however, showed a considerably larger value when compared to the other life stages (Table 4.2, Figure 4.11).

Table 4.2. Estimated parameters for Taylor's (1961) power law, $s^2 = a + \bar{x}^b$, calculated for each life stage of *Aleyrodes proletella*.

Stage	Parameter	Estimate	Standard Error	P-value
Adults	b	2.82	0.444	<0.01
	a	-2.45	1.364	N.S.
Eggs	b	1.69	0.011	<0.01
	a	2.58	0.015	<0.01
Nymphs	b	1.97	0.351	<0.05
	a	1.08	1.491	N.S
Pupae	b	1.50	0.115	<0.01
	a	3.49	0.540	<0.05

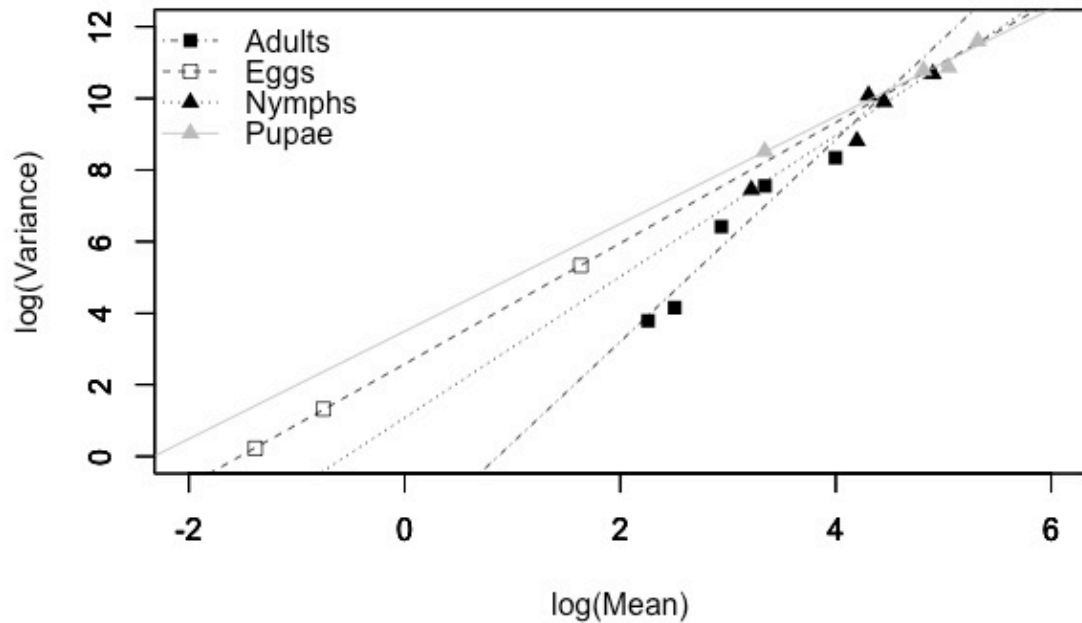


Figure 4.11. Plot of log-variance against log-mean of number of *Aleyrodes proletella* of each stage per leaf for the five plants surveyed. Data are omitted if none of that stage of was found on a plant. The regression line to estimate parameters for Taylor's power law (1961), is plotted for each life stage (Table 4.2).

4.3.2 Experiment 4.2. Distribution of *Aleyrodes proletella* in commercial crops of kale.

There was a decrease in the numbers of *A. proletella* found further into the field (Figure 4.12) and this trend was statistically significant (ANOVA, $F_{(5,23)}=7.55$, $P<0.001$), indicating that on average there were over three times as many adult *A. proletella* on the field edge as on plants located 115 plants (45 m) into the field. There was no interaction between distance from the field edge and the crop surveyed; this pattern, with the highest numbers found at the field edge and the lowest numbers furthest into the field, was consistent between fields (ANOVA, $F_{(1,23)}=1.15$, $P=0.36$). Transects with less than two adult *A. proletella* in total were removed from analysis as each individual adult had a large leverage effect on the model. The numbers of adult *A. proletella* differed between the field edges (north, south, east and west) (ANOVA, $F_{(3,28)}=56.75$, $P<0.01$). The most pronounced

difference was between the northern and southern edges of Field A, where there was an approximately five-fold difference in the numbers of adult *A. proletella* (Figure 4.13).

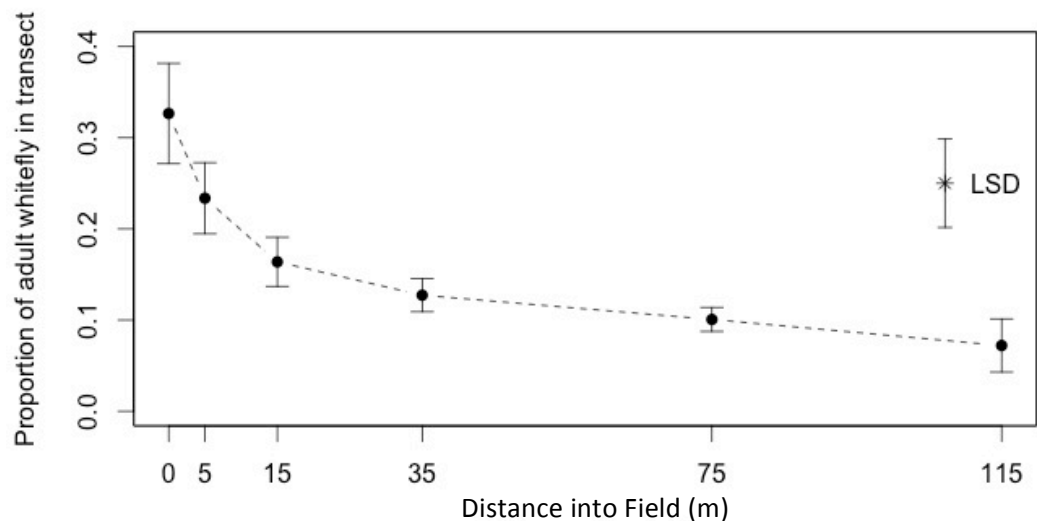


Figure 4.12. Mean (\pm SE) proportion of *Aleyrodes proletella* sampled at for each distance (m) into the field from the edge averaged over the three crops.

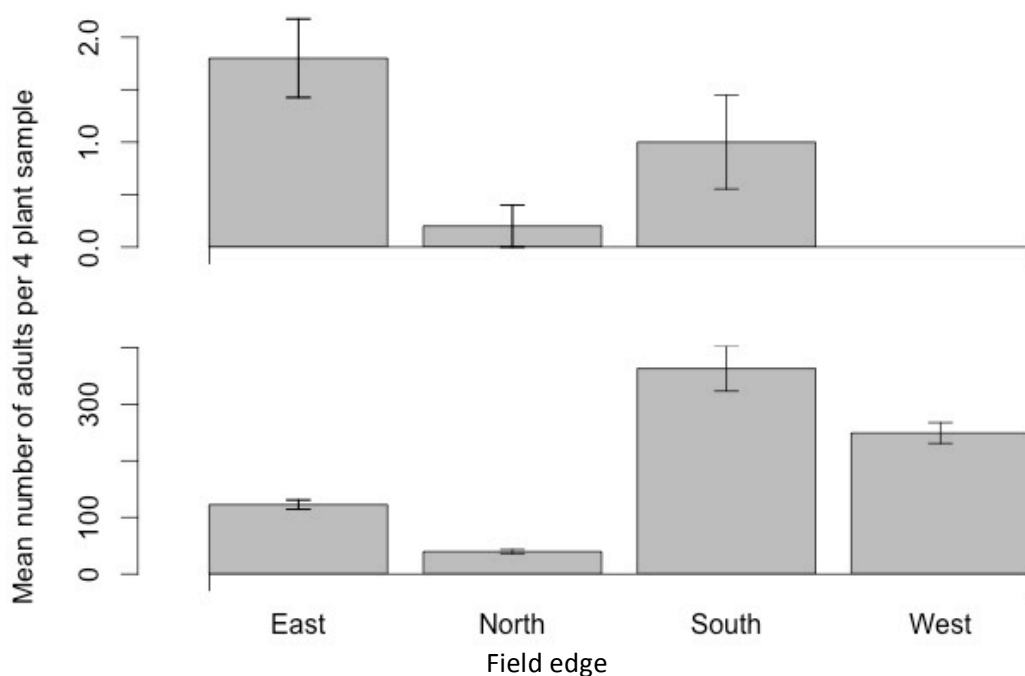


Figure 4.13. Mean (\pm SE) number of adult *Aleyrodes proletella* per 4-plant sample on the field edges. Field B –above and A –below.

The three Fields (A-C) differed significantly from each other in the number of adult *A. proletella* (ANOVA, $F_{(2,28)}=85.5$, $P<0.001$). Field C, the most recently planted field, supported the lowest numbers, while Field A, which was planted first, supported the highest numbers (Figure 4.14). Interestingly there was also a statistically-significant effect on the numbers of *A. proletella* found at each of the edges sampled. Both Fields A and B had up to a four-fold difference in numbers of adult *A. proletella* between field edges, although there was no consistency in the pattern with respect to aspect i.e. north, east, south, west (Figure 4.13).

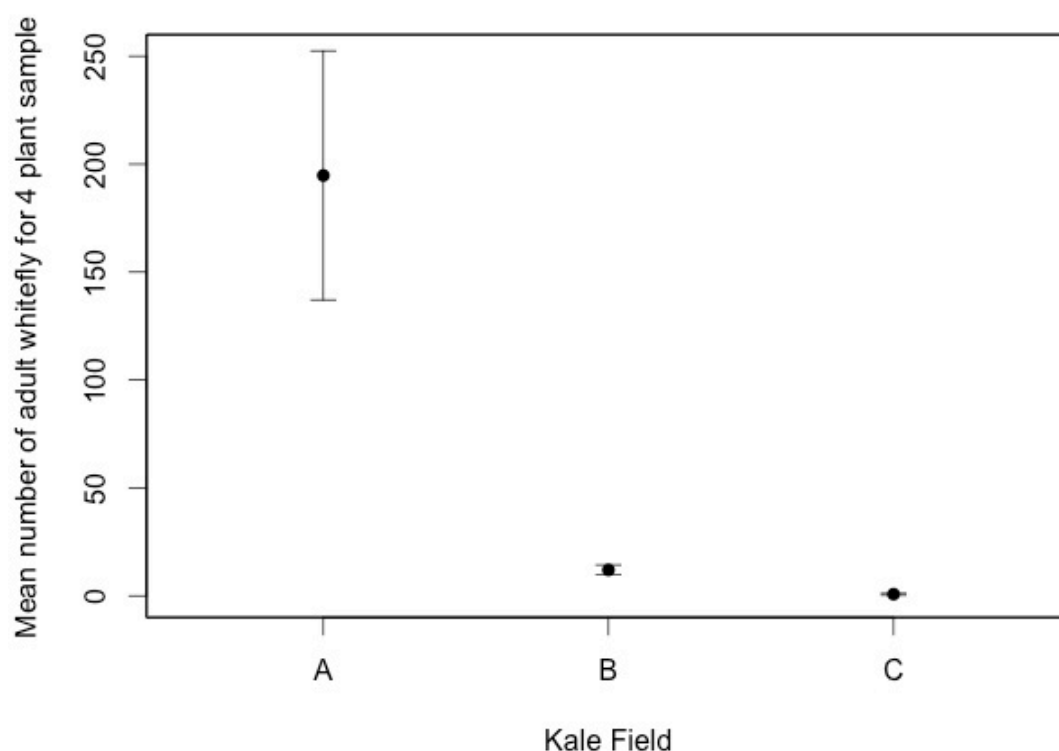


Figure 4.14. Mean (\pm SE) number of adult *Aleyrodes proletella* per four-plant sample for three commercial kale Fields A-C.

4.3.3 Experiment 4.3. Survey of wild host plants for the presence of *Aleyrodes proletella* in uncultivated fields in 2014 and 2015.

Of the wild hosts of *A. proletella* mentioned in Mound and Hasley (1978) only *Sonchus*, *Taraxacum*, *Euphorbia* and *Lactuca spp.* were found (Table 4.3). No *A. proletella* were found on any of these potential host plants, either in the 2013 or 2014 survey. It is likely that the population was too low for them to be detected at this time.

Table 4.3. Mean number of wild host plants and their percentage coverage per 0.25 m² from 40 x 0.25 m² quadrats in uncultivated fields for surveys conducted in 2013 and 2014 at Warwick Crop Centre, Wellesbourne.

Wild host plant	Mean (\pm SE) number of plants 0.25 m ² (% cover)	
	2014	2015
<i>Sonchus</i> spp.	0.46 \pm 0.12 (1%)	1.35 \pm 0.32 (13%)
<i>Taraxacum</i> spp.	0.24 \pm 0.11 (0.9%)	1.6 \pm 0.03 (12%)
<i>Euphorbia</i> spp.	0.05 \pm 0.03 (0.2%)	0 (0%)
<i>Lactuca</i> spp.	0 (0%)	0.2 \pm 0.1 (1%)

Ad. Hoc. Surveys of wild host plants around the Wellesbourne campus and Kenilworth was more successful in detecting the presence of *A. proletella* on wild hosts. The largest populations were present on hosts growing in disturbed ground and tarmacked, car park, habitats (Table 4.4).

Table 4.4. Results of *Ad Hoc* survey of wild host plants taken at Warwick Crop Centre (W) or Kenilworth (K). The date, height of plant and host plant species are given with number of each development stage of *Aleyrodes proletella*.

Date	Location *	Habitat **	Height	Hostplant	Adults	Eggs	Nymphs
26/07/2014	W	G	30	<i>Sonchus</i>	0	0	0
26/07/2014	W	G	30	<i>Taraxacum</i>	0	0	0
26/07/2014	W	T	60	<i>Sonchus</i>	25	70	120
26/07/2014	W	T	10	<i>Sonchus</i>	0	0	0
26/07/2014	W	H	15	<i>Sonchus</i>	0	0	0
26/07/2014	W	H	20	<i>Sonchus</i>	0	0	20
26/07/2014	W	T	40	<i>Sonchus</i>	10	35	25
26/07/2014	W	G	50	<i>Sonchus</i>	1	20	0
26/07/2014	W	G	20	<i>Sonchus</i>	0	10	0
22/08/2014	K	T	70	<i>Euphorbia</i>	15	30	140
22/08/2014	K	T	150	<i>Lactuca</i>	4	10	40

Date	Location *	Habitat **	Height	Hostplant	Adults	Eggs	Nymphs
26/07/2014	W	G	30	<i>Sonchus</i>	0	0	0
26/07/2014	W	G	30	<i>Taraxacum</i>	0	0	0
26/07/2014	W	T	60	<i>Sonchus</i>	25	70	120
26/07/2014	W	T	10	<i>Sonchus</i>	0	0	0
26/07/2014	W	H	15	<i>Sonchus</i>	0	0	0
26/07/2014	W	H	20	<i>Sonchus</i>	0	0	20
26/07/2014	W	T	40	<i>Sonchus</i>	10	35	25
22/08/2014	K	T	50	<i>Lactuca</i>	25	150	140
22/08/2014	K	T	60	<i>Papaver</i>	3	15	5

* Kenilworth, Warwickshire, UK.

** Habitat types, T = Tarmac, (i.e. car parks), G=Grassland, H=Hedgerow.

4.3.4 Experiment 4.4. Survey of commercial oilseed rape fields for *Aleyrodes proletella*.

4.3.4.1 2013 Survey

No *A. proletella* were found during the surveys in April or June. The final survey in July showed an average of 0.1 adults per 0.25 m²; as a total of 4 adults were found from 40 x 0.25 m² quadrats (Table 4.5). It should be noted that all *A. proletella* found during the survey were within 40 m of the field edge.

Table 4.5. Mean number of oilseed-rape (OSR) plants per 0.25 m² and number of *Aleyrodes proletella* at different life stages from 40 x 0.25 m² quadrats on three different dates.

Survey date	Mean (\pm SE) number 0.25m ⁻²				
	OSR plants (% cover)	<i>Aleyrodes proletella</i>			
		Adults	Eggs	Nymphs	Pupae
20 April 2013	9.66 \pm 0.84 (10%)	0	0	0	0
5 June 2013	5.67 \pm 0.34 (64%)	0	0	0	0
17 July 2013	3.62 \pm 0.34. (66%)	0.10 \pm 0.05	3.25 \pm 1.66	0.15 \pm 0.1	0.0 \pm 0.13

4.3.4.2 2015 Survey

The numbers of adult *A. prolella* found in the five OSR Fields surveyed were significantly different from each other (ANOVA, $F_{(4,173)}=2.81$, $P<0.05$). OSR Fields A and D were found to have lower populations when compared to OSR Field B. Even though a higher mean number of *A. prolella* were found on the eastern edge of OSR Field C no significant difference was found between the field edges, north, south, east, west, of the fields (ANOVA, $F_{(3,173)}= 2.02$, $P=0.11$), nor was there a significant interaction of field edge with OSR Field (ANOVA, $F_{(12,173)}=1.44$ $P=0.15$, Figure 4.15). Although there was a slight decrease in numbers of *A. prolella* found from 15-105 m into the field, this was not found to be a significant effect (ANOVA, $F_{(1,173)}=0.99$, $P=0.32$, Figure 4.16)

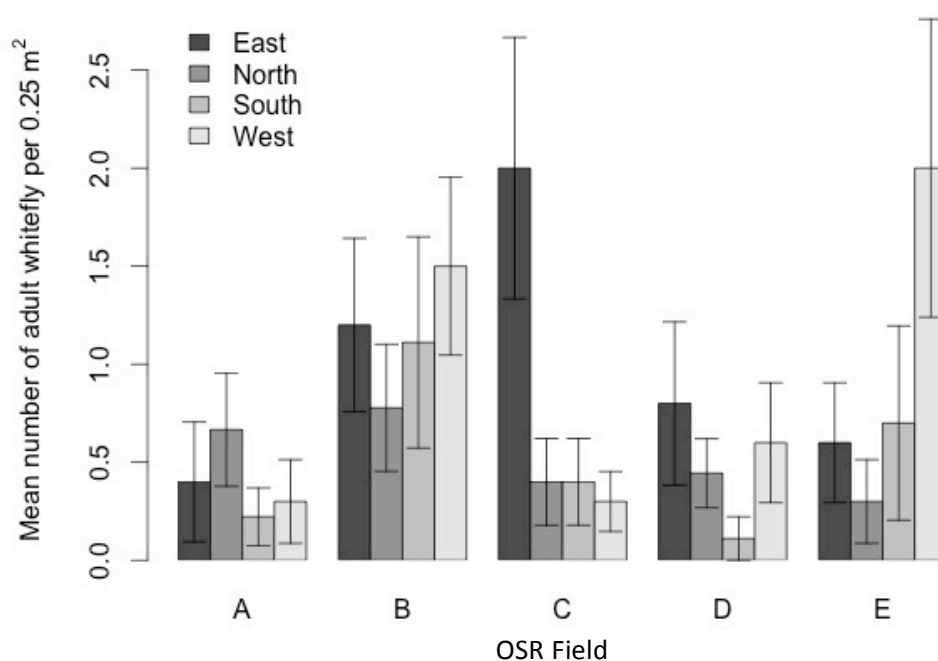


Figure 4.15. Mean number (\pm SE) of adult *Aleyrodes prolella* observed in each OSR Field (A-E) from the four field edges.

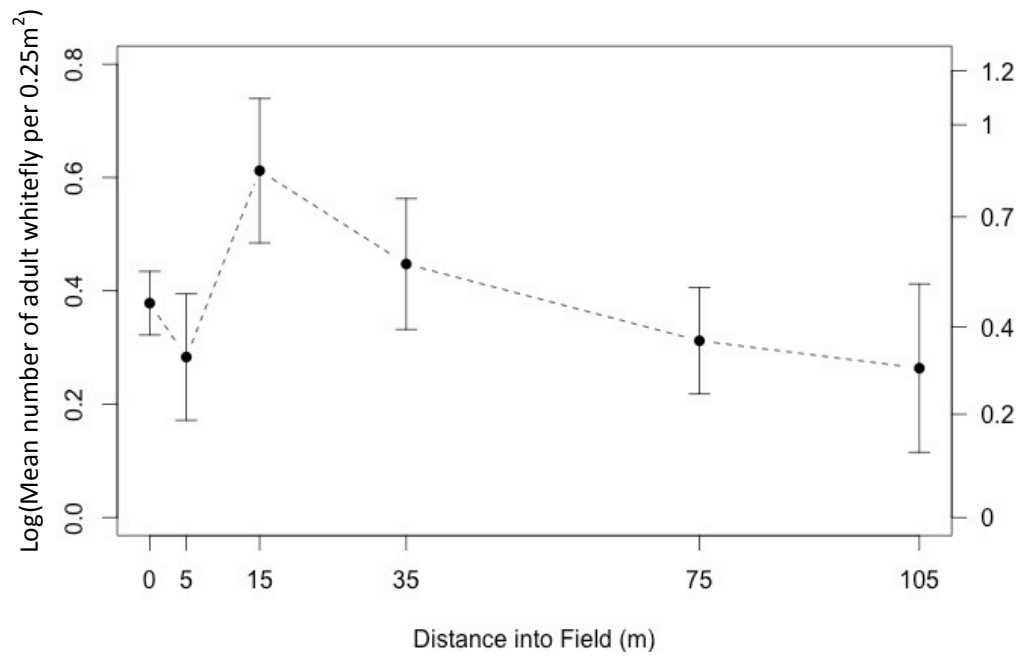


Figure 4.16. Mean (\pm SE) number of adult *Aleyrodes proletella* at each distance into the OSR Field. The right axis shows back transformed data.

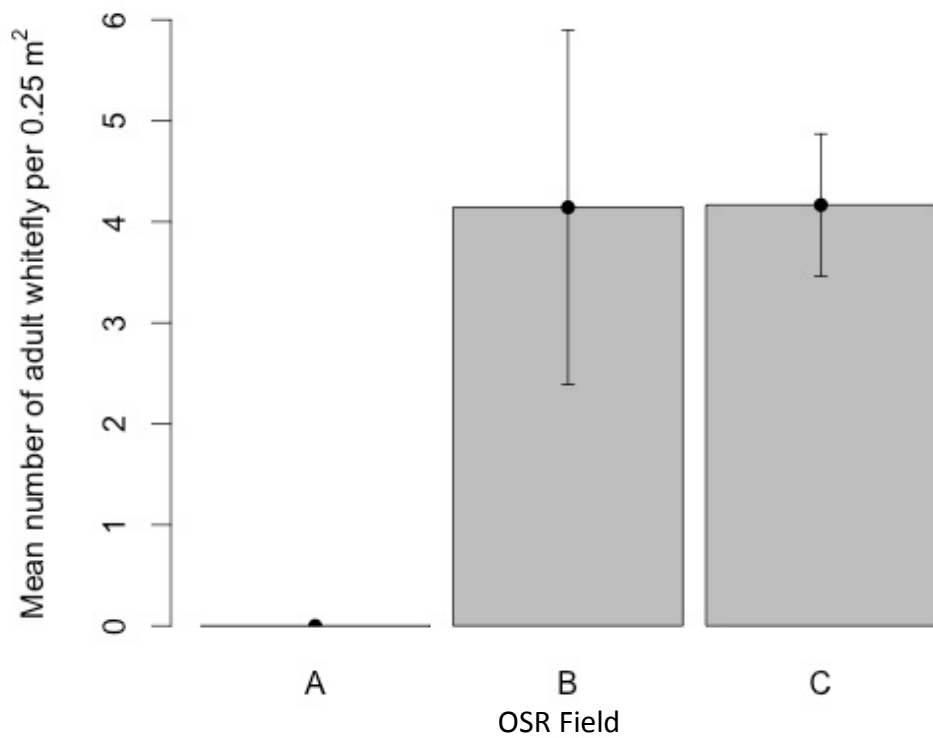


Figure 4.17. Mean number (\pm SE) of adult *Aleyrodes proletella* observed in each OSR field for the survey conducted in April 2015.

Of the three OSR Fields sampled for a second time in April 2015, a considerable difference was observed. No adult *A. proletella* were found from the 8 x 0.25 m² quadrats sampled from OSR Field A, while approximately four adults *A. proletella* per 0.25m² were present in OSR Fields B and C, (Figure 4.17). This difference was found to be significantly different (Kruskal-Wallis =11.19, d.f.=2, P<0.01) With OSR Field A significantly lower than both OSR Fields B and C (Kruskal-Wallis chi-squared = 11.1882, df = 2, P<0.01). OSR Fields B and C however were not found to be significantly different (Wilcoxon ranked sum W=26.5, P=0.46, Figure 4.17).

4.3.5 Experiment 4.5. Monitoring *Aleyrodes proletella* on vulnerable field crops (Brussels sprout and kale) throughout the season.

The length of time before the plots planted in May 2013 were colonised by *A. proletella* ranged from ~2 weeks for the kale plants in Plot C, to nearly 50 days for the kale plants in Plot B. The length of time prior to colonisation was not found to be significantly different between kale and Brussels sprout plots for the five planting locations, Plots A-E (ANOVA, $F_{(1,8)}=0.027$, P=0.87, Figure 4.18).

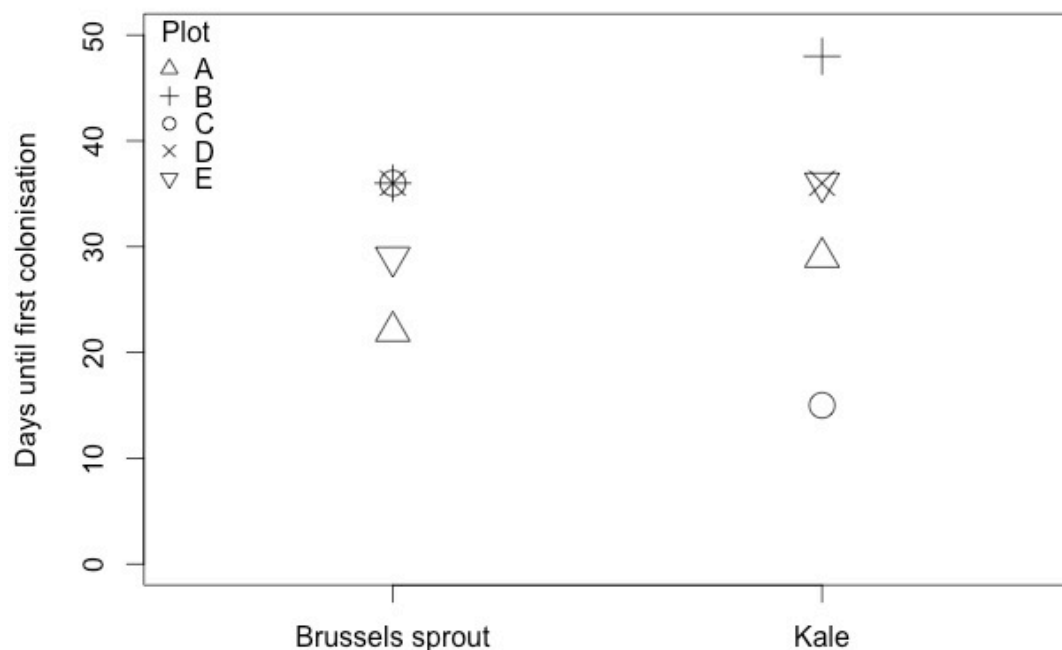


Figure 4.18. Days from planting until first colonisation by *Aleyrodes proletella* in Plots A-E, for Brussels sprout and kale.

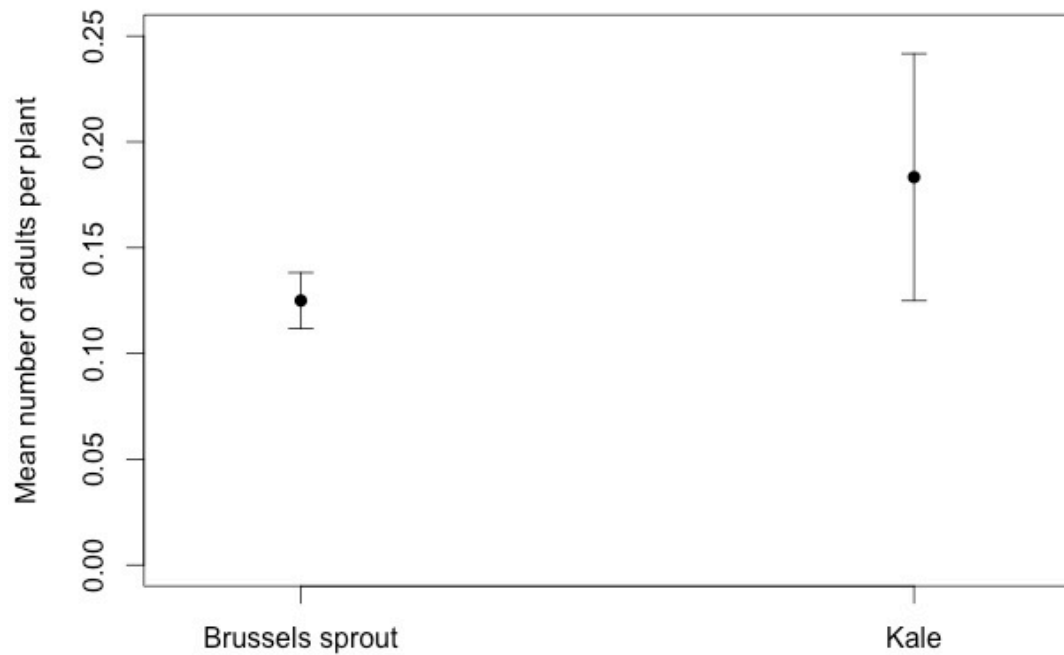


Figure 4.19. Mean (\pm SE) number of *Aleyrodes proletella* adults 50 days after transplanting for Brussels sprout and kale plots.

After 50 days the kale plots showed a slightly higher mean number of adult *A. proletella* per plant when compared with the Brussels sprout plots but this was not found to be statistically significant for the five Plots A-E (ANOVA, $F_{(1,8)} = 0.951$, $P = 0.358$, Figure 4.19).

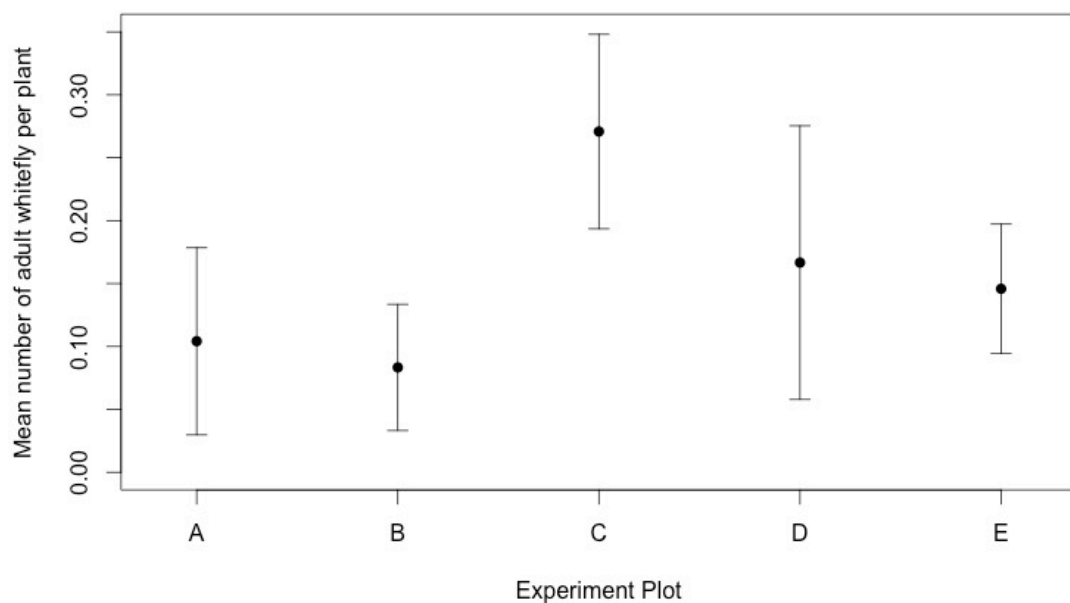


Figure 4.20. Mean (\pm SE) number of adult *Aleyrodes proletella* per plant 50 days after transplanting for the 5 study plots, A-E.

After 50 days Plot C supported nearly three times as many adult *A. proletella* as Plots A and B, (Figure 4.20). The immigration rates between the plots was significantly different (Kruskal-wallis, chi-squared = 10.4695, df = 4, $P < 0.05$), with Plot C having a significantly higher population of adult *A. proletella* at 50 days when compared to Plots A and B (Wilcoxon ranked-sum, A-C $W = 946$, $P < 0.05$, B-C $W = 963$, $P < 0.05$, all other combinations N.S.).

Populations of *A. proletella* on the five monitoring plots remained very low for the first few weeks after transplanting. It was not until June 2013 that an increase in the numbers of eggs occurred, and soon after that all other life stages increased considerably in numbers (Figures 4.21-4.25). This increase in the numbers of all life stages continued until mid-September when numbers ceased to increase. Subsequently, all populations decreased from November 2013 until January 2014 when a large increase in egg numbers was observed. A corresponding increase in the numbers of nymphs did not occur until March 2014 and finally, an increase in the numbers of pupae occurred in mid May. The last 'peak' in numbers observed on these plots was the dramatic increase in adult numbers in early June 2014, after which the numbers of all life stages declined to zero in most cases. This decline coincided with the senescence of the plants after flowering.

There were significantly higher numbers of adults (ANOVA, $F_{(1,286)} = 117.74$, $P < 0.001$, Figure 4.21), eggs (ANOVA, $F_{(1,286)} = 120.40$, $P < 0.001$, Figure 4.22), nymphs (ANOVA, $F_{(1,286)} = 96.14$, $P < 0.01$, Figure 4.23), pupae (ANOVA, $F_{(1,286)} = 52.5$, $P < 0.001$, Figure 4.24) and exuviae (ANOVA, $F_{(1,286)} = 15.70$, $P < 0.001$, Figure 4.25) on kale plants when compared with Brussels sprout plants. This difference was only observed from ~September onwards with regard to all life stages. The scale of the difference in numbers between crop types decreased in the order: adults>eggs>nymphs>pupae>exuviae, with the numbers of exuviae being significantly higher on kale plants in February 2014 only (Figure 4.25).

Using the mean number of adults and eggs per plant, the ratio of eggs to adults at each sampling time point, for each plot and crop type (Brussels sprout and kale) was calculated:

$$\frac{\text{Mean no. eggs per plant} + 1}{\text{Mean no. adults per plant} + 1}$$

A value of 1 was added to prevent zero values causing difficulties with interpretation.

There were no statistically-significant differences in the number of eggs per adult between kale and Brussels sprout plants throughout the entire season. However, Brussels sprout plants had slightly higher mean numbers of eggs per adult during July-August 2013. The onset of diapause in the population is signified by eggs per females falling from September. It was noted that the increase in the numbers of eggs per adult, signifying the start of egg laying, began earlier on kale plants than it did on Brussels sprout plants (Figure 4.26).

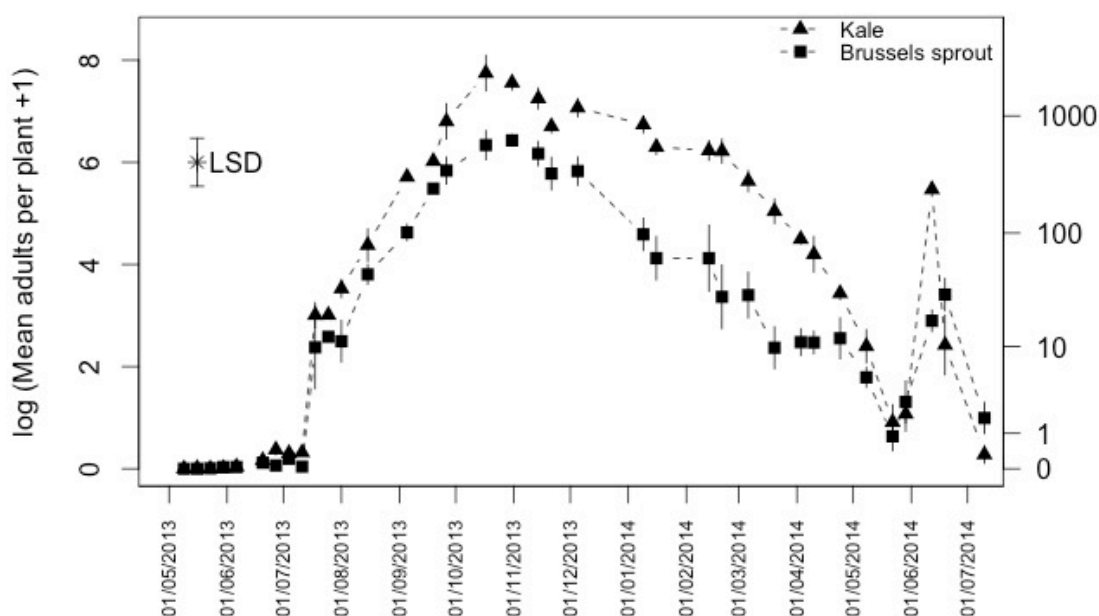


Figure 4.21. Mean (\pm SE) number of adult *Aleyrodes proletella* per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA ($P < 0.01$) is also shown. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

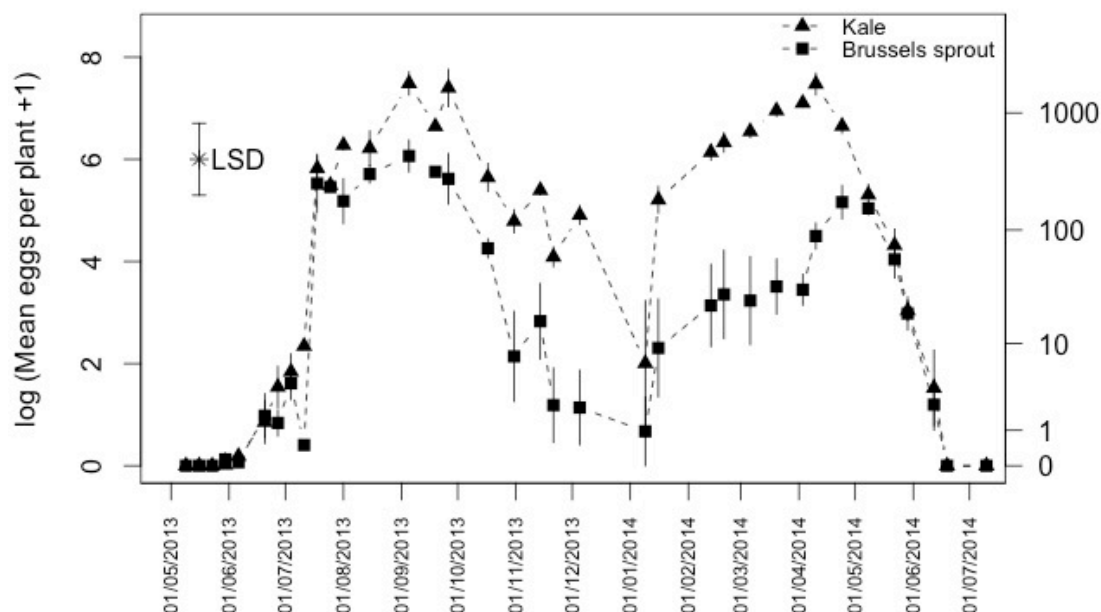


Figure 4.22. Mean (\pm SE) number of *Aleyrodes proletella* eggs per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA ($P < 0.01$) is also shown. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

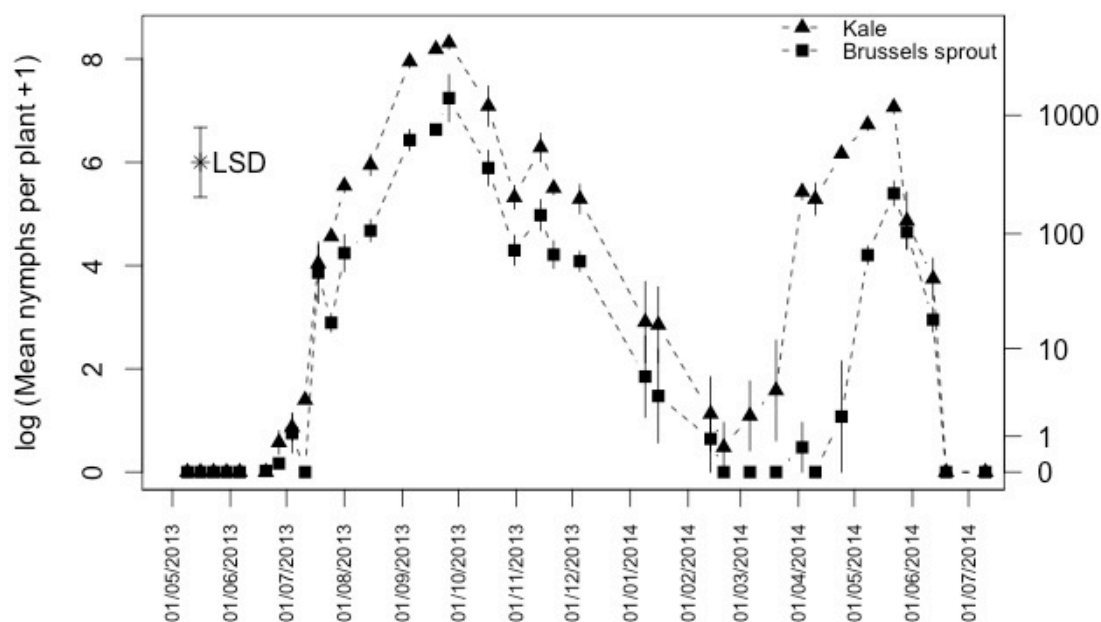


Figure 4.23. Mean (\pm SE) number of *Aleyrodes proletella* nymphs per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA ($P < 0.01$) is also shown. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

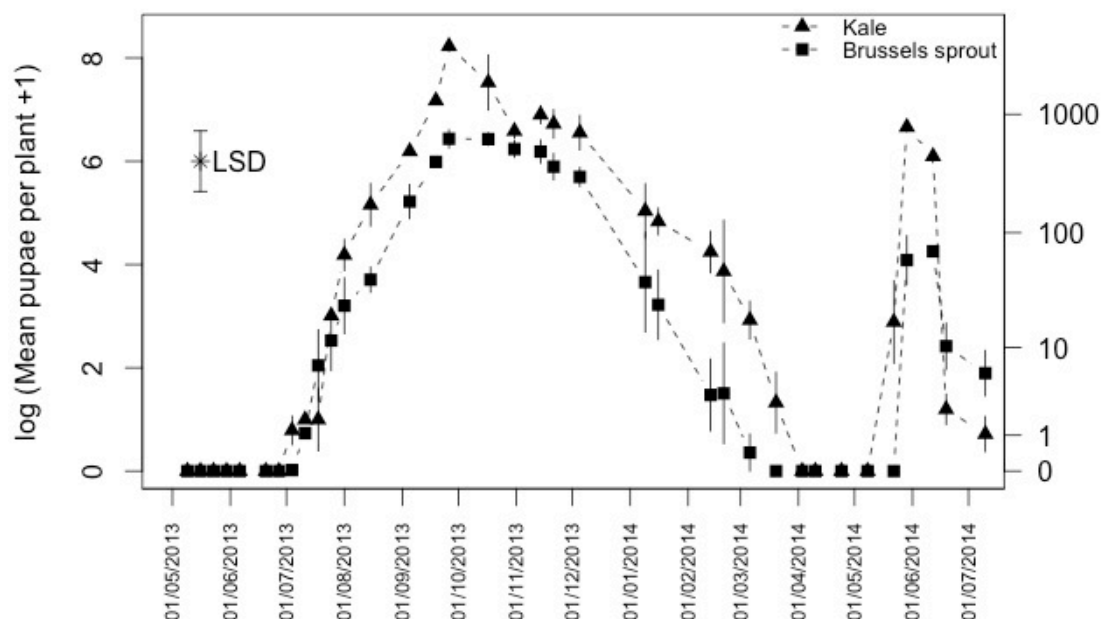


Figure 4.24. Mean (\pm SE) number of *Aleyrodes proletella* pupae per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA ($P < 0.01$) is also shown. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

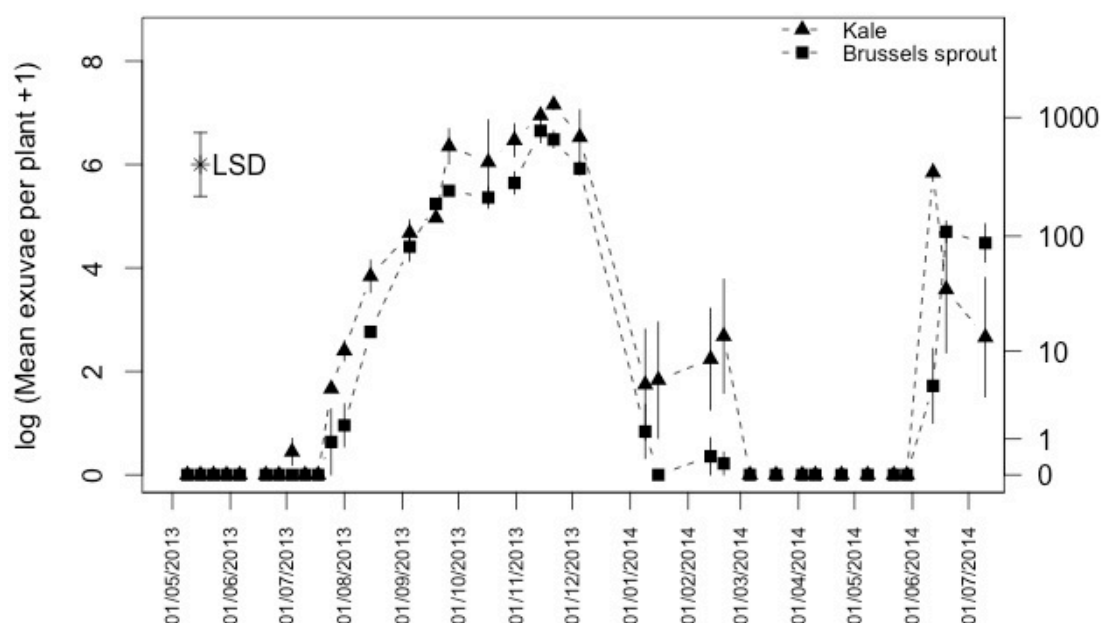


Figure 4.25. Mean (\pm SE) number of *Aleyrodes proletella* exuviae (empty pupal cases) per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA ($P < 0.01$) is also shown. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

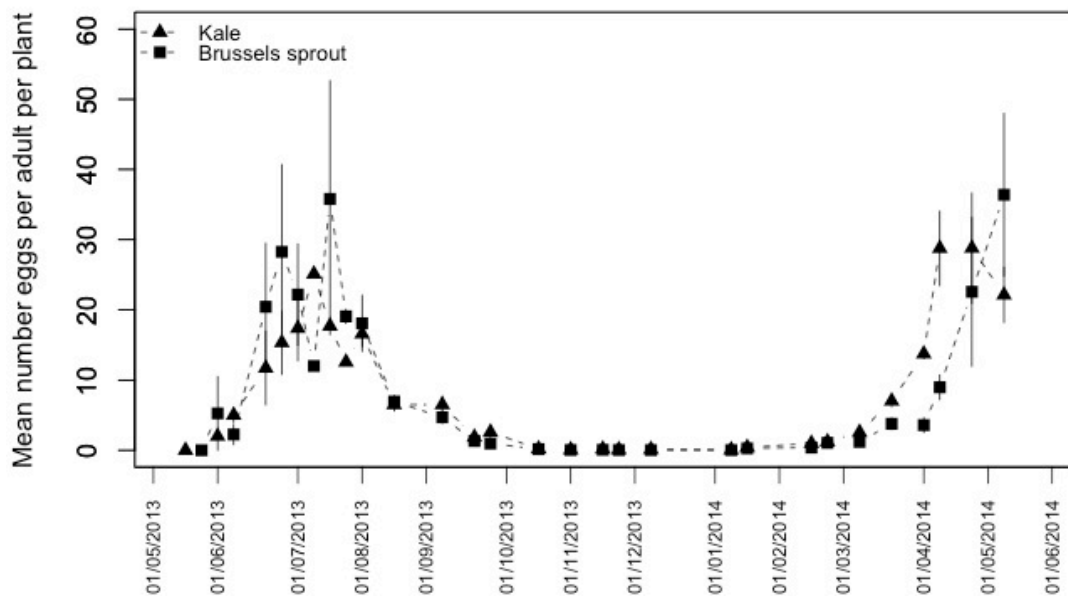


Figure 4.26. Mean (\pm SE) number of eggs per adult for the 5 Brussels sprout and kale plots. Differences were not statistically-significant.

4.3.6 Experiment 4.6. Monitoring immigration and establishment of *Aleyrodes proletella* on spatially- and temporally-separated plantings of kale.

Plots planted in June and August 2014 were colonised most rapidly by adult *A. proletella*; all plots were infested with adults within a week of transplanting. Colonisation was generally slowest in plots planted in May, with one plot, Plot I, not being colonised until 26 days after planting. The time until first colonisation was found to be significantly longer in this May date planting (Kruskal-Wallis ranked sum, $K=14.40$, d.f.=4, $P<0.01$, Figure 4.27).

Mean immigration rates (measured two weeks after planting) were greatest in August and lowest in May, with an approximately three-fold difference between them. There was also a markedly higher immigration into the plots planted in June compared with those planted in either May or July. The immigration rates were, however, not significantly different between planting dates (ANOVA, $F_{(4,18)}=2.31$, $P=0.097$, Figure 4.28)

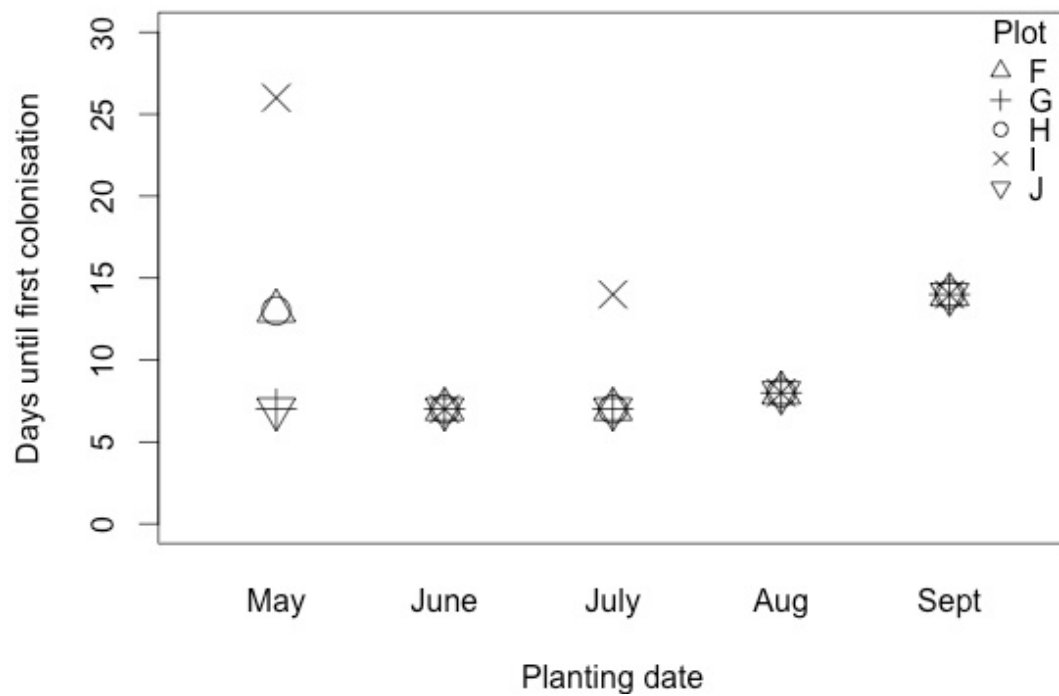


Figure 4.27. Days until first colonisation of plots by adult *Aleyrodes proletella* for each planting date in 2015 and Plot (F-J). The median day of first colonisation was significantly different between planting dates, $P < 0.01$.

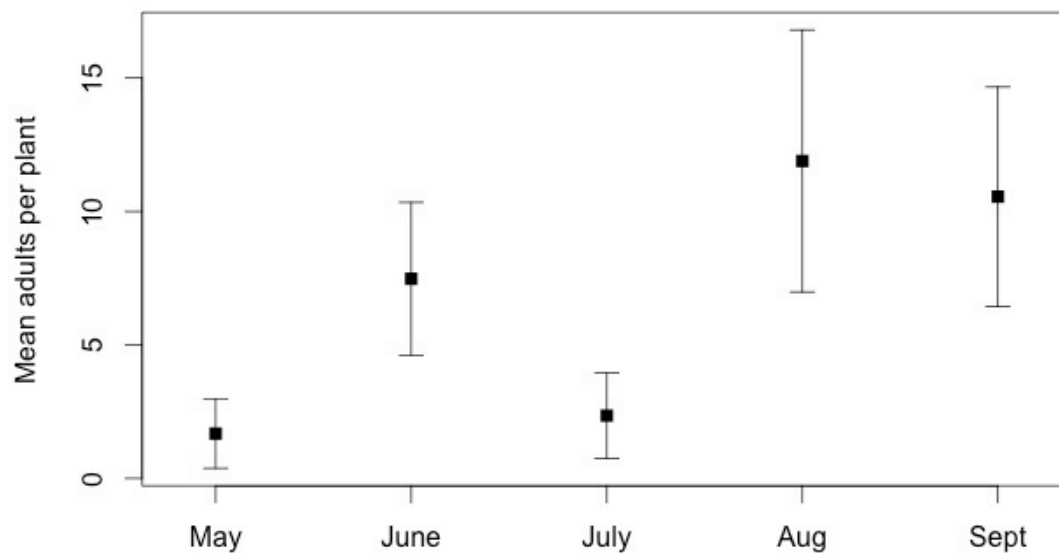


Figure 4.28. Mean (\pm SE) number of adult *Aleyrodes proletella* per plant two weeks after transplanting for each planting date: 19th May, 17th June, 19th July, 16th August and 16th September 2014.

The pattern of population increase did not differ between plots within a single planting date. Numbers of adult *A. prolella* increased gradually until October 2014, after which they began to decline (Figure 4.29). The date by which the maximum number of adults in each plot was reached did not differ between planting dates and ranged from 18th September until 15th November (Rank sum Kruskal-Wallis, $K=5.28$ d.f.=4, $P=0.26$, Table 4.6). So, for example, the plots planted in May achieved their maximum numbers of adult *A. prolella* at a similar time to those planted in September. However, the maximum size of the population was affected by planting date (ANOVA, $F_{(4,16)}=8.091$, $P<0.001$, Figure 4.30). A general trend in the maximum size of the population at the end of the season in each location was: May>June>July>August>September (Figure 4.30 and Table 4.6)

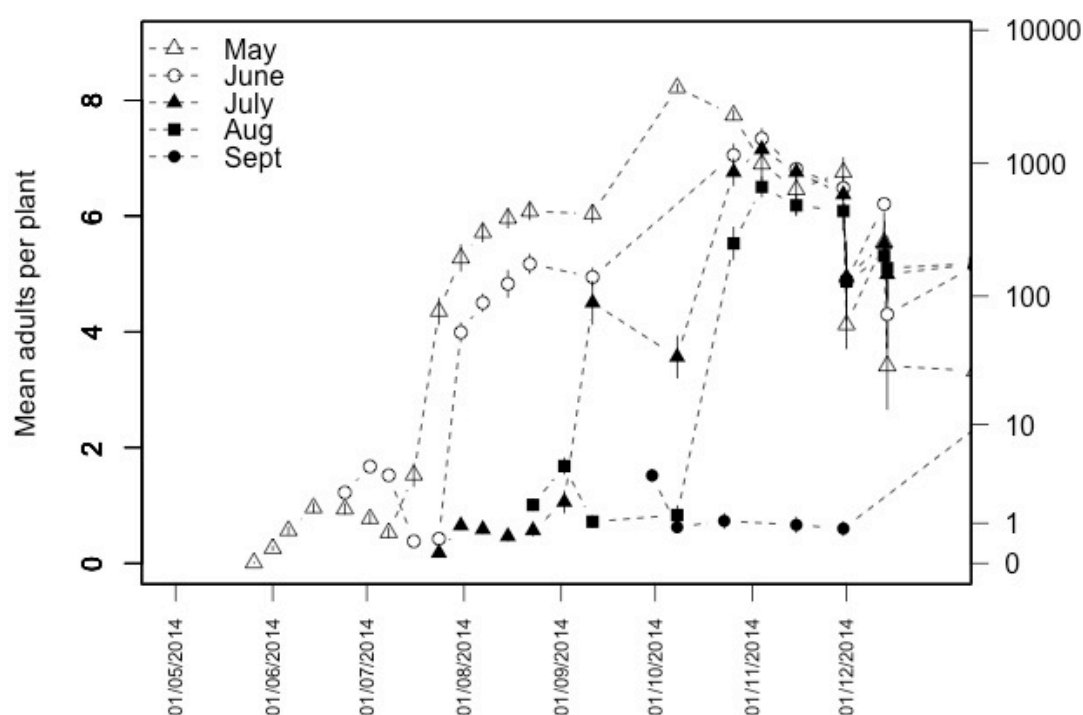


Figure 4.29. Mean (\pm SE) number of adult *Aleyrodes prolella* per plant for each planting date: 19th May, 17th June, 19th July, 16th August and 16th September. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

Table 4.6. Maximum number of adults per plant for each of the Plots (F-J) and the corresponding planting dates with the date at which this value was achieved. (All dates are for 2014, day/month)

Plot	May		June		July		August		September	
	Max	Date	Max	Date	Max	Date	Max	Date	Max	Date
F	1945	26/10	1575	26/10	938	04/11	595	04/11	42.5	23/08
G	1085	15/08	1777	04/11	2099	04/11	1200	26/10	18.75	30/09
H	2527	04/11	3040	04/11	1115	15/11	1565	15/11	61.67	23/10
I	3706	08/10	1878	26/10	466	30/11	261	15/11	21.25	30/11
J	3035	26/10	912	15/11	1822	26/10	612	04/11	3.5	30/11

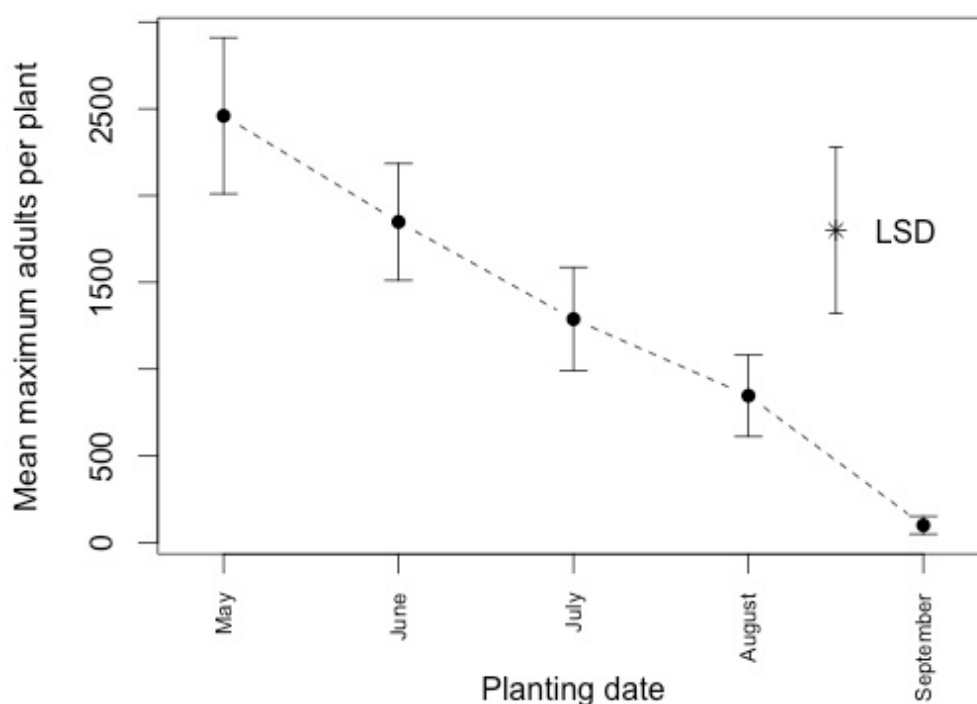


Figure 4.30. Mean (\pm SE) maximum number of adult *Aleyrodes prolella* per plant for each of the planting dates. The 5% LSD from ANOVA ($P<0.001$) is also shown.

A ‘nearly significant’, ($P<0.1$), negative regression was found between the mean number of adult *A. prolella* per plant 3 weeks after planting and the distance the plot was from a source of overwintering *A. prolella*, for both May and June plantings (Figure 4.31). This relationship was not apparent for the plantings in July,

August or September. When May and June data points were combined this relationship became highly significant ($P < 0.001$, Table 4.7).

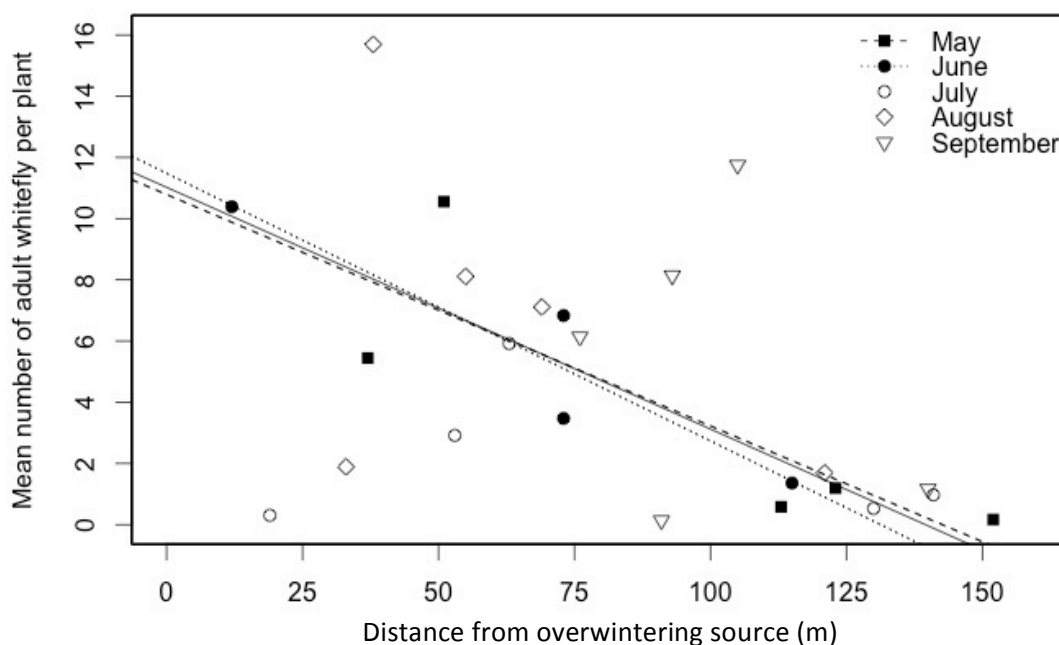


Figure 4.31. Mean number of adult *Aleyrodes proletella* per plant after 3 weeks against distance (m) from the nearest location of an overwintering population, for each plot and planting date. Regressions are shown for June (dotted line) and May (dashed line), $P < 0.1$. The solid line shows statistically-significant linear regression for the May and June combined $P < 0.001$.

For the plots planted in May, a small number of immigrant *A. proletella* were observed within a few weeks and a subsequent rise in the number of eggs was seen soon afterwards. Nymphs were the next stage to increase in numbers, followed by pupae. In mid-June the numbers of adult *A. proletella* rose rather dramatically, reaching ~100 per plant by August. This dramatic increase coincided with the first occurrence of exuviae, empty pupal cases (Figure 4.32). This pattern of population increase was common for all planting times. The length of time before exuviae appeared and the level of immigration did, however, differ between them (Figures 4.32-4.36). There was no sharp increase in *A. proletella* numbers for the September plantings and no pupae or exuviae were present before winter (Figure 4.36).

Table 4.7. Linear regression analysis of mean number of adult *Aleyrodes proletella* per plant (x) and distance from overwintering source (y) for each planting date, $y = mx + c$

Planting date	Parameter		S.E	R ²	P value
May	c	10.78909	2.96829	0.6054	0.0359
	m	-0.07563	0.02831		0.0756
June	c	11.48261	1.77520	0.829	0.0231
	m	-0.08745	0.02290		0.0623
July	c	3.05481	2.35614	0.064	0.286
	m	-0.01142	0.02514		0.681
August	c	11.71236	5.83675	0.221	0.138
	m	-0.07615	0.08261		0.425
September	c	11.0018	11.5294	0.074	0.410
	m	-0.0548	0.1116		0.657
May and June combined	c	11.02332	1.48895	0.748	0.000149
	m	-0.07905	0.01589		0.001612

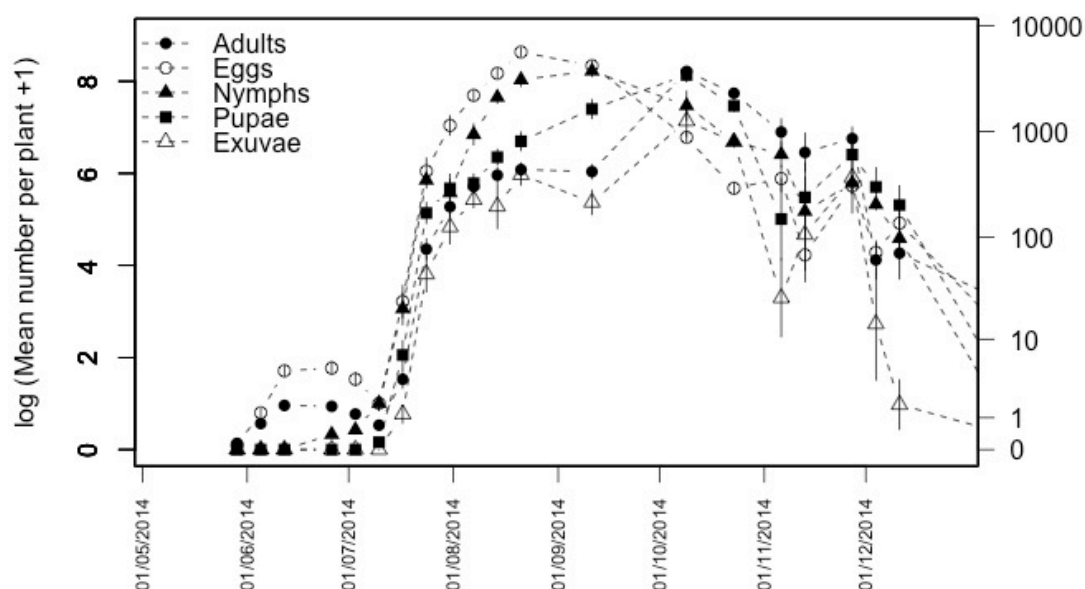


Figure 4.32. Mean (\pm SE) number of all life stages of *Aleyrodes proletella* per plant for the plots planted on 19th May 2014. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

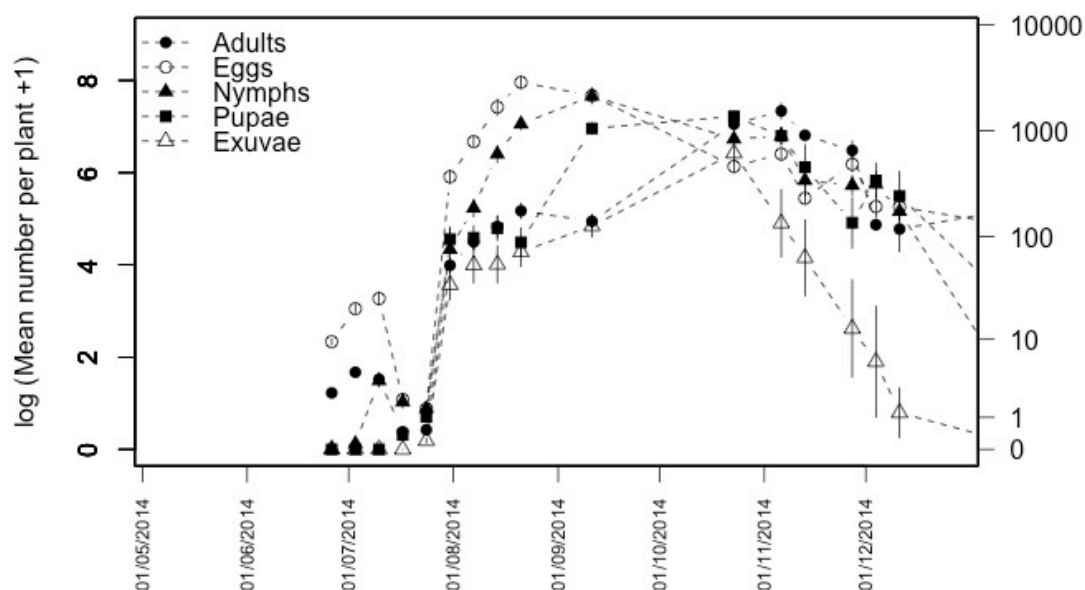


Figure 4.33. Mean (\pm SE) number of all life stages of *Aleyrodes proletella* per plant for the plots planted on 17th June 2014. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

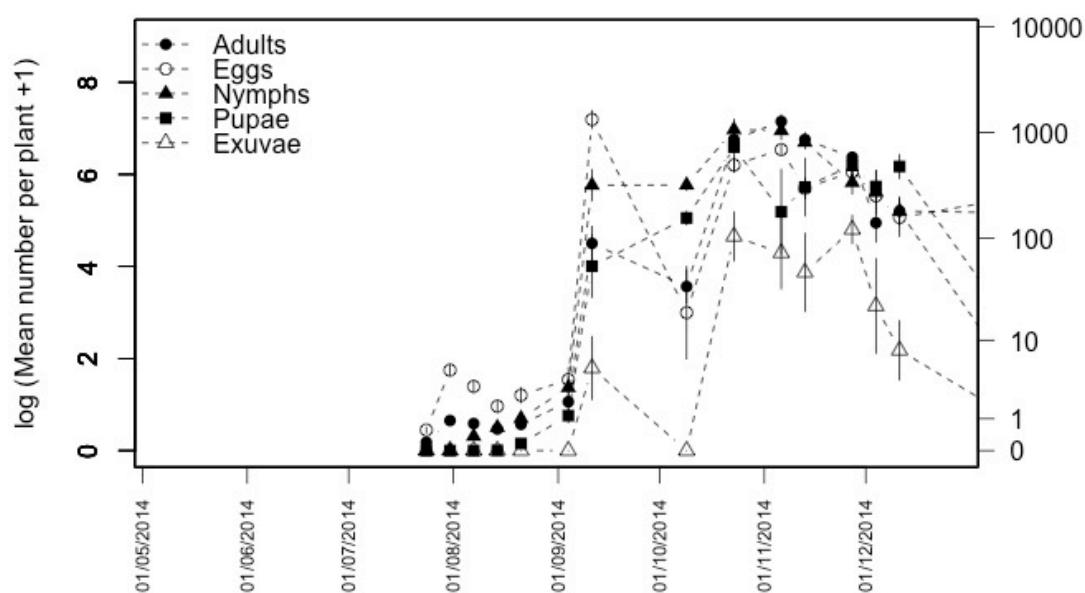


Figure 4.34. Mean (\pm SE) number of all life stages of *Aleyrodes proletella* per plant for the plots planted on 19th July 2014. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

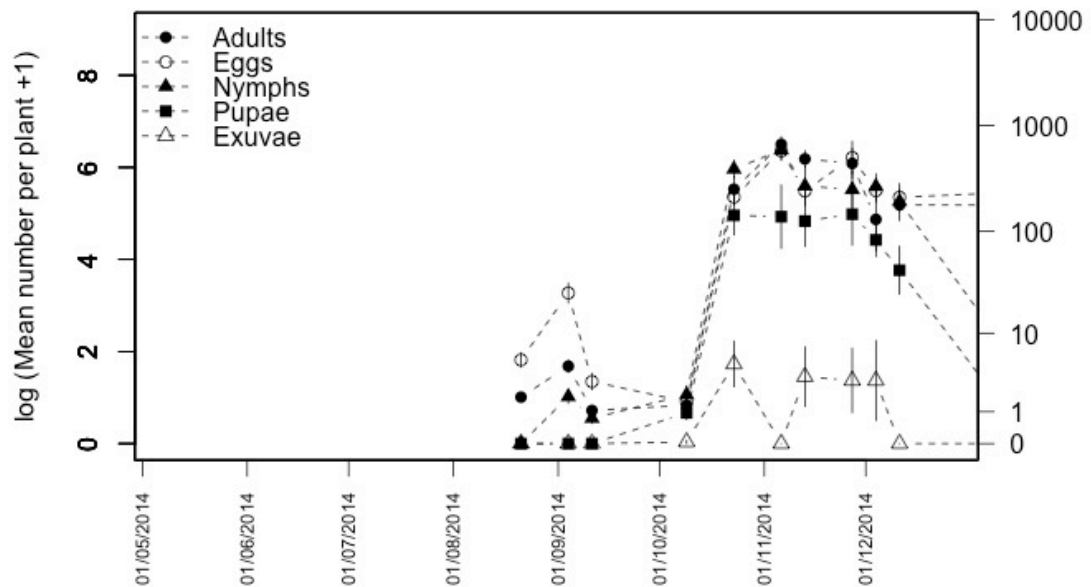


Figure 4.35. Mean (\pm SE) number of all life stages of *Aleyrodes proletella* per plant for the plots planted on 16th August 2014. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

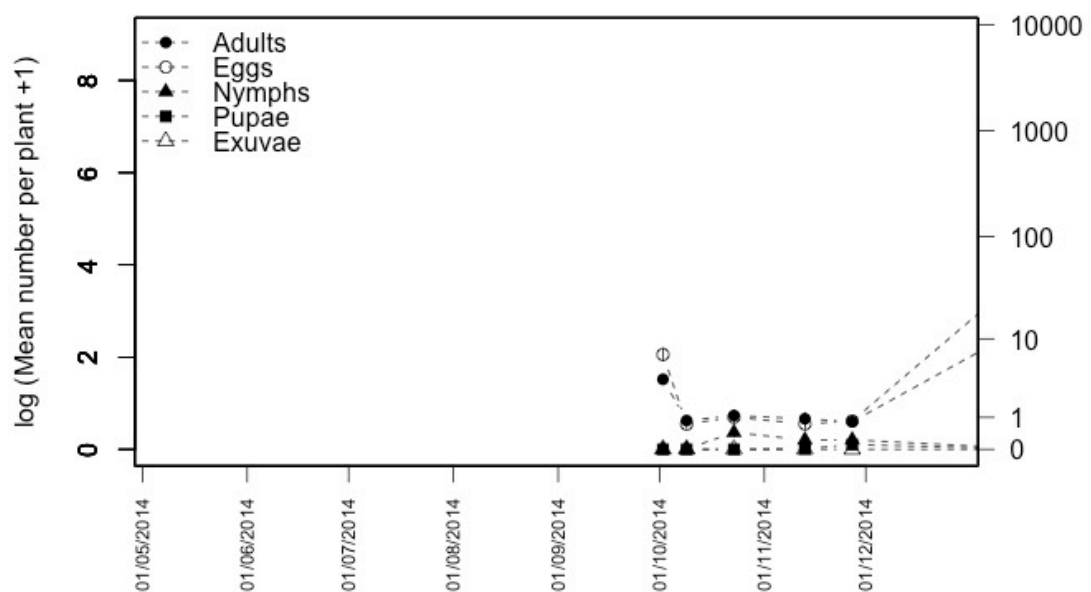


Figure 4.36. Mean (\pm SE) number of all life stages of *Aleyrodes proletella* per plant for the plots planted on 16th September 2014. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

4.3.7 Experiment 4.7. Monitoring *Aleyrodes proletella* populations on kale, 2015.

The first adult colonisers of Plots K, L, N and O were observed 11 days after planting, on 21st April 2015. Plot N was not colonised until 15th June; 66 days after planting. The populations in all five plots followed a similar pattern of increase to the populations observed in 2014, with a dramatic increase in numbers starting in mid-June. This period of increase continued until mid-July, when a slight decrease was observed, after which the population continued to rise until October. The numbers of adults had started to decrease in all plots by November (Figure 4.37).

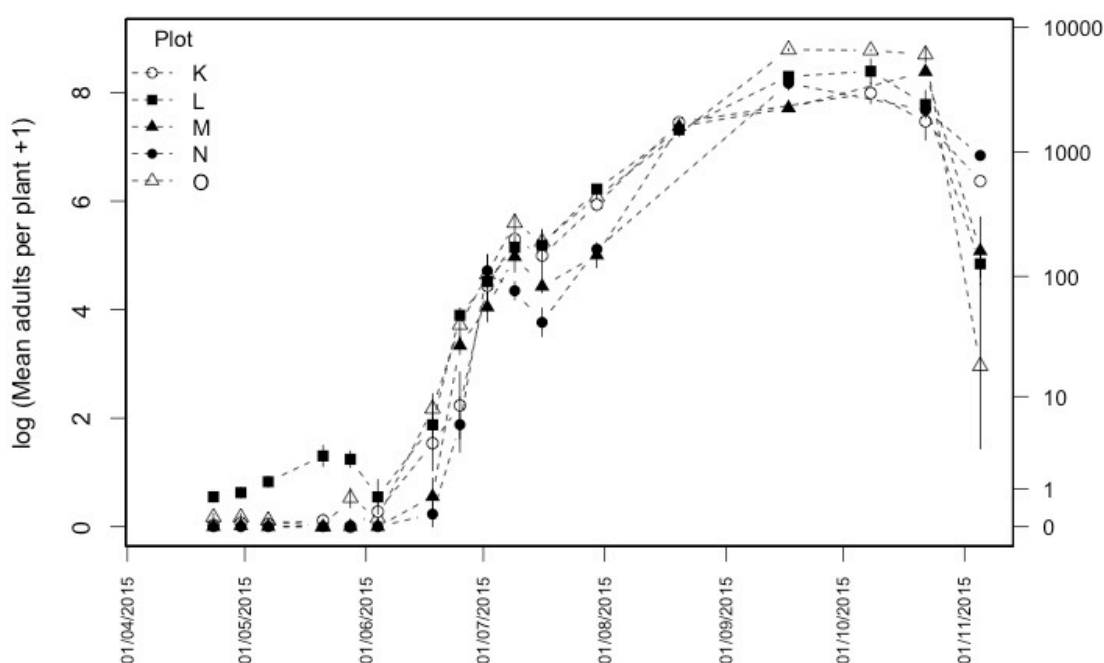


Figure 4.37. Mean (\pm SE) number of adult *Aleyrodes proletella* per plant from April 2015 until November 2015 for the 5 study Plots (K, L, M, N, O). The left-hand axis shows log+1transformed data while the right-hand axis shows back-transformed data.

Numbers of adults began to increase in all Plots in the beginning of June, with all plots exceeding 10 adult *A. proletella* per plant by July. The first exuviae were not recorded on the plants until July, when a sudden increase from 0 to ~10 per plant was recorded, (Figures 4.38-3.42).

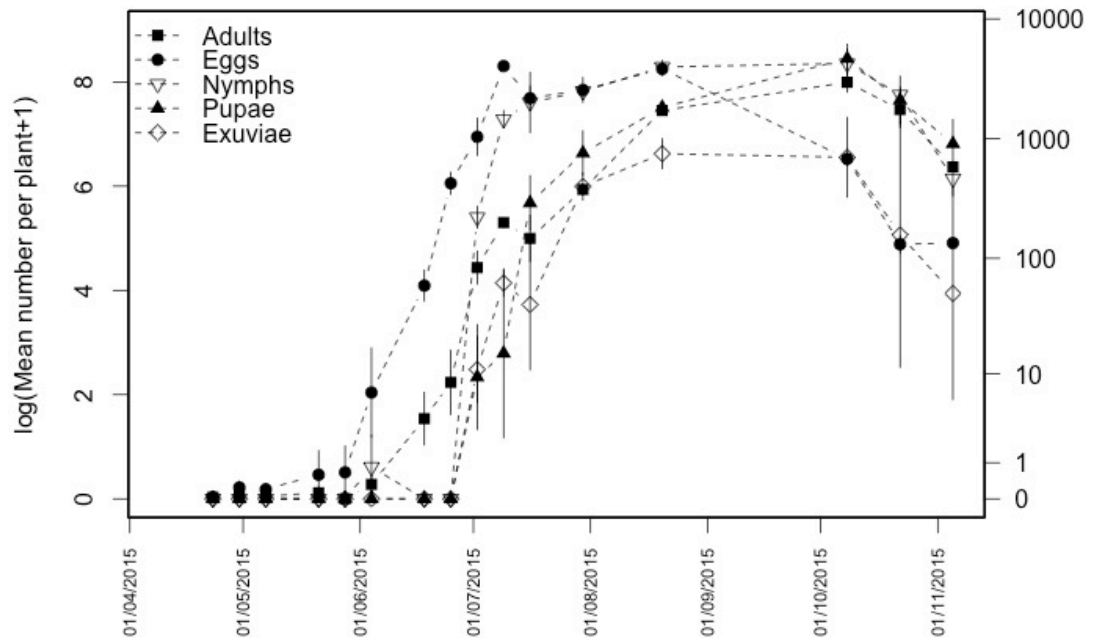


Figure 4.38. Mean (\pm SE) number of each life stage per plant for Plot K in 2015. The left axis shows $\log+1$ numbers while the right hand axis shows back- transformed data.

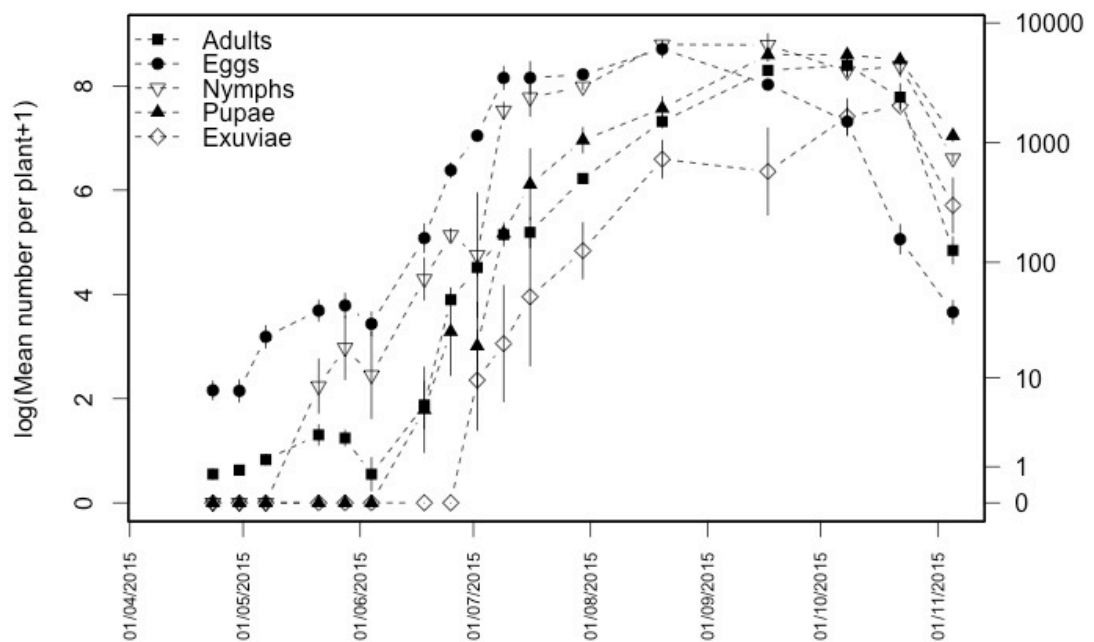


Figure 4.39. Mean (\pm SE) number of each life stage per plant for Plot L in 2015. The left axis shows $\log+1$ numbers while the right hand axis shows back- transformed data.

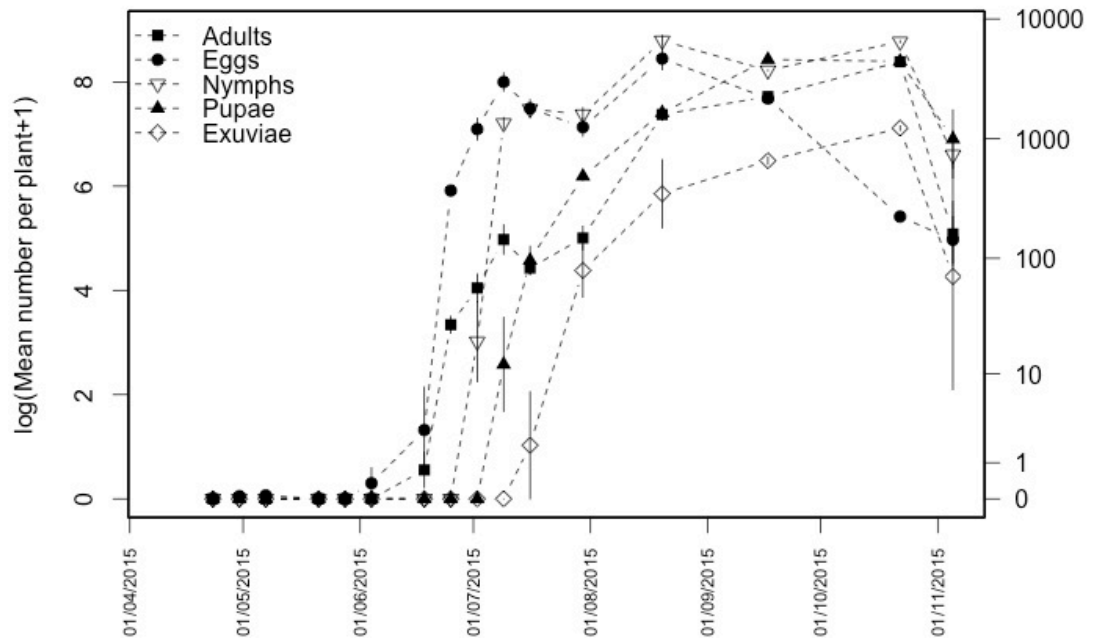


Figure 4.40. Mean (\pm SE) number of each life stage per plant for Plot M in 2015. The left axis shows log+1 numbers while the right hand axis shows back- transformed data.

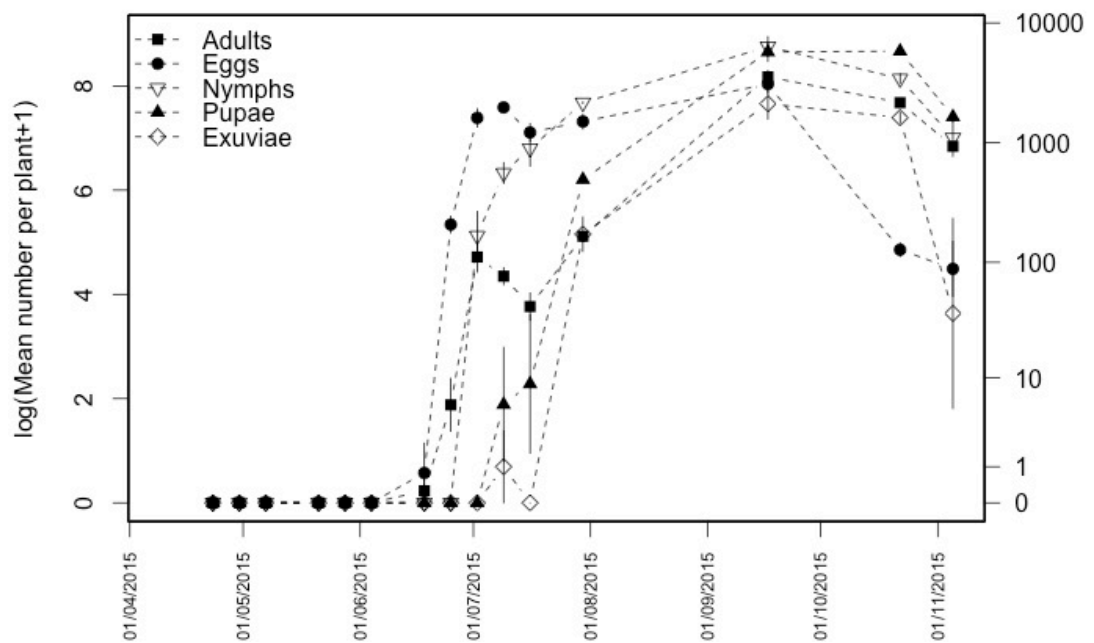


Figure 4.41. Mean (\pm SE) number of each life stage per plant for Plot N in 2015. The left axis shows log+1 numbers while the right hand axis shows back- transformed data.

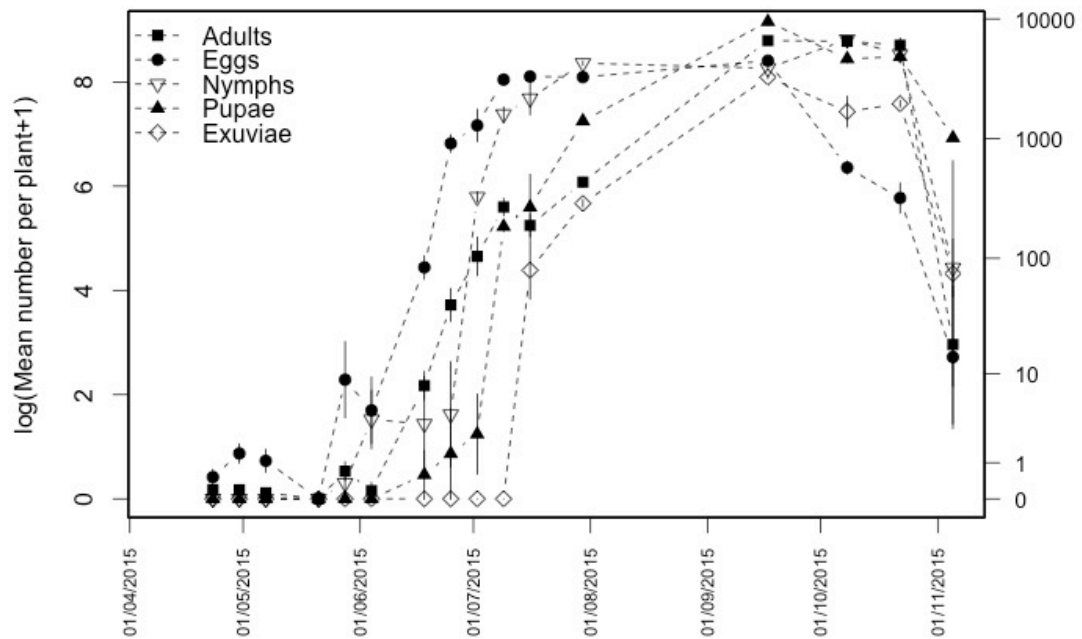


Figure 4.42. Mean (\pm SE) number of each life stage per plant for Plot O in 2015. The left axis shows log+1 numbers while the right hand axis shows back- transformed data.

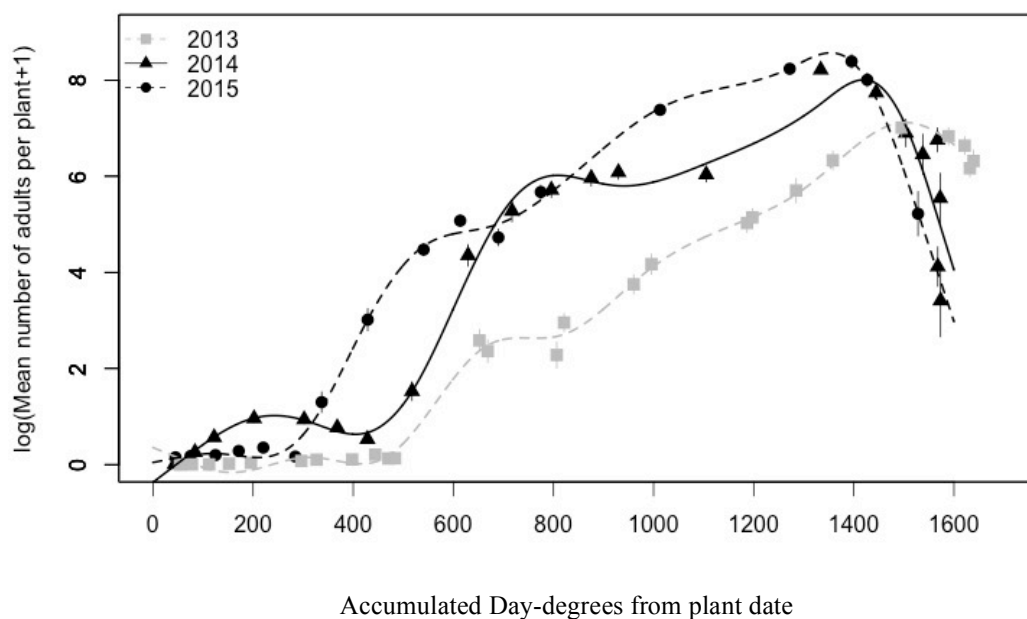


Figure 4.43. Log-mean (\pm SE) number of adults per plant plotted against accumulated day-degrees from planting date. A smoothed trend line is shown for each experimental year. 2013-Experiment 4.5, 2014-Experiment 4.6, 2015 -Experiment 4.7.

Figure 4.43 shows the log-mean number of adults per plant from all the monitoring plots in Experiment 4.5, Experiment 4.6 (May plantings only) and Experiment 4.7 plotted against accumulated day-degrees (Lower threshold 6.3°C, Experiment 3.2) from the planting date with a minimized residual smoothed curve created by a general additive model (Wood, 2006). These smoothed curves show an initial small increase in adult numbers with a marked increase after approximately 400-500 day-degrees had been accumulated for 2013 and 2014 plots. This increase in numbers continued as day-degrees were accumulated throughout the season. A less defined increase in numbers of adults can also then be seen after ~1000 accumulated day-degrees. The increase in numbers of 2015 plots increases slightly earlier at ~300 day-degrees and then the second at ~700 day-degrees. A decline in all plots was noted after ~1600 day-degrees that coincided with the onset of winter, with the decline in 2014 and 2015 at a much more dramatic rate.

4.4 Discussion

4.4.1 Host plants.

4.4.1.1 Wild hosts

Determining the density of wild host plants in different habitats will potentially give an estimate of populations of *A. proletella* that might be supported in these areas and such plants may be important reservoirs for females that could colonise newly-planted *Brassica* crops. Newly-disturbed ground is likely to support relatively large numbers of wild hosts of *A. proletella*, (e.g. *Sonchus* spp., Fenner, 1978), which may in turn support large populations of *A. proletella*.

The surveys of wild hosts undertaken suggest however that the populations of *A. proletella* were too low to be detected through the survey methods described in this chapter, as no *A. proletella*, at any development stage, were found in either 2013 or 2014 in the surveys of wild hosts conducted on uncultivated fields. In 2012, the weather conditions were considered to be very unfavourable for the development and survival of *A. proletella* and this may account for the generally low numbers overwintering on wild hosts in 2013 (Springate and Colvin, 2013).

The field used in this investigation had been uncultivated since 2008 and all plants were the product of natural colonisation. Grass species dominated the area and these are not known to be hosts of *A. proletella*. Some of the most numerous wild hosts of *A. proletella* found during this investigation were *Sonchus* species. These latter species are known to be one of the first colonisers of bare soil (Fenner, 1978). It is likely that higher densities of this host plant would be found if this study were to be repeated using land that was cleared approximately a year previously, or on ‘disturbed’ ground, and this might lead to the development of larger populations of *A. proletella*.

Again, no *A. proletella* were found in the second survey of wild host plants in 2014. The previous year, 2013, could not be described as a poor year for *A. proletella* as the plots, which were part of Experiment 4.5 supported populations exceeding 1,000

adults per plant and showed very high levels of population growth. The survey field was also in close proximity to Plot B (~100m) which supported a high population of *A. proletella* at the time of the survey, >100 adults per plant. It is therefore interesting that no wild host plants surveyed supported any stage of *A. proletella*.

Aleyrodes proletella do, however, occur on wild hosts at Wellesbourne as spot checks of *Sonchus* and *Euphorbia* plants in locations other than uncultivated fields showed that they supported this species. The fields, that were fallow for at least 2 years, seemed to have had lower populations of *A. proletella* per host plant than host plants found in hedgerow or roadside conditions. A number of *Sonchus* spp. plants supported populations of whitefly exceeding 30 individuals but these were often in locations isolated from other areas of dense vegetation, such as car parks.

The data collected in this study suggest that *A. proletella* is not always present where there are wild hosts and, wild hosts in close proximity to known populations on cultivated hosts did not harbour *A. proletella*. *Sonchus* plants in a car park locations did however regularly support many individuals.

These observations suggest that the distribution of *A. proletella* in the environment is not determined purely by the distribution of wild host plants. Other factors are likely to govern their distribution, either environmental conditions or increased predator pressure in areas of high vegetation density limit their abundance in such areas. It should be noted that the wild host surveys were conducted in April, as the intention was to determine the potential number of overwintering *A. proletella* that could be supported by wild hosts; however this time of year also corresponds to the period in the season when populations of whitefly are at their lowest. To increase the likelihood of finding wild hosts that support *A. proletella* it is suggested that a survey of wild hosts should be conducted late in the season, (e.g. September), as larger populations of *A. proletella* would be expected, giving a higher probability of finding them in such a survey.

4.4.1.2 Oilseed rape

When a commercial crop of oil-seed rape (OSR) was surveyed in 2013, no *A. proletella* were found within the sampling areas in April or June. In April, the

percentage of ground covered by OSR was very low (10%), a result of heavy feeding damage by pigeons earlier on. The field was nearly completely defoliated over the winter, providing little foliage that could support overwintering female *A. proletella*. Furthermore, any *A. proletella* that were overwintering on the OSR would have been disturbed by the pigeons.

The final survey in July 2013 showed an average of 0.1 adults per 0.25 m²; a total of 4 adults were found from 40 x 0.25 m² quadrats. These adult whiteflies could have either been immigrants from nearby wild hosts, or there is the possibility that they were the progeny of very low numbers of overwintering females within the crop. Adult *A. proletella* show an aggregated distribution (Experiment 4.1) and therefore more sampling points would be needed to reliably estimate the mean population size at low densities (Cochran, 2007).

Taking this as an estimate for the entire field indicated a potential for the 10 ha field to support approximately 40,000 adult *A. proletella* [(0.1 x 4) x 100,000]. This is likely to be an over-estimate since all of the insects were found within 40 m of the field margin and sampling in commercial fields of kale (Experiment 4.2) indicated that adult *A. proletella* were more abundant close to field edges. As such low numbers of *A. proletella* were observed it was not possible to analyse their distribution in more detail.

The numbers of overwintered *A. proletella* on OSR during 2015 were variable across the Wellesbourne Campus. There was considerable variation in numbers over the relatively small area. This difference was further increased later in the season when no adult *A. proletella* were found in OSR Field A, but ~4 per 0.25 m² were found in OSR Field C. Thus there was an increase in *A. proletella* in one field and a reduction in another. This suggests that local conditions are having an impact on the potential of OSR to act as a reservoir for overwintering *A. proletella*. The increase in *A. proletella* numbers in OSR Field C could not have occurred due to reproduction within the crop as the first generation had not yet developed from the earliest eggs laid, shown by data from Experiment 3.3. The most likely cause for this increase would be immigration by individuals performing trivial dispersal from the nearby experimental Plot M (kale), which, in April supported over 200 adult *A. proletella*

per plant. In contrast, Plot G (also kale) adjacent to OSR Field A supported only 20 adult *A. proletella* per plant.

4.4.1.3 Brussels sprout and kale.

The initial adult populations on kale and Brussels sprout plots were similar in size, suggesting no difference in the ‘attractiveness’ of the host to the immigrating *A. proletella*. The numbers of eggs were also similar on both crops, suggesting a similarity in host quality for influencing the fecundity of adult females, supporting the result found from Experiment 3.1. Differences between the two crops became apparent after August, by when an entire generation would have developed on the plots and at this time kale appeared to be a slightly better host. Differences were most pronounced over winter, suggesting that overwintering females may prefer or survive better on kale plants. It could be that the structure of kale plants provides more protection for *A. proletella*, causing them to be less easily disturbed by rain or wind, for example. Activity of *A. proletella* was shown to be higher on Brussels sprout plants (Experiment 2.2) and this may have led to more adults being dislodged when compared when kale, leading to a net decrease in the numbers of adults on Brussels sprout plants when compared with kale.

4.4.2 Spatial distribution

All life stages of *A. proletella* showed aggregated distributions. Estimations of field populations on crops are likely to have large error when the insects are at low densities: large numbers of plants should be sampled to ensure an accurate estimate of population size (Cochran, 2007). The vertical distribution of life stages shows that sampling should incorporate all leaf ages. Inspecting only young leaves, for example, will only provide information on adults and eggs. Information on pupal numbers would only be acquired by sampling some of the oldest leaves, and such data could be important as it will indicate the potential size of the next adult generation.

A clear ‘edge effect’ was shown when kale crops were surveyed. The numbers of *A. proletella* were significantly higher on plants on the edge of the crop than on those towards the centre. Indeed, nearly four times as many adult *A. proletella* were found

at the edge of a field when compared to a distance of 45 m into the crop (75 plants into the field at 60 cm spacing). The sizes of populations of *A. proletella* were different between fields and also significantly different between field edges within fields. Field A (Experiment 4.2) supported the largest population and was planted the earliest, and this supported 10 times as many adult *A. proletella* as the other fields.

The data from only two of the three fields could be analysed with respect to the aspect of the field edges sampled and it seemed that aspect had no consistent effect on the numbers of *A. proletella* found. For example, the northern edge had largest numbers in Field A but not in Field B. Such distributions have not been found for *B. brassicae*, since field edges often supported some of the lowest populations, which was likely to be due to the increased levels of predation by natural enemies from nearby areas (Van Emden, 1965).

Information on the distribution of *A. proletella* may help growers to survey fields more effectively and they should be aware that if counts are always made in the same location then they may not provide a true representation of the field as a whole as populations of *A. proletella* are likely to be localized with high levels of variation within and between fields in the same general locality.

No edge effect was observed for the OSR fields surveyed in January 2015. The fields were, however, shown to support different numbers of *A. proletella*. Adults that were present within OSR in January were likely to have arrived in late Autumn when temperatures were warm enough to support flight, as the lower threshold temperature for flight has been estimated at 9°C in *A. proletella* (Butler, 1938b). The *A. proletella* are likely to have been within the field for at least a month and in this time it is possible that trivial movement between plants had occurred.

The lowest numbers of *A. proletella* were found towards the field edge, contrary to the pattern found in kale crops in August. It could be that *A. proletella* feeding on plants at the immediate edge of an OSR crop are subject to increased disturbance from pigeons, or increased predation from generalist predators that are living within the field margins, as found for *B. brassicae* (Van Emden, 1965). The dispersal behaviour of *A. proletella* has been shown to be different over winter and summer

(El Khidir, 1963), it is likely that this difference in behaviour, short trivial dispersal over summer and a higher proportion of long range migration from September, would lead to a difference in their distribution in crops during the respective seasons. All OSR fields surveyed in 2015 were surrounded by hedgerows, such ‘barriers’ to aerially flying insects have been shown to create a lower population of colonising insects in the ‘shadow’ of the hedge and this may have impacted on the distributions within the field (Lewis, 1969).

4.4.3 Immigration

Although they were very low in number (0.04 adults per plant 36 days after planting), the first adult *A. proletella* arrived in the plots planted in 2013 in mid-May 2013. This was before the first adult generation of the year had emerged on an overwintering crop in the same location; on 28th June (Experiment 3.3). This provides evidence that the first females to colonise the plots were overwintering females and not newly-emerged first generation females as proposed by Butler (1938a). In addition, the first colonisation of plots occurred before the first generation had developed in both 2014 and 2015, further supporting the notion that overwintering females can, and do colonise plots.

In 2014 the immigration rates were much more varied and those in the plots planted in May and June seemed to be influenced by the distance of these new plots from plots supporting overwintering females; such plots were not present in 2013. There was an approximate decrease in the number of immigrants of 25% for an increase in distance of 100m from a source of overwintering females. This relationship no longer applied from July onwards, probably because the May and June plantings began to act as a source of adult *A. proletella*. The May 2014 planting of Plot I supported ten adults per plant when the July plot was transplanted, and could have influenced the immigration rate considerably.

4.4.4 Population Trends

In 2013 there was, in general, little difference in the number of all life stages of *A. proletella* between the five plots, although a lower number of adults were evident in Plot C on the Brussels sprout plants. This plot was particularly damaged by caterpillars, which significantly reduced the overall leaf area of the plants. This is likely to have had an impact on the availability of locations for *A. proletella* to populate. Brussels sprout plants in general were more damaged as a result of herbivory by caterpillars and this reduced leaf surface area may have partly contributed to lower numbers of *A. proletella* seen on them. This may not be the complete reason for the difference in numbers, as *A. proletella* never populated 100% of the leaves of the Brussels sprout plants which indicates that leaf surface area may not be the limiting factor.

Numbers of *A. proletella* increased quickly in this study; the mean number of *A. proletella* per plant in Plot F in Experiment 4.6 exceeded 10 after only 36 days. In contrast, this did not happen in 2013 (Experiment 4.5) until after 2 months. One factor that may have contributed to this difference was the larger overwintering population of *A. proletella* in the Wellesbourne campus in 2013-14 than in 2012-13. The plots from the 2013 study were still present in early 2014 and were likely to have been the main source of immigrating *A. proletella* into the 2014 plots.

The statistically-significant model relating the numbers of *A. proletella* on each new plot, after 22 days, to its distance from the nearest source of *A. proletella* supports this suggestion. The closer a 2014 plot was to a plot planted in the previous year, the higher the numbers of *A. proletella* it received. Doubling the distance from the source from 50 m to 100 m seemed to lead to a 75% reduction in the numbers of *A. proletella* arriving. This suggests, a very reasonable conclusion, that the rate of colonisation by *A. proletella* into new crops is highly influenced by the distance of the new crop from sources of overwintering females. Important sources are likely to be overwintered brassica crops such as OSR, kale and Brussels sprout.

In all the plots planted in 2013 and 2014, populations of *A. proletella* adults increased dramatically in July and this coincided with an increase in the numbers of

exuviae. It is highly likely that this increase in the number of adults was due to the emergence of the progeny of earlier immigrants rather than to a sudden immigration of adults from elsewhere.

Such a pattern of population development did not occur in 2015; all plots showed an increase in the numbers of *A. proletella* in June that did not coincide with the first occurrence of exuviae. This increase in numbers was unlikely to be due to the development of a complete generation within the plot. The increase in numbers occurred when the plots planted in 2014 and the commercial OSR fields on site had begun to flower and senesce. Early 2015 supported a dramatically higher area of commercial OSR at the Wellesbourne campus, ~25ha. Compared to only ~5ha in 2014. As mentioned previously, the oilseed rape was known to be populated with *A. proletella* early in the year (Experiment 4.4) and it is highly likely that the increase in numbers in June in the 2015 study plots was due to immigration from the nearby surrounding OSR fields.

Following the 2013 plots through to flowering and senescence showed that the numbers of all life stages began to decrease dramatically from June 2014. The declining leaf area provided little or no habitat for any life stage and this is likely to have been the cause of the decline. As new adults were known to have emerged, as the numbers of exuviae had briefly increased from May onwards, the reduction in the numbers of adults is unlikely to have been caused by mortality. It is likely that newly-emerged adults left the plants in June, due to the lack of available new leaves to feed on. They probably moved onto new hosts in the surrounding area. As such, the reduction in the numbers of adults in the plots planted in June 2013 coincided with immigrations into the plots planted in June 2014 (Experiment 4.6). It was the June plantings in 2014 that showed the highest rates of immigration. The negative relationship between the immigration rate into the new plots planted in 2014 with their distance from the old plots planted in 2013 suggests that the *A. proletella* were performing trivial dispersal, possibly alighting on the first host plant they encountered, which shows a similarity to the dispersal characteristics of *B. tabaci* (Gerling and Horowitz, 1984).

When plotting all the populations in all the study plots against accumulated day-degrees, a pattern common to the 2013 and 2014 experiments appeared. The populations remained relatively low for the first ~400 day-degrees, and then began to increase rapidly. The population continued to increase throughout the season, with a slight second change in the rate of increase after ~1000 day-degrees. These population trends suggest that the first increase in numbers occurs after development of the first generation, and emergence of adults of the second generation can also be seen, to a lesser extent, at 1000 day-degrees. The timing of such population increases are very similar to those that would be expected from the day-degree model (Experiment 3.2) when the first generation would occur at ~455 day-degrees and the second at ~910 day-degrees. It would be sensible to conduct further population monitoring of *A. proletella* to test the reliability of the day-degree model for forecasting population trends of *A. proletella* in vulnerable crops. Such a forecasting system may inform growers of the times when numbers of *A. proletella* might increase and could be used to suggest when they should monitor their crops for pests to indicate potential timings for pesticide applications.

It should be noted, however, that in 2015 the plots showed an increase in the size of the *A. proletella* population earlier than predicted by the day-degree model. The reason for this is discussed above. The second increase in numbers, potentially the second generation, did however, occur at ~700 day-degrees, approximately 400 day-degrees after the first population increase. This value is close to the theoretical timing for completion of a generation following the period of immigration at 300 day-degrees, $[300 + 455 = 755]$.

Interestingly the plots planted in 2013 appeared to have consistently lower populations throughout the season than those planted in 2014 or 2015, but the numbers of *A. proletella* seemed to increase at approximately a similar rate. It is likely that final population size reached in any year is related to the initial level of immigration and the number of generations developing subsequently within the crop, with little variation in the rate at which the population grows.

5 Natural Enemies of *Aleyrodes proletella*

5.1 Introduction

5.1.1 Whitefly predators

Whitefly predators have been documented from the orders Coleoptera: Coccinellidae, Hemiptera: Anthocoridae, Miridae, Diptera: Drosophilidae, Dolichopodidae, Empididae, Syrphidae, Neuroptera: Coniopterygidae, Chrysopodidae, Acarina: Phytoseiidae and Aranae. A number of these species have been recorded feeding on whitefly species that were recorded outside of their normal endemic ranges and are generalist 'scale predators' (Gerling, 1990). Outbreaks of pest whitefly (e.g. *Bemisia tabaci*) are often linked with use of indiscriminate insecticides that kill polyphagous predators and it is believed that these predators often exert the most control on whitefly (Gerling, 1990). Although whitefly have been recorded to be consumed by the above predators, numbers of whitefly consumed are often lower when aphid prey are also available, suggesting that preference for many whitefly predators is towards the aphid prey (Ekbom, 1981). Whitefly predator biological control has often had little success when compared with parasitoid control. Few studies show whitefly specific predators having much control on whitefly populations (Gerling, 1990).

5.1.2 Parasitoids.

These include parasitoid wasps from the Families Platygasteridae, Aphelinidae and Eulophidae. The most studied group of parasitoid wasps of whitefly are the *Encarsia* wasps with ~150 species known to develop within the nymphal stages of species of Aleyrodidae. Parasitoids have been studied to a great extent with regards to whitefly population control with a number of biological control methods available for control of whitefly pests namely for protected crops in glasshouse conditions (Gerling, 1990). Mass rearing of *Encarsia formosa* began as early as the 1920s and introductions of this parasitoid have shown to be very effective in controlling pest numbers of *B. tabaci* and *Trialeurodes vaporariorum* (Hoddle *et al.*, 1998).

5.1.3 Pathogens

Only fungal pathogens have been recorded in whitefly, the cuticle offers great protection from bacteria and viral infection, which likely only infect through damaged cuticle walls. The whitefly specific fungal pathogen *Aschersonia* produces *conidia* (spores) within pycnidia and are fusoid or narrow-oval with pointed ends. The species are widespread, however, often prefer warm climates near to the tropics (Meekes, 2001). Broad-spectrum fungal pathogens infecting a diverse range of invertebrate hosts, including whitefly, include; *Beauveria bassiana*, *Cladosporium*, *Erynia racidans* (now *Zoophthora radicans*), and *Verticillium*. Naturally occurring fungal pathogens have been documented impacting the Viburnum whitefly (*Aleurotrachelus jelinekii*) in the UK, likely *Cladosporium spp.* This pathogen has been documented as being the main factor in adult mortality, however these infections were not seen to have an impact on populations of the species and were not causing a controlling effect (Hassel *et al.*, 1987).

5.1.4 *Aleyrodes proletella* specific natural enemies.

Very low rates of predation have been seen on this species within the field in the UK and of those witnessed they are likely feeding upon *A. proletella* when it is very abundant and their normal diet of aphids is scarce. Larvae of *Syrphus cinctus* and *Syrphus auricollis* have been observed feeding upon the pupae but the degree to its selectivity to *A. proletella* is unknown (Butler 1938b). The coccinellid *Clitostethus arcuatus* has been shown to develop completely on a diet consisting only of eggs and nymphs of *A. proletella*, feeding on 10,000 eggs over a lifetime of 150 days. (Loi, 1978; Mota *et al.*, 2008). In contrast, *Coccinella undecimpunctata* had a significant reduction in survival and longevity when given a diet of solely *A. proletella*. Mature females are unable to produce eggs when offered only *A. proletella* suggesting it was unsuitable and most likely an alternative prey species (Cabral, *et al.*, 2006).

Documented parasitoids of *A. proletella* include; *Encarsia inaron*, *E. japonica*, *E. lutea*, *E. partenenoepae*, *E. pergandiella*, *E. melanostoma*, *E. noahi*, *E. orientalis*, *E. tricolor*, *Eretmocerus orientalis*, *Euderomphale cerris*, *E. chelidonii*, *E. gomer* *E.*

insularis and the Dipteran parasitoid, *Acletoxenus formosus* (Butler, 1938b; Mound and Hasley, 1978; Gerling, 1990).

The two species of chalcid parasites *E. partenenoepae* and *E. tricolor*, occur within the UK however the latter was not recorded until 1938. Very few of these chalcids have been found within the field in the UK, cold winters are likely to reduce populations significantly as both species do not survive low winter temperatures well (Butler, 1983b; Springate and Arnold, 2012). It is possible that introductions from warmer parts of Europe or, very warm localities allowing overwintering, keep the species present.

A record of a fungal pathogen was made infecting *A. proletella* in Rep. of Georgia, where the pathogen *Aschersonia placenta* was released to control a pest whitefly species (Ponomarenko *et al.*, 1975). To the author's knowledge no naturally occurring fungal pathogen of *A. proletella* has been recorded in the field within Europe.

The aims of the experiments described in this chapter are to document the natural enemies acting on *A. proletella* in the field, including predators, parasitoids and pathogens. The potential each of the natural enemies may control field populations are investigated. Understanding natural enemies that may control population growth in *A. proletella* would be valuable to growers, allowing them to encourage those with a large impact on population growth and may lead to the development of a novel biological control strategy for this pest species.

5.2 Methods

5.2.1 Experiment 5.1. Prevalence of natural enemies

The numbers of naturally-occurring predators were recorded when conducting population sampling using the sampling methods described for Experiment 4.5, Experiment 4.6 and Experiment 4.7. Predators recorded included, earwigs (*Forficula* spp., Dermaptera), ladybirds (adults and larvae, Coccinellidae), pirate-bugs (Anthocoridae), hoverfly larvae (Syrphidae) and lacewing larvae (Neuroptera). Signs of the presence of other invertebrates were also recorded, for example slugs. A conscious effort was made to record any parasitism, indicated by blackening of the scales (Butler, 1938b). In order to identify hoverfly larvae to species, 10 hoverfly pupae were removed from the field in July 2015 and kept separately in Petri dishes in laboratory conditions (20°C, 16h light, 8h dark), until they emerged as adults and could be identified more easily from adult morphology (Gilbert, 1986).

5.2.2 Experiment 5.2. Predator feeding assays – slug, earwig, hoverfly larvae and ladybird larvae.

An experiment was set up to test the predation rates of slugs, earwigs, hoverfly larvae and ladybird larvae on *A. proletella* using Petri dishes as arenas. Earwigs were collected within the Wellesbourne Campus. Refuges, 30 x 9 cm corrugated cardboard rolled into a cylinder, were placed in hedgerows and left overnight to ‘collect’ earwigs. All earwigs were removed from the refuges the next morning and kept individually in empty Petri dishes lined with dampened filter paper (Whatman[®], Grade 1, 90mm). The most common species of slug observed within kale plants as part of Experiment 4.5 was identified as *Limax marginatus* using keys from Cameron, (2003). These slugs were numerous on the Wellesbourne Campus and were chosen to be used as the study species for the slug feeding assay. All experimental slugs were found in and around pots of Brussels sprouts plants in the Dutch Lights area (Figure 2.1).

Hoverfly larvae of the same species, later identified as *Episyrphus balteatus* (Gilbert, 1986), were hand-collected from the kale plants used in Experiment 4.7, Plot L. Care

was taken to only include third instar larvae, using size as an indicator of developmental age.

Ladybird larvae were hand-collected from kale plants in Experiment 4.6, Plot F. Care was taken to only include larvae of *Coccinella 7-punctata* in the second or third instar. Identification to species was achieved using the distinctive orange markings of larvae.(Roy *et al.*, 2013)

The tests were set up to determine how many, if any, *A. proletella* nymphs each predator would eat over a 24 hr period. The *A. proletella* were offered as second to third instar nymphs, feeding on an 11 mm diameter leaf disc (cauliflower). Control treatments containing no *A. proletella* were set up for both slugs and earwigs, as both species are known to be omnivorous, together with a choice test to ascertain if feeding on *A. proletella* was preferred to feeding on an uninfested leaf disc. All experimental treatments/conditions are shown in Figure 5.1.

Leaf discs infested with *A. proletella* were taken from a laboratory culture of *A. proletella* where second to third instar nymphs were developing on leaves at 20°C 16h light, 8h dark. Leaf discs were used only if >70% of the surface area was covered with nymphs, which approximated to 80-120 individuals. Uninfested leaf discs were taken from the leaves of plants grown in 'clean conditions' with no pests. All leaf discs were of cauliflower cv. Skywalker (Elsoms Seeds Ltd), from plants of at least the eighth true leaf stage and using leaf discs from the fourth youngest leaf. All study predators were collected, starved for 12 h and then used in the feeding assay. All earwigs and slugs were weighed prior to starting the assay $\pm 0.001\text{g}$ (Mettler, AM100).

5.2.2.1 Experimental conditions

Filter paper was used to cover the bottom of a 9 cm Petri dish, with 1 ml of distilled water added to provide moisture for the test predator and to prevent the leaf disc from drying out. Prior to starting each test a count was made of the *A. proletella* present on each leaf disc using x40 microscope. The leaf discs were put in place before the study predator was added to the centre of the Petri dish.

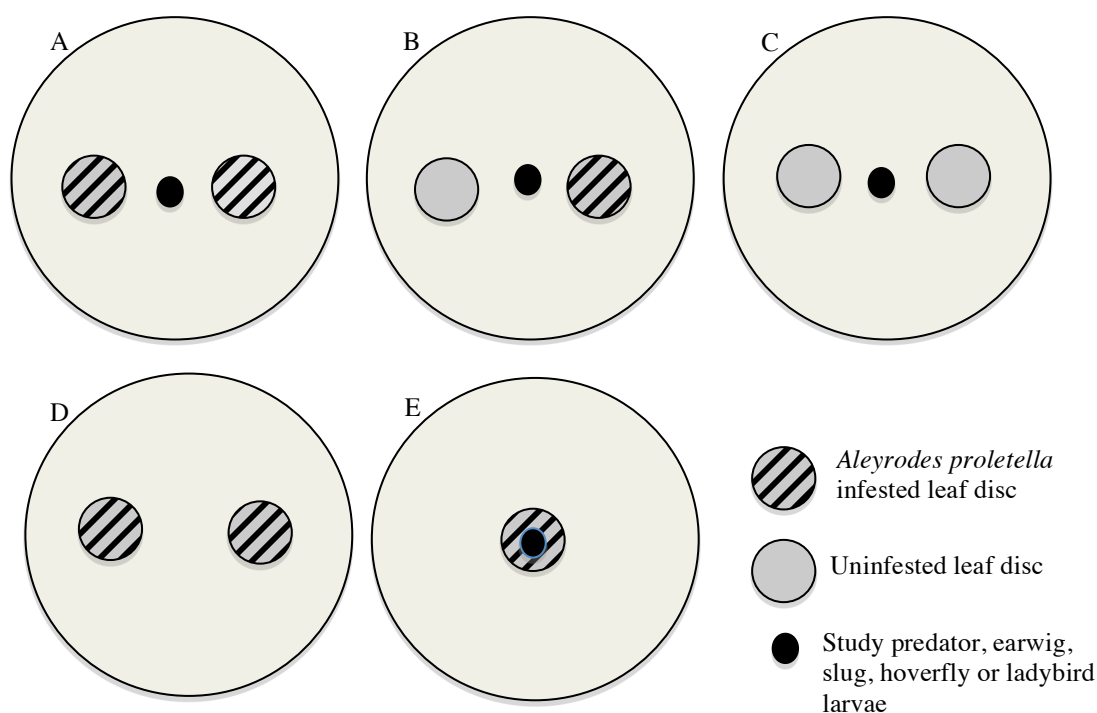


Figure 5.1. Diagram showing Petri dish arena treatment conditions (A-E) for Experiment 5.2, A-C for slug and earwig assay. A- Two *Aleyrodes proletella* leaf discs, B – Choice, *A. proletella* and uninfested leaf disc, C – uninfested leaf discs only. D – No predator Control – *A. proletella* leaf discs no predator. E – Hoverfly or Ladybird assay – *A. proletella* leaf disc only.

After adding the predator, the Petri dishes were all placed in a controlled environment room for 24h at 20°C, 16h light, 8h dark. After this time each leaf disc was assessed, when a second count of *A. proletella* was taken. For the slug and earwig assays, the percentage of each leaf disc remaining was recorded. The control discs were also assessed in case any *A. proletella* died during the test, or the leaf discs desiccated, appearing to be eaten. Slugs and earwigs were tested in conditions A, B and C (Figure 5.1) with eight replications in each. Hoverfly and ladybirds were tested in treatment E (Figure 5.1), with 8 replicates. There were 8 replicates of the *A. proletella*-only leaf discs, treatment D (Figure 5.1). Initially the intention was to leave each hoverfly larva *in situ* for 24h. However, very few nymphs were consumed, therefore it was decided to leave the hoverfly larva *in situ* for a further 24h, 48h in total.

5.2.3 Experiment 5.3. Monitoring the occurrence of fungal pathogens in field plots infested with *Aleyrodes proletella*.

Dead adult *A. proletella* (an epizootic) were observed in the field from October 2014 onwards. They were first noticed within Plot G where a large number of dead adults were attached to the leaves with outspread wings. The numbers of dead *A. proletella* showing signs of fungal growth and outstretched wings were counted in conjunction with the normal population monitoring conducted in Experiment 4.6 and Experiment 4.7, following an identical procedure as for the *A. proletella* sampling, to give mean values per plant for each study plot.

5.2.4 Experiment 5.4. Isolation of fungal pathogen

Live adult *A. proletella* were brought into the laboratory from a Plot F (Experiment 4.6), May planting, where *A. proletella* were known to be infected with a fungus. Twenty-two live adults were surface-sterilized individually by immersing them in sodium hypochlorite (0.5% solution), then ethanol, both for 1 minute. They were then rinsed three times in sterile water. Each surface-sterilized adult was then placed upon a SEMA; Sabouraud dextrose, Egg yolk (11.5%), whole Milk (8.5%), agar plate (Lacey, 1997) and incubated at 20°C for 6 days. After 6 days, 8 of the 22 adults had begun to grow fungus of a similar creamy orange/yellow colour. The remaining 14 samples were left for a further 10 days but no fungal growth was seen.

Spores were naturally discharged from the fungus onto water agar where they were subsequently stained using lactophenol blue. Morphological traits were observed using a microscope at x100 magnification and measurements taken with an eye piece graticule calibrated at 160 EPU = 5 µm

5.2.5 Experiment 5.5. Genetic identification of fungal pathogens

Entomopathogen spp. cultures were initiated from stock isolated from surface sterilized *A. proletella* from the field, Experiment 5.4. An agar plug was removed and the mycelial mat was washed four times in sterilized RO water and blotted dry

with tissue paper. The mycelium was freeze dried overnight after which the DNA was extracted using a DNeasy Mini Plant Kit (Qiagen, Ltd. UK) using the manufacturers guidelines. Primers used are given in Table 5.1 using the PCR reaction mix shown in Table 5.2 and PCR conditions for each primer pair are given in Table 5.3.

Table 5.1. Primers used in methods to genetically identify Entomopathogenic fungus from Experiment 5.4.

ITS5 ^a	Forward	5'-GGAAGTAAAAGTCGTAACAAGG
ITS1 ^a	Forward	5'-TCCGTAGGTGAACCTGCGG
ITS4 ^a	Reverse	5'-TCCTCCGCTTATTGATATGC
ITS1-F	Forward	5'-CTTGGTCATTTAGAGGAAGTAA
Zf ^b	Forward	5'-GTATGCTCTCGGGTRTATTGTTGG
Zr ^b	Reverse	5'-TAGACTAATCYAWAACAATAATGCTC

^a White *et al.* 1990

^b Guzmán-Franco *et al.* 2008

Table 5.2. Generalised PCR reaction mix used for different primer combinations

REDTaq (Sigma-Aldrich, UK)	12.5µl
Forward primer	1µl
Reverse primer	1µl
DNA	1µl
Sterilised water	9.5µl
Total	25µl

Table 5.3. Primer pairs and conditions used for DNA replications to be used for genetic identification of entomopathogenic fungus.

Primer pair	PCR conditions
ITS1/ITS4	98°C 30s, 38 x (98°C 10s, 55°C 20s, 72°C 60s), 72°C 10m
ITS1/ITS4	94°C 2m, 40 x (94°C 35s, 55°C 55s, 72°C 60s), 72°C 10m
ITS1-F/ITS4	94°C 2m, 40 x (94°C 35s, 55°C 55s, 72°C 60s), 72°C 10m
Zf/Zr	95°C 3m, 35x (95°C 30s, 50°C 1m, 72°C 1.5m) 72.5°C 5m.

5.3 Results

5.3.1 Experiment 5.1. Prevalence of natural enemies

During Experiment 4.6 and Experiment 4.7 very few predators fed on the *A. proletella* nymphs. During 2014, the first predators observed in the plots were Coccinellidae and these were only present during June 2014 and at very low densities, less than a mean of 0.04 per plant. Apart from the presence of hoverfly larvae at a mean density per plant of less than 0.02, during most of the next 9 months no predators were seen whilst sampling Experiment 4.6 (Figure 5.2).

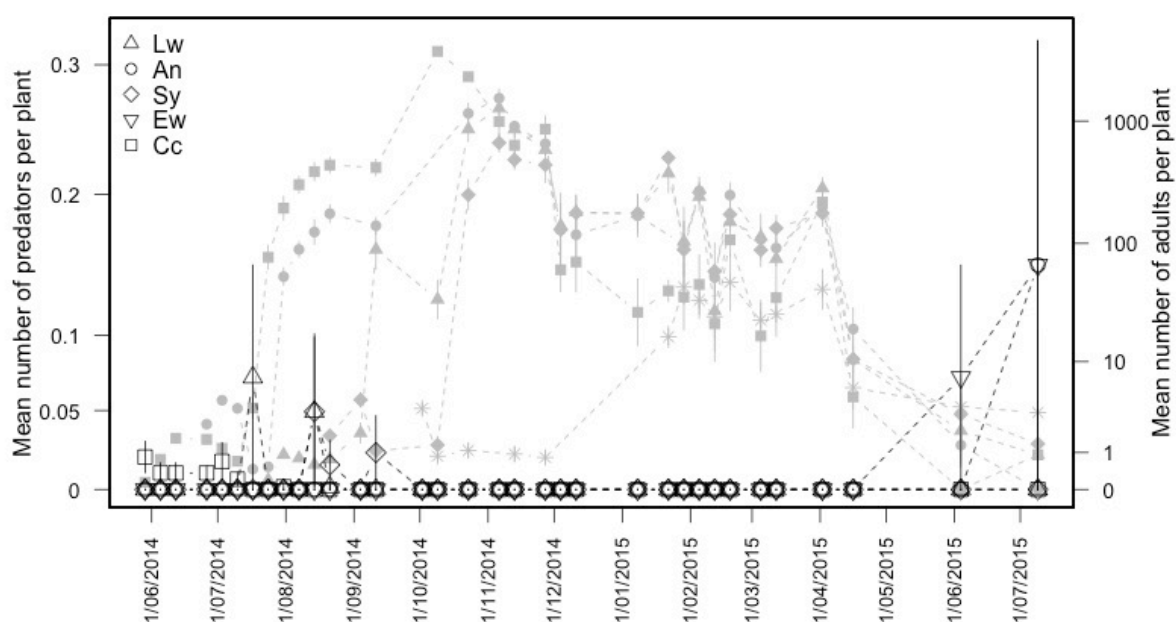


Figure 5.2. Mean (\pm SE) number of predators per plant per plot from Experiment 4.6 Lw = lacewing, An = anthorcorids, Sy = hoverfly larvae (Syrphids), Ew = earwigs, Cc = Coccinellidae. Mean (\pm SE) number of Adult *Aleyrodes proletella* from Experiment 4.6 is given in grey for plantings May (Squares), June (Circles), July (Triangles), August (Diamonds), September (Asterisks).

A rather sudden appearance of earwigs and anthorcorids occurred late into Experiment 4.6 from June 2015. There were approximately 1 per 10 plants assessed; the highest number of predators seen per plant during the entire experiment (Figure 5.2). Slugs were recorded relatively frequently while conducting Experiment 4.6

with mean numbers per plant often exceeding 0.2; greater than the average numbers of all other predator species sampled during the entire year.

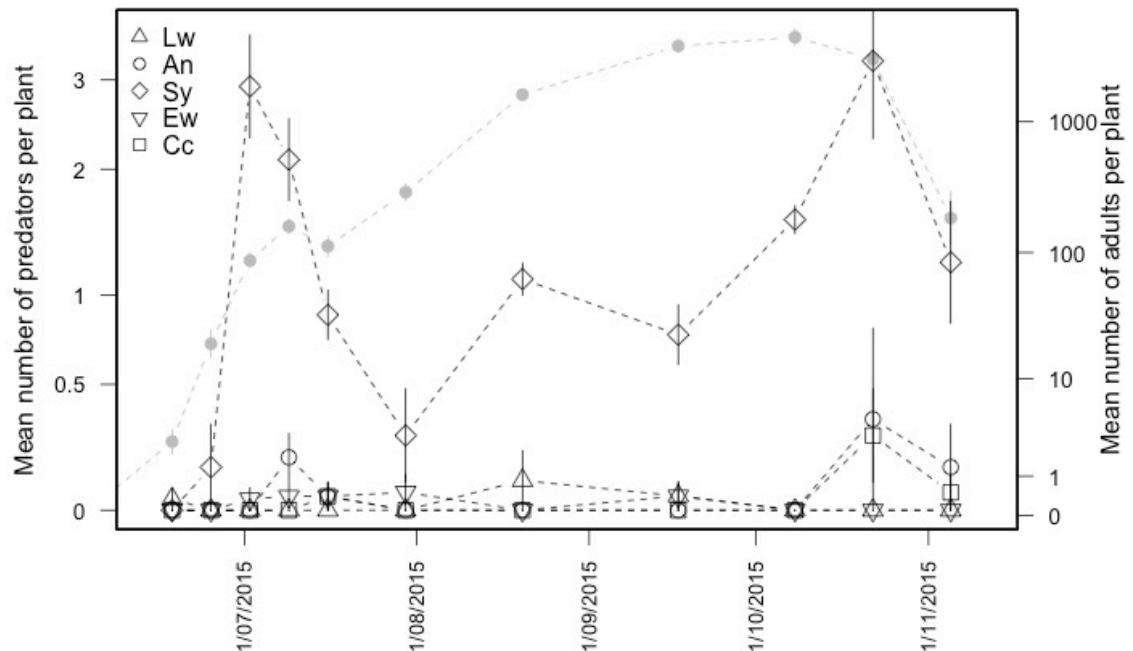


Figure 5.3. Mean (\pm SE) number of predators per plant for each plot from Experiment 4.7. Lw = lacewing, An = anthorcorids, Sy = hoverfly (Syrphids), Ew = earwigs, Cc = Coccinellidae. Mean (\pm SE) number of Adult *A. proletella* for the 5 plots in Experiment 4.7 is given in grey.

Relatively high numbers of predators were recorded in Experiment 4.7 when compared with Experiment 4.6. In Experiment 4.7 numbers of hoverfly larvae rose from late June to a mean of 3 per plant relative to a population of adult *A. proletella* of ~100 adults per plant. The numbers of hoverflies then declined, but they were present throughout the entire sampling period (August–November). Lacewing larvae were present during August in mean numbers per plant of less than 0.2; whilst adult *A. proletella* numbers exceeded 1000 per plant. Anthocorids appeared late in the season, together with Coccinellidae but at mean numbers less than 0.5 per plant when adult *A. proletella* numbers were over 100 per plant (Figure 5.3). All of the 10 hoverfly pupae removed from the field in July 2015 and kept separately in Petri dishes emerged as adults that were identified as *Episyrphus balteatus* (Gilbert, 1986).

5.3.2 Experiment 5.2. Predator feeding assays – slug, earwig, hoverfly larvae and ladybird larvae.

The mean number of *A. proletella* eaten by a slug over the course of 24h was 89. This, however, differed considerably between the treatments. Slugs given two leaf discs infested with *A. proletella* ate significantly more nymphs than those given one infested leaf disc. It should, however be noted this may be related to the fact that more nymphs were available to eat. A linear relationship was found, $y \sim 0.65x$, (ANOVA, $F_{(1,15)}=389.8$, $P<0.001$, Figure 5.4) showing that, in effect, an average of 65% of nymphs were eaten regardless of how many were present. The number of leaf discs available containing *A. proletella* (i.e. treatment) did not influence the linear model and could be removed (ANOVA, $F=0.63$, $df=2$, $P=0.55$, Figure 5.4), showing that treatment (i.e. the number of *A. proletella* infested leaf discs) had no impact on the percentage of *A. proletella* eaten.

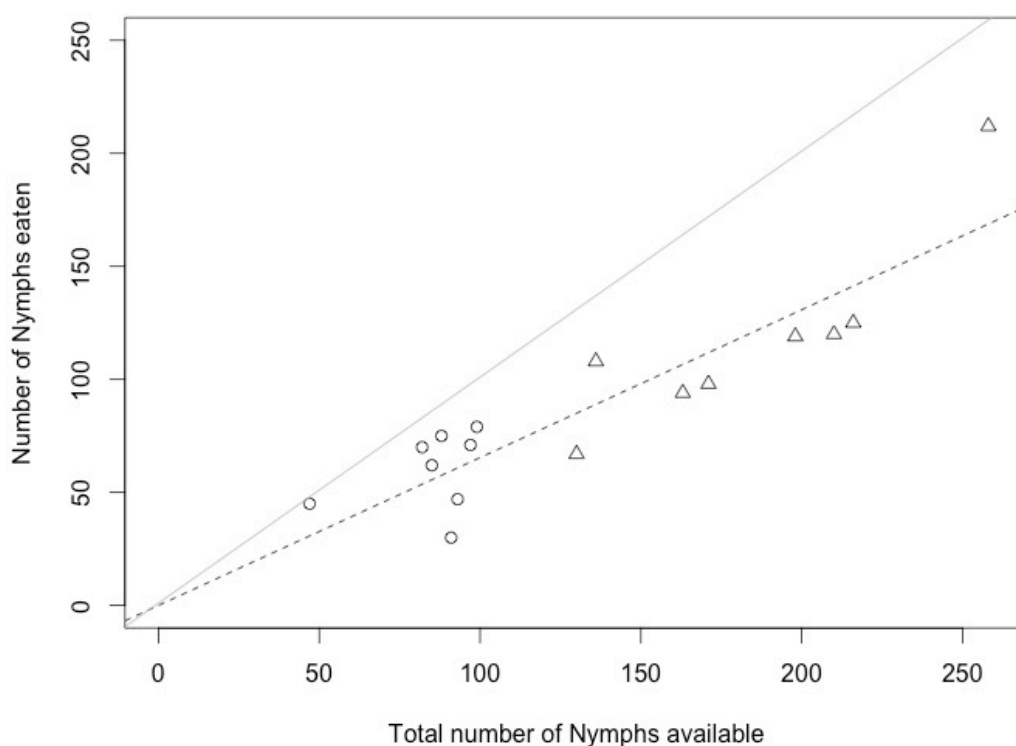


Figure 5.4. Number of *A. proletella* nymphs eaten by slugs plotted against the total number of nymphs available within the arena. The dashed line represents the statistically significant linear regression, $y=0.65x$ ($P<0.05$). The solid line represents the theoretical regression where 100% of nymphs were eaten regardless of the number available. Triangles = Treatment A, circles = Treatment B (Figure 5.1).

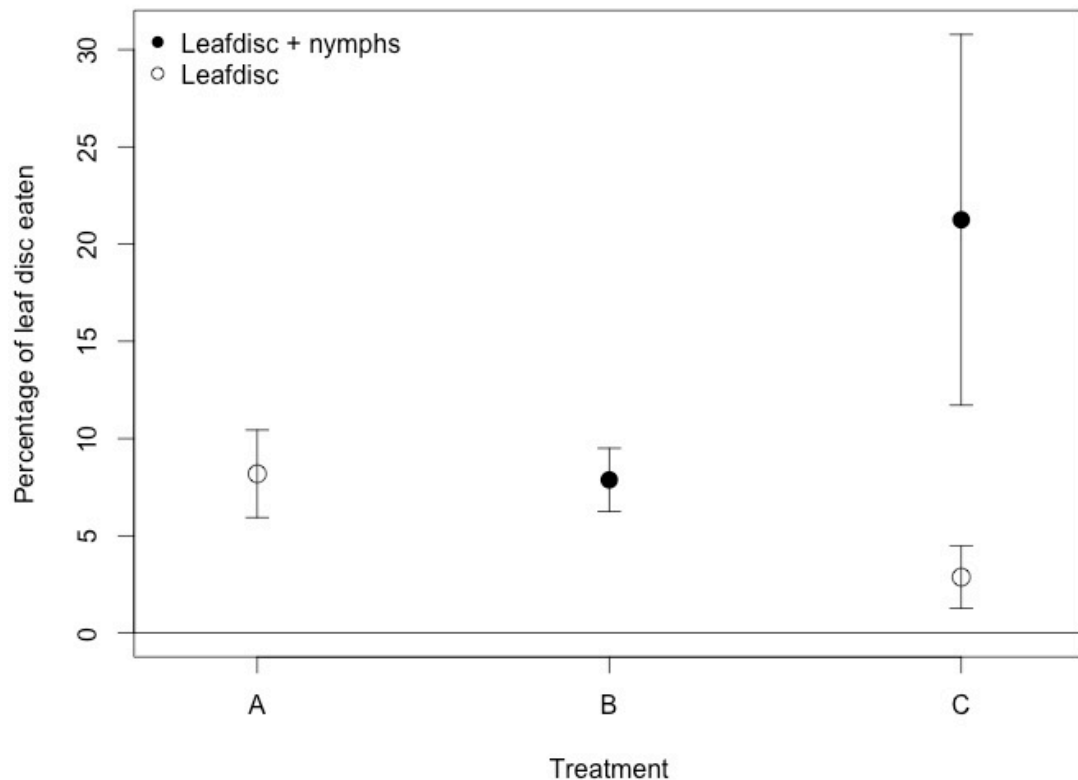


Figure 5.5. Mean (\pm SE) percentage of leaf disc eaten by slugs for each of the treatments. A – 2 x uninfested leaf discs, B – 2 x *A. proletella* infested leaf disc, C – 1 x uninfested leaf disc and 1 x *A. proletella* infested leaf disc.

The percentage of the leaf disc consumed did not differ between the treatments (ANOVA, $F_{(2,27)}=0.47$, $P=0.47$). Although the slug consumed over twice the amount of leaf discs infested with *A. proletella* compared with the uninfested leaf discs in the choice test (Figure 5.5) and this was found to be a statistically significant difference, slugs consumed more leaf discs infested with *A. proletella* than uninfested leaf discs (ANOVA, $F_{(1,27)}=9.05$, $P<0.05$, Figure 5.5).

There was a significant linear relationship between the proportion of nymphs eaten and the proportion of the leaf discs eaten by slugs ($y= 0.55*x+0.59$, ANOVA, $F_{(1,22)}=12.58$, $P<0.01$, Figure 5.6). A predicted value of ~60% of nymphs eaten was calculated when no leaf disc was eaten by a slug (i.e. y-intercept), indicating that a slug would still consume, on average, 60% of nymphs on a leaf disc even if none of the leaf itself was consumed.

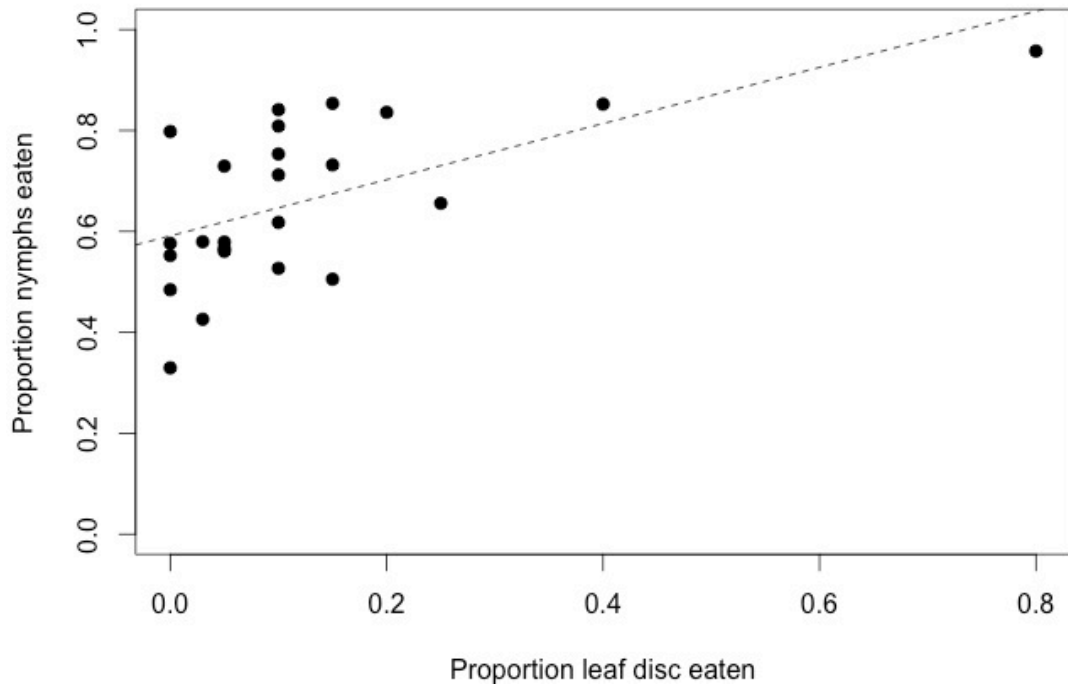


Figure 5.6. Proportion of *Aleyrodes proletella* nymphs eaten plotted against the proportion of leaf disc eaten by slugs. The dashed line represents the statistically significant linear regression, $y = (0.56 \cdot x) + 0.59$, $P < 0.01$.

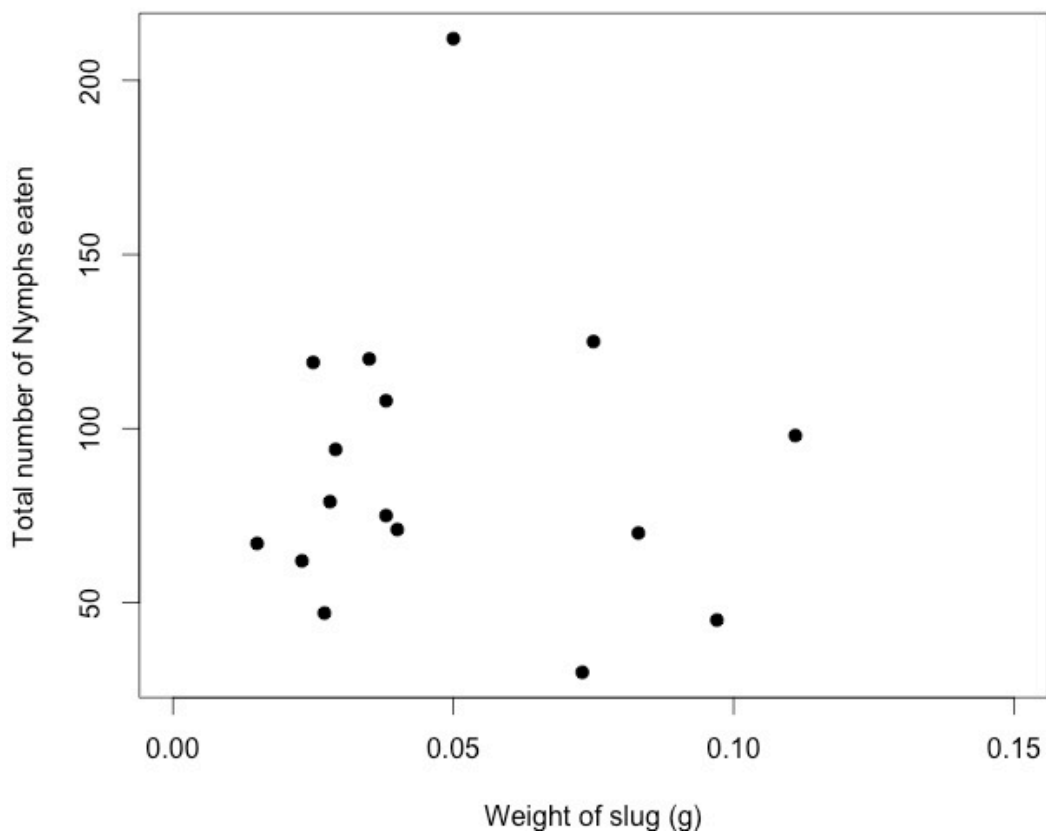


Figure 5.7. Total number of nymphs eaten plotted against the weight of the slug which consumed them.

There was no relationship between the weight of the slug and the number of *A. proletella* nymphs they consumed (ANOVA, $F_{(1,14)}=0.05$, $P=0.83$; Figure 5.7), heavier slugs did not consume more nymphs than lighter ones.

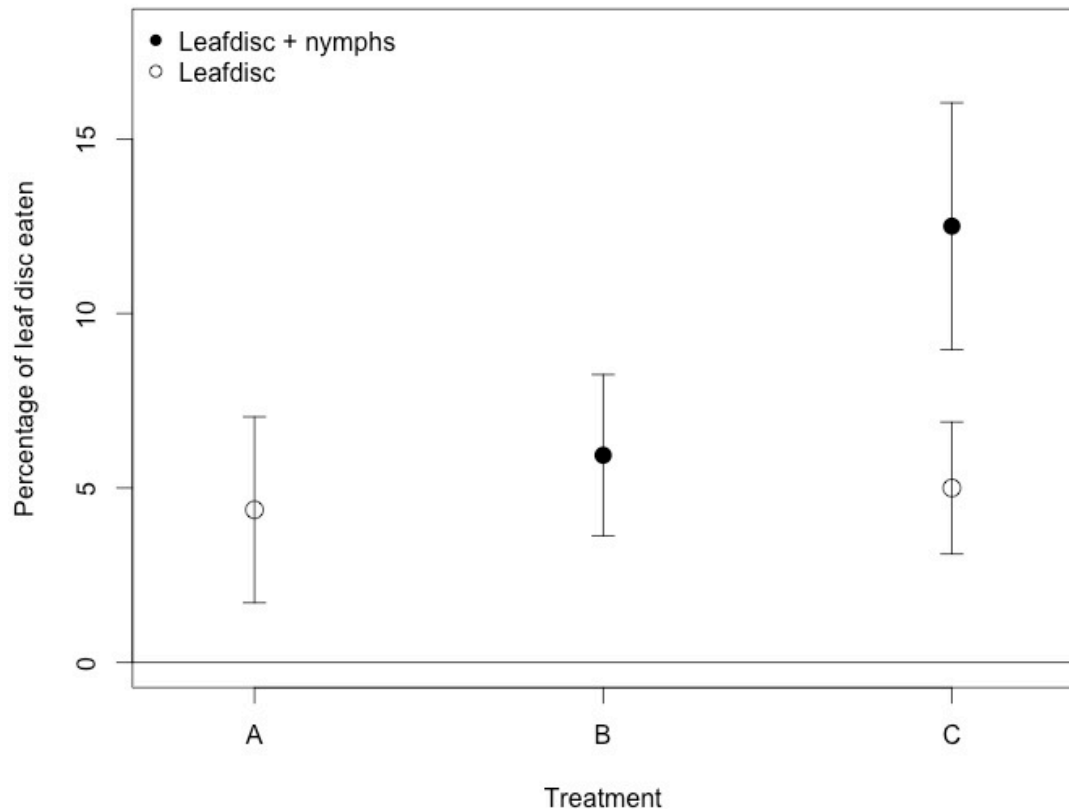


Figure 5.8. Mean (\pm SE) percentage of leaf disc eaten by earwigs for each of the treatments. A – 2 x uninfested leaf discs, B – 2 x *A. proletella* infested leaf disc, C – 1 x uninfested leaf disc and 1 x *A. proletella* infested leaf disc.

The percentage of the leaf disc consumed by earwigs did not differ between the treatments (ANOVA, $F_{(2,28)}=0.99$, $P=0.38$, Figure 5.8). Although similar with the slug feeding assays, the earwigs consumed over twice the amount of leaf discs infested with *A. proletella* compared with the uninfested leaf discs in the choice tests. This was, however, not found to be a statistically significant difference (ANOVA, $F_{(1,28)}=3.95$, $P=0.06$, Figure 5.8).

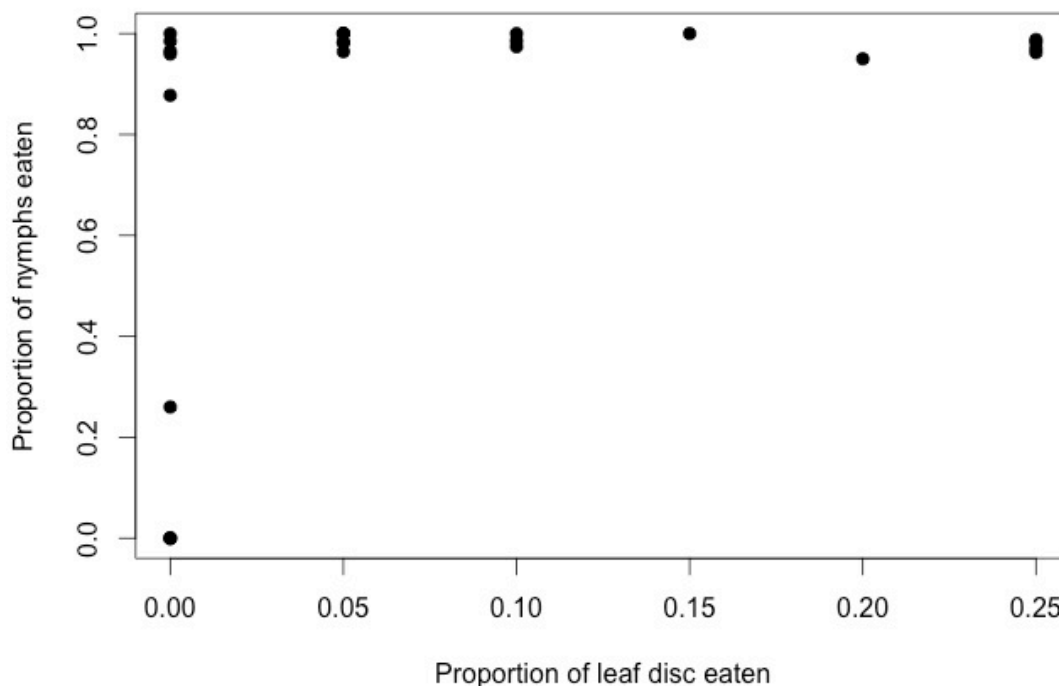


Figure 5.9. Proportion of *Aleyrodes proletella* nymphs eaten against proportion of leaf disc eaten by earwigs.

There was no relationship between the percentage of leaf disc eaten and the percentage of nymphs eaten by earwigs; nearly 100% of nymphs were eaten regardless of the amount of leaf disc eaten (ANOVA, $F_{(1,22)}=4.20$, $R^2=0.12$, $P=0.053$, Figure 5.9). The maximum percentage of leaf disc eaten was only 25% for earwigs compared to 80% for slugs.

A linear relationship, $y \sim 0.81x$, (ANOVA, $F_{(1,15)}=82.76$, $R^2=0.84$, $P<0.001$, Figure 5.10) was found, showing, in effect, that an average of 81% of nymphs were eaten regardless of how many were present. The number of leaf discs containing *A. proletella* (i.e. treatment) available did not influence the linear model and could be removed (ANOVA, $F=0.57$, $df=2$, $P=0.58$, Figure 5.10), showing that treatment (i.e. number of *A. proletella* infested leaf discs, had no impact on the percentage of *A. proletella* eaten). The majority of earwigs consumed close to the maximum number of nymphs available regardless of number available. This suggests that earwigs could potentially eat more nymphs than indicated by the assay, the earwigs that ate the most nymphs nearly ate 100% of the nymphs available on both infested leaf discs (Figure 5.10).

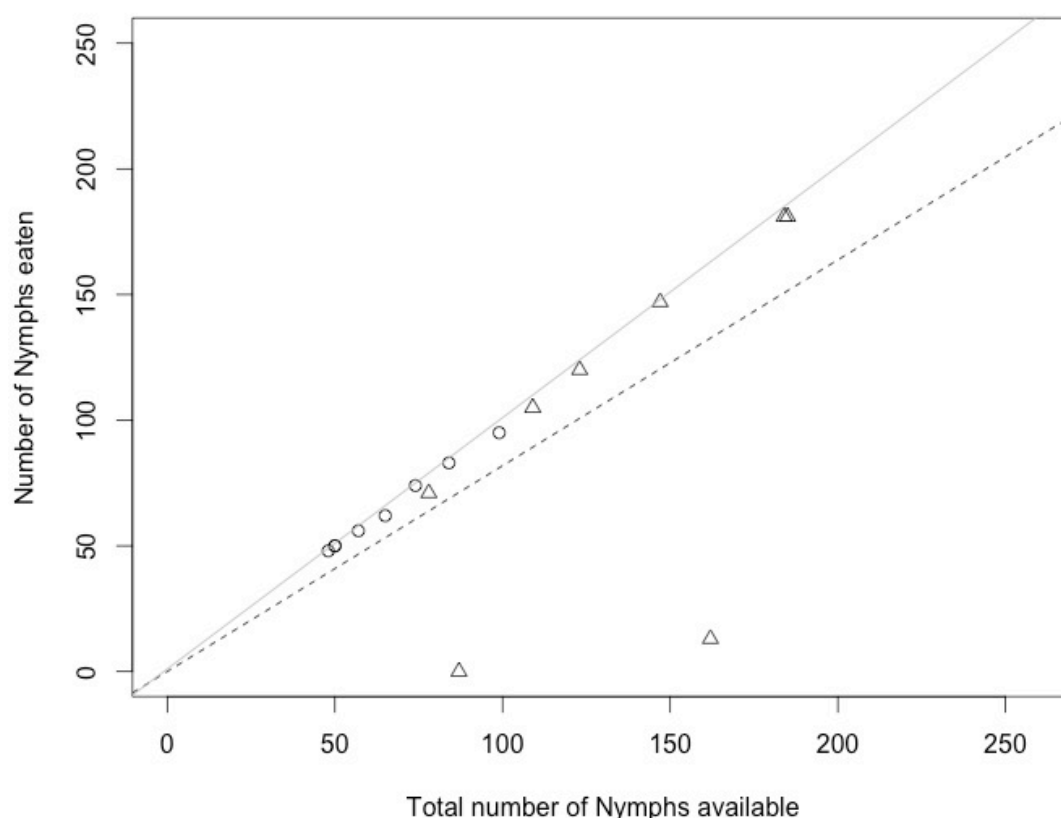


Figure 5.10. Number of *Aleyrodes proletella* nymphs eaten by slugs against the total number of nymphs available within the arena. The dashed line represents the statistically significant linear regression, $y=0.81x$. The solid line represents the theoretical regression where 100% of nymphs were eaten regardless of the number available. Triangles = Treatment A, circles = Treatment B (Figure 5.1).

There was no relationship between the weight of the earwig and the number of nymphs that it ate in total (ANOVA, $F_{(1,14)}=0.36$, $R^2=0.03$, $P=0.56$, Figure 5.11); smaller earwigs did not eat less nymphs.

As hoverfly larvae have no potential to consume leaf discs, this test was designed to see how many *A. proletella* nymphs a hoverfly larva would eat. Over 24h very few nymphs were eaten and it was decided to leave the test *in situ* until the following day. The variation in the numbers of *A. proletella* nymphs eaten by the hoverfly larvae was considerable, ranging from 5 to 67 (Figure 5.12). There was no

relationship between the number of nymphs offered and number consumed; the percentage consumed ranged from 7-75% (Figure 5.12).

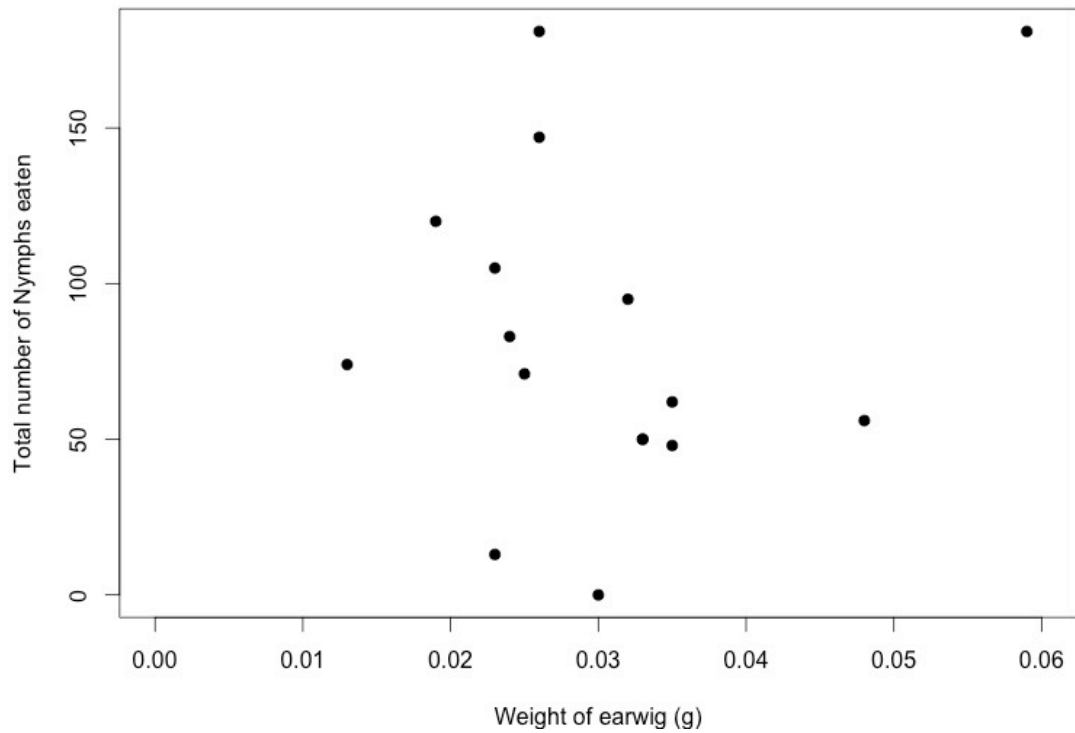


Figure 5.11. Total number of nymphs eaten plotted against the weight of the earwig.

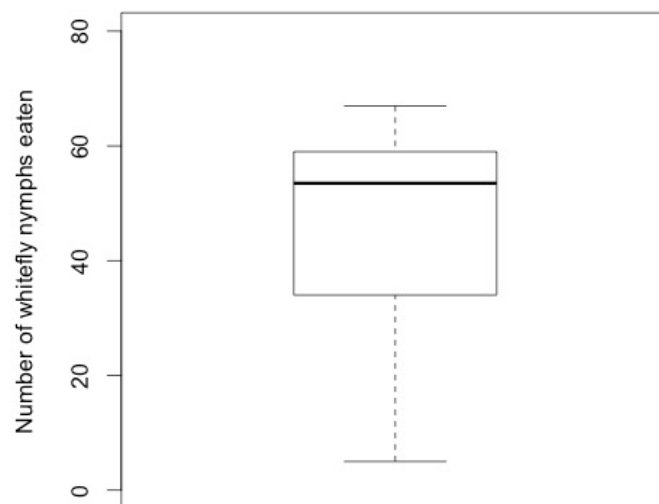


Figure 5.12. Number of *Aleyrodes proletella* nymphs eaten by 3rd instar hoverfly larvae over 48 h, n=8.

When the study hoverfly larvae were left to continue their development on a diet consisting exclusively of *A. proletella* nymphs, none completed development to adulthood, all died before pupation. The assay investigating the feeding rates of ladybird larvae on *A. proletella* did not produce encouraging results. No nymphs were consumed by the larvae over the 24h. When the larvae were allowed to feed exclusively on *A. proletella* under laboratory conditions, again as with the hoverfly larvae, all ladybird larvae died and none developed to adulthood.

5.3.3 Experiment 5.3. Monitoring the occurrence of fungal pathogens in field plots infested with *Aleyrodes proletella*.

Mortality of adult *A. proletella* due to infection with a fungal pathogen was recorded in Experiment 4.6 and Experiment 4.7. Fungal growth could be seen on the thorax and abdomen of some individuals sampled in October 2014 (Figure 5.13).



Figure 5.13. Left: Dead *Aleyrodes proletella* adults on underside of kale leaf. Right: Adult *A. proletella* showing typical symptoms of mortality due to a fungal pathogen, with fungal growth visible on thorax and abdomen and outstretched wings.

The symptoms of death caused by the fungal pathogen were first recorded in Plot G, August planting in October 2014 and within a month the pathogen was seen in all Plot G plantings, and in most other plots. Infection rates differed across the site, most notably, infection was almost absent from Plot I, whilst it caused almost complete mortality in Plot G (Figures 5.14-5.18). No relationship was found between the

proportion of dead whiteflies and the total number of whiteflies present on the plant, (Figure 5.19).

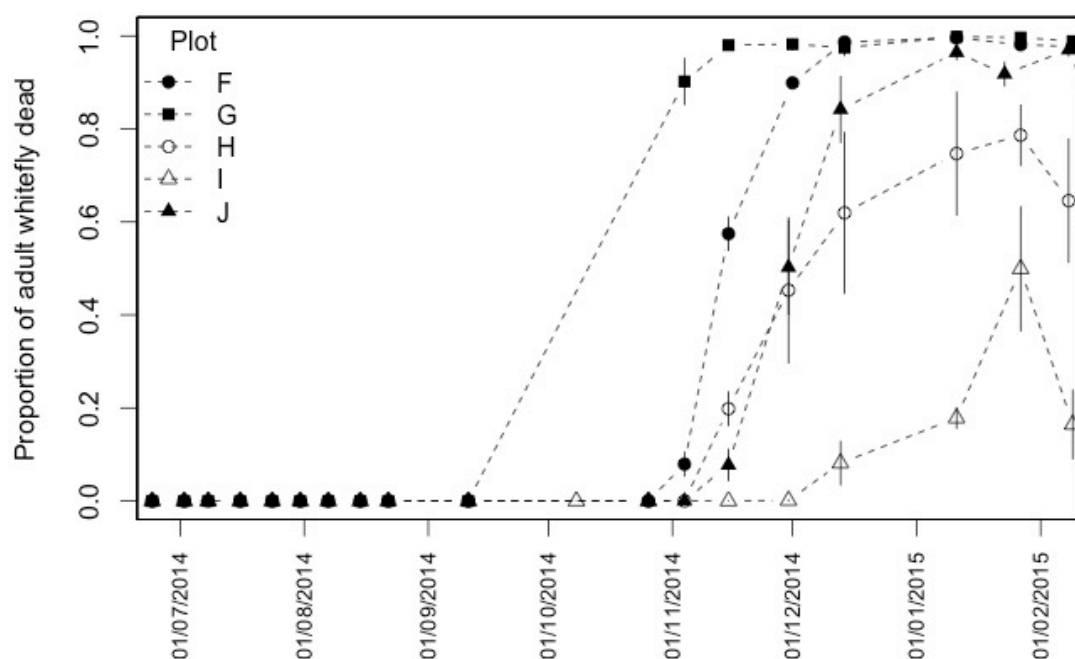


Figure 5.14. Proportion of adult *Aleyrodes proletella* dead on plants for Plots F-J for May plantings of Experiment 4.6.

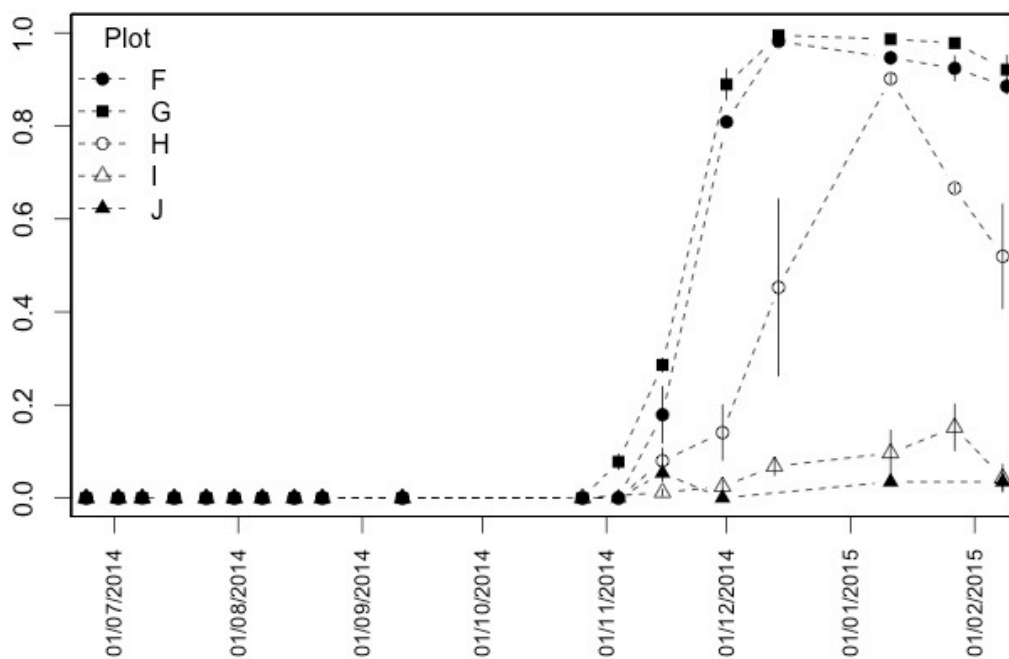


Figure 5.15. Proportion of adult *Aleyrodes proletella* dead on plants for Plots F-J for June plantings of experiment Experiment 4.6.

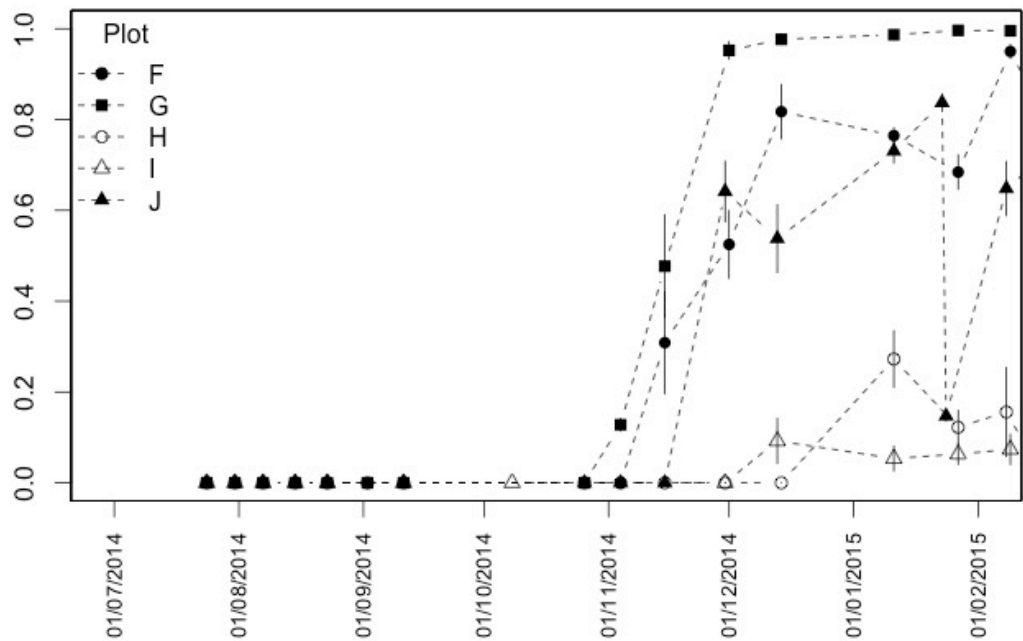


Figure 5.16. Proportion of adult *Aleyrodes proletella* dead on plants for Plots F-J for July plantings of Experiment 4.6.

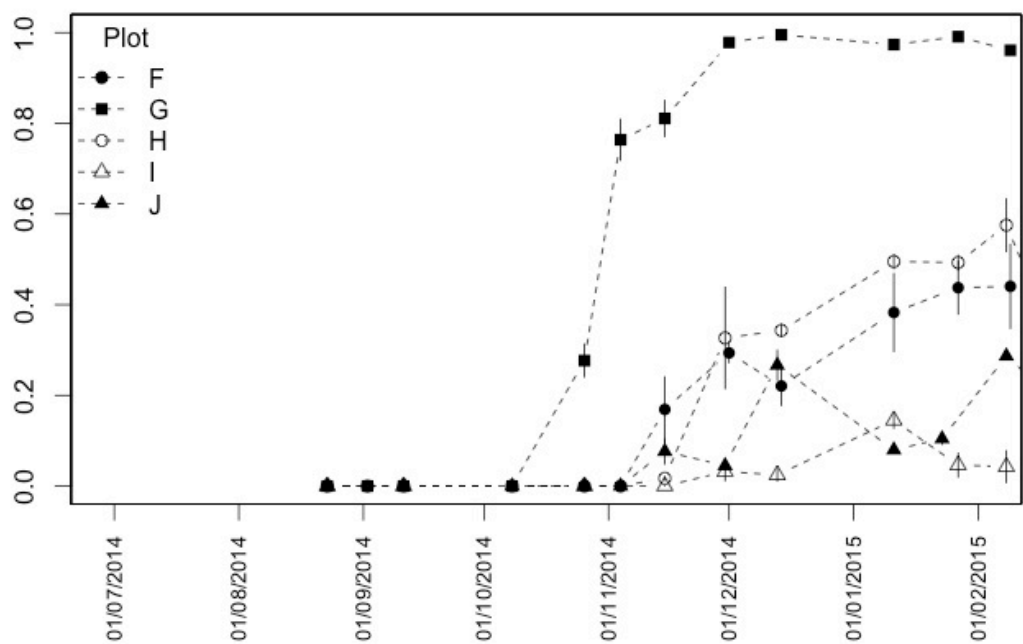


Figure 5.17. Proportion of adult *Aleyrodes proletella* dead on plants for Plots F-J for August plantings of Experiment 4.6.

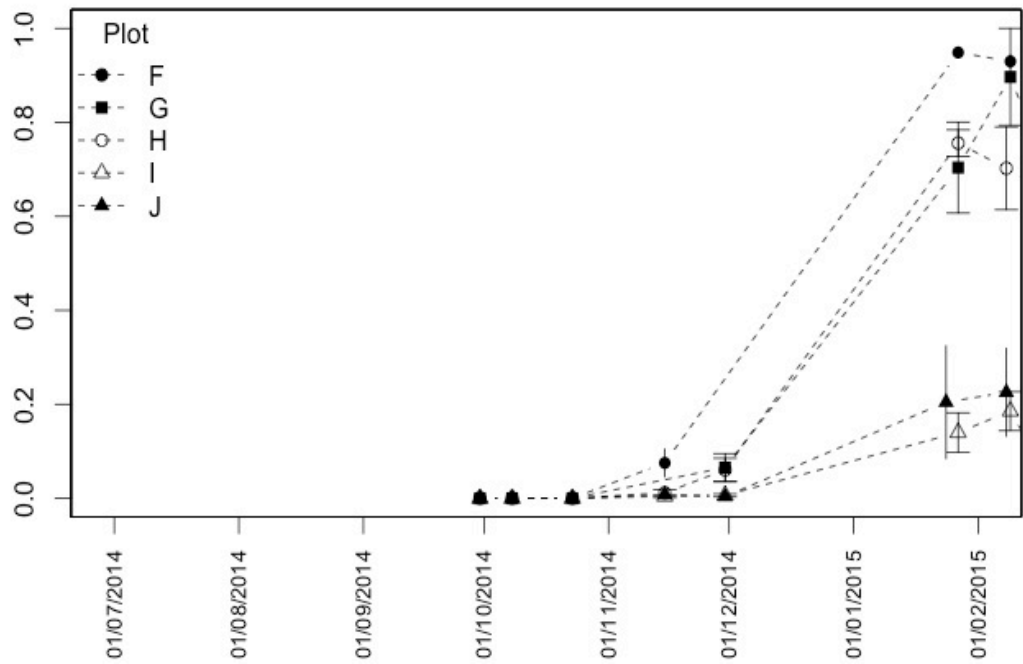


Figure 5.18. Proportion of adult *Aleyrodes proletella* dead on plants for Plots F-J for September plantings of Experiment 4.6.

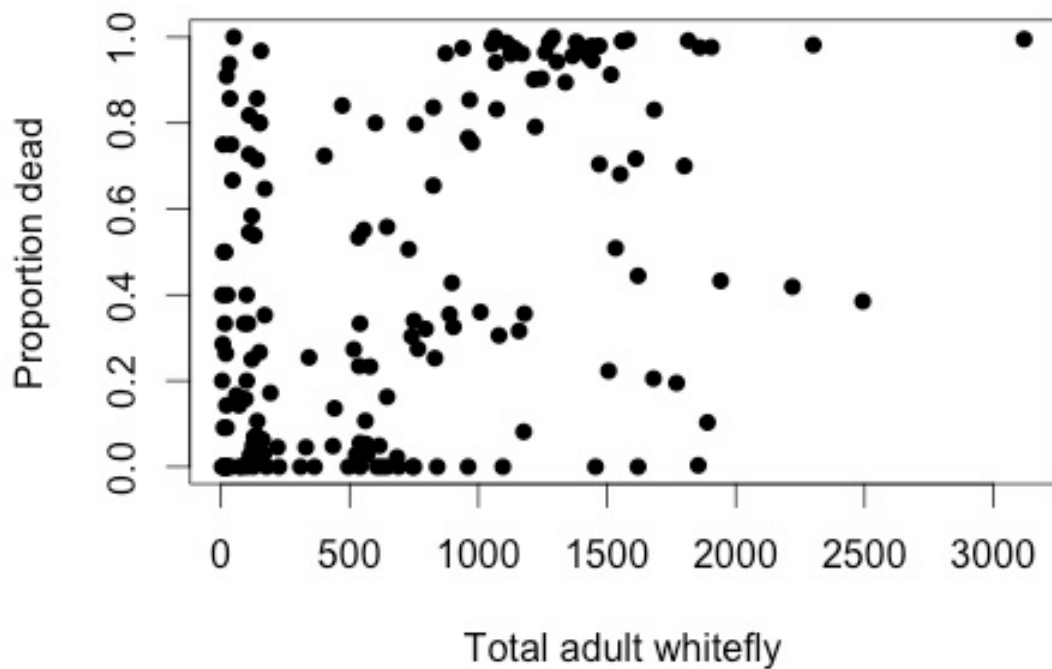


Figure 5.19. Proportion of dead *Aleyrodes proletella* adults plotted against the total number of *A. proletella* on the plant. No statistically-significant relationship was found.

Patterns of infection by the fungal pathogen in 2015 were very similar to 2014 with the first signs of the pathogen occurring in Autumn. Variation between the plots was also seen with some plots showing close to 100% infection while others showed very low percentages of infection not exceeding 25% (Figure 5.20).

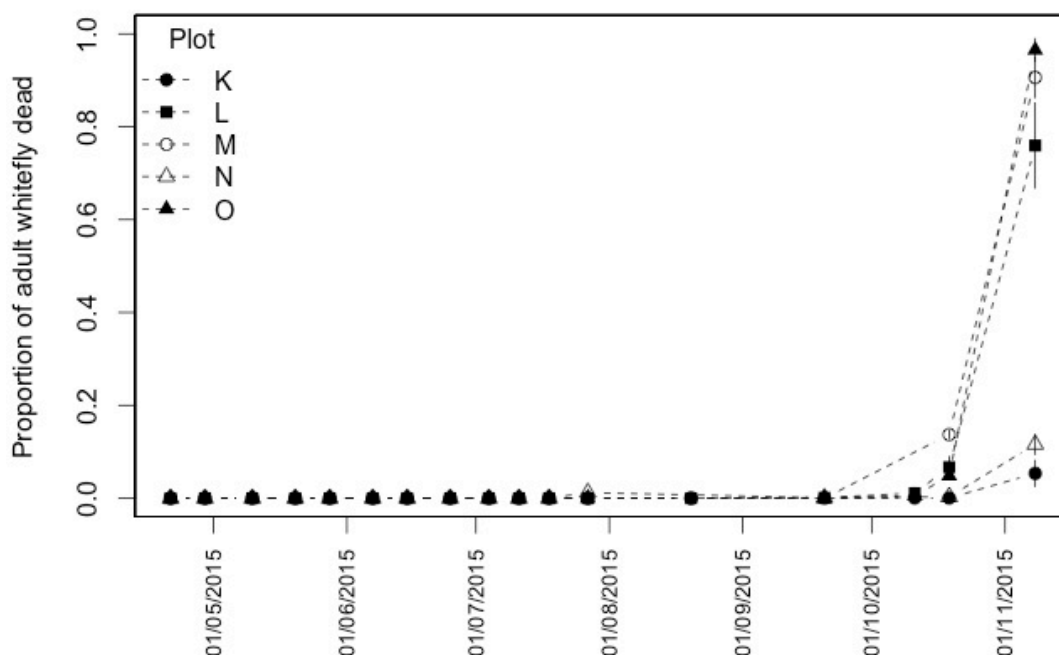


Figure 5.20. Proportion of adult *Aleyrodes proletella* dead on plants for Plots K-O for Experiment 4.7.

5.3.4 Experiment 5.4. Isolation of fungal pathogen

Primary, secondary and capillary conidia were all present, which is a feature of the Zoophthora, Entomophthorales. The presence of unicellular primary conidia confirms that the pathogenic fungus is a member of the genus Zoophthora (Humber, 1997). The primary spores measured had a mean length of $23.02\mu\text{m} \pm 1.42\text{SD}$ and a mean width of $9.79\mu\text{m} \pm 0.91\text{SD}$ (Figure 5.21).

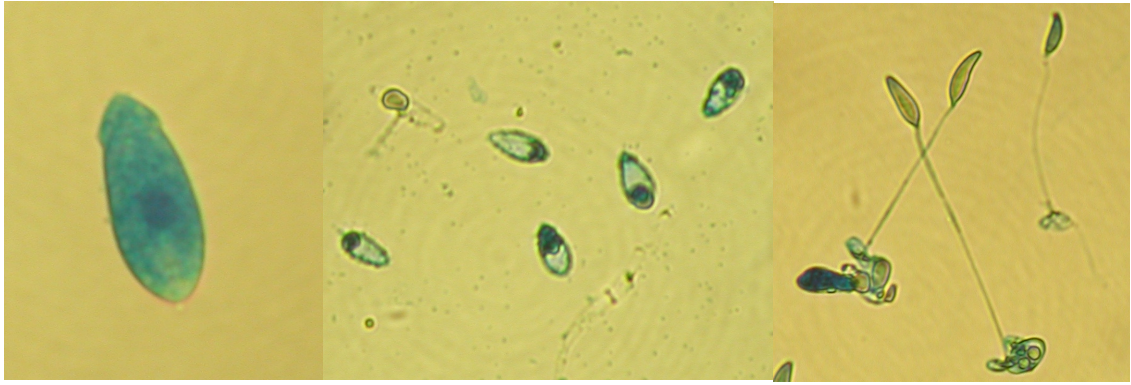


Figure 5.21. Left to Right. Primary unicellular conidia, secondary conidia, capilicondia still attached to capillary condiphore.

5.3.5 Experiment 5.5. Genetic identification of fungal pathogens

Genetic identification of the fungal pathogen responsible for the epizotic observed in the field of Experiment 4.6 could not be made from the PCR reactions described. The ITS4/5 primers did not successfully replicate the ITS regions of the DNA to allow genetic phylogenetic identification. A positive control was successful with the primer pairings ITS4/ITS5, ITS4/ITS1 and PnCNf/PnCNr, (Table 5.3). The *Zoophthora radicans* specific primer pair Zf/Zr (Table 5.3), was not successful either. No positive control for a known *Zoophthora radicans* specimen was available, and therefore the reliability of the PCR reaction for multiplication of the ITS region could not be validated.

5.4 Discussion.

5.4.1 Predators

5.4.1.1 Hoverfly

Very low numbers of predators were recorded from Experiment 4.6 and Experiment 4.7 in 2014 and 2015 with hoverfly larvae the most abundant predators in both experiments. The results of Experiment 5.2 do however suggest that the feeding rate of hoverfly larvae is low, and that each is only able to feed on 30 nymphs over 24h, which is half the number of aphids that *E. balteus* was found to feed upon in the same duration (Jalilian, 2015). The fact that no hoverfly larvae were able to complete development to adulthood supports the notion that *A. proletella* is not a good source of food for hoverfly larvae. The same was true for the ladybird larvae assay described, which supported similar work conducted on *Coccinella undecimpunctata* (Cabral *et. al.*, 2006).

5.4.1.2 Slugs

Slugs were seen in high numbers in Experiment 4.6 and it was shown that the species *Limax marginatus* does feed on nymphs of *A. proletella*. Feeding on the nymphs was shown to be an active process, not merely because the slugs were feeding upon the plant tissue and accidentally consuming the nymphs. It was predicted that 60% of nymphs would have been consumed when no direct consumption of the leaf occurred, taken from the y-intercept value for the linear regression (i.e. proportion of nymphs eaten when no leaf disc was consumed) was 0.6. There was a tendency for more nymphs to be consumed with the consumption of leaf discs. Interestingly, more leaf tissue was consumed from a leaf disc infested with *A. proletella* when compared with an uninfested leaf disc, suggesting that a slug may prefer to spend its time on a leaf disc that is host to *A. proletella*. Further research would be needed to determine whether slugs are actually attracted to *A. proletella* and to understand their potential as predators within the field. Approximately 65% of nymphs were eaten regardless of the numbers available, suggesting that more nymphs could have been eaten if more were available. The limitation on the percentage of the nymphs consumed could have been due to the foraging behaviour of the slugs whereby the slug did not investigate the entire leaf disc and only came in contact with 60% of the leaf disc.

Slugs have rarely been studied with respect to their role as predators, especially within the UK. However, some limited studies have shown the potential for slugs to consume pest insects, especially sessile species. *Deroceras laevae* was shown to feed on the Florida wax scale (*Ceroplastes floridensis*), so much so that it nearly wiped out a laboratory culture of this species (Mienis, 1989). A similar instance of predation on the citrus mealy bug (*Planococcus citri*) was recorded in England (Quick, 1951). Interestingly when analysing the diet of the land snail, *Wainuia urnula*, Efford (2000), found that over 80% of the gut contents contained remains of amphipods and later studies showed that the snails actively preyed on these species, which was an unexpected outcome. Another study indicating the carnivorous behaviour of the ‘herbivorous’ slug *Deroceras hilbrand*, showed that it actively searched for carcasses of invertebrates killed by the carnivorous plant, *Pinguicula vallisneriifolia* (Zamora and Gomez, 1996). The study showed that the slugs had a preference for feeding on plants supporting carcasses of invertebrates, similar to the results of the present study, whereby slugs fed more on leaf discs supporting *A. proletella* when given a choice between these and uninfested leaf discs. Although studies investigating the carnivorous behaviour of slugs typically believed to be herbivorous are few, such relatively recent discoveries suggest that slugs have a more complex feeding biology than was once believed.

5.4.1.3 Earwigs

Earwigs showed high feeding rates on *A. proletella* nymphs. Nearly all the earwigs ate close to 100% of the *A. proletella* available, even at the highest number of nymphs available, 117. This suggests that the maximum rate of feeding was not determined for earwigs in these experiments. Further work would be needed, increasing the number of *A. proletella* available; to determine the maximum number that might be consumed and to really understand the potential earwigs have as natural predators of *A. proletella*.

There was no direct field evidence of earwigs feeding on *A. proletella* nymphs, however they were seen hiding within the crop foliage on a number of occasions indicating that they may have been feeding on *A. proletella*. Experiment 5.2 showed that earwigs can feed on *A. proletella* nymphs, consuming numbers exceeding 100 in

24h. Earwigs are nocturnal predators and their presence within the crop is likely to be underestimated, as all monitoring was conducted during daylight hours

Generalist predators such as earwigs are often in low densities in crop settings and have been shown to be negatively impacted by a number of insecticides and tillage, suggesting that a *Brassica* crop system would likely dramatically negatively impact earwig populations (Fountain and Harris, 2015; Moerkens *et al.*, 2012). These predators are more likely to be numerous in a more stable environment and may in fact be more effective in regulating *A. proletella* in such conditions. Future work could investigate the mortality of *A. proletella* due to predation in different habitats, e.g. stable climax conditions and recently cleared land, to ascertain if predation rates are different, which may be due to differences in the presence of predators.

5.4.2 Parasitism

Throughout Experiment 4.5, Experiment 4.6 and Experiment 4.7 there was no evidence of parasitism of *A. proletella* by any species. Suggesting that there is little evidence that natural populations of *E. tricolor* can regulate *A. proletella* infestations in a commercial setting.

From Experiment 4.3, however, during spot checks of *Euphorbia lathyris*, a wild host plant of *A. proletella*, pupae parasitized by *E. tricolor* were found in a car park in Kenilworth (Table 4.4). The *Euphorbia* plant supported 140 *A. proletella* nymphs, 35 showing signs of parasitism by *E. tricolor*, with adult parasitoids visible on the leaves. Overwintering survival of *E. tricolor* is negatively impacted by low temperatures and therefore it is likely present in localities with warm winter microclimates, such as created in towns (Parry, 1950). *Encarsia tricolor* has been seen to parasitise the whitefly, *Aleurotrachelus jelinekii*, in the UK, however, rates of parasitism were not sufficient to successfully control infestations (Hassel *et al.* 1987), suggesting that even if the parasitoid was present within a commercial setting it is unlikely to have an impact on the levels of population growth of *A. proletella*.

5.4.3 Fungal pathogen epizootic

In this study, predators and parasitoids have been shown to have little impact on populations of *A. proletella*. However, an epizootic caused by an entomopathogenic

fungus caused mortality rates >90%. The absence of a relationship between the proportion of *A. proletella* killed by fungus and the total population of *A. proletella* suggests that the fungal pathogen did not spread in a density-dependent manner. The first incidence of the disease was recorded in Plot G (August planting) in October. However, it is likely that this was not the actual first instance of the disease. As this was the first time the disease was encountered, the symptoms had probably been overlooked previously, especially if the proportion of infected individuals was small. Plot G supported the highest populations of *A. proletella* in September and was the first plot to experience the epizootic. After its first appearance in October, the disease was observed within a month on all sub-plots (planting dates) within Plot G. Whether the disease was present in all sub-plots or spread to them from the initial infestation cannot be determined. Future work would need to be designed to fully study the ecology of the fungal pathogens as has been conducted on other insect pathogenic species (Glare and Milner, 1991).

The high level of mortality of *A. proletella* in a number of plots showed how virulent the pathogen can be. The pathogen may be important in regulating numbers of *A. proletella* throughout the years and may cause population crashes, preventing large overwintering populations from developing. For example, suction trap captures at Kirton fell from 292 in 2011 to 6 in 2012 (Experiment 2.5); a dramatic change in population size, which could not be attributed to differences in the number of generations that were likely to have developed in the two years. A highly virulent fungal pathogen, such as the one seen in this study, could account for such a decrease in numbers of *A. proletella* from year to year. The prevalence of the pathogen in wild hosts and commercial crops would need to be known to understand its potential importance in population regulation. The size of infestations of *A. proletella* in the plots in this study are uncharacteristically high compared with those seen in commercial crops, or even on wild host plants, and this may have been important for the establishment of the epizootic. The outbreak of the fungal pathogen occurred after September in both 2014 and 2015, suggesting that this is the natural timing of such an epizootic, which is also the time when *A. proletella* is most abundant in the field. It would also be interesting to determine whether epizootics occur in commercial field settings. Sampling of whitefly should focus at times near to October as the results suggest that this is the typical time for epizootics to occur in

field populations of *A. proletella* and would likely provide the most data to confirm the presence of the fungus within the field.

Although the pathogen could not be identified to species, its morphology indicates that it is a member of genus *Zoophthora* within the order Entomophthorales. This is the first recorded occurrence of a fungal pathogen on *A. proletella* within the UK. The work on the genetic identification of the pathogen should be repeated as none of the PCR reactions described above successfully multiplied ITS regions of it's DNA to allow a genetic phylogenetic comparison with documented species.

6 Discussion

The overall aim of this project is to understand the population biology of *A. proletella* infesting the most vulnerable commercial crops, Brussels sprout and kale. This includes understanding how and when populations increase, including when the colonisation of new crops occurs. This information can then be used to inform the development of an integrated control strategy for *A. proletella* using insecticides and other tools, which might include biological control agents and methods of cultural or physical control.

6.1 A further understanding of *Aleyrodes proletella* as a pest.

At the beginning of the year adult female *A. proletella* are overwintering in a state of ovarian diapause. Overwintering sites will include winter oilseed rape crops, horticultural *Brassica* crops and wild hosts. This project has shown that oviposition can begin as early as January in years, such as 2014, when temperatures are high enough to elicit this. Butler (1938a) suggested that the low temperature threshold for oviposition was 10°C. Further work in controlled environments with field validation could ascertain if this value is valid and would allow the prediction of the start of oviposition in subsequent years.

6.1.1 Colonisation of new vulnerable crops.

New crops that are vulnerable to colonisation by *A. proletella* are present from early spring, with crops planted from April until July (Elsoms 2015). Some of these crops are not harvested until early in the following year so there is a long period over which infestations can establish and develop. It has been suggested that *A. proletella* colonises new crops following the development of adults from eggs laid in the spring (Butler, 1938a).

It would be useful for growers to know when first colonisation by adult *A. proletella* is likely to occur. Over a number of years, efforts have been made to forecast the arrival of several important pest species in newly-planted *Brassica* crops. For example, the first arrival of both *Brevicoryne brassicae* and *Myzus persicae* is often

timed in accordance with the development of alates on overwintering hosts (Hafez, 1961) and day-degree models predicting the development of overwintering stages and migration into new crops can be used to forecast first occurrence with differing levels of accuracy (Collier and Finch, 1992; Cividanes *et al.*, 2012; Nematollahi *et al.*, 2016).

Colonisation of new crops by these species of pest aphid is often achieved through long-range migration (Kring, 1972) and such migration can be highlighted by captures of alate aphids in the network of suction traps coordinated by the Rothamsted Insect Survey. In addition, the long term monitoring data collected by the Rothamsted Insect Survey have been used to determine relationships between the start of aphid migration from overwintering sites in the spring and parameters such as temperature (Zhou *et al.*, 1995). Output using these statistical models is now released annually by the Rothamsted Insect Survey to provide forecasts of aphid migration and the colonisation of new crops (Woiwod *et al.*, 1984).

Once it had been determined in the present project that *A. proletella* performed flights in excess of 4 m (Experiment 2.4), which was greater than the previous highest capture record of 9ft (El Khidir, 1963), it seemed likely they might be caught by the suction traps operated as part of the Rothamsted Insect Survey (12.2 m high). Stored samples from the suction traps at Wellesbourne and Kirton were obtained from the Insect Survey to determine whether and when *A. proletella* were captured in these traps. Such information was important in understanding when long-range migration might occur.

Analysis of the samples for the presence of *A. proletella* indicated that long-range migration appears to occur, for the most part, from September onwards, after diapause has been elicited. Indeed increased flight behaviour of females in ovarian diapause, when compared with summer egg-laying morphs, has been demonstrated in the laboratory (Iheagwam, 1977b) and El Khidir, (1963) showed that females were caught in significantly higher numbers in the autumn in 9ft high suction traps located above crop plants.

The data on captures of *A. proletella* by the Rothamsted suction traps at Wellesbourne in 2014 and 2015 also supports El Khidir's (1963), suggestion that there is a short period in mid-summer when a small proportion of the population undertakes long range migration, during July-August. It is likely that this is because these individuals have developed on senescing hosts. Such an increase in migratory behaviour has also been seen in *Trialeurodes vaporariorum* when developing on senescent hosts (Bonsignore, 2015).

In both 2014 and 2015, the first suction trap captures of *A. proletella* occurred at the time when plants in the study plots were senescing, as were nearby crops of oilseed rape. It should be noted, however, that the numbers captured were relatively low and were, for example, lower than the numbers of many species of migrating aphid caught in the suction trap. Sadly there was no time to ascertain the factors determining the increased migratory behaviour by *A. proletella* at this time. A small study on *A. proletella* showed that flight behaviour could be increased through starvation (Iheagwam, 1976), and development on senescent hosts may, in effect, result in starvation. The hypothesis, that senescing hosts lead to the emergence of migratory morphs of *A. proletella*, would need to be tested, together with determining the degree to which this occurs.

Since the suction trap data and data from the Experiment 2.4 at Wellesbourne suggest that much of the long-range migration is concentrated in the autumn months, with a short period of migration in mid summer, it seems unlikely that the initial colonisation of new crops planted from April onwards is the result of long range migration. In contrast, the evidence gained throughout this project strongly supports the hypothesis that the initial colonisation of vulnerable crops is the result, in the main, of trivial flight by individuals originating from the immediate or close vicinity.

In 2014, when new plots were planted at different distances from known sources of infestation (plots planted in 2013) and sampled 3 weeks later, there was a highly significant negative correlation between the mean numbers of adult *A. proletella* per plant and the distance of the plot from a source of overwintering *A. proletella* (Figure 4.31). To corroborate this, studies undertaken at the same time to investigate the dispersal of *A. proletella* using traps and trap plants placed at different distances

from an infestation showed that *A. proletella* had rather poor dispersal potential (Experiment 2.3). This was described through modeling using the dispersal models proposed by Taylor (1978) and Gregory and Read (1949). In addition, the vast majority of *A. proletella* were trapped close to the ground and responded strongly to a potential super-host stimulus of yellow. A similar poor propensity to disperse was found in the Viburnum whitefly (*Aleurotuba jelinekii*), which aggregates around the population source and has a very low probability of performing migratory flights (Reader and Southwood, 1984).

It was thought previously that colonisation of new crops by *A. proletella* followed the emergence of the first generation of adults that had developed from eggs laid upon overwintering hosts (Butler, 1938a, Al-Houty, 1979). The results from several experimental studies in this project indicate this is not the case. First colonisation of plots in each year 2013, 2014 and 2015 occurred before adults of the first generation had emerged with the evidence suggesting that it was performed by overwintering females dispersing from their winter hosts.

Although initial colonisation of newly-planted crops may be due to overwintering females, there is a further opportunity for colonisation once the first generation of adults emerge, in June. The activity of adult *A. proletella* was at a peak at this time (Experiment 2.2), and, as discussed above, some further colonisation was likely to be undertaken by new adults emerging on plants that had begun to senesce. However, it is unlikely that a large number of *A. proletella* undertake long range migration at this time, as during June a very small proportion of *A. proletella* were caught on traps located more than 60 cm above ground, with the vast majority attracted to a super-host stimulus, yellow. Again no *A. proletella* were caught in the Rothamsted Suction Trap at Wellesbourne at this time.

The spatial distribution of *A. proletella* in commercial crops further supports the notion that immigration into new crops is achieved through trivial dispersal from hosts in the immediate area, e.g. wild hosts in headlands and hedgerows (Lewis, 1969) or nearby crops (Wright and Ashby, 1946; Lewis, 1969). The within-field distribution of *A. proletella* appears to be very similar to that found for carrot fly (*Psila rosae*) where immigration into new crops has been shown to be the product of

low and short-distance flights with the flies likely originating from habitats surrounding the field e.g. headlands, hedgerows and nearby crops (Wright and Ashby, 1946; Lewis, 1969; Finch and Collier, 2004). Again, comparing *A. proletella* to the intensively studied pest Aphididae, the apparent differences are informative. Aphids often have within-field distributions that are influenced greatly by potential windbreaks, e.g. hedgerows, whereby a ‘shadow’ of low infestation exists immediately behind them; and wind direction can have a dramatic effect on distribution (Lewis, 1969). To further cement the differences between pest aphids and *A. proletella* it would be sensible to complete more studies on their distribution in commercial fields or large scale experimental plots where variables can be controlled to test specific hypotheses. A considerable amount of information on the migratory behaviour of immigrating insects can be inferred from such data (Wright and Ashby, 1946; Lewis, 1965; Lewis, 1969). Sadly such data are lacking in contemporary research in agricultural entomology.

All of the factors discussed above point to a conclusion that overwintering female *A. proletella* can colonise new crops early in the season, achieving this by low, short-range trivial dispersal. As such the distance of a new crop from the site of a substantial overwintering population of *A. proletella* is highly influential in determining the initial size of an infestation, although further immigration is likely to occur throughout the season, with a potential influx occurring when overwintering hosts in the vicinity no longer offer fresh foliage for newly-emerged adults.

To further support this hypothesis, additional studies could investigate the use of physical barriers to deter colonisation by *A. proletella*, such as those deployed to reduce colonisation by carrot fly (Jukes *et al.*, 2009). These would be expected to reduce colonisation considerably. One would also expect migrant pest aphids to be hindered less by this approach and again differences, or lack of differences, in the colonisation rates of these pests could be extremely informative.

Winter oil seed rape is planted in the autumn and the new crop is therefore available for colonisation by *A. proletella* at this time. In January 2015, when a survey of the distribution of *A. proletella* within oil seed rape fields on the Wellesbourne Campus was conducted there was no distinct ‘edge effect’. The distribution resembled that

described for pest insects that have colonised through immigration caused by long-range aerial flight (Lewis, 1969). Therefore the colonisation of oil seed rape by whitefly at this time is likely to be due to long-range migration signified by the suction trap data and the capture of relatively more *A. proletella* at greater heights in Experiment 2.4.

Interestingly, the numbers of *A. proletella* within the oil seed rape fields changed between January and April. This change in numbers could not be due to the emergence of new adults, as the first generation had not developed. One field close to a highly populated plot of kale (Experiment 4.7, Plot N) showed an increase in numbers of adult *A. proletella* from 1.2 to 4 per 0.25m², whereas, in contrast, another field without a highly populated plot in the immediate vicinity showed a population decline. This shows a potential for short-range trivial movement to be influencing populations on oilseed rape at this time. It should be noted however that although the data suggests this effect, more research would need to be conducted to investigate the potential for trivial movement overwinter to lead to further colonisation of oilseed rape crops adjacent to sites of high populations of *A. proletella*. This certainly suggests that oilseed rape can act as a green-bridge when located near to horticultural *Brassica* crops.

6.1.2 Population trends within the crop.

The aggregated distribution of *A. proletella*, Experiment 4.1, suggests that small populations may be easily overlooked and underestimated if the sampling intensity is insufficient to ascertain the presence of the pest. As such, sample size would need to increase dramatically at low populations to achieve a good level of accuracy in population estimates or even the confirmation of presence (Cochran, 2007). It is therefore likely that the first immigrant *A. proletella* are often overlooked in commercial crops. In the present project, all leaves of all plants in each plot were surveyed during the early stages of immigration to determine accurately when immigration first occurred. Random sampling of a small number of plants would not have been sufficient.

Infestations in 2013 and 2014 showed rapid growth once the first colonisers had completed a generation; the coincidence of an increase in adult numbers with the

first occurrence of exuviae is strong evidence of this. The population trend for 2013 and 2014 (Figure 4.43) fits a smoothed curve of growth with a considerable increase in numbers after approximately 400 day-degrees ($T^t=6.3^{\circ}\text{C}$). This also suggests, as does the concurrent increase in the number of exuviae, regardless of timing of planting, that the increase in numbers is due to reproduction within the crop, rather than substantial immigration. The increase in numbers of *A. proletella* does occur slightly earlier in 2014 and it is likely this was due to the faster rates of colonisation of crops in that year.

In contrast, in 2015 (Experiment 4.7), all plots showed an increase in numbers of *A. proletella* in June and this was unlikely to have been due to emergence of adults from pupae within the plots, as development of a complete generation had not yet occurred at this time. This increase in the numbers of adults occurred at a time when there was likely to have been increased activity, especially on overwintering hosts.

A much greater area of overwintering hosts of *A. proletella* were present at Wellesbourne in 2015. This would probably have supported a considerably larger overwintering population of whitefly at that time than in previous years, which may have increased the amount of trivial dispersal into new crops (or plots). It is certainly possible that senescence of the oilseed rape led to a sudden increase in the numbers of *A. proletella* undertaking trivial dispersal and seeking new hosts at this time.

It appears that the growth of *A. proletella* infestations continues very much undeterred, with very few predators feeding on any life stage. *Aleyrodes proletella* are likely to be alternative prey for many predators, such as syrphids and coccinellids, that feed primarily on aphid species and laboratory studies showed that these predators did not develop through to adult-hood when given exclusively *A. proletella* as a diet (Experiment 5.2). Of the predators tested, the most efficient were earwigs, but these predators are generally uncommon in field vegetable crops, with regular plowing of fields dramatically reducing local populations (Moerkens *et al.*, 2012). From observations made over the course of this project there is no evidence that any natural enemy, parasitoid or predator, will regulate population growth in this species to levels that would be acceptable commercially. Little evidence exists for introduced predators or parasitoids offering effective control of *A. proletella*

populations in the field. The parasitoid *Encarsia tricolor* was not encountered once during this project in monitoring experiments, suggesting that natural populations are very low. Control of populations of *A. proletella* by introductions of *E. tricolor* would therefore likely need to be a very large inundative release, which would be at a high cost. In contrast, a number of studies have shown that aphid infestations can be regulated by a range of natural enemies including parasitoid wasps and specialist predators (e.g. Raworth *et al.*, 1984). In the UK, the growth of infestations of the Viburnum whitefly (*Aleurotrachelus jelinekii*) was poorly regulated by natural enemies (Hassel *et al.*, 1987) and as such they believed naturally-occurring predators had little impact on population growth, with adult mortality contributing mostly to the death rate, which is likely to be similar for infestations of *A. proletella* in field crops.

Although naturally-occurring predators and parasitoids of *A. proletella* have been shown to be very rare, a newly-identified fungal pathogen provided the highest levels of control, often killing >90% of adult *A. proletella*. Year-to-year differences in the size of the *A. proletella* population may be strongly influenced by this naturally occurring pathogen. As epizootics are usually triggered by a particular combination of environmental conditions (Fuxa and Tanada, 1987), it is possible that the fungal pathogen is a key factor influencing the size of the *A. proletella* population from year to year. Further work on historical suction trap samples, to provide a larger data set, would make it possible to look for relationships between the abundance of *A. proletella* and environmental conditions. The prevalence of the fungal pathogen in field populations is unknown, as this is the first record of its occurrence. The sizes of the *A. proletella* infestations achieved in this project are likely to be unnaturally high and this may have exacerbated the outbreak, however the apparent lack of density dependence suggests this may not be the case.

The discovery of a naturally-occurring entomopathogen leading to high levels of control of *A. proletella* populations is encouraging. The pathogen did, however, only occur at times when numbers of *A. proletella* were high, after which the crop would probably be irretrievably damaged by the pest; thus natural infection is unlikely to provide sufficient levels of control. Although the pathogen could not be identified to species level, it is a member of Entomophthrales and likely to be in the genus

Zoophthora. Sadly the development of such pathogens into commercial products is incredibly costly due to the high nutritional requirements of *in-vivo* production of the Entomophtrales and high costs of registration (Butt *et al.*, 2001). As such, this fungal pathogen is unlikely to offer a commercially viable biocontrol solution in the near future. However, the establishment of a field epizootic caused by a fungal pathogen in a field population of *A. proletella* is encouraging. Documentation of the fungal biological control pathogen, *Aschersonia placenta*, killing *A. proletella* populations in the field (Ponomarenko *et al.*, 1975) suggests that this biological control, or similar, may offer potential for field control in brassicas. As a consequence the use of a currently commercially available whitefly-specific fungal control agents should be further investigated. Such fungal control methods have been developed for, and effectively control *Bemisia tabaci* (Faria and Wraight, 2001) and *Trialeurodes vaporararium* (Osborne and Landa, 1992) and such methods may offer promising results in *Brassica* field crops. Data on the effectiveness of currently available fungal biological control products against *A. proletella* is lacking and should be investigated to ascertain their potential for uses on field populations.

6.2 Suggestions for improved pest control.

6.2.1 Locate new vulnerable crops at distance from overwintering crop sources.

Overwintering host crops are likely to support the highest numbers of *A. proletella* as numbers would have built up during the previous season and a large proportion of these individuals would overwinter within this crop. Females would colonise new crops by trivial movement early in the season and the distance between the old and new crops is likely to be critical. Oilseed rape would likely act as a source of overwintering whitefly; however it seems that the degree to which this occurs can vary. Crops of oilseed rape in the near vicinity would provide a green-bridge if new crops are planted in the same location as the previous years crop. When oilseed rape starts to senesce resident *A. proletella* are likely to undertake trivial movement and infest new crops in close proximity; long range migration may occur at this time, but to a lesser extent.

6.2.2 Late season planting

This would prevent early immigration of overwintering females, which would begin oviposition very early in the season. The multiplicative nature of generations means that the earlier the onset of reproduction within a crop the larger the size of the infestation before the onset of diapause, when reproduction is halted. The more generations that can develop within a crop the larger the population achieved.

6.2.3 Pesticide application.

As populations of *A. proletella* seem to develop within the crop from the time of first colonisation, the development of large infestations is likely to be prevented by reducing the rate of multiplication through control measures. In particular, when large populations of *A. proletella* are in the near vicinity, immigration may peak in June and it may be a good strategy to avoid pesticide applications until after this potential ‘wave’ of immigration has passed. The aim would be to reduce the potential number of *A. proletella* produced as a consequence of generations developing within a crop. Targeted applications based on a forecasting model predicting generation times could be useful. In an AHDB-funded project to investigate approaches to controlling *A. proletella*, applications of pesticides in July significantly reduced pest infestations compared with applications in June. The application of an insecticide treatment in early June may have been too early to control an influx of new immigrants arriving later that month. Furthermore, waiting to apply the treatment until August led to a larger infestation. A much larger population had probably developed within the crop by this time and the insecticide application was likely to be insufficient to control such a large population effectively (Collier and Springate, 2016).

6.3 Comparison with Aphids.

Aleyrodes proletella and aphids such as *B. brassicae* and *M. persicae* fill a very similar niche and feed on some of the same hosts. Although both *B. brassicae* and *M. persicae* may have a holocyclic life-cycle in certain parts of the world, in the UK most individuals are anholocyclic. In this respect they are similar to *A. proletella* in that they can remain on the ‘same’ host throughout the year, however, in some

respects their population biology seems to be very different. Firstly it seems that aphids use long-range migration to colonise new hosts in the spring and early summer whereas *A. proletella* undertakes most of its long-range migration in the autumn and colonises new hosts in spring and early summer through trivial movement.

Aphid populations have been shown to support a large guild of predators and parasitoids, several of which are specialists, that often regulate infestations (Raworth *et al.*, 1984). The large numbers of aphid-specific predators/parasitoids lead to considerable regulation of populations and have been known to create mid-season population crashes (Hughes, 1963; Raworth *et al.*, 1984; Collier and Finch 1996). Few specialist predators were shown to feed on *A. proletella*, with any predation potentially coming from predators feeding on them as secondary prey. Hoverflies, for example were shown to be unable to develop through to adulthood when fed exclusively *A. proletella*. Therefore the growth of infestations of *A. proletella* can continue unchecked throughout the season.

On the whole even though as described above *A. proletella* and aphid pests of Brassicas, such as *B. brassicae* feed on the same hosts and are subject to the same environmental conditions the differences in their biology are such that they exhibit differences in population trends. The assumption of whitefly biology closely resembling that of aphids is unlikely a valid one and the experiments within the thesis have been used to highlight such disparity. *Brevicoryne brassicae* has been estimated at completing a generation in as little as 150 day-degrees, ($T^L=6.7^{\circ}\text{C}$), (Raworth *et al.*, 1984) when work from Experiment 3.2 estimated a value of 455 day-degrees for the *A. proletella* ($T^L=6.3^{\circ}\text{C}$). The generation time in *B. brassicae* is approximately half as long as *A. proletella* and would be likely to have large impacts on the population growth of these two pests in the field.

6.4 Conclusions

The first colonisation by *A. proletella* of new crops planted from April onwards is mostly achieved by low, short distance flights through trivial dispersal. The distance of newly planted vulnerable crops from sources of large populations of overwintering females will likely have a large impacts on rates of immigration rates.

The first colonisation of crops is often achieved by overwintering females and the date of first colonisation of newly vulnerable crops is not determined by the emergence of the first generation on overwintering hosts.

A potential larger ‘wave’ of immigration, again most likely achieved by trivial dispersal, is likely to occur when adults are emerging on senescing hosts in June, such hosts are likely to be winter oilseed rape or overwintered *Brassica* crops.

Long-range migration is achieved mainly after the onset of ovarian diapause, with potentially vast distances achieved by individual insects. Dispersal heights regularly exceed 12 m, as such they are regularly caught in suction traps used by the Rothamsted Insect Survey, with peak numbers of individuals often caught in October. To a lesser extent smaller numbers migrate in mid-summer, around July, coinciding with the senescence of overwintered hosts.

As immigration into crops is likely to be achieved by short distance trivial flights, within-field distributions are likely to show larger numbers at field edges, similar to that of the carrot fly. Large differences in numbers are likely to occur at different field edges, especially if the habitats in the immediate surroundings support varying populations of *A. proletella*. Areas supporting high populations of *A. proletella* are likely to be other *Brassica* crops, oilseed rape, and habitats that can support high densities of wild hosts, e.g. disturbed ground.

Populations of whitefly in vulnerable crops, such as kale, often increase with little regulation from predators or parasitoids. Very few predators feed on the juvenile stages of *A. proletella* under field conditions and the parasitoid *E. tricolor* is very

rare, likely to be present only in localised areas, with little evidence it will be able to regulate field populations.

A newly identified Entomophthrales fungal pathogen, likely a *Zoophthora* spp. was found to have significant regulatory potential in the field with rates of infection exceeding 90% of *A. proletella*. The ability for an fungal epizootic to establish in field conditions field shows there may be potential for biocontrol with fungal entomopathogens in the future.

Development of *A. proletella* from the egg to adult stages has been estimated to require 455 day-degrees above a lower-thermal threshold of 6.3°C and has been shown to predict the emergence of the first generation of adult *A. proletella* with varying degrees of accuracy. The trends in errors, however, suggest that inaccuracies are unlikely due to an inaccurate lower thermal threshold but are potentially due to the methods of recording the dates of first emergence of the generations.

The development of field populations on vulnerable crops follows patterns that appear to be predictable through the development of successive generations within the crop and the population growth of *A. proletella* within crops is likely to be due to the development of generations within the crop following colonisation early in the season. The size of the infestation that can develop within vulnerable crops during a season is likely to be strongly related to the number of generations that can develop before the onset of diapause in September.

Screening of a range of host plants for development rates and egg laying showed little difference in the quality of *Brassica* hosts to *A. proletella*. The data shows that the vulnerability of kale hosts, such as Reflex, is unlikely to be due to an intrinsic quality of the host. More work would need to be conducted to ascertain the cause for the observed high infestations of field kale crops.

Brassica incana potentially provided a level of resistance to *A. proletella* due to the presence of trichomes preventing normal feeding behaviour. Although this quality is an unattractive trait to breed into kale crops, this quality may provide a benefit to Brussels sprouts and if present in oilseed rape it could reduce the degree it would act

as an overwintering crop. The screen showed a trend of lower development and egg laying rates in non-*Brassica* wild hosts, especially *Chelidonium majus*. The study population of *A. proletella* may have been adapted to *Brassica* hosts, future research should investigate this further.

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