Project title:	Biology of the cabbage whitefly, *Aleyrodes proletella.*
Project number:	CP 091
Project leader:	Dr Rosemary Collier
Report:	Final report, December 2015
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Key staff:	Spencer Collins (PhD student)
Location of project:	Warwick Crop Centre School of Life Sciences University of Warwick Wellesbourne CV35 9EF
Industry Representative:	Andy Blair, T.H. Clements, UK.
Date project commenced:	1st October 2012
Date project completed (or expected completion date):	31st December 2015

[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name]
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[Organisation]

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[Name]
[Position]
[Organisation]

Signature .... Date ....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature .... Date ....

[Name]
[Position]
[Organisation]

Signature .... Date ....

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GROWER SUMMARY

Headline

In the field, the generations of cabbage whitefly were clearly distinguishable. The first generation of adults emerged after approximately 455D° above a base temperature of 6°C had elapsed, confirming laboratory studies. The fit was not so close for subsequent generations. During the early summer, adults make short flights within and around their host plants so that crops close to heavy infestations are most at risk. Longer range flights occur in the autumn. An entomopathogenic fungus appeared to be the biotic factor causing the highest levels of natural mortality during the study period.

Background

The cabbage whitefly, *Aleyrodes proletella,* has become an increasing problem for the Brassica industry in recent years, especially on Brussels sprout and kale. The reason for this 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. Little research has focused on this species as, historically; it has been regarded as a minor pest. Knowledge about the biology of the cabbage whitefly is limited and most of what is currently understood about its ecology has been inferred from minimal anecdotal evidence.

The overall aim of this project was to understand population trends of *Aleyrodes proletella* in the most vulnerable crops, Brussels sprout and kale. This includes understanding the key times of population/generation increase and colonisation of a new crop. 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. Information from this project is being used to inform experimental control programmes in AHDB Horticulture project FV 406a.

Summary

Host crops of cabbage whitefly

- Potential wild hosts were surveyed at the start of the project but no cabbage whiteflies were found. Whiteflies were found subsequently on *Sonchus*, *Taraxacum* and *Euphorbia spp.,* all known to be wild hosts.
- An oilseed rape crop sampled in July 2013 was infested with an average of 0.1 adults 0.5m$^{-2.}$ Taking this as an estimate for the entire field, there was potential for the 10ha field to support approximately 40,000 adult whiteflies.

- Adult cabbage whiteflies were counted on individual plants within a commercial crop of kale in Lincolnshire. Average numbers of cabbage whitefly on plants at the edge were approximately 4 times greater than those at a distance of 115 plants into the field. There was as much as a five-fold difference in numbers of adult whitefly between the four edges of the crop.

Headline: infested crop plants are likely to be the main sources of cabbage whitefly.

Monitoring dispersal of cabbage whitefly

- It is believed that yellow sticky traps are attractive to adult cabbage whiteflies making short flights within and around their host plants and that blue traps are attractive to female cabbage whiteflies undertaking longer-range dispersal flights. In preliminary field tests, yellow sticky traps caught more whiteflies than blue traps and traps on the ground caught more whitefly than those positioned 1m above the ground.
- Yellow and blue sticky traps were used to determine the vertical distribution of flight. Early in the year (April – August), yellow sticky traps caught significantly more whitefly than blue traps, but this trend was only evident for heights up to 120cm above the ground; above these heights captures on blue and yellow traps were very low and did not differ. Yellow sticky traps at ground level caught more adult whitefly than any other trap colour/height combination. Relatively more whiteflies were caught on blue sticky traps in September – November than in the earlier part of the year. At this point, female whiteflies had entered ovarian diapause (the overwintering stage).
- Yellow sticky traps and cauliflower trap plants were deployed in transects at a range of distances from plots infested with whitefly. There was a statistically-significant decrease in the number of adult whiteflies found on trap plants between 0 and 5 m from infested plots, but the numbers found on plants placed at distances of 5 and 15 m did not differ. At 25 m, the number of whitely was nearly 5 times less than that at 5 m. The numbers of whitefly caught on yellow sticky traps at distances of 0 and 5 m from the population source differed significantly from each other and from all other traps within the transect. The numbers captured on traps located at the remaining distances of 10m, 20m and 30m caught very similar numbers of whitefly and did not catch significantly different numbers from each other.
- In a further experiment, yellow sticky traps were placed horizontally and 1cm above the ground on the north, south, east and west side of infested plots. 'Activity indices' were calculated using the log (number of whitefly caught/mean number of adult whitefly per plant) in the plot. The indices varied during a year (August 2013 – July

2014). A slight, but statistically-significant peak in activity occurred in October 2013. Another peak in November was followed by a period where the activity was at its lowest (January), increasing in February-March. Activity indices during the period April 2014 – July 2014 were significantly higher than at all other times, with a large, statistically-significant peak occurring in late May – June.

- Samples from Rothamsted Insect Survey suction traps at Wellesbourne and Kirton (12.2 m high) were assessed for the presence of cabbage whitefly. With the exception of 2010 at Kirton, the median date of capture of adult whiteflies by the suction traps was between 11th October and 12th November. Whiteflies were caught about a month earlier at Kirton in 2010.

Headline: the results together suggest that cabbage whitefly undertake short low-level flights, within and around their host plants, during the first part of the summer (yellow sticky traps) and that female cabbage whiteflies undertake longer-range dispersal flights at higher altitudes in the autumn once they enter ovarian diapause (captures in suction traps and on blue sticky traps).

Development of cabbage whitefly

- The time required for cabbage whiteflies to complete development from egg to adult was monitored under controlled conditions for six 'constant' temperatures. The duration of development ranged from 79 days at 11.9°C to 23 days at 25.5°C.
- A straight line was fitted to the relationship between the rate of development and rearing temperature. The extrapolated lower development threshold was 6.3°C and it was estimated that 455 day-degrees (D°) above this threshold were required to complete development. The model was shown to predict accurately the emergence of the first generation of the season; however subsequent generations were predicted with less accuracy. Development of generations within a new crop was predicted accurately.

Development of field populations

- Plots of Brussels sprout and kale were planted on 2nd May 2013 to investigate the natural pattern of colonisation and increase in cabbage whitefly numbers over a season. Five replicate plots were planted in different locations on the Wellesbourne site. Numbers of whitefly remained very low, but were similar in all plots, for the first few weeks after transplanting. It was not until June that egg numbers increased, and soon after that all other life stages increased dramatically in numbers. This trend continued until September when numbers ceased to increase. All populations decreased from November until January 2014 when a large increase in egg numbers was observed. However, a corresponding increase in numbers of nymphs did not occur until March, followed by pupae in mid-May and adults in early June, after which numbers declined, coinciding with the senescence of the plants after flowering. Statistically-significant differences between plots in the numbers of whitefly were for a short duration only and most of the differences occurred over the winter months.

- Immigration rates of whitefly onto kale and Brussels sprouts were not significantly different nor were the rates of initial population increase, suggesting that they do not differ in 'attractiveness' to immigrant whitefly or their quality as a host plant. Differences in populations did occur later in the season, particularly during the winter, suggesting that kale may have provided more shelter from poor weather conditions.
- In 2014, plots of kale were planted in 5 locations at Wellesbourne. Each plot consisted of 5 sub-plots separated by ~18m. A single sub-plot was planted at each location on 19th May, 17th June, 19th July, 15th August and 16th September. In contrast to 2013, the numbers of adult whitefly differed between locations from as early as 2 weeks after the first plots were transplanted and there was a statistically significant relationship between the adult population on each new plot and the distance of the plot from the nearest highly-infested plot planted in 2013. Doubling the distance from 50m to 100m led to an approximate reduction in the adult population of 75%. There was also a strong relationship between the mean population size after one generation and the initial immigration rate into each plot (number of adults 2 weeks after planting). Generally, although the size of the infestation varied, the pattern of population increase did not differ between locations. The size of the infestation at the end of the season was related to planting date and was greatest in plots planted in May. The whitefly counts were also plotted on a day-degree scale (threshold 6.3^{o}C) and this indicated that whitefly numbers increased considerably once one generation (455D^{o}) had been completed following planting. This pattern occurred in all plots for all of the planting dates, except the plots planted in September, where ovarian diapause occurred and, over time, temperatures decreased below the lower development threshold of 6.3^{o}C, preventing further development.
- Egg laying began a month earlier in 2014 than in 2013. Mean January maximum daily temperatures were 2°C warmer in 2014 than 2013 and this may have been the reason for the earlier egg laying.

Headline: in 2013 there were no highly infested plants near to the newly-planted plots and initial infestation rates were low and similar between plots. The similarity between plots in the *size of the whitefly infestation persisted throughout the year. In 2014, some of the new plots were closer to infestations of whitefly (in the overwintered 2013 plots) than others and the immigration rate was related to the distance from an overwintering site. This, together with the results of the experiments on dispersal, suggests that colonisers of new crops in spring are likely to be from the immediate vicinity. The development of cabbage whitefly infestations could be clearly separated into 'generations'. In the field, the duration of the first generation in particular could be predicted using the day-degree sum estimated from laboratory experiments.*

Natural control of cabbage whitefly

Relatively few potential natural enemies (predators or parasitoids) of cabbage whitefly were seen in commercial crops or in field plots. However, dead adult whiteflies were observed in some of the field plots from October 2014. These dead adults were attached to the foliage and had outspread wings. A fungal growth could be seen on the thorax and abdomen of some individuals indicating that a fungal epizootic had occurred in some (but not all) of the plots. No relationship was found between the proportion of dead whiteflies and the total number of whiteflies present on a plant. Observations of the fungus in the laboratory confirmed that it is a member of the genus Zoophthora. Confirmation that the fungus was *Zoophthora radicans* could not be made as subsequent magnification of the specific primers for *Zoophthora radicans* was not successful.

Conclusions

- The rate of colonisation of new crops by whitefly is highly influenced by the distance of the new crop from sources of overwintering females. An increase in distance from 50m to 100m led to a 75% reduction in immigration rate.
- Kale and Brussels sprout plants appear to have the same level of 'attractiveness' to colonising cabbage whitefly.
- Field edges supported, on average, 4 times as many whitefly as areas towards the centre of the field. Populations of whitefly at each field edge also differed significantly from each other.
- Whiteflies are caught most effectively on yellow sticky traps at ground level, suggesting they are dispersing near to the ground. Increasing the height to 60cm led to, on average, a ten-fold reduction in catch rate.
- Activity of overwintering female whitefly increased during February-March showing potential for these females to move into new crops. Peak activity of adult whitefly was

in May-June when first generation of adults were emerging. It is likely to be the time of greatest colonisation of new crops.

- Female cabbage whiteflies undertake longer-range dispersal flights at higher altitudes in the autumn once they enter ovarian diapause (captures in suction traps and on blue sticky traps).
- It was estimated that 455 day-degrees above a threshold of 6.3°C are required to complete development from egg to adult. This was shown to be accurate in predicting the emergence of the first generation in 2013 and 2014; subsequent generations in 2014 were predicted with less accuracy.
- No parasitoids and very few natural predators were seen feeding on cabbage whitefly in the field. An entomopathogenic fungus was seen to cause an epizootic often killing >90% of the adult whiteflies.

Financial Benefits

Contamination of fresh produce by cabbage whitefly and associated damage can lead to rejections by retailers. Improved control of whitefly will have considerable financial benefits for growers through an improvement in crop quality.

Action Points

- Growers should be aware that new crops planted close to crops already infested by cabbage whitefly are likely to be most at risk of infestation.
- When planning their strategy for crop walking, growers should be aware that field edges are likely to support higher numbers of cabbage whitefly than areas towards the centre of the field. Whitefly numbers may also vary from edge to edge.
- A sum of 455 day-degrees above a threshold of 6.3°C are required for cabbage whiteflies to complete development from egg to adult. This information can be used to predict emergence of the first generation.
- Action points presented in the Final Report for Project FV 406a will be based on biological observations made in this project.

SCIENCE SECTION

Introduction

Historically the cabbage whitefly (*Aleyrodes proletella*) has been a minor pest of *Brassica* crops (Butler, 1938a), but more recently it became 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 cabbage whitefly with insecticides is difficult due to the abaxial 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. Since approximately 2010 the levels of cabbage whitefly infestation in Britain have declined due to use of the systemic insecticide Movento® (spirotetramat) (Richter, 2010; Collier, 2012). Although this pest is relatively easy to control at present there is a risk of it developing resistance to spirotetramat. Resistance to pyrethroid insecticides has been documented in *A. proletella* (Springate & Colvin, 2011), indicating that resistance management is very important.

In the scientific literature, whiteflies (Aleyroidae) are a particularly under-represented taxa, including 1556 described species, with potentially many more unknown to science (Martin & Mound, 2007). Research has focused mainly on two species: *Bemisia tabaci* and *Trialeurodes vaporariourm.* This is due to their significant economic importance, and very few other species have been studied to such an extent. As relatively little is known about the family as a whole, care should be taken not to generalise the findings from these species to all members of the Aleyroidae, as these species are unlikely to be typical of the family (Gerling, 1990).

The basic biology of *A. proletella* was investigated during the 1930s and this gave one of the first insights into this species (Butler, 1938a; 1938b). Females begin laying eggs when temperatures rise in the spring and increasing temperatures are responsible for stimulating egg laying after a winter diapause (Iheagwam, 1978). The cabbage whitefly is a multivoltine species and between 3 and 5 generations occur in England. Field temperatures have the largest influence on the number of generations in a season (Butler, 1938a; El-Khidir, 1963, Al-Houty, 1979). The first generation is thought to develop on the overwintering host and then the emergent adults migrate to summer hosts. This is believed to be the case as new colonisation by whitefly on crops usually occurs when the adults of the first generation emerge (Butler, 1938a; Al Houty, 1979; El-Khidir, 1963). Reproduction continues until late September. Diapause is elicited when the second instar develops in a decreasing photoperiod of less than

15¾:8¼ (L:D), which occurs in late July/early August in the UK. This generation of adults emerges in late September and consists of adults that overwinter (Adams 1985a; 1985b). Females that emerge at this time no longer have fully-developed ovarioles, and egg laying ceases (El-Khidir, 1963). Overwintering is achieved primarily by these females who can tolerate temperatures as low as -18°C for short periods of time. Adult males have been shown to have a considerably lower tolerance of cold, and after mating they have a life expectancy of only 10 days (Butler, 1938a; 1938b). A suggestion that the later nymphal instars can overwinter was made by Iheagwam (1977a) and this is supported by the fact they can withstand sub-zero temperatures, which would be experienced through winter (Butler, 1938b). The degree to which this occurs has not been substantiated, as most pupae are likely to perish when the plant sheds its older leaves late in the winter (El-Khidir, 1963). The factors leading to the termination of diapause in *A. proletella* have not been elucidated. Chilling has been shown to shorten diapause and temperatures exceeding 25°C to prevent diapause (Iheagwam, 1977a). It is likely that a number of factors interact to bring females out of diapause. If a phenological model was to be developed for this species, factors involved in the termination of diapause and the lower threshold temperature for egg laying should be determined.

It is very important to understand the pattern of crop colonisation by *A. proletella*. As the immature stages of whiteflies are sessile, dispersal and therefore colonisation of new crops is achieved by the winged adults. Females show a vastly higher rate of dispersal than males (El-Khidir, 1963). It is likely that males respond to a cue from females, as males have been recorded 'waiting' for females to emerge from their puparia (Butler, 1938a). Dingle (1986) considered that there are two distinct forms of dispersal; trivial flight and migration in insects. Trivial flights can be regarded as short duration flights, usually between hosts, where flight is directed to host plants and elicited by attraction to green/yellow light wavelengths (400-600nm). Migration is undistracted flight, whereby the insect ignores vegetative cues and is attracted to other cues such as sky light, causing the insect to fly up, out of vegetation. The distribution of captured whiteflies from a source follows a bimodal pattern supporting the notion of trivial and migratory flight morphs (Byrne *et al.* 1996). Two seasonal morphs of *Aleyrodes proletella* have been discovered, that differ in their dispersal behaviour (El-Khidir, 1963). The summer morph has been shown to be reluctant to fly and, when it does fly, this is only for short durations, and it quickly returns to vegetation. It has been shown that the summer morph of *A. proletella* is attracted to yellow-green light (500-600nm), which is close to the wavelength of light reflected from vegetation and this has been documented for other whitefly species (Bulter, 1938a; Mound, 1962). This information suggests that during the summer, whiteflies stay within a localised region with minimal immigration from long distances into crops. This ‘morph’ can be regarded as performing mainly trivial dispersal. The autumn diapausing morph has shown increased flight behaviour with long flight durations, potentially reaching up to 40m in height. Diapausing individuals have shown positive phototaxis to an overhead light source, which was not observed in non-diapausing individuals (Iheagwam, 1977b).

Differences in phototactic responses between migratory and non-migratory morphs of whitefly have been observed in *Bemisa tabaci.* Migratory morphs have shown a clear attraction to ‘Sky light’, specifically wavelengths towards the ultraviolet part of the spectrum. This is believed to attract the whitefly to fly upwards toward the sky eliciting the higher flights needed for migration (Mound, 1962). The distance of migration by cabbage whitefly has not been quantified and although whitefly have been shown to migrate distances over 2km, this is likely to be a conservative estimate, as prevailing winds influence their migration (Byrne *et al.* 1996). Although whitefly migration is likely to be 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 (Byrne *et al.* 1996). It seems that active flight to achieve height, along with prevailing winds, provides the potential for an individual to travel vast distances. This longer range migration is likely to be achieved by the overwintering females.

The lower threshold temperature for flight for this species has been found to be 9°C (El-Khidir, 1963). As a result, overwintering females stay within the same location once temperatures fall in November and are unlikely to take flight again until temperatures warm in spring. Being able to predict peaks of this migration by developing a phenological model would be useful to the Brassica industry allowing a targeted timing of control to eradicate the first colonisers to a new crop. Knowledge of the window of immigration of the pest into the crop would be invaluable as control may be best held back until immigration has stopped, allowing the control of whitefly without the risk of new colonisers occurring afterwards.

The overall aim of this project is to understand population trends of *Aleyrodes proletella* in the most vulnerable crops, Brussels sprout and kale. This includes understanding the key times of population/generation increase and colonisation of new crops. This information will be used to indicate periods when the application of control methods would be most effective in reducing the overall pest load.

The key objectives are to:

- Develop a phenological model for *Aleyrodes proletella* development within a crop.
- Understand the dispersal potential of *Aleyrodes proletella.* Is proximity to overwintering populations important?
- Understand the natural pattern of crop colonisation. Are there distinct periods when colonisation occurs?
- Identify factors limiting population growth. Which factors contribute to overall pest load in a crop.

Materials, Methods and Results

Study Site

Field studies were conducted at, or near, Warwick Crop Centre, Wellesbourne, England, CV35 9EF (UK Grid reference SP 27320 56936) unless otherwise stated.

Host crops of cabbage whitefly

Experiment 1.1 Survey of wild host plants for the presence of whitefly in an uncultivated field.

Materials and methods

Potential wild hosts of cabbage whitefly were sampled within a field at Warwick Crop Centre, Wellesbourne (approx. 1ha) for the presence of whitefly. 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). Forty randomly generated co-ordinates were used as sample locations, where 0.5m^2 quadrats were used to survey for all known wild host plants (Mound & Hasley, 1978). These plants were checked thoroughly for the presence of cabbage whitefly. The survey was conducted on 20th May 2013. The percentage ground cover in each quadrat and the numbers of each host plant present were also recorded.

Results

Of the wild hosts of the cabbage whitefly mentioned in Mound and Hassle (1978) only *Sonchus*, *Taraxacum* and *Euphorbia spp* were found (Table 1.1.1). No whiteflies were found on any of these potential host plants. It is likely that the population of cabbage whitefly was too low for them to be detected from this study.

Table 1.1.1 Mean number of wild host plants and their percentage coverage per 0.5m^2 from 40 x 0.5m^2 surveyed quadrats from an uncultivated field at Wellesbourne.

Wild host plant	**Mean number 0.5m^{-2}** (% cover)
Sonchus spp.	0.46 (1%)
Taraxacum spp.	0.24 (0.9%)
Euphorbia spp.	0.05 (0.2%)

Experiment 1.2 Survey of a commercial oil seed rape crop for whitefly.

Materials and methods

A commercial field of oilseed rape (OSR), *Brassica napus*, approximately 10ha in size and located close to Warwick Crop Centre, was surveyed for the presence of whitefly on three occasions during 2013: 20th April, 5th June and 17th July. A sampling grid 40m x 40m was used within the field. At each sampling point all plants within a 0.5m^2 quadrat were investigated for the presence of whitefly. An estimate of the percentage ground cover by OSR plants and their distance from the field margin were also recorded.

Results

No whiteflies were found from surveys in April or June. The final survey in July showed an average of 0.1 adults 0.5m^{-2}; a total of 4 adults were found from 40 survey quadrats (Table 1.2.1). Taking this as an estimate for the entire field, there is potential for the 10ha field to support approximately 40,000 adult whitefly [(0.1 x 4) x 100,000)]. It should be noted that all whitefly found during the survey were within 40m of the field edge.

Table 1.2.1 Mean number of OSR plants per 0.5m^2 and number of whitefly at different life stages from 40 x 0.5m^2 survey quadrats on three different survey dates.

Survey date	Mean number 0.5m^{-2}				
	OSR plants (% cover)	Cabbage whitefly			
		Adults	Eggs	Nymphs	Pupae
20 April 2013	9.66 (10%)	0	0	0	0
5 June 2013	5.67 (64%)	0	0	0	0
17 July 2013	3.62 (66%)	0.10	3.25	0.15	0.20

Experiment 1.3. Distribution of whitefly in a commercial crop of kale.

Materials and methods

Adult whitefly were counted on individual plants within a commercially grown field (~17 hectares) of kale, cv. Reflex, in Lincolnshire, (Grid reference, TF 33530 21723). Transects were taken from the middle of each side of the four field edges (North, South, East and West). Whitefly were counted at distances of 0 (edge plants), 5, 15, 35, 75 and 155 plants into the field where the plant spacing was ~60cm. Four plants at each distance were sampled for whitefly using a similar approach to Experiment 4.2. As the numbers of whitefly in each transect seemed to differ, each field edge was sampled in more detail. The field edges were divided into potential sampling sections of 2m and four were selected at random to become replicates for each field edge. The ‘‘0” point of the 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 50m of an adjacent field edge. Figure 1.3.1 shows a schematic of the sampling plan. Sampling was conducted on 18th August 2014 and the crop had been in the field for ~3months. The crop had not been treated with insecticide beforehand. The resulting data were analysed using Analysis of Variance (ANOVA).

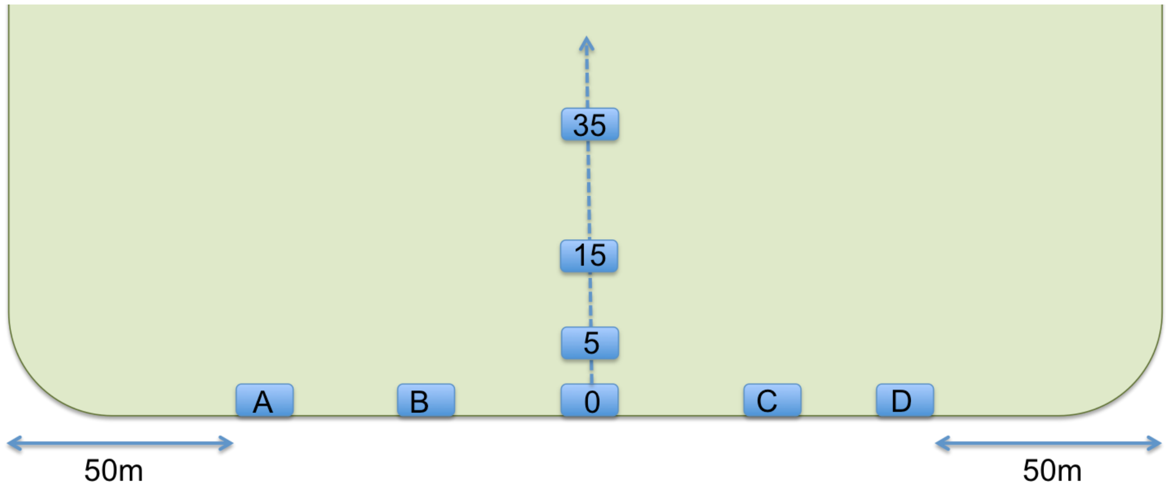


Figure 1.3.1 Sampling schematic for Experiment 1.3. Transects into field are represented by numbers 0, 15, 35 etc. A, B, C and D represent the randomised sampling points on each field edge. No sampling occurred within 50m of an adjacent field edge.

Results

Sampling into the field showed that whitefly numbers near the edge were significantly different from those nearer the centre (ANOVA, P<0.01, Figure 1.3.2). Average numbers of cabbage whitefly on plants at the edge were approximately 4 times greater than those at a distance of 115 plants into the field.

Comparing populations between each of the field edges (North, South, East and West) showed that numbers of adult whitefly differed between them (ANOVA, P<0.01). The most pronounced difference was between the northern and southern edges where there was an approximately a five-fold difference in numbers of adult whitefly (Figure 1.3.3).

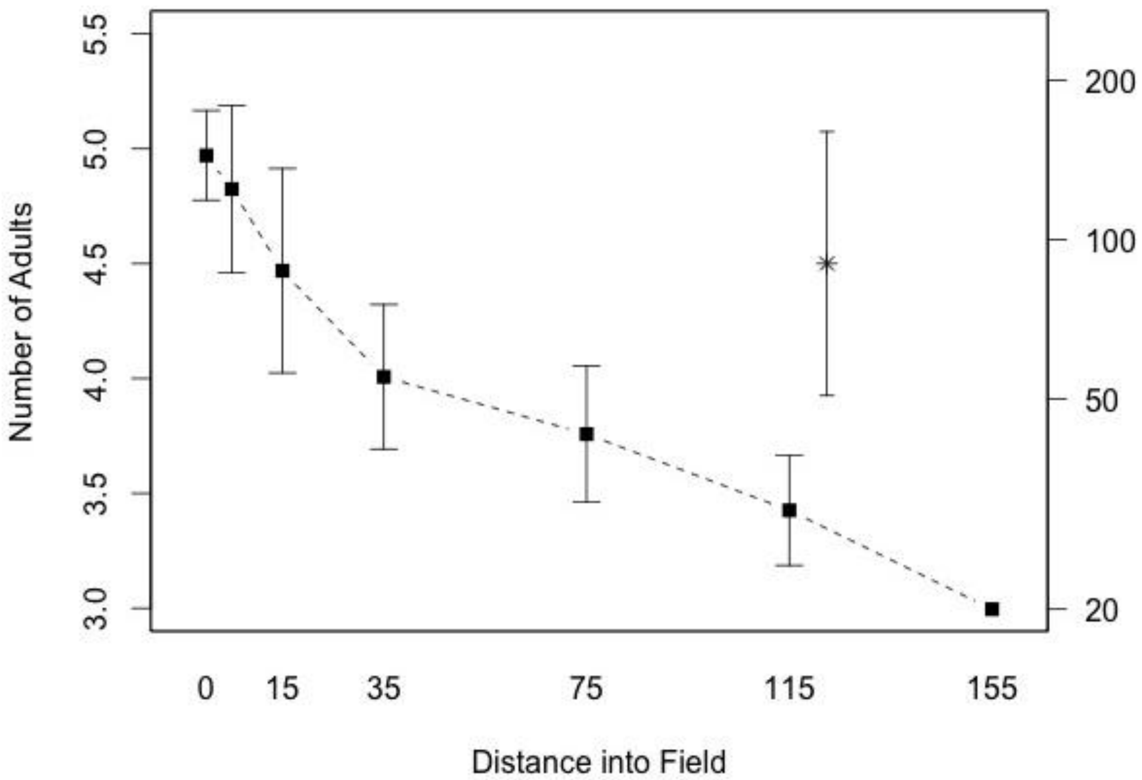


Figure 1.3.2 Mean (±SE) number of adult whitefly on 4 plants at different distances from each edge of a commercial crop of kale. The 5% LSD from ANOVA ($P<0.01$) is shown as an asterisk. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

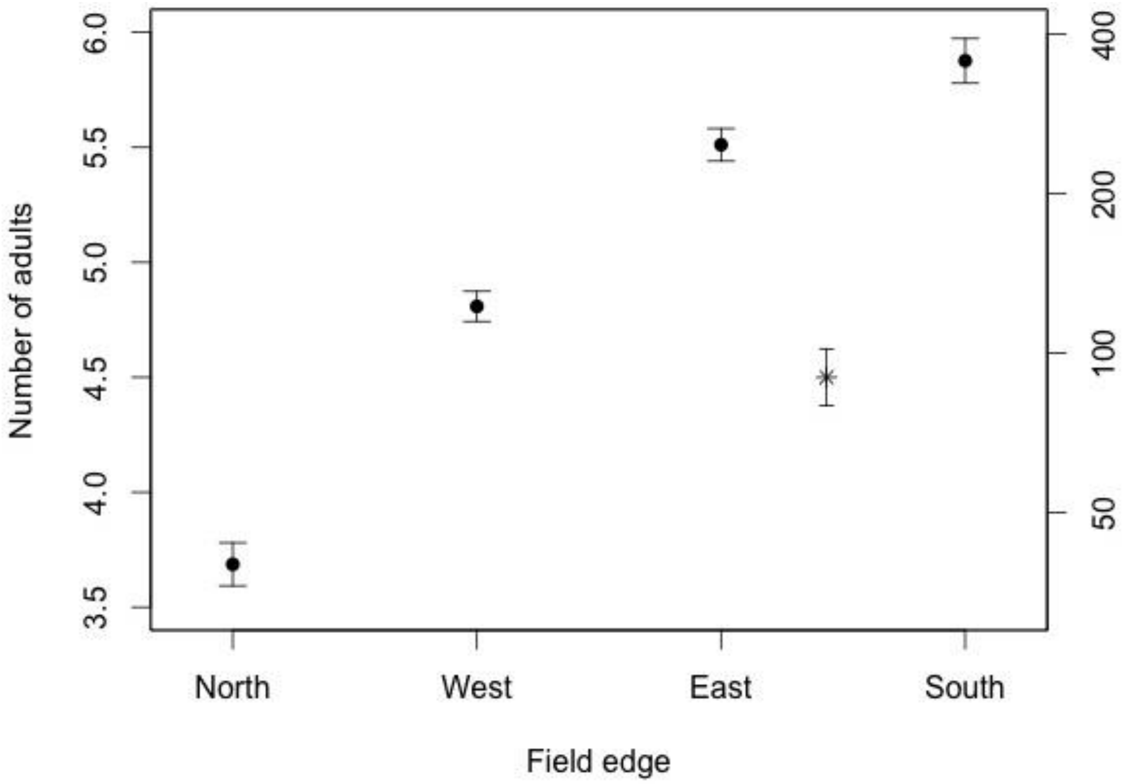


Figure 1.3.3 Mean (±SE) number of adult whitefly found on plants along each of the field edges (North, West, East and South). The 5% LSD from ANOVA (P<0.01) is also shown as an asterisk. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

Monitoring dispersal of cabbage whitefly

Experiment 2.1 Developing an effective method for trapping active adult whitefly in the field.

Materials and methods

This experiment was undertaken to investigate the preferences of the cabbage whitefly for landing on sticky traps of different colours (yellow/blue) and heights (ground/1m high). A small plot of Brussels sprout plants (3m x 10m, 30 plants) with a heavy infestation of cabbage whitefly was used to investigate any preferences for colour and height. Replicate treatments (4) were set out in a randomized-block design. The sticky traps (22cm x 10cm) were either yellow or blue (BHGS Ltd, UK). The traps close to the ground were placed upon a plastic base (9cm diameter) to suspend them approximately 1cm above soil level. The traps at 1m above ground were attached between two 1m bamboo canes to secure them horizontally. All sticky traps were placed perpendicular to the ground. Traps were set out on 26th October

2012 and collected on 29th October 2012. The numbers of whitefly caught were recorded on the day of collection. The resulting data were analysed using Analysis of Variance (ANOVA).

Results

Yellow sticky traps caught more adult whitefly than blue traps (ANOVA, F=18.6, n=4 P<0.001) and traps on the ground caught more whitefly than those positioned 1m above the ground (ANOVA, F=47.9, n=4, P<0.001) (Figure 2.1.1).

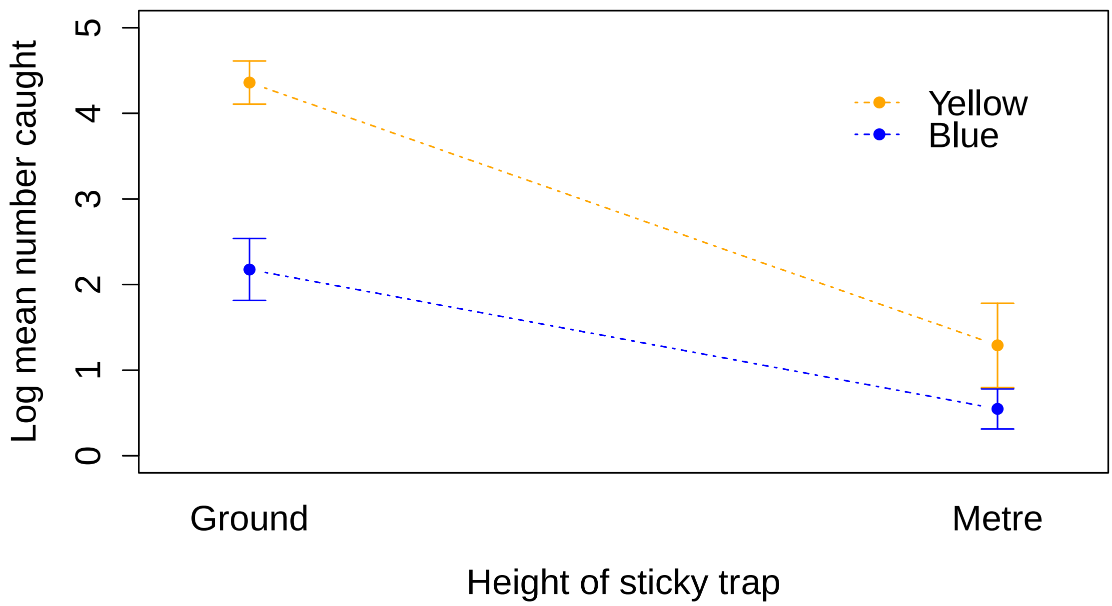


Figure 2.1.1 Log mean number of whitefly caught for each colour of sticky trap (yellow/blue) at each height (ground/1m). (± 1 S.E.).

A significant interaction of colour and height of trap was found (ANOVA, F=4.5, n=4, P<0.05). There was no statistically significant difference between the numbers caught on the upper or lower sides of the traps.

Experiment 2.2 Height at which adult cabbage whitefly disperse.

Materials and Methods

Sticky traps deployed at various heights from the ground were used to monitor adult whitefly to determine the vertical distribution of flight. Telescopic 7 metre-high ‘flagpoles’ (Newquay Camping, UK) were erected within 5m of plots with large cabbage whitefly populations (to increase the probability of capture), using the plots in Experiment 4.2. The apparatus was set up as shown in Figure 2.2.1.

The sticky traps (22cm x 10cm) were either yellow or blue (BHGS Ltd, UK) and were rolled into a cylinder (10cm tall, 8cm diameter) to provide a coloured sticky surface that covered 360°. Four replicate flagpoles were set up on the same day and left for 7 days. The traps were then collected and the numbers of whitefly were counted using a microscope, as damaged individuals were easily missed with the naked eye. The trapping periods began on 13th April, 28th April, 28th May, 14th June, 28th June, 1st August, 18th September, 28th October, 4th November and 18th November. The resulting data were analysed using Analysis of Variance (ANOVA) and Chi-squared tests.

Results

Between April and August yellow sticky traps caught significantly more whitefly than blue traps, but this trend was only evident for heights up to 120cm above the ground (ANOVA, $P<0.01$) and above these heights catches from blue and yellow traps did not differ. Yellow sticky traps at ground level caught significantly more adult whitefly than any other trap colour/height combination, catching on average ~50 individuals during the study period. The next highest catch rate was by yellow traps at 60cm and here the average catch rate fell to only ~5 individuals (Figure 2.2.2).

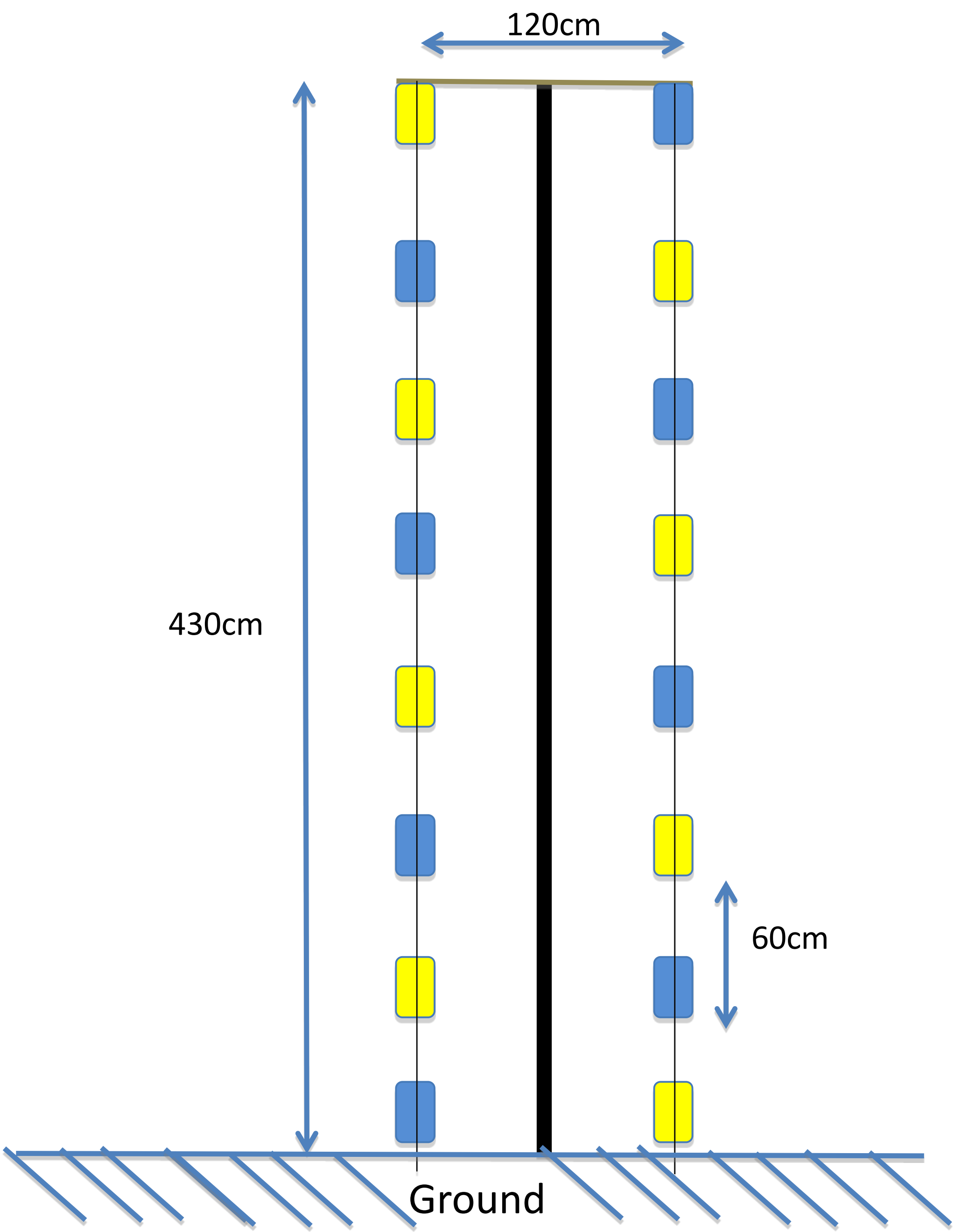


Figure 2.2.1 The flagpole supporting yellow and blue sticky traps used in Experiment 2.2.

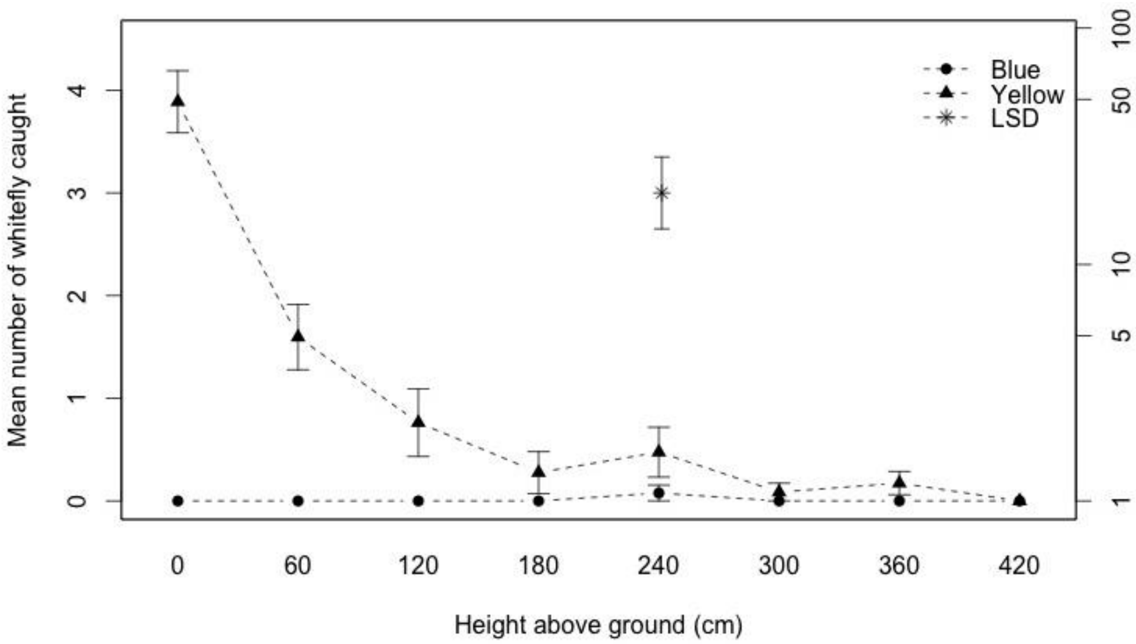


Figure 2.2.2. Mean (±S.E.) number of whitefly caught on yellow or blue sticky traps at different heights above ground (April-August). The 5% LSD from ANOVA is also shown (P<0.01). The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

Significantly more whitefly were caught on blue sticky traps in September – November (yellow = 902, blue = 80) than the earlier period before (June-August, yellow = 713, blue= 2), (Chi-square=53.98, df = 1, p<0.01) (Figure 2.2.3).

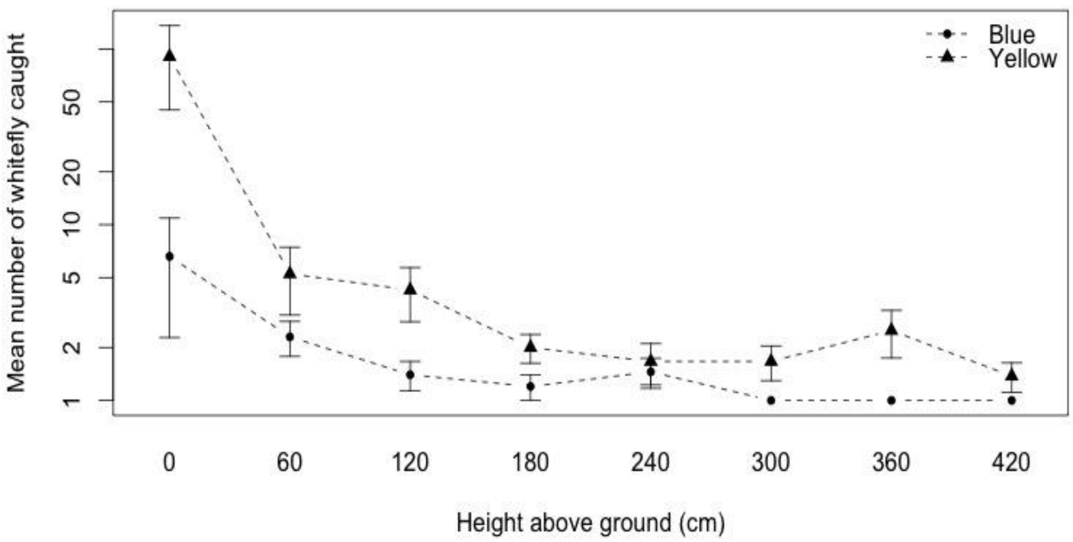


Figure 2.2.3. Mean (±S.E.) number of whitefly caught on yellow or blue sticky traps at different heights above ground September-November.

Experiment 2.3 Estimating the abundance of adult whitefly at different distances from a field infestation.

Materials and methods

Yellow sticky traps and 'trap plants' were deployed in 'transects' at a range of distances from plots infested with whitefly; which were those used in Experiment 4.2. Each sticky trap was placed horizontally on a plastic tray ~1 cm above the ground. This prevented ground active insects (e.g. carabid beetles) being caught on the trap, which quickly reduced their efficiency to catch other insects. For the approach using 'trap plants', groups of three cauliflower plants (7^{th} true leaf stage) were placed at the required distances along the adjacent hedge line of the infested plot. The plants were placed on a 30 x 20cm white plastic tray to hold water. Both the sticky trap 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 microscope. The sticky trap method was used in each of 3 locations beginning on 3^{rd} and 30^{th} April, 2014. The trap plant method was used in each of 3 locations beginning on 24^{th} April and 9^{th} June 2014. The data were analysed using Analysis of Variance (ANOVA).

Results

Trap plants

The numbers of whitefly found on trap plants placed at various distances from a known population source were significantly different (ANOVA, P<0.01, Figure 2.3.1). There was a significant decrease in the number of adult whitefly found between 0 and 5m, but the numbers found on plants placed at distances of 5 and 15m did not differ. At 25m, the number of whitefly was nearly 5 times less than that at 5m.

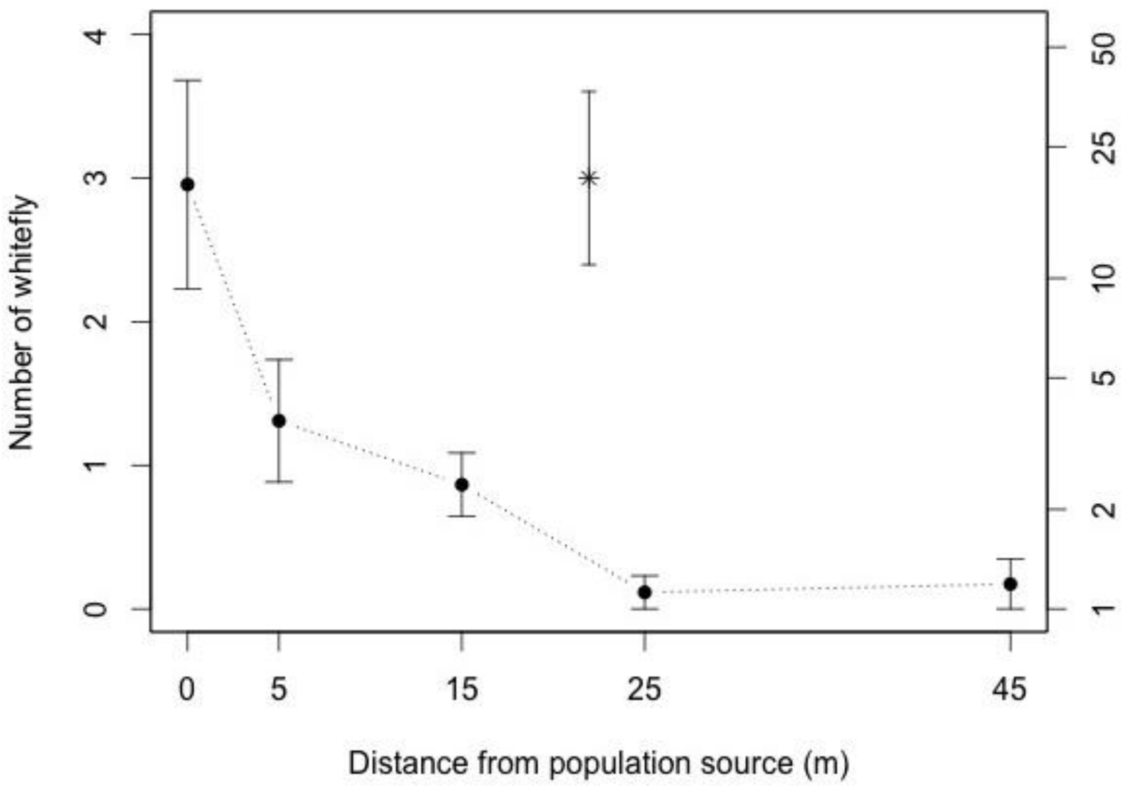


Figure 2.3.1 Mean (±S.E.) number of whitefly found on trap plants placed at different distances from a population source. The 5% LSD from ANOVA (P<0.01) is also shown as an asterisk. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

Yellow sticky traps

The numbers of whitefly caught on yellow sticky traps at distances of 0 and 5 m from the population source differed significantly from each other and from all other traps within the transect. The numbers captured on traps located at the remaining distances of 10m, 20m and 30m caught very similar numbers of whitefly and did not catch significantly different numbers from each other (ANOVA, P<0.01, Figure 2.3.2).

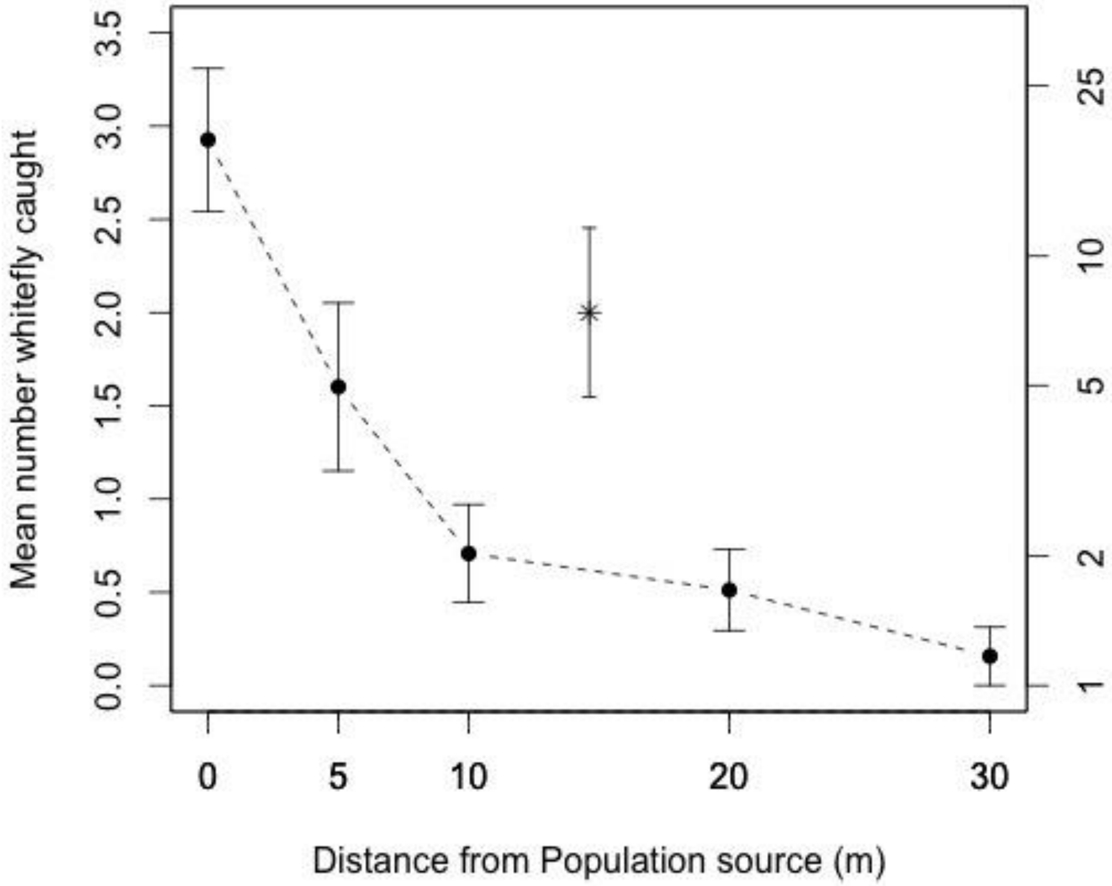


Figure 2.3.2 Mean (±S.E.) number of whitefly caught on yellow sticky traps placed on the ground at different distances from a population source. The 5% LSD from ANOVA (P<0.01) is also shown as an asterisk. The left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

Experiment 2.4 Monitoring whitefly activity using yellow sticky traps on the ground.

Materials and methods

Yellow sticky traps, 22cm x 10cm (BHGS Ltd, UK) were placed horizontally and 1cm above the ground on the North, South, East and West side of the plots used in Experiment 4.2. 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 microscope. Weather data were collected for all days when traps were present in the field. To reduce the potential for dead whiteflies to fall onto the traps, the traps were placed 30cm from the nearest plant, since the aim was to capture individuals flying actively.

Results

An 'activity index' was calculated. This was defined as the log (number of whitefly caught/mean adult whitefly number per plant in the plot). The 'activity index' varied during the year (August 2013 – July 2014). A slight, but statistically-significant peak occurred in October 2013. A large increase in activity was seen in November which was followed by a period where the activity index was at its lowest (January and February). The activity indices during the period April 2014 – July 2014 were significantly higher than at all other times, with a large, statistically-significant peak occurring in late May – June (Figure 2.4.1).

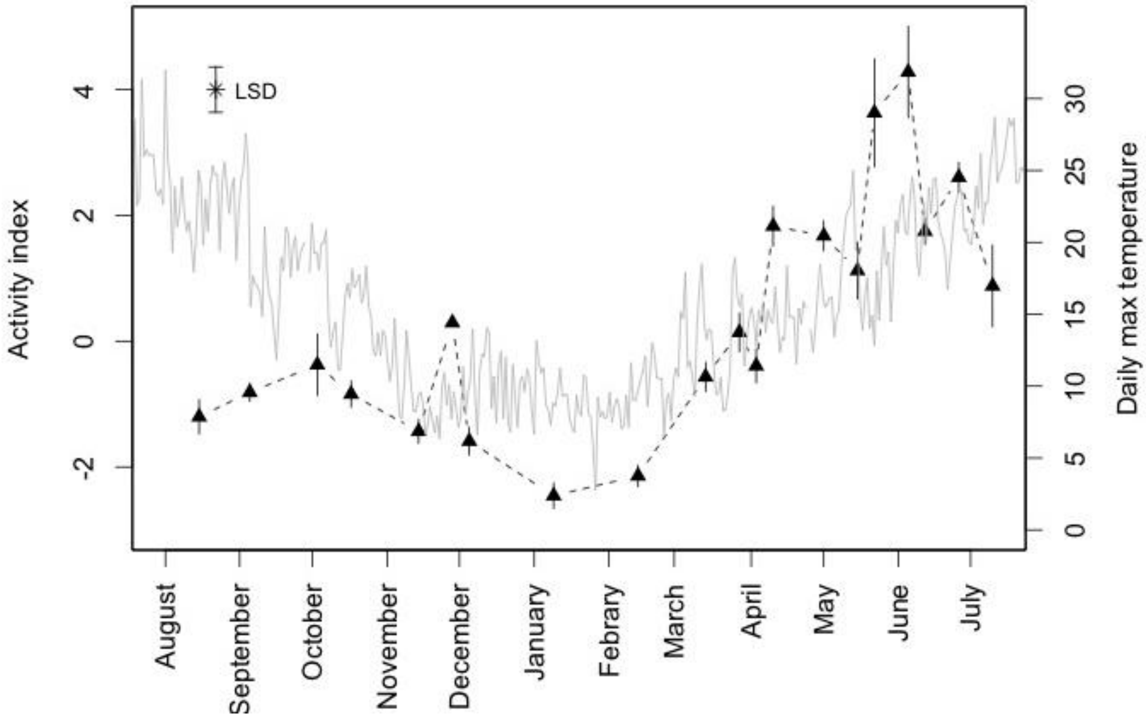


Figure 2.4.1. Mean (±S.E.) whitefly activity index [log (Number whitefly caught on traps/numbers per plant in plot)] throughout a year (August 2013 – July 2014). The 5% LSD from ANOVA (P<0.01) is also shown. The grey line represents daily maximum air temperatures (°C) for Wellesbourne.

Throughout most of the year (August 2013 – July 2014) the catch rates for sticky traps to the North, South, East or West of the plots did not differ significantly from one another. There was a long period from December 2013 until March 2014 when catch rates were significantly higher on the traps to the North or East (ANOVA, P<0.01, Figure 2.4.2) than those to the South and West.

There was a statistically significant relationship between the log sticky trap catch and the log mean adult population (log-log linear model y=0.9173x, P<0.01, R^2=0.88, y-intercept constrained to 0, Figure 2.4.3).

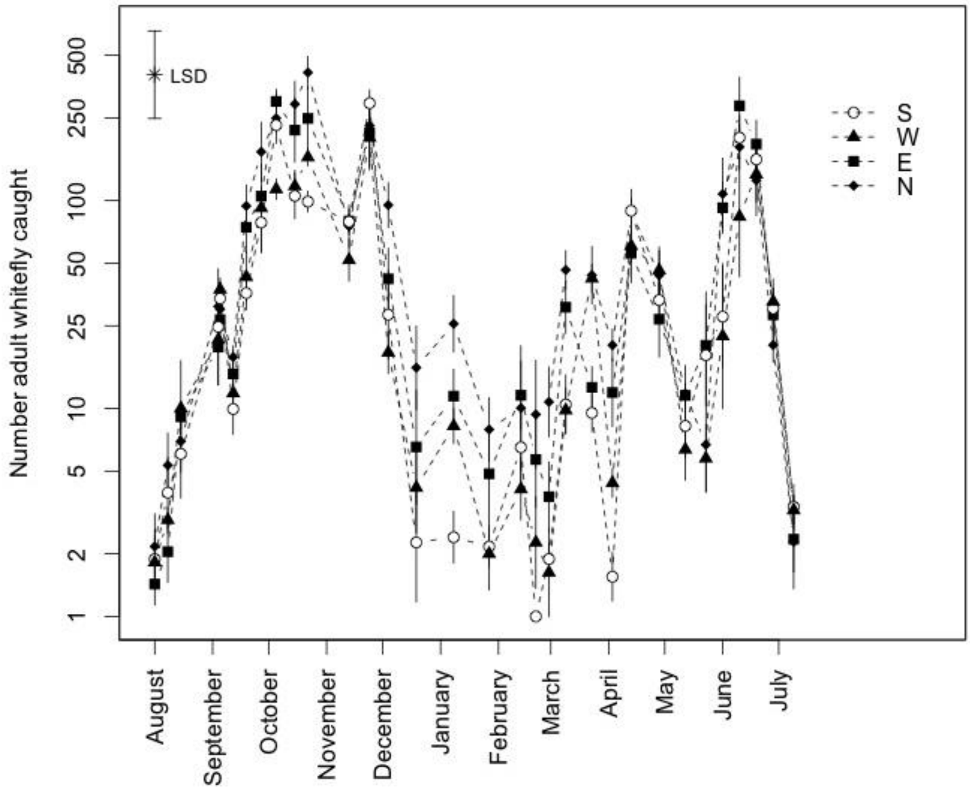


Figure 2.4.2. Number of whitefly caught on traps placed in different directions from plots (N-North, S-South, E-East, W-West) The 5% LSD from ANOVA (P<0.01) is also shown.

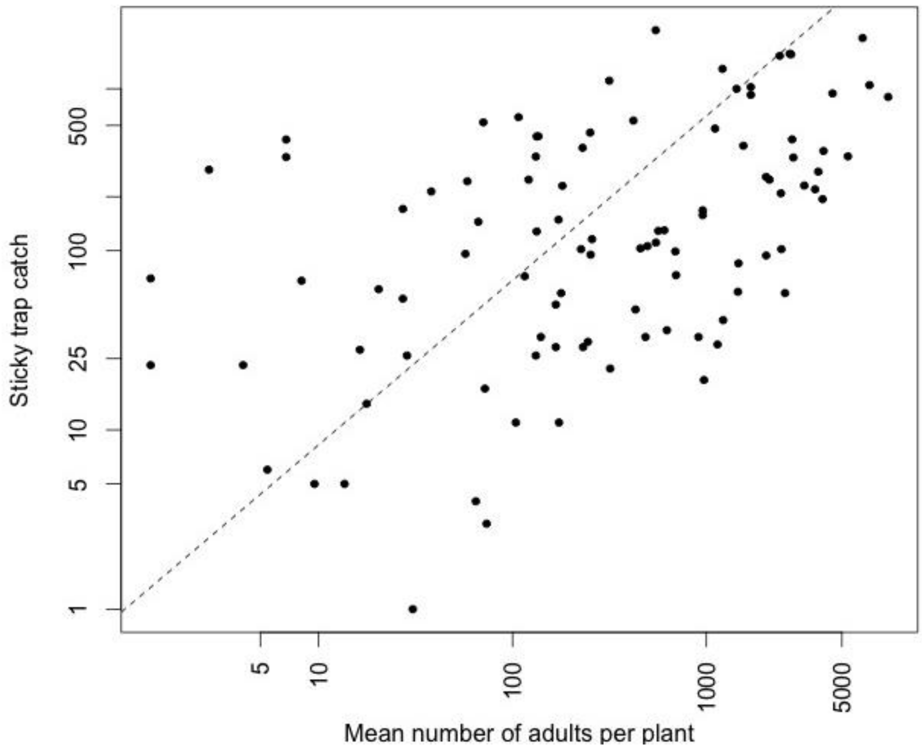


Figure 2.4.3 Mean number of whitefly caught on sticky traps versus the mean number of adults per plant. A fitted logged linear model (y=0.9173x) is shown as a dotted line (P<0.01).

Experiment 2.5 Assessing Rothamsted suction trap samples for presence of cabbage whitefly.

Materials and methods

Samples from Rothamsted Insect Survey suction traps were assessed for the presence of cabbage whitefly. The trap is 12.2m high and samples 45m^3 of air per minute. All animals sucked into the trap are automatically collected in bottles containing 70% alcohol and are then transferred to 95% ethanol, 5% glycerol solutions for storage in small vials. The samples assessed are summarized in Table 2.5.1.

Table 2.5.1. 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.

Each sample was assessed for the presence of *Aleyrodes proletella.* Samples were viewed under x40 magnification. Identification of whitefly species was confirmed using wing venation.

Results

Figure 2.5.1 shows cumulative catches of whiteflies between August and December for Kirton (2010-2014) and Wellesbourne (2012-2015). Gaps in cumulative catches represent samples that were missing due to trap failure. The dashed line shows the point by which 50% of the total whiteflies captured were caught each year (median date of capture).

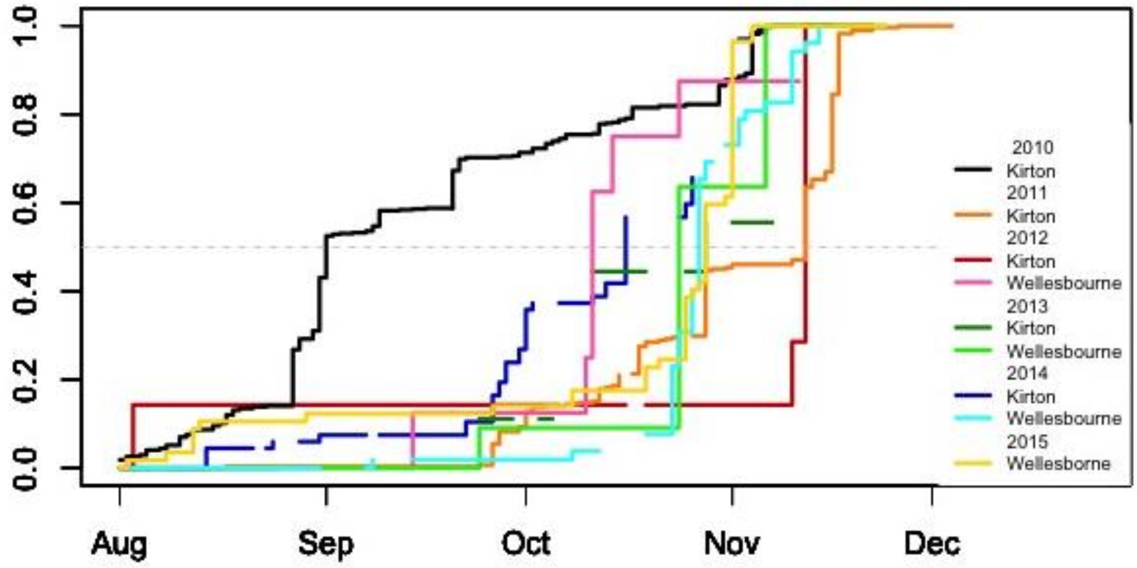


Figure 2.5.1 Cumulative catches from suction traps between August and December for Kirton (2010-2014) and Wellesbourne (2012-2015). Gaps in cumulative catches represent samples that were missing due to trap failure. The dashed line shows the point by which 50% of the total whiteflies captured were caught each year (median date of capture).

Table 2.5.2 shows total captures (Aug-Dec) for each trap location and date. The date of median catch is shown with the accumulated day degrees from 1 January to that date, together with the accumulated day degrees from 1st August (base 6.3ºC).

With the exception of 2010 at Kirton, the median date of capture of adult whiteflies by the suction traps was between 11th October and 12th November. Whiteflies were caught about a month earlier at Kirton in 2010.

Table 2.5.2 Total captures (Aug-Dec) for each trap location and date. The median capture date is shown with the accumulated day degrees (base 6.3ºC) from 1 January to that date, together with the accumulated day degrees to 1st August.

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

**Numbers in brackets represent total catches for the year.*

In the years where samples from both sites were assessed, the total numbers of whitefly captured were very similar. No relationship was found between median time of capture and accumulated day-degrees from 1 January to 50% catch or to 1st August.

During 2014 at Wellesbourne, the majority of samples for the year did not contain any whitefly. Most of catches were concentrated in October and November. Only 3 whiteflies were caught outside this time period, 1 in September and 2 in June (Figure 2.5.2).

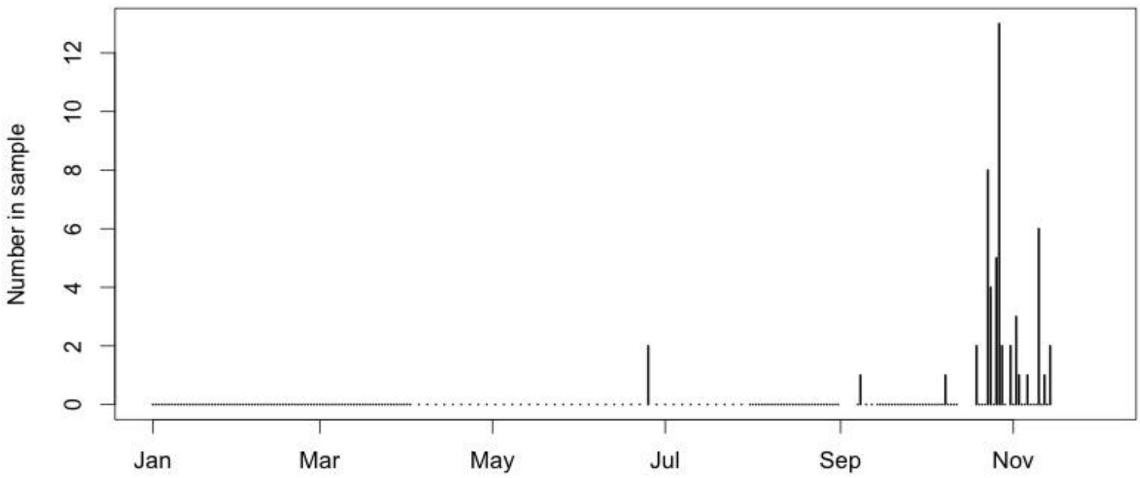


Figure 2.5.2 Number of *Aleyrodes proletella* caught in the Wellesbourne suction trap, April-December 2014.

The numbers 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 2.5.3).

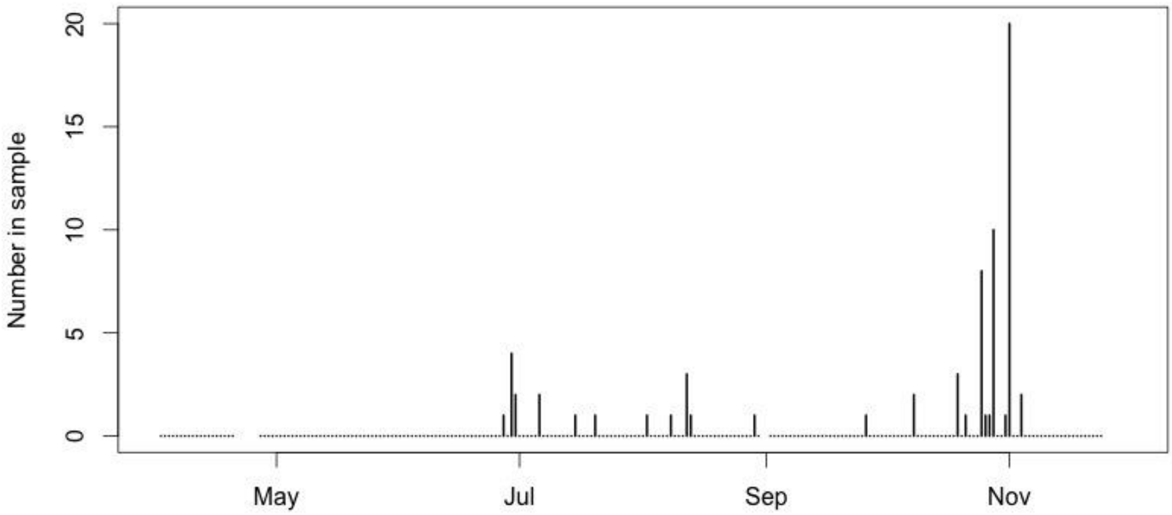


Figure 2.5.3 Number of *Aleyrodes proletella* caught in the Wellesbourne suction trap April – December 2015.

Development of cabbage whitefly

Experiment 3.1 Egg laying rate and duration of egg laying on three different Brassica oleracea crops: Brussels sprout, kale and cauliflower.

Materials and methods

Three types of *Brassica oleracea* crop were tested to determine the fecundity of female whitefly and the duration of egg laying. These were cauliflower (cv. Skywalker), Brussels sprout (cv. Revenge) and kale (cv. Reflex). All the whitefly 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 whiteflies were collected by taking a sample of leaf from the laboratory culture, that contained pupae, and keeping it overnight in a closed petri dish to prevent newly emerged adults from escaping. The next day any adult whitefly that had emerged were sexed, males could easily be identified by the presence of two claspers on the abdomen. One adult of each sex was then placed onto a leaf using a fine paint brush. This was performed over ice, providing chilled conditions to prevent individuals flying. The newly-infested leaf was enclosed within a 'clip-cage' to prevent the whiteflies from escaping and to ensure feeding occurred on that particular leaf. All the leaves infested consisted of the second youngest leaf at the time of infestation. The number of eggs laid was 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 was dislodged from the leaf, she was immediately placed back in the same location. Plants were kept at 20°C with a photoperiod of 16:8h (L: D). A total of 5 replicates were tested on each type of host plant. Females were moved onto new leaves if the leaves showed signs of senescence.

Results

The mean duration of egg laying was 33 days, with females producing a mean of 89 eggs in this time. There was no significant difference between the mean number of eggs laid per female over a 2 day period for the three crops tested, Brussels sprout, cauliflower and kale (ANOVA, $F=3.7$, $P>0.05$, Figure 3.2.1).

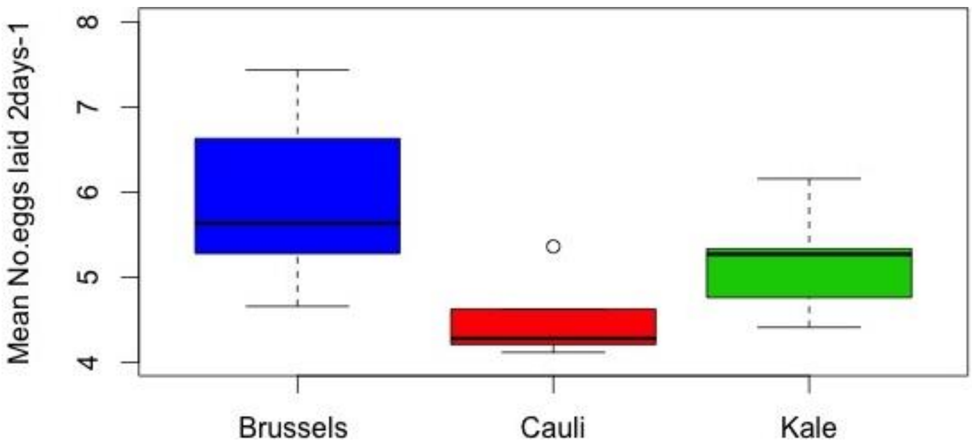


Figure 3.2.1 Mean number of eggs laid per female in 2 days on three different host plants, Brussels sprout, cauliflower and kale. ANOVA shows no statistically significant difference, n=5.

Experiment 3.2 Development rate of Aleyrodes proletella in constant temperatures.

Materials and methods

The time required to complete development from egg to adult was monitored under controlled conditions for 6 ‘constant’ temperatures between 11° and 26°C. Adults were allowed to lay eggs for a period of 24 hours at 20°C on the foliage of cauliflower plants (cv Skywalker) at the 5th true leaf stage**.** If more than twenty 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 development of the 20 eggs was monitored until adult emergence. The rearing temperature was monitored at plant height using ibutton® thermochrons (DS1921G).

Field data on the development of generations was gathered from the overwintered plots from Experiments 4.1 and 4.2. The date when the first pupal cases were present, evidence that adults had emerged from pupae, was taken as the observed date of emergence of the first generation. When the first generation adults emerged, the development of the subsequent generation was determined by transferring adults onto a fresh kale plant (7 true leaf stage) on the same day. The adults were allowed to lay eggs and 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.

Results

Table 3.2.1 shows the duration of development in days of whitefly held at each temperature. This ranged from 79 days at 11.9°C to 23 days at 25.5°C. The same data are plotted in Figure 3.2.1. A straight line was fitted to the data and the estimated lower development threshold (LDT), i.e. the point where development is zero, is 6.3°C. The accumulated day-degrees (D°) above this threshold required to complete development were estimated to be 455.

Table 3.2.1. Mean temperature and mean length of development in days for *Aleyrodes proletella* reared on cauliflower (cv Skywalker).

Mean Temperature	*Mean length of development, in days (egg-adult)*
11.9±0.5	78.6
14.8±0.5	48.2
18.5±0.5	43.6
21.5±0.5	29.1
22.7±0.5	27.7
25.5±0.5	23.1

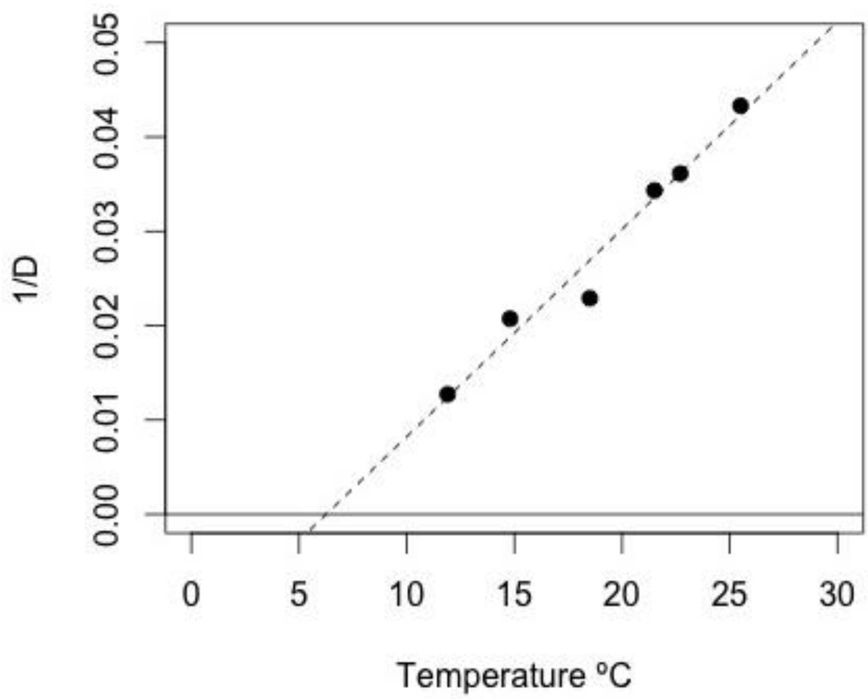


Figure 3.2.1 Development rate (1/time to complete development, egg-adult) against temperature for *Aleyrodes proletella* in controlled conditions. Dashed line shows significant (P<0.05) linear regression analysis (Y= 0.002199*x-0.013764).

Table 3.2.1. Predicted and observed generation emergence times for cabbage whitefly for the field population at Wellesbourne (2013-2015)

Year	Generation		Date	Accumulated D°	Difference
2013	First	Observed	28/06/2013	511.9	-6days, 57D°.
		Predicted	22/06/2013	455	
2014	First	Observed	29/05/2014	452.7	1 day, 2D°
		Predicted	30/05/2014	455	
	Second	Observed	09/07/2014	828	8 days, 82D°
		Predicted	17/07/2014	910	
	Third	Observed	07/08/2014	1186.4	21 days, 179D°
		Predicted	28/08/2014	1365	
	Fourth	Observed	12/09/2014	1504.8	42 days, 315D°
		Predicted	24/10/2014	1820	

The predicted emergence date for the first generation was within a week of the observed emergence date showing good accuracy. The subsequent predictions of generations decreased in accuracy with the prediction for the fourth generation being out by 42 days

Development of field populations

Experiment 4.1 Monitoring of whitefly on overwintering Brussels sprout plants.

Materials and methods

A small plot of overwintering Brussels sprout plants (30 plants) was sampled for whitefly presence over the winter of 2012-13. Initial observations showed a distinct distribution of whitefly life stages across the plants (leaves of different ages), showing surveys needed to incorporate this vertical distribution. The plot was attacked by birds and rabbits in December, considerably reducing the number of overwintering whitefly. The plants were then covered with bird netting to prevent further damage. The remaining population of whitefly was surveyed monthly until March when fortnightly samples were taken.

Results

Egg laying began as early as February. An initial 'spike' in egg numbers in early February was followed by a nearly month-long period when egg numbers remained constant, which coincided with a reduction in the mean air temperate below that of early February. It is not clear whether the eggs laid in early February were still viable and were responsible for the appearance of nymphs in May. The number of adults decreased during May but it could not be determined whether this was due to their death or to dispersal from the plants.

Experiment 4.2 Monitoring whitefly on newly-planted vulnerable field crops (Brussels sprout and kale) throughout season.

Materials and methods

Plots of Brussels sprout and kale were planted on 2nd May 2013 to investigate natural colonisation and population increase of the cabbage whitefly over a season. Plants were sown in modules and raised in glasshouse conditions for 7 weeks prior to transplanting in the field. Plots consisted of 24 plants (3 x 8, 50cm spacing) of both kale (Reflex) and Brussels sprout (Revenge) (Figure 4.2.1). Five replicate plots were planted in different locations on the Wellesbourne site as represented in Figure 4.2.2. (A-E). A root 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 4.2.1. Study plot of Brussels sprout and kale covered in netting to prevent damage by pigeons.

To determine the date of first colonisation to within a week, all leaves of all plants were surveyed weekly for a month after planting. As the sampling effort increased significantly later in the season, due to increased numbers of leaves per plant and numbers of whitefly, a method was developed to optimise sampling. Cabbage whiteflies have a distinct distribution on plants. Juvenile stages are sessile and age with their host leaves. Adults and eggs are found primarily on young leaves, while pupae are found on older leaves further down the stem (Gould & Naranjo 1999). Such a distribution requires leaves of varied ages to be sampled in order to assess all whitefly life stages without bias. Figure 4.2.3 shows a schematic of the sampling approach that was used to assess plants. 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. All whitefly were surveyed using the naked eye.

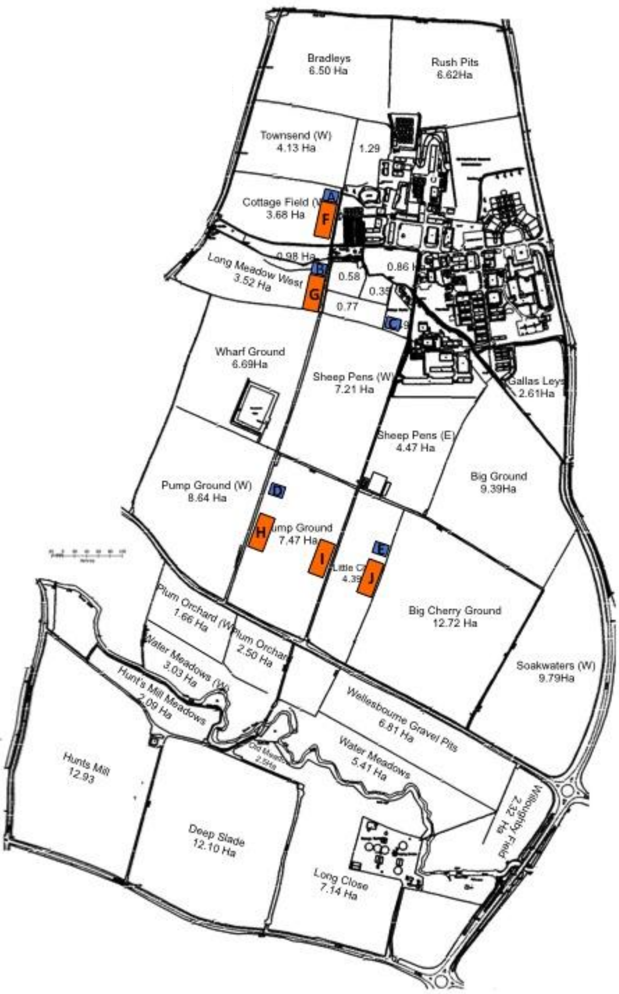


Figure 4.2.2. Locations of experimental plots for Experiment 1 (A – E) and Experiment 6 (F-J) at Warwick Crop Centre, Wellesbourne.

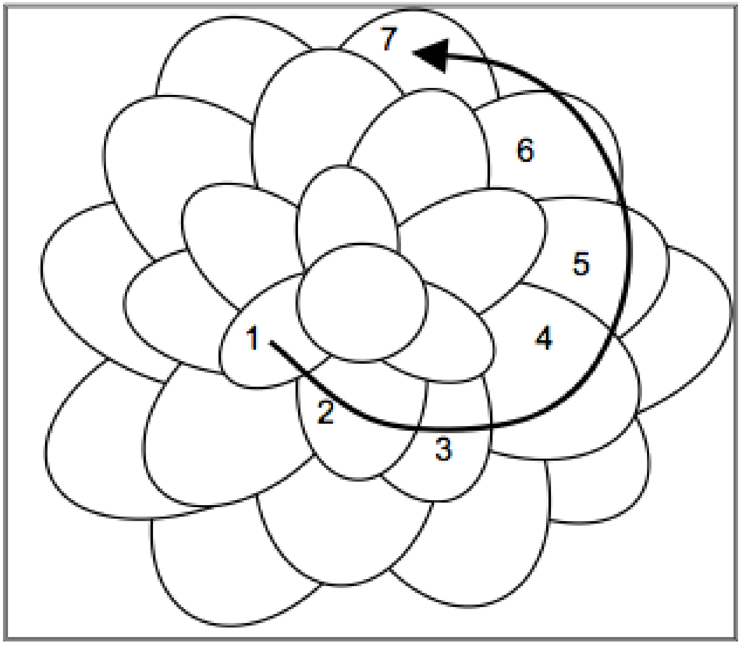


Figure 4.2.3. Schematic of sampling plan of plant to incorporate leaf-age distribution of whitefly (From Schultz *et al,* 2010).

Results

Populations of whitefly remained very low for the first few weeks after transplanting. It was not until June that an increase in the numbers of eggs occurred, and soon after that all other life stages increased dramatically in numbers (Figures 4.2.4-4.2.7). This trend continued until September when numbers ceased to increase. All populations decreased from November until January when a large increase in egg numbers was observed. The corresponding increase in numbers of nymphs did not occur until March 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, after which all life stages declined to zero in most cases. This decline coincided with the senescence of the plants after flowering.

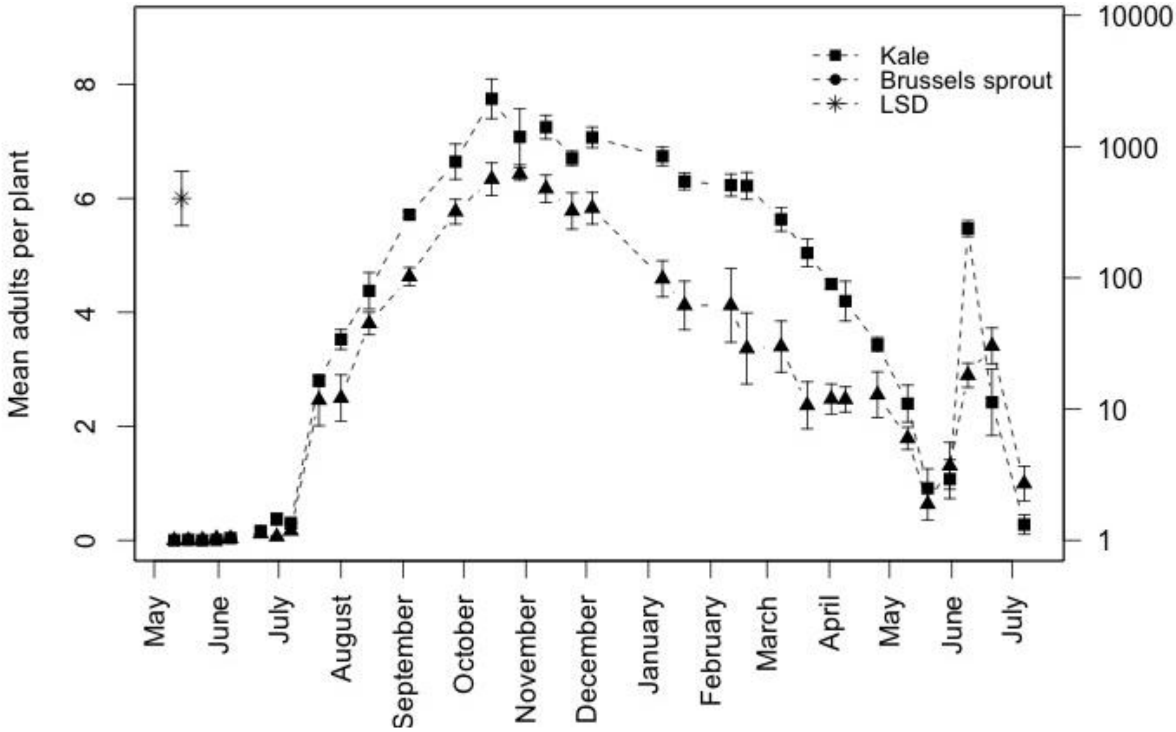


Figure 4.2.4. Mean (±S.E.) number of adults per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA (P<0.01) is also shown. Left- hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

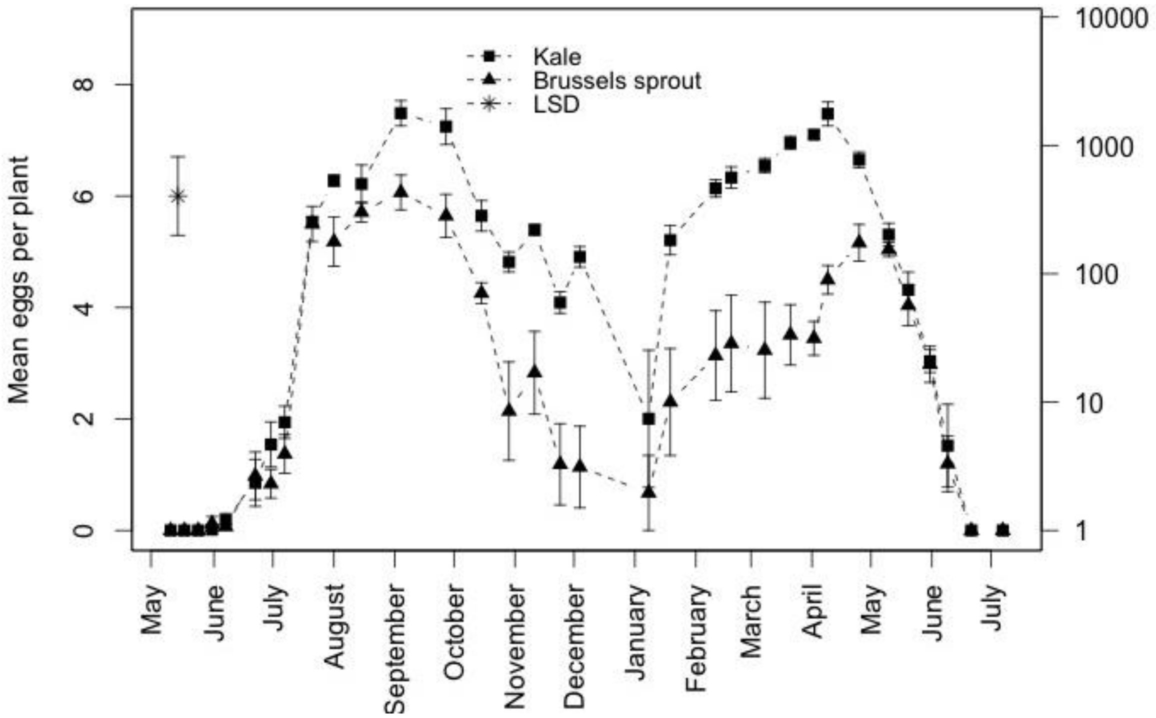


Figure 4.2.5. Mean (±S.E.) number of eggs per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA (P<0.01) is also shown. Left- hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

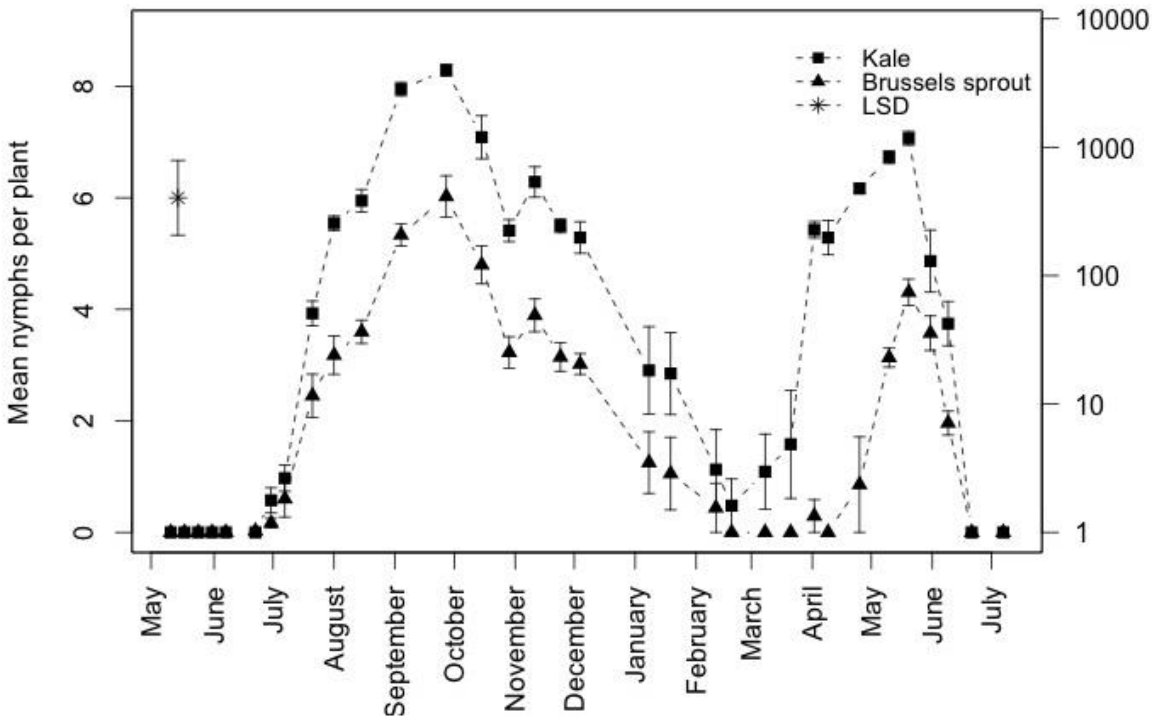


Figure 4.2.6. Mean (±S.E.) number of nymphs per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA (P<0.01) is also shown. Left axis shows log+1 transformed data while the right axis shows untransformed data.

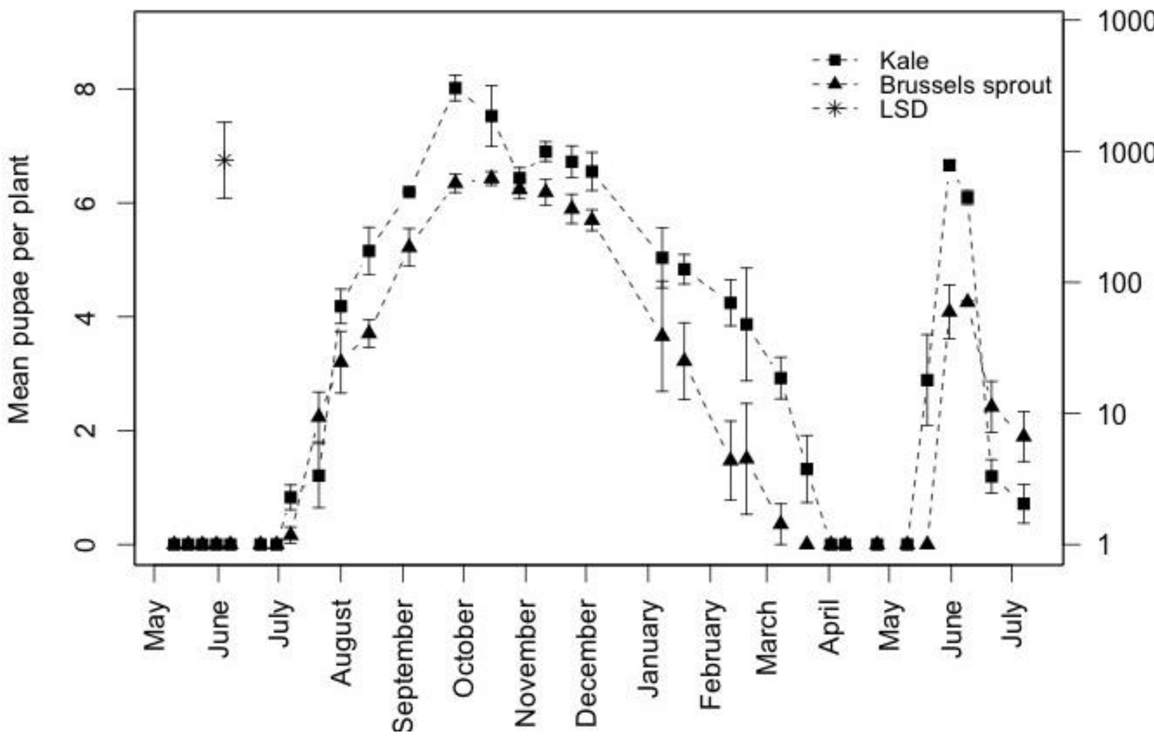


Figure 4.2.7. Mean (±S.E.) number of pupae per plant on kale and Brussels sprout from May 2013 until July 2014 for the 5 study plots. The 5% LSD from ANOVA (P<0.01) is also shown. Left axis shows log+1 transformed data, the right axis shows untransformed data.

Comparing cultivars.

Initial populations on Brussels sprout and kale did not differ significantly but subsequently, kale had consistently higher populations of all life stages; adults (Figure 4.2.4), eggs (Figure 4.2.5), nymphs (Figure 4.2.6) and pupae (Figure 4.2.7) after August.

Comparisons between plots.

One the whole, when considering each crop separately and comparing the numbers of each life stage, there were few differences between the plots.

Brussels sprout

The numbers of pupae found on the Brussels sprout plots were generally similar, with occasional statistically-significant differences between plots. For example, in August 2013, Plot B had fewer pupae than the other plots, but this difference was no longer evident at the next sampling date (Figure 4.2.8). For adults, the only significantly different plot was Plot C, which had a lower population in February 2014 (Figure 4.2.9).

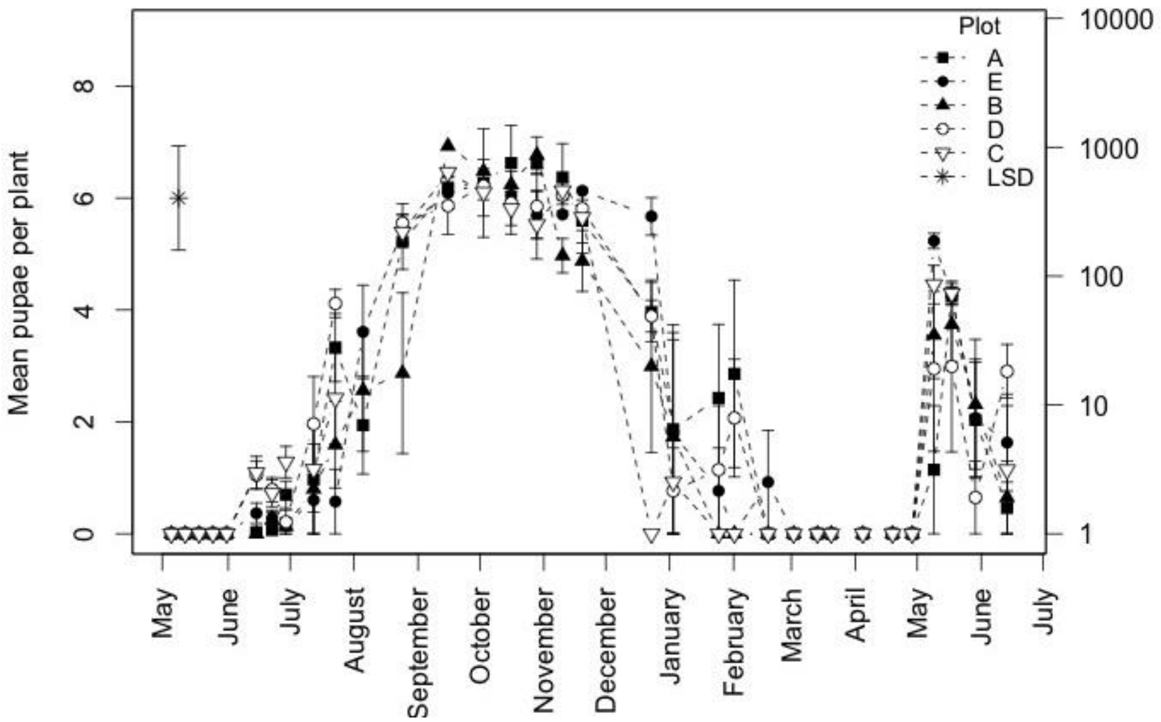


Figure 4.2.8. Mean (±S.E.) number of pupae per plant on Brussels sprout from May 2013 until July 2014 for the 5 study plots, A,B,C,D,E. The 5% LSD from ANOVA (P<0.01) is also shown. Left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

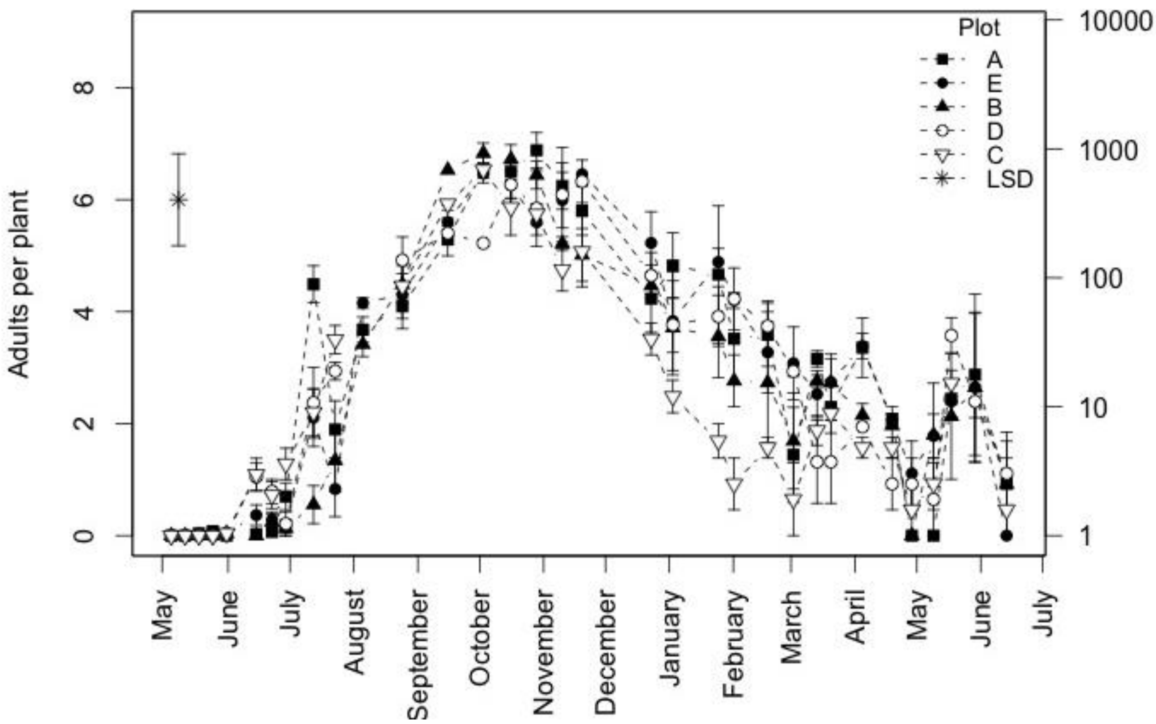


Figure 4.2.9. Mean (±S.E.) number of adults per plant on Brussels sprout from May 2013 until July 2014 for the 5 study plots, A,B,C,D,E. The 5% LSD from ANOVA (P<0.01) is also shown. Left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

Kale

There were some differences in the numbers of adults on the 5 plots of kale between May 2013 and July 2014 but these were not consistent. The most notable difference was between Plots D and E in October 2013, but this difference had reduced by the next sampling date (Figure 4.2.10).

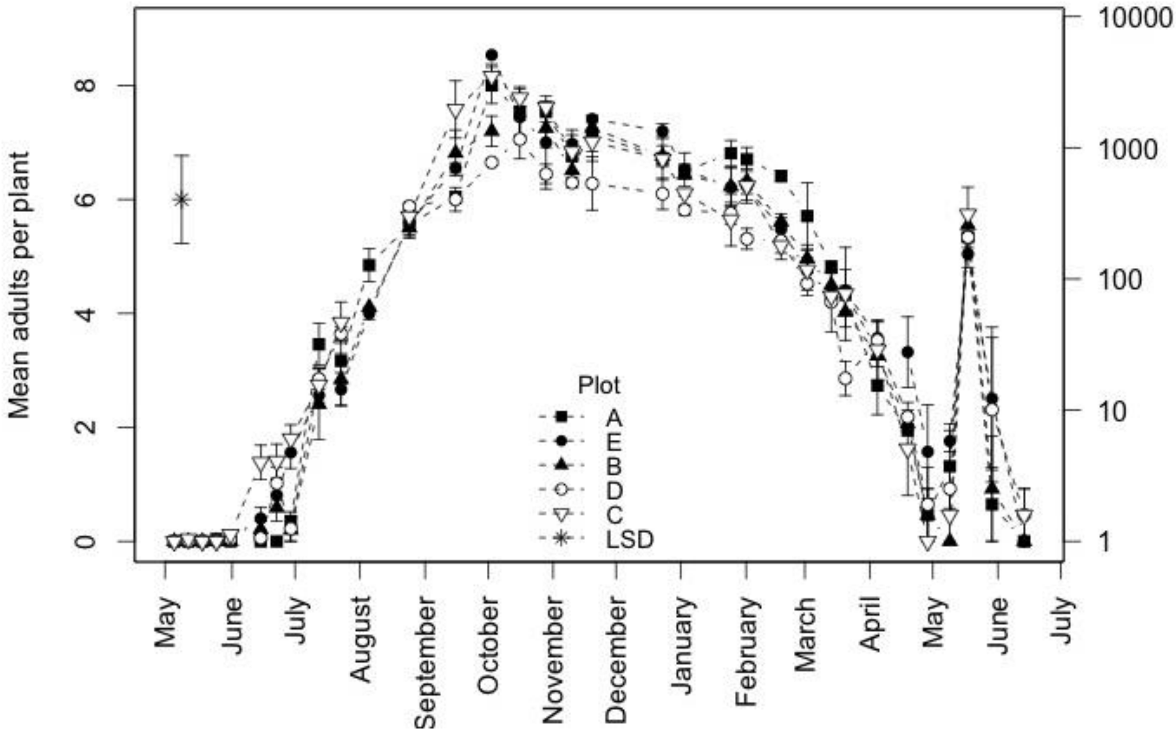


Figure 4.2.10. Mean (±S.E.) number of adults per plant on kale from May 2013 until July 2014 for the 5 study plots, A,B,C,D,E. The 5% LSD from ANOVA (P<0.01) is also shown. Left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

Egg numbers on the plots of kale were very similar for the majority of the sampling dates and there were generally no statistically-significant differences. The only significant differences between plots were over the winter period when eggs were still present on Plots C and E but the other plots had none (Figure 4.2.11). All plots then showed an increase in egg numbers in January when diapause terminated in overwintering females and egg laying began.

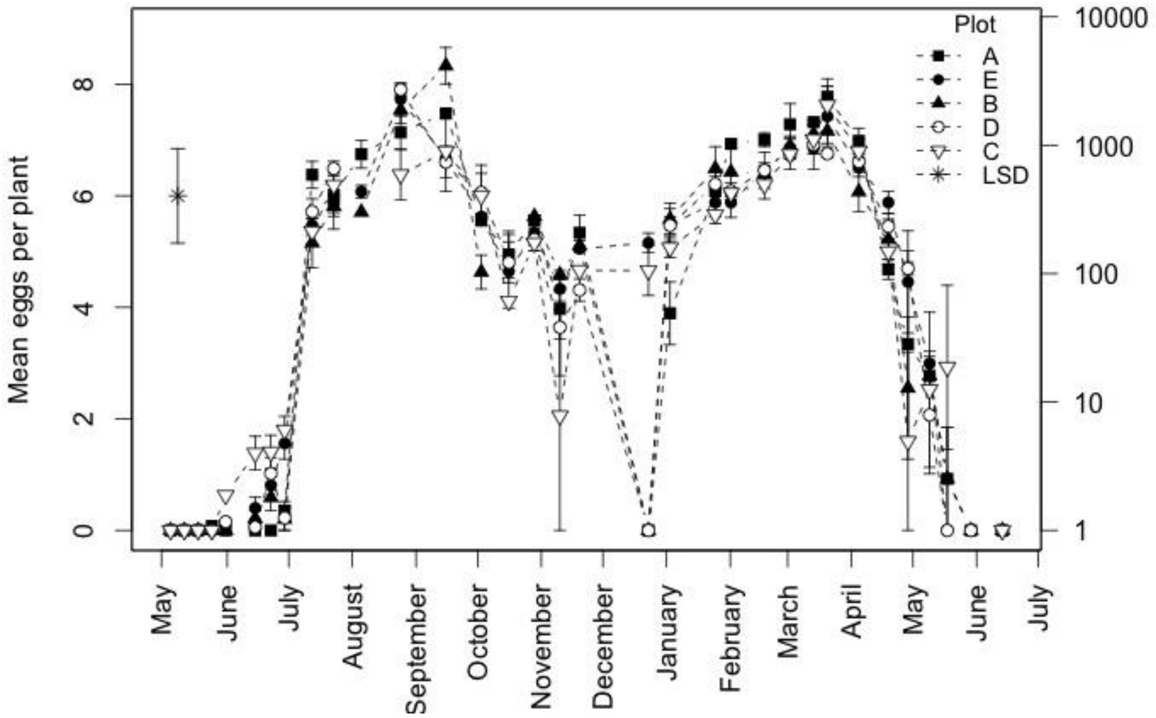


Figure 4.2.11. Mean (±S.E.) number of eggs per plant on kale from May 2013 until July 2014 for the 5 study plots, A,B,C,D,E. The 5% LSD from ANOVA (P<0.01) is also shown. Left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

On kale, the numbers of pupae per plant were very similar on all 5 plots between May 2013 and September 2013. It was not until the winter months that Plot D had significantly lower numbers than 3 of the other plots (Figure 4.2.12). After March 2014 the numbers of pupae on each plot were again very similar, showing no significant differences.

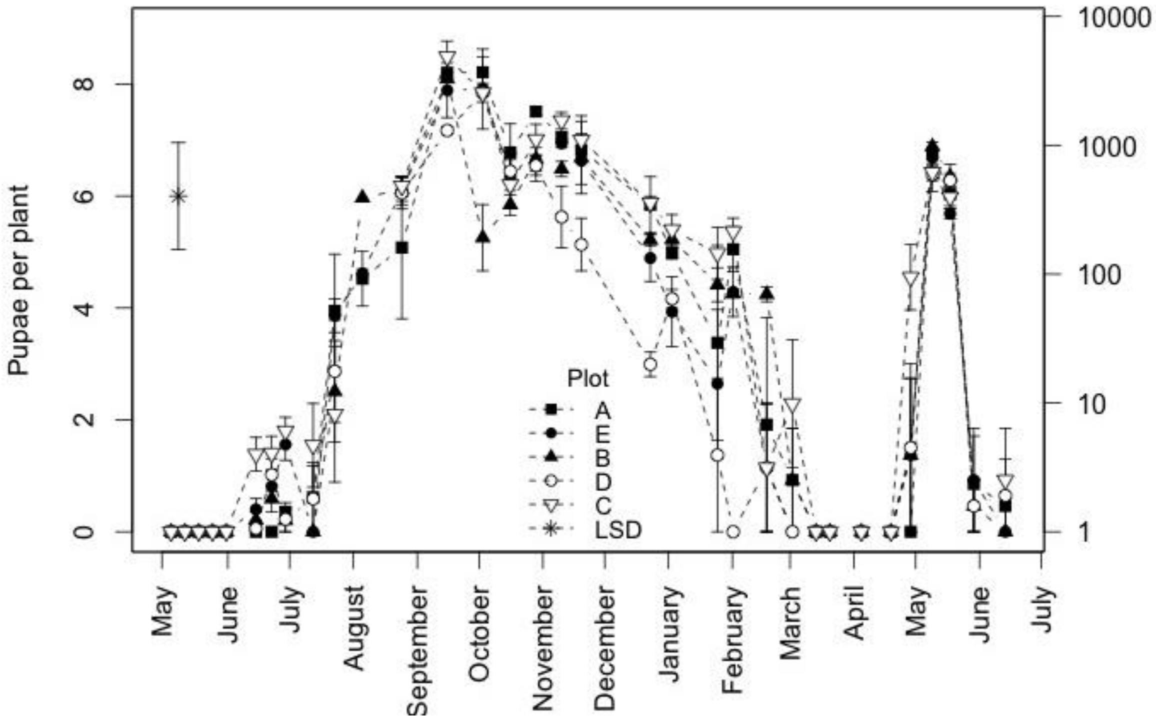


Figure 4.2.12. Mean (±S.E.) number of pupae per plant on kale from May 2013 until July 2014 for the 5 study plots, A,B,C,D,E. The 5% LSD from ANOVA (P<0.01) is also shown. Left-hand axis shows log+1 transformed data while the right-hand axis shows back-transformed data.

To summarise, no plot had consistently lower numbers of any life stage than the others. Statistically-significant differences between plots in the numbers of whitefly were usually for only a short duration and most of the differences occurred over the winter months.

Experiment 4.3 Monitoring immigration and establishment of whitefly populations on spatially- and temporally- separated plantings of kale.

Materials and Methods

In 2014, plots of kale (cv. Reflex) were planted in 5 locations at Warwick Crop Centre, Wellesbourne, represented in Figure 5.3.1 (F-J). Each plot consisted of 5 sub-plots of 6x6 kale plants separated by ~18m. Figure 4.3.1 shows a plan of how each plot was set up. A single sub-plot was planted at each location on 19th May, 17th June, 19th July, 15th August and 16th September 2014. Plants were sown in modules and raised in glasshouse conditions 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 cabbage root fly; no other pesticides were applied. For the first month, all leaves of all plants were surveyed for the presence of whitefly using the naked eye. When plant size increased, together with the size of the infestation, 10 randomly-selected plants were sampled completely. However, when the plants consisted of 9 or more leaves, the sampling method shown in Figure 4.2.3 was adopted.

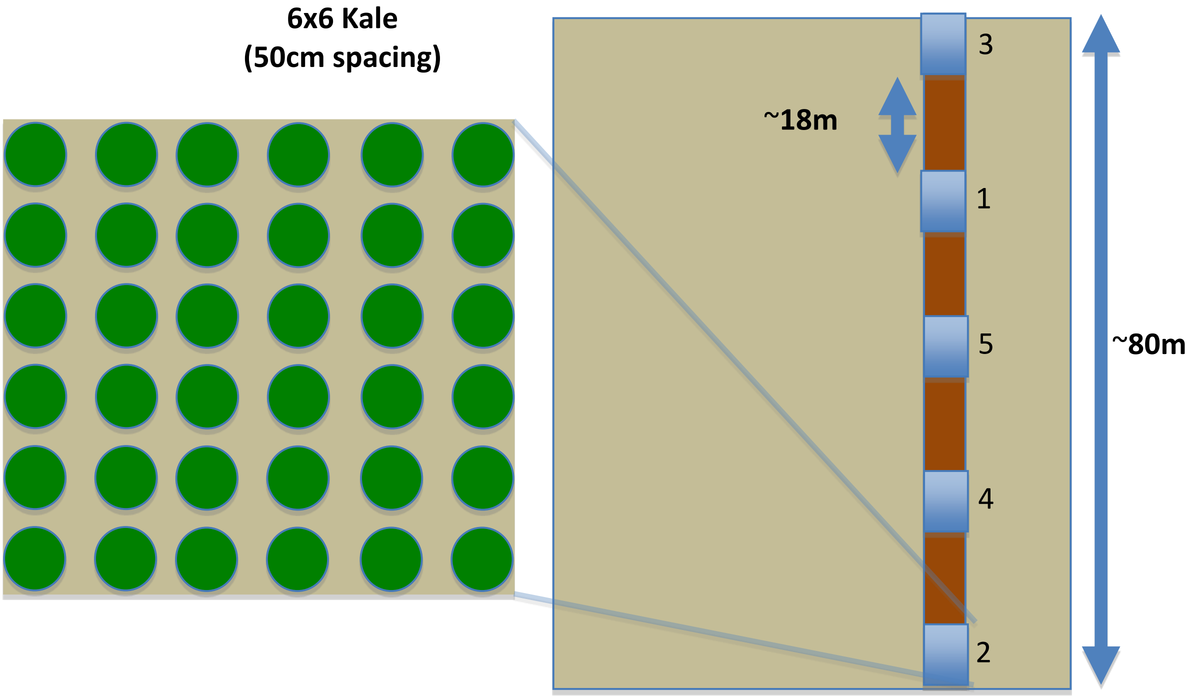


Figure 4.3.1. Schematic for plantings of kale plots at each of the five locations at Warwick Crop Centre, Wellesbourne. The numbers 1-5 represent the randomized positions of the sub-plots (planted May-September).

Results

The numbers of whitefly found on the kale plants transplanted in May 2014 differed between plots from as early as Day 13 (ANOVA, $P<0.01$, Figure 4.3.2.). Plots H and I had the lowest numbers and were only significantly different from each other on days 26 and 36. Plot G had the highest number of whitefly on most occasions and this was often significantly higher than all other plots. A decline in the number of adults was observed in Plot F on Day 26 due to a huge loss of foliage as a result of herbivory by vertebrate pests. All plants in this plot were replaced on day 30.

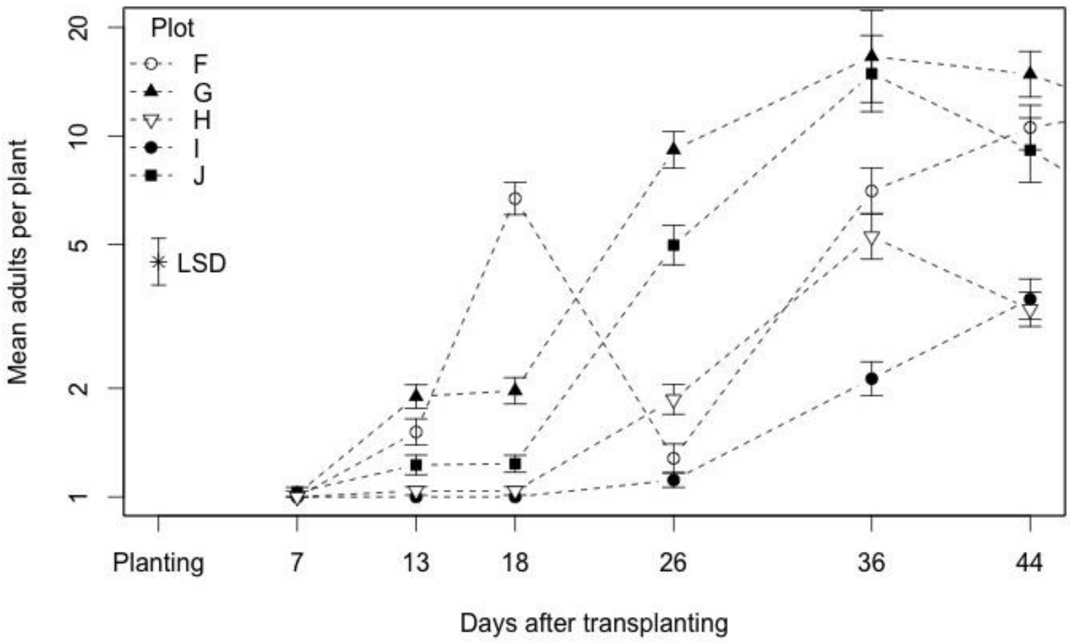


Figure 4.3.2. Mean (±S.E.) number adults per kale plant for the five study plot plantings made in May 2014. The 5% LSD from ANOVA (P<0.01) is also shown.

There was a statistically significant relationship between the adult population on the newly transplanted plot at Day 13 and the distance of the plot from the nearest highly infested plot planted in 2013 (Figure 4.3.3.). Doubling the distance from 50m to 100m led to an approximate reduction in the adult population of 75%.

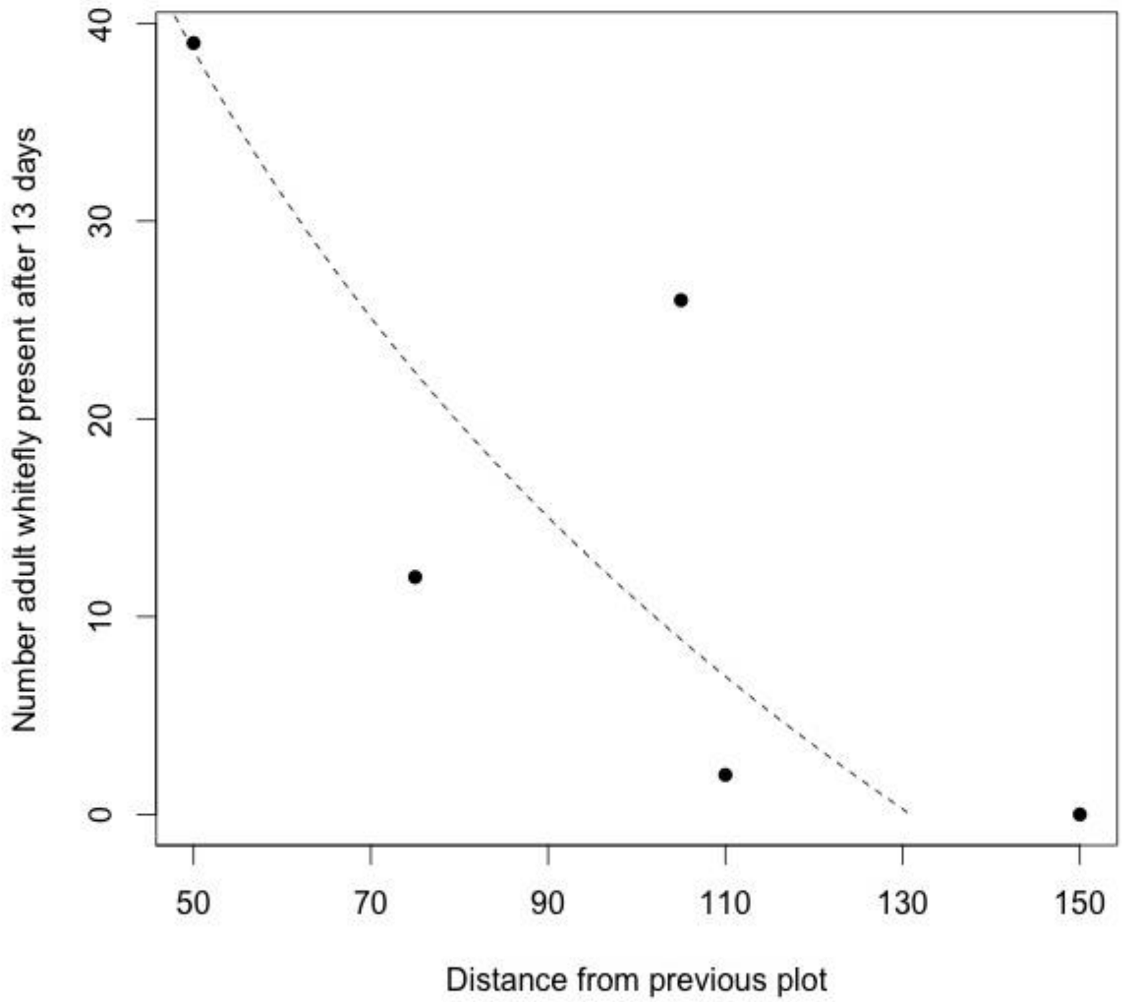
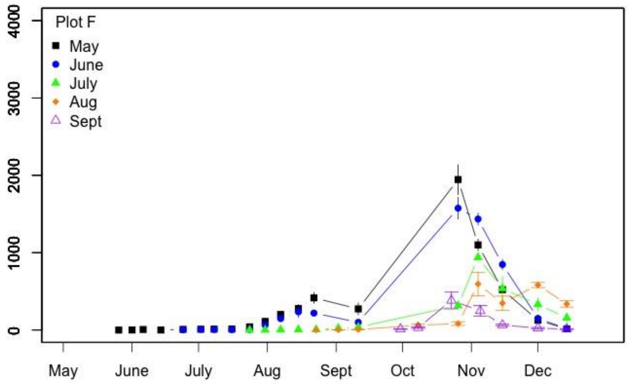
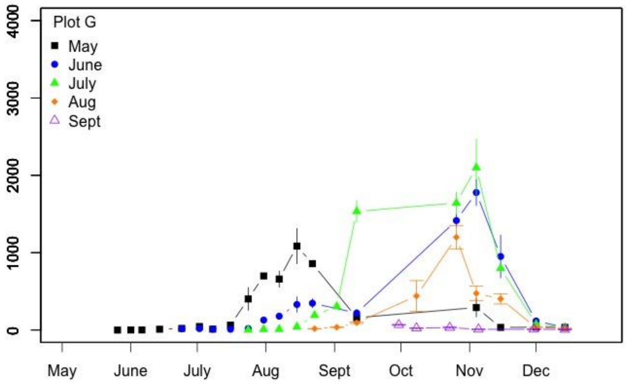
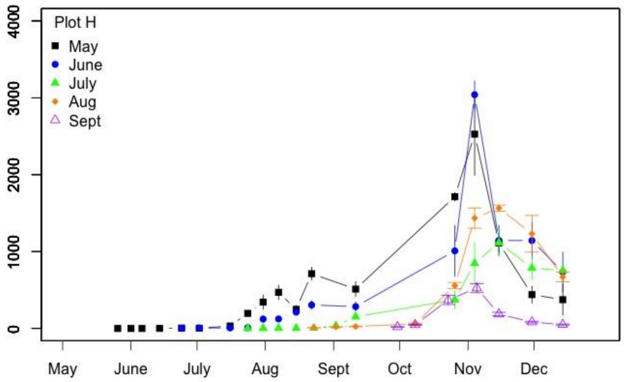


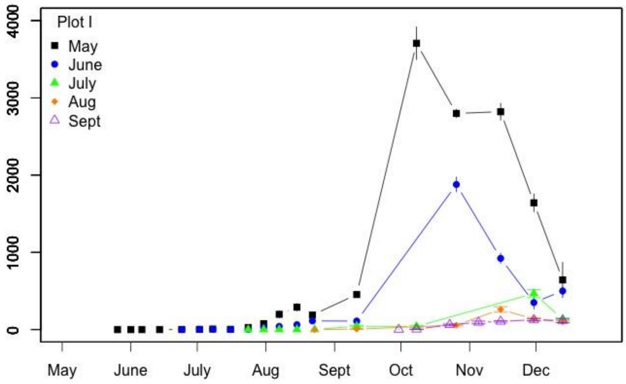
Figure 4.3.3. Number of whitefly present within the newly-transplanted kale plots, 13 days after transplanting, versus the distance of each plot from the nearest plot planted in 2013. The dashed line shows a statistically-significant GLM (Population after 13 days ~Distance, family= poisson, y=-0.02491x+4.87437, P<0.01).

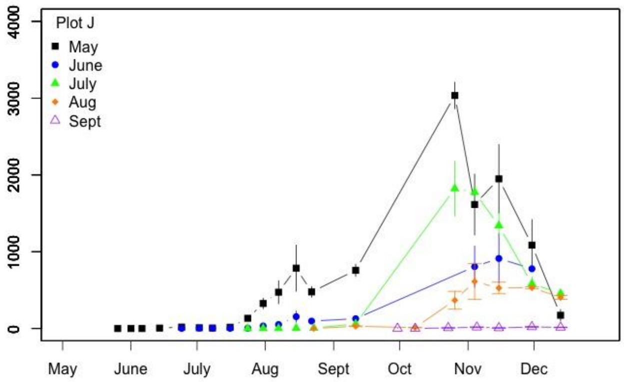
Figures 4.3.4-4.3.8 show the mean number of adults per plant in each plot for each of the planting times. Generally the plots did not differ in the pattern of population increase for each of the planting dates. A general trend in the size of the populations in each location at the end of the season was May>June>July>Aug>Sept. Plot I showed data contrary to this pattern, with the May planting having the largest population of adults in August with a drop in numbers in September. This plot had the largest population of caterpillars causing defoliation that may have impacted on the subsequent numbers.





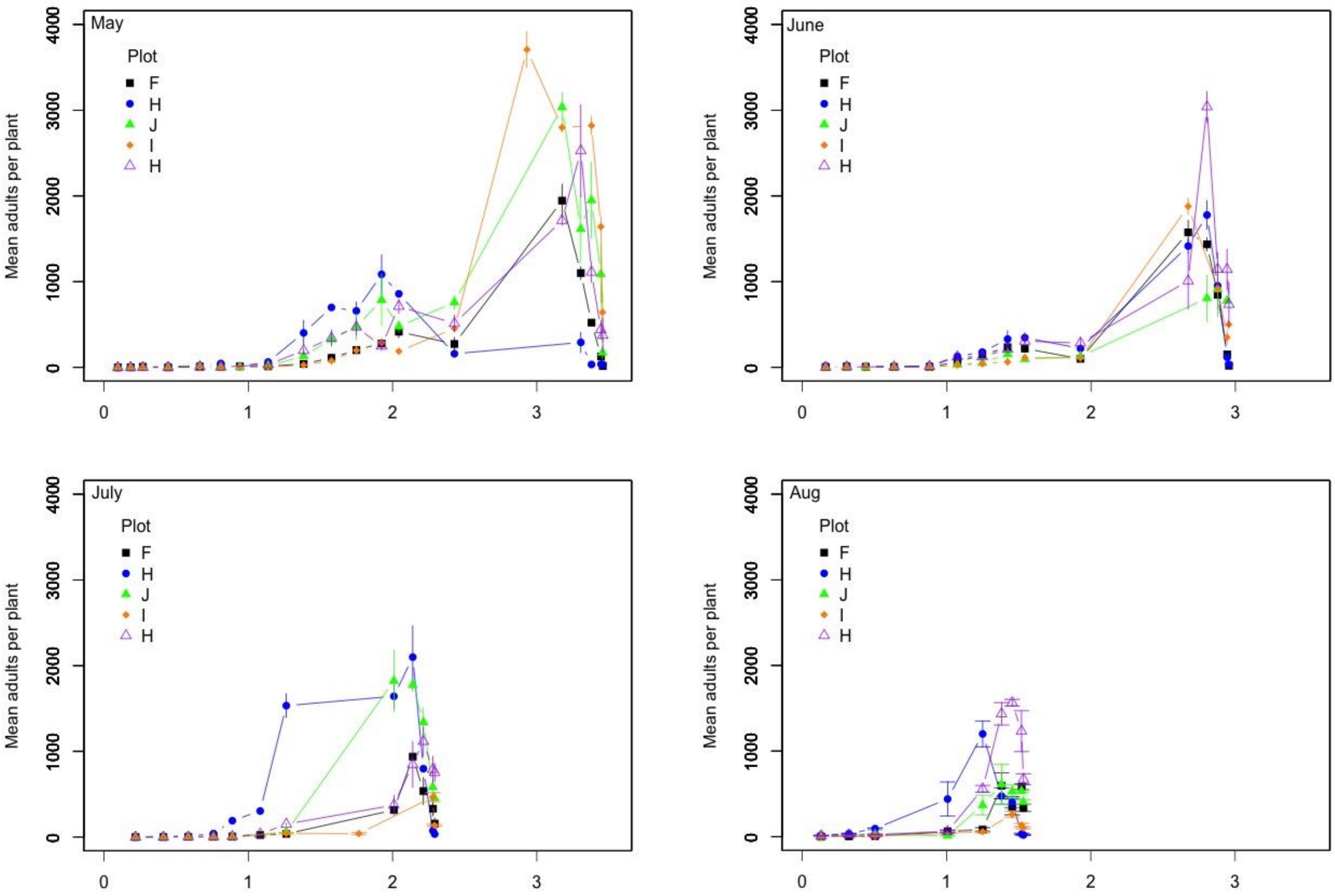


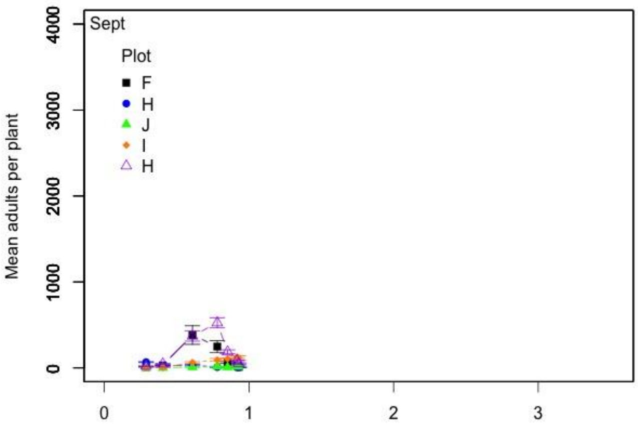




Figures 4.3.4-4.3.8. Mean number of adults per kale plant in each plot for each of the planting times (May-September).

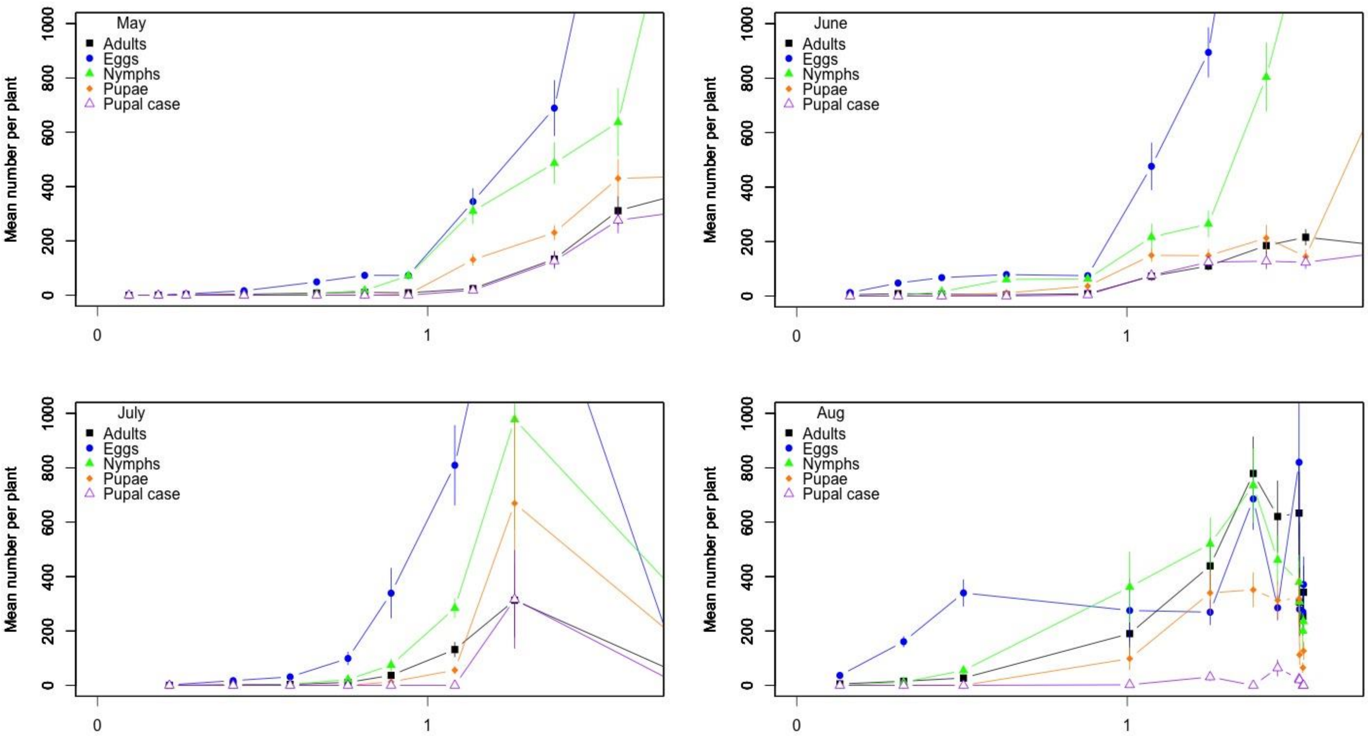
Figures 4.3.9-4.3.13 show the mean number of adults per plant for each planting date plotted against accumulated day degrees (expressed as generation times from planting date: one generation = 455 D°, lower development threshold = 6.3°C). These graphs indicate that whitefly numbers increased considerably once one generation had been completed following planting. This pattern occurred in all plots for all of the planting dates, except September where a 'full generation' of day-degrees was not accumulated before ovarian diapause was induced and temperatures decreased below the lower development threshold of 6.3^{o}C, to prevent further development by December. There were sufficient accumulated day-degrees to allow two generations to develop within the May and June plantings. This is denoted by a further increase in the number of adults within the plot, with most plots reaching approximately 3,000 adults per plant. Although sufficient day-degrees were accumulated for a third generation, this total was reached in December when females would be in diapause, preventing a third increase in numbers.

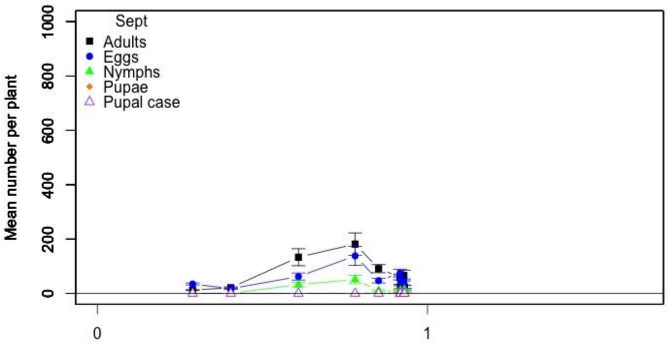




Figures 4.3.9-4.3.13. Mean number of adults per kale plant for each planting date plotted against accumulated day degree generations from planting date (one generation = 455 D°, lower development threshold = 6.3 °C).

Figures 4.3.14-4.3.18 show the mean number (averaged over the 5 locations) of adults, eggs, nymphs, pupae and pupal cases for each planting date (May-September) against generations (in accumulated day-degrees). The increase in adults appears to be a direct result of emergence from pupae on the plants since empty pupal cases appear at the time that adult numbers rise.





Figures 4.3.14-4.3.18 Mean number of adults, eggs, nymphs, pupae and pupal cases for each planting date of kale (May-September) against accumulated day degree generations. Data presented to focus on first generation only.

Figure 4.3.19 shows that there is a strong relationship between the initial immigration rate into each kale plot (number of adults 2 weeks after planting) and the mean population size after an accumulation of one generation of day degrees.

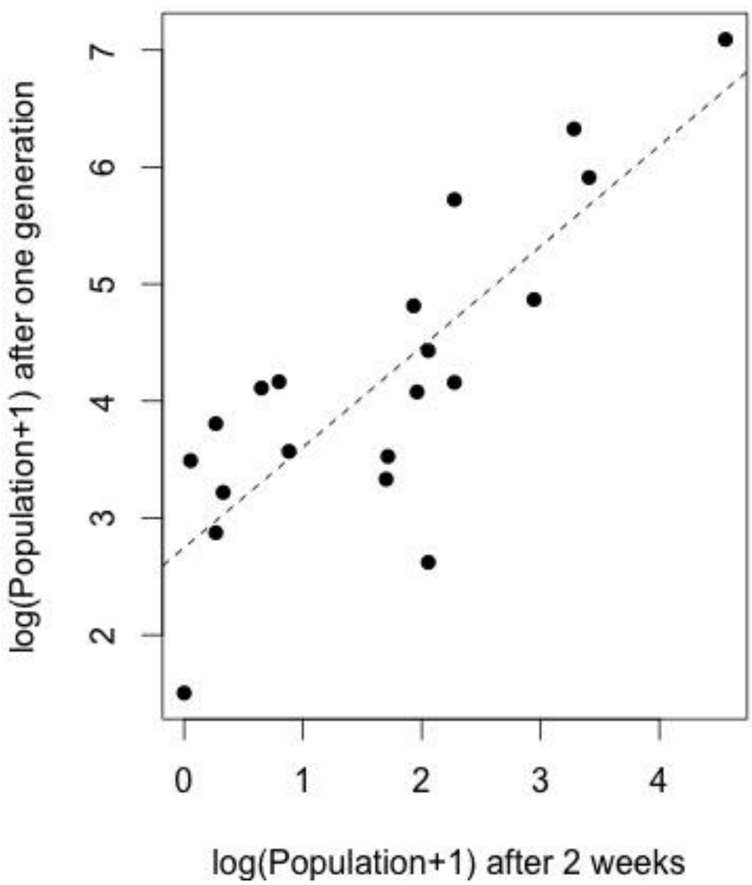


Figure 4.3.19 Log (Population+1) of adults after 2 weeks against log(population +1) after one generation of day degrees. (one generation = 455 D°, lower development threshold = 6.3 °C). The dashed line shows a significant linear relationship (Y=0.859x + 2.7464, P<0.01).

The greatest immigration rates into new plots were seen in Plot G (Figure 4.3.20) and rates of immigration were highest in August followed by September.

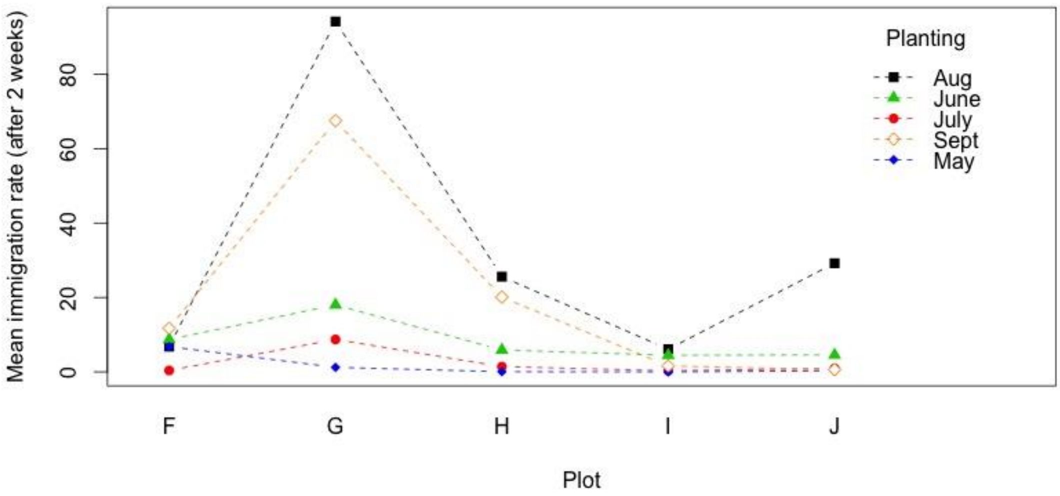


Figure 4.3.20 Mean number adults per plant after 2 weeks from planting, for each plot of kale and for each of the planting dates (May-September).

Natural control of cabbage whitefly

Experiment 5.1 Monitoring fungal pathogen epizootic in field population of whitefly.

Materials and Methods

Naturally-occurring predators were recorded when conducting population sampling for Experiment 4.2. Dead adult whiteflies (an epizootic) were observed in the field from October 2014. They were first noticed within Plot G where a large number of dead adults were attached to the leaves with outspread wings. Numbers of dead whitefly showing signs of fungal growth and outstretched wings were counted in conjunction with normal population monitoring performed in Experiment 4.3.

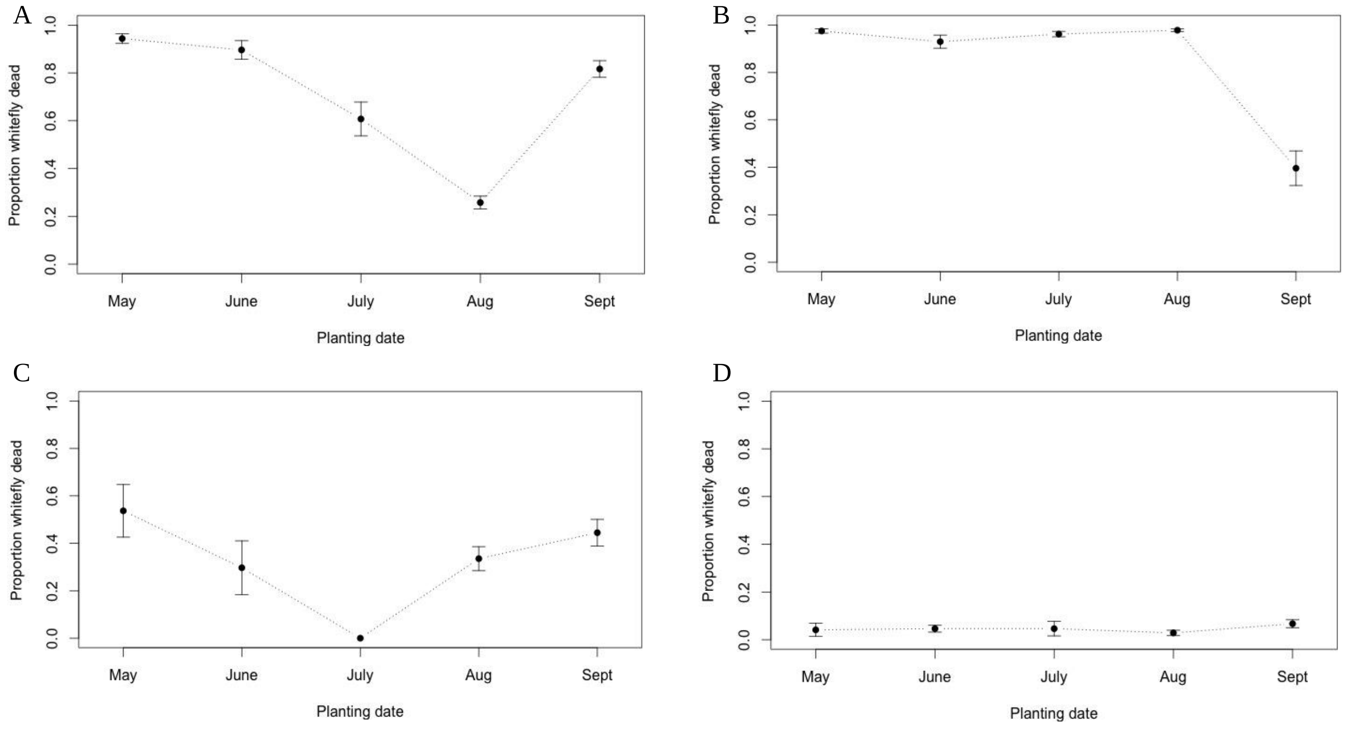
Results

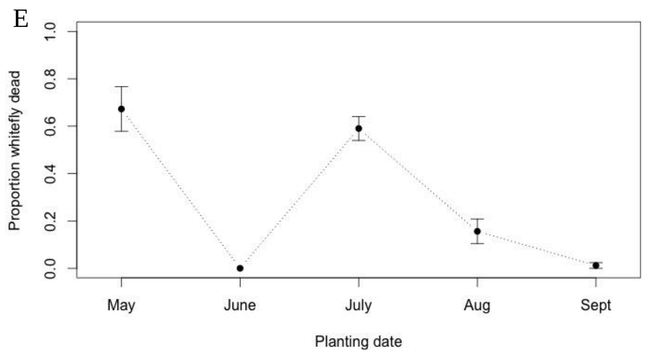
Very few natural predators were observed within the field. For the entire duration of Experiment 4.2 no whitefly-specific predators or parasitoids were encountered. Ladybirds were observed; however they were highly likely to be feeding on infestations of aphids on the plants. Lacewing larvae were seen feeding on whiteflies however this was only on three occasions.

A fungal growth could be seen on the thorax and abdomen of some individuals sampled in October 2014 (Figure 5.1.1).

Figure 5.1.1 Left: Dead cabbage whitefly on underside of kale leaf. Right: Adult cabbage whitefly showing typical symptoms of mortality due to a fungal pathogen, with outstretched wings and fungal growth visible on thorax and abdomen

The symptoms of death caused by the fungal pathogen were first recorded in Plot G, August planting in October 2014. Within a month the pathogen was recorded in all Plot G plantings, and most other plots. However, infection rates differed across the site. Most notably infection was nearly completely absent from Plot I, whilst it caused almost complete mortality in Plot G **(**Figures 5.1.2-5.1.6**).** No relationship was found between the proportion of dead whiteflies and the total number of whiteflies present on the plant (Figure 5.1.7).





Figures 5.1.2-5.1.6. Proportion of dead whiteflies in each plot (with planting dates) in December. A=Plot F, B=Plot G, C=Plot H, D=Plot I E= Plot J

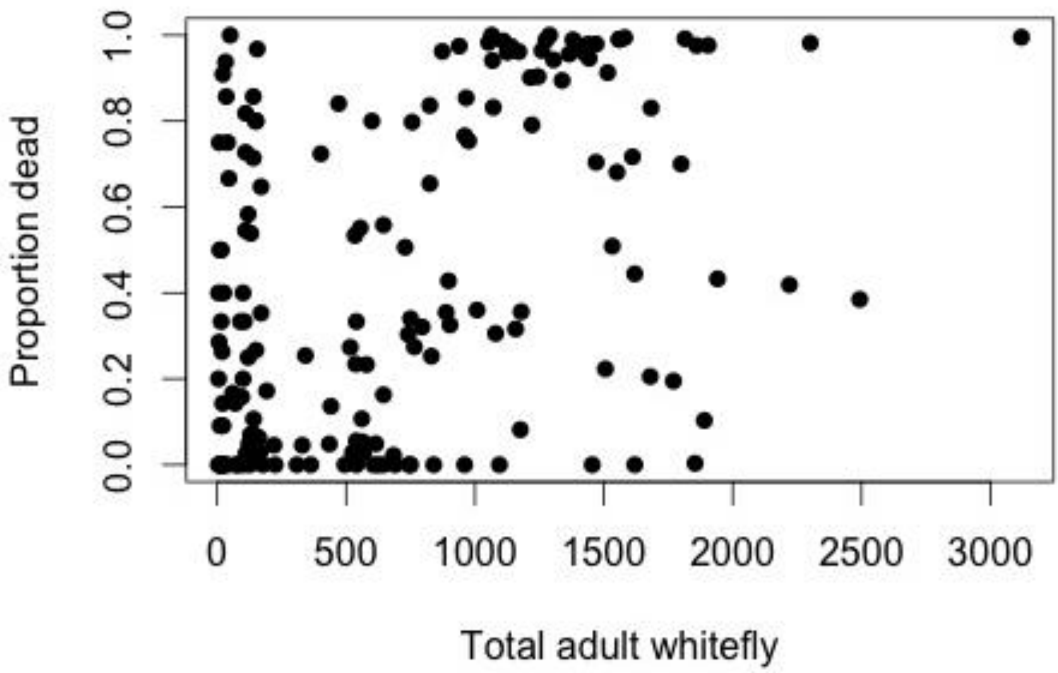


Figure 5.1.7. Proportion of dead whitefly plotted against the total number of whitefly on the plant. No statistically-significant relationship was found.

Experiment 5.2. Isolation of the fungal pathogen

Materials and methods

Live adult whiteflies were brought into the laboratory from a plot with a known fungal infection of whiteflies (Plot F, May planting).

Twenty-two live adults were individually surface-sterilized by immersing them in sodium hypochlorite (0.5%), 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 plate and incubated at 20° 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.

Primary, secondary and capillary conidia were all present, which is a feature of Zoophthora, Entomopthorales. The unicellular primary conidia confirm that the pathogenic fungus is a member of the genus Zoophthora. Confirmation that the fungus was *Zoophthora radicans* could not be made as subsequent magnification of the specific primers for *Zoophthora radicans* was not successful.

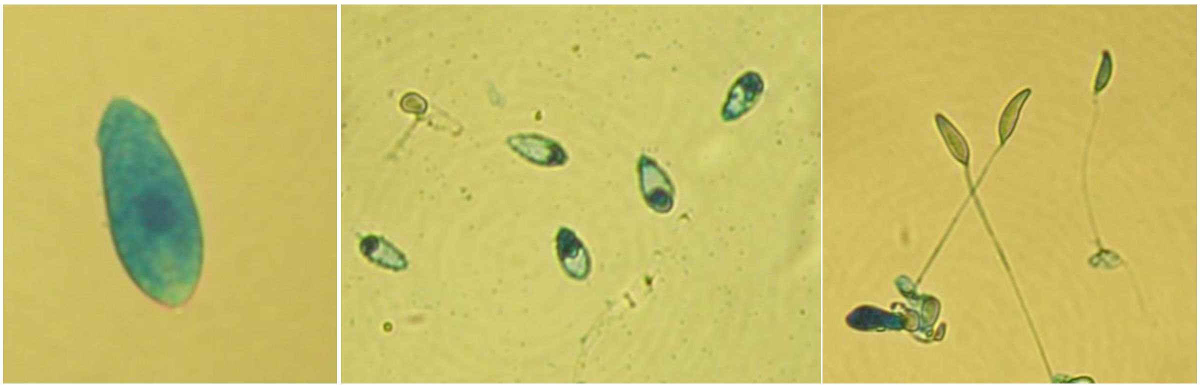


Figure 5.2.1. Left: Primary unicellular conidia, Centre: Secondary conidia, Right: Capiliconida still attached to capillary conidiophore.

Discussion

Host crops of cabbage whitefly

Experiment 1.1 Survey of wild host plants for the presence of whitefly in an uncultivated field.

The results of the study suggest that in 2013, whitefly populations on wild hosts were at densities too low to be detected. In 2012, weather conditions were considered to be very unfavourable to the development and survival of whitefly populations and this may account for the generally low numbers of overwintering whitefly (Springate & Colvin, 2013). However, whitefly do occur on wild hosts at Wellesbourne as spot checks of *Sonchus* plants near the car park in February 2013 found overwintering females (unpublished data).

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 the cabbage whitefly. Some of the most numerous wild host plants of cabbage whitefly found during this investigation were *Sonchus* species*. Sonchus* species are known to be one of the first colonisers of bare soil (Fenner, 1978). Higher densities of this host plant would be likely to be found if this study is repeated using land that was cleared approximately a year previously, or on 'disturbed' ground.

Determining the density of wild host plants in different habitats will give an estimate of populations of whitefly that can be supported in these areas, which may be important reservoirs for females that could colonise newly-planted vulnerable *Brassica* crops. Newly-disturbed ground is likely to support wild hosts of whitefly (e.g. *Sonchus* spp) which are likely to, in turn, support larger populations of whitefly.

Experiment 1.2 Survey of a commercial oil seed rape crop for whitefly.

No whiteflies were found within the sampling areas in April or June. In April, the percentage ground cover by OSR was incredibly low (10%), which was caused by heavy feeding damage by pigeons early in the year. The field was nearly completely defoliated over the winter, providing few leaves that could support overwintering female whitefly. Furthermore, any whitefly that were overwintering on the OSR would have been disturbed by the pigeons.

The adult whiteflies that were found within the field in July could have either been immigrants from nearby wild hosts or there is the possibility that they were the progeny of very few overwintering females within the crop. The estimate of a population of 40,000 whiteflies within this field is likely to be an over-estimate. Only 4 adult whiteflies were found from 40 quadrats and all of these were within 40m of the field margin. Thus multiplying the mean up to the whole field area might be incorrect as whitefly may only be present within 40m of the field margin. As such low numbers of whitefly were observed it was not possible to analyse the distribution of the whitefly statistically. This survey does not support the suggestion that the OSR was acting as a reservoir for overwintering females, in that year at least. It was not until July that whitefly were found on the crop and it is possible that the whitefly present at this time entered the OSR field from nearby wild hosts.

Experiment 1.3. Distribution of whitefly in a commercial crop of kale

A clear 'edge effect' was shown in this crop. The numbers of whitefly in a field were significantly higher on plants on the edge of the crop than on those towards the centre. This study showed that 4 times as many adult whiteflies were found at the edge of a field when compared to a distance of 45m into the crop (75 plants into field at 60cm spacing). The numbers of whitefly on each field edge were very different. The southern edge had a mean number of whitefly which was ten times greater than that of the northern edge.

Over the year the average direction of the wind in Holbeach, Lincolnshire is South-South-West (Met Office, 2015). Whitefly that may have been moved with the wind would be likely to land at the southern field edges. This conforms to the results of the sampling. The southern edge of the field was adjacent to a residential area, with some wooded areas, while the northern edge was next to a dyke leading onto a potato field. No other host crops surrounded the area of the study field so differences in populations may have been due to differences in the density of wild host plants.

Synthesis.

Whitefly numbers appear to be higher on crop plants at the edges of fields and numbers may also differ between the different field edges. Field edges adjacent to host crops or to wild hosts are likely to have larger populations of whitefly and shelter/aspect may also play a part. Information on the distribution of cabbage whitefly 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.

Monitoring dispersal of cabbage whitefly

Experiment 2.1 Developing an effective method for trapping active adult whitefly in the field.

Yellow sticky traps at ground level were the most efficient in catching adult whitefly. This shows the importance of colour and height of sticky traps on the efficiency of catch.

Experiment 2.2. Height at which adult cabbage whitefly disperse.

Yellow sticky traps placed at ground level were the most successful at capturing adult cabbage whitefly. These results again show the importance of trap height in terms of the efficacy of yellow sticky traps for monitoring the cabbage whitefly. A difference of 60cm can reduce catch rates of whitefly by ten-fold. Any sticky traps used for monitoring cabbage whitefly are likely to be much more effective at ground level. Due to the interaction of colour and height seen in Experiment 2.1 it was hypothesised that blue might become a more preferred colour at greater heights above the ground, which was not the case. However, there was a slight increase in the numbers of whitefly captured on blue sticky traps suspended at a height of 240cm.

There was a change in the distribution of whitefly catches later in the season (September – November), with relatively more whitefly caught on blue sticky traps. The attraction of short wavelength light (blue/ultraviolet) is a behaviour that has been shown to signal long range migration in aphids (Kring, 1972), and the increase in catch rates on blue traps signifies a change in the proportion of whiteflies likely to be undertaking long range migration.

Experiment 2.3 Estimating the abundance of adult whitefly at different distances from a field infestation.

As expected, counts of whitefly closest to areas of infestation were significantly higher than those at greater distances. What is particularly interesting, however, is the fact that for the trap plant study, the catches at distances of 25 and 45m did not differ from each other. A 20m increase in distance of the trap from the source of whitefly had no impact on numbers caught. Determining the distance at which a population source no longer impacts on catch rates would be important for indicating a potential minimum distance at which vulnerable crops should be planted from currently-infested crops to reduce the impact of immigration from these sources. A large decrease in catch rates within a relatively short distance (0-25m) shows how increasing distances can have a measurable impact on immigration rates. Vulnerable new crops that are within a short distance of overwintering populations of whitefly are likely to have a markedly higher immigration rate than those with a greater separation of just 45m.

Experiment 2.4 Monitoring whitefly activity using yellow sticky traps on the ground.

The graph showing the activity index of whitefly throughout the year (Figure 2.4.1) indicates some interesting differences. First of all, there was an increase in activity in October with a large increase in activity in November. At this time all adults emerging would be in ovarian diapauses; a morph that has been shown to have stronger flight behaviour (Iheagwam, 1977b).

As expected, the coldest months of December and January were also those showing the lowest levels of activity. At these times whitefly are likely to remain on their overwintering hosts. Activity of overwintering females increased through March until May. The higher activity of females early in the season shows the potential, at this time, for immigration into new crops from overwintering populations. A sharp increase in activity in May-June occurred at a time when the first generation of adults emerged (progeny of the overwintering females). The considerable increase in activity at the time that the first generation adults emerged suggests that first generation adults are likely to move onto new hosts and it signals a time when new crops are likely to be colonised.

For the majority of the year, the direction in which the sticky trap was placed (i.e. northern, southern, eastern and western sides of the plot) had no effect on the catch rate. However, during December and January more whiteflies were captured on the traps placed to the North and the East. This also corresponds to the period when the whitefly had the lowest activity index (Figure 2.4.1). It is likely that trap captures at this time of year are whitefly that have been dislodged from their host rather than actively leaving the crop. The wind direction at

Wellesbourne is predominantly south-westerly (Met Office, 2015); this would cause any whitefly that are dislodged to be blown towards the northern and eastern traps more often.

Although there was a statistically-significant relationship between the numbers of whitefly caught on sticky traps and the numbers of whitefly per plant (both in log numbers), sticky trap catches are unlikely to provide reliable estimates of the size of infestations in crops, particularly because they give no indication of the numbers of nymphs within the crop. Physically checking leaves is the only method that could provide information on the numbers of nymphs present within the crop, which informs the grower of both current adult numbers and potential future numbers.

Experiment 2.5 Assessing Rothamsted suction trap samples for presence of cabbage whitefly.

Very few whiteflies were caught before September, with the exception of 2010 where the median catch date was considerably earlier than the other years. In addition, the number caught was much greater for 2010 than for the other years, indeed more than all the others combined. No relationship could be found to explain this; it was not an exceptionally warm year. At the time of diapause induction (~1st August), similar numbers of day-degrees above 6.3°C had been accumulated compared with the other years. Interestingly the suction trap data supports reports that 2010 was a year when particularly high numbers of whitefly were present on kale crops and it was considered to be a problematical year for growers (Springate & Colvin, 2013). More suction trap data would need to be acquired to establish patterns between environmental factors and whitefly numbers and the timing of catches. The suction trap data may provide useful relative estimates of whitefly numbers regionally.

In 2015, the increase in suction trap captures in late June coincided with the senescence of oilseed rape in the Wellesbourne locality, which was known to support populations of cabbage whitefly. Senescent host plants have been shown to elicit long distance flight behaviour in *Trialeurodes vaporariorum* (Bonsignore, 2015). This could explain the increase in catches of the cabbage whitefly for this time of the year. The larger number captured in 2015 compared to 2014 might also be explained, as the Wellesbourne site had over twice the area of oilseed rape in 2015 when compared with 2014.

Two periods of dispersal, one caused by senescence of host plants in mid-summer and the other caused by the induction of diapause in late autumn were hypothesised by El Khirdir (1969) studying flight activity at 9ft. The data from the Rothamsted Suction Trap for Wellesbourne in 2014 and 2015 supports this hypothesis.

Synthesis.

An increase in activity during November was seen in Experiments 2.4 and 2.5, supporting earlier laboratory studies indicating that females in diapauses (those emerging after September) show stronger flight behaviour (Iheagwam, 1977b). The increase in attraction to blue at this time supports the hypothesis that more whitefly are undertaking long range migration at this time. The high numbers of catches in the Rothamsted suction traps at 12.2m provide evidence that whitefly are flying at heights appropriate for long-range migration.

All these studies strengthen the evidence for females performing low, short distance, trivial host-seeking flights during the reproductive period (April-August), and activity studies in Experiment 2.4 show how females may begin flying these short distances in seek of new hosts as early as March. This suggests that any immigration onto newly planted crops is likely to be achieved by females overwintering nearby (e.g. females on overwintering crops in an adjacent field, or on wild hosts). There is little or no evidence that females are undertaking long range flights at this time.

Development of cabbage whitefly

Experiment 3.1. Egg laying rate and duration of egg laying on three different Brassica oleracea crops: Brussels sprout, kale and cauliflower.

The average duration of egg laying was 33 days, meaning that, in this species, the duration of egg laying is long in relation to its generation time. At 21.5°C the length of time it takes for an egg to develop to an adult is 29 days and, as such, a female could be continuing to lay eggs after her first eggs have emerged as adults. This study suggests that generations of cabbage whitefly may overlap significantly.

This laboratory study also showed how the reproductive potential measured by egg laying rate did not differ between kale and Brussels sprout. Differences in infestation levels between these crops are likely to be caused by other features of the host, perhaps extra shelter provided by certain plant structures. This study did not investigate potential host quality effects that may affect the fecundity of females raised on different hosts. Further studies would need to be conducted to investigate this.

Experiment 3.2.Development rate of Aleyrodes proletella in constant temperatures.

The day-degree model predicted the emergence of the first generation in 2013 and 2014 to within a few days. The subsequent generation predictions generally become less accurate, suggesting that the extrapolated lower temperature threshold may be inaccurate. The model was useful for predicting the development of the first generation on newly planted crops. For all planting dates and plots the first pupal cases did not appear until one generation of day-degrees had been accumulated.

Synthesis

The laboratory studies showing that there were no differences in the fecundity of females feeding on kale or Brussels sprout hosts provide corroborating evidence for the field data which showed that populations of whitefly on Brussels sprout and kale did not differ after the development of a generation in the field. The field data did show higher populations on kale later in the season, which might have been due to differences in the fecundity of the females raised on the different crops. The day degree model gave a good prediction of the emergence of the first generation of whitefly on new crops with a range of planting dates, providing a good validation for the model.

Development of field populations

Experiment 4.1 Monitoring of whitefly on overwintering Brussels sprout plants.

The initial ‘spike’ in egg numbers in early February was followed by a nearly month-long period when egg numbers remained constant, which coincided with a reduction in the mean air temperate below that of early February. Air-temperatures may have fallen below the lower thermal threshold for egg laying and it is not clear whether the eggs laid in early February were still viable and were responsible for the appearance of nymphs in May. The first generation of adults appeared on overwintering Brussels sprout plants on 28th June. The day-degree model of development from egg to adult predicted this date to within a week. The number of adults decreased during May but it could not be determined whether this was due to their death or to dispersal from the plants.

Experiment 4.2 Monitoring whitefly on newly-planted vulnerable field crops (Brussels sprout and kale) throughout season.

The sizes of the initial adult populations were very similar between crops (kale and Brussels sprout), suggesting no difference in attractiveness of the host to the immigrating whitefly. The number of eggs remained similar on both crops, suggesting a similarity in host quality for determining fecundity of arriving adult females. Differences between the two crops became apparent after August, by when an entire generation would have developed on the crop. Differences were most pronounced over winter, suggesting that overwintering females may prefer kale to Brussels sprout plants. It could be that the structure of kale plants provides more protection to the whitefly, causing them to be less easily disturbed by rain or wind, for example. It would be interesting to test whether females, especially those overwintering, are less likely to be dislodged by rain on kale when compared with Brussels sprout plants.

Although it was a very small number (0.04 adults per plant 36 days after planting), the first adult whitefly arrived on the plots in mid-May 2013. This was before the first generation emerged on an overwintering crop in the same location; on 28th June (Experiment 4.1). This provides evidence that the first females to colonise the plots were overwintering females. Thus the date of the first migration into crops does not depend on the date of emergence of the first generation adults. If there is a nearby source of overwintering females, i.e. overwintered brassicas, immigration is likely to occur early in the season.

In general there was little difference in the number of all life stages of cabbage whitefly between the five plots. A lower number of adults were evident in Plot C on the Brussels sprout plants. This plot was particularly damaged by caterpillars that significantly reduced the overall leaf area of the plants. This is likely to have had an impact on the availability of locations for whitefly 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 whitefly seen on the Brussels sprouts. This cannot be the complete cause of the difference in numbers as whitefly never populated 100% of the leaves of the Brussels sprout plants and therefore leaf surface area was unlikely to be the limiting factor.

Egg laying by overwintering females first occurred in January 2014. This was a month earlier than in 2013, when the first eggs were seen in mid-February (Experiment 4.1). The winter of 2013-2014 was considerably warmer than 2012-2013 with mean January maximum temperatures of 6.8°C in 2014, compared with 4.6°C for 2013. This is likely to be the reason for earlier egg laying.

Populations of whitefly adults increased dramatically in late July-August. This was after a time when pupal numbers had begun to increase. It is highly likely that this increase in adults was due to the emergence of the progeny of earlier immigrants rather than a sudden immigration of adults. The numbers of all life stages began to decrease dramatically from June 2014 as the plants were undergoing senescence following flowering and seed production. The declining leaf area provided little or no habitat for any life stage and this is likely to have been the cause for the decline.

Experiment 4.3 Monitoring immigration and establishment of whitefly populations on spatially- and temporally- separated plantings of kale.

Whitefly numbers increased quickly in this study; the mean number of whiteflies per plant in Plot F exceeded 10 after only 36 days. In contrast, this did not happen in 2013 (Experiment 4.2) until after 2 months. One factor that may have contributed to this difference was the larger overwintering whitefly population at Wellesbourne 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 immigration into the 2014 plots. The statistically-significant model relating the numbers of whitefly on each new plot after 13 days to its distance from the nearest source of whitefly (plots planted in 2013) supports this (Figure 4.3.3).

The closer a 2014 plot was to a plot planted in the previous year the higher the rates of immigration it received. Doubling the distance from the source from 50m to 100m seemed to cause a 75% reduction in the numbers of whitefly (after 13 days). This suggests, a very reasonable conclusion, that the rate of colonisation by whitefly onto new crops is highly influenced by the distance of the new crop from sources of overwintering females. Such locations are likely to be overwintered brassica crops such as kale and Brussels sprout. There was also a significant relationship between the log population size after 2 weeks (immigration rate) and the subsequent population 455D° (one generation) later. This, in conjunction, with the number of empty pupal cases first appearing when the adult population rises, indicates that this adult population increase is due to a proliferation of whitefly within the crop rather than an influx of adults due to immigration. Although the immigration rate has been shown to increase with time of the year (highest in August and September), this increase does not account for the dramatic increase in numbers seen in the plots. The day-degree model was effective in describing the development of generations on newly planted crops as the first pupal cases, a sign that one generation has developed within the crop, appeared approximately 455D° after the planting date, signaling a time when populations of adults are likely to rise dramatically. A temperature sum of 455D° did not accumulate following the

September plantings, suggesting that a full generation was unable to develop on the crop and the absence of the appearance of pupal cases validates this assumption.

Synthesis.

These results show that early immigration can be accomplished by overwintering females before emergence of the first generation of adults. The increase in activity of overwintering females in response to increasing temperatures in March suggests that warm years may support early immigration. These females are likely to be performing low, short distance, trivial, flights.

Females immigrating onto new vulnerable crops are likely to have come from the immediate location, either adjacent overwintering host crops or wild hosts near to the new crop. Even small changes in the distance between overwintering population sources and a new vulnerable crop may influence immigration rates.

Following immigration, populations generally remain low until a generation of whitefly has developed on the crop, leading to a large increase in numbers. The numbers dramatically increase again after a further generation has developed.

Natural control of cabbage whitefly

Experiment 5.1 Monitoring fungal pathogen epizootic in field population of whitefly

Very few predators were seen during Experiment 4.2 and those seen, e.g. ladybirds, were likely to be feeding predominantly on aphids in the crop. Natural predators did not provide an observable level of control. However, an epizootic caused by an entomopathogenic fungus, seen in October, often caused mortality rates >90%. The absence of a relationship between the proportion of dead whitefly and the total population of whitefly suggests that the fungal pathogen is not spreading in a density-dependent manner. The first incidence of the disease was recorded in Plot G (August planting) in October. 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 whitefly in September. After its first appearance in October, the disease was noted, within a month, in all planting dates of Plot H. Whether the disease was present in all sub plots or spread to them from the initial infestation cannot be determined.

The high level of whitefly mortality in a number of plots showed how virulent the pathogen can be. The pathogen may be important in regulating whitefly numbers throughout the years and may cause population crashes, preventing large overwintering populations. For example Kirton suction trap catches fell from 292 in 2011 to 6 in 2012; a dramatic population change which could not be attributed to differences in accumulated generations in the years. A highly virulent fungal pathogen such as the one seen here could account for such a drop in numbers 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 infestation rates on the plots in this study are uncharacteristically high compared with those seen in commercial crops, or even on wild host plants, and may have been important for the establishment of the epizootic.

Experiment 5.2. Fungal pathogen isolation.

Although the pathogen could not be identified to species level the morphology indicates that it is a member of *Zoophthora* genus within the order Entomophthorales. This is the first recorded occurrence of a fungal pathogen on *Aleyrodes proletella* within the UK.

Conclusions

- The rate of colonisation of new crops by whitefly is highly influenced by the distance of the new crop from sources of overwintering females. An increase in distance from 50m to 100m led to a 75% reduction in immigration rate.
- Kale and Brussels sprout plants appear to have the same level of ‘attractiveness’ to colonising cabbage whitefly.
- Field edges supported, on average, 4 times as many whitefly as areas towards the centre of the field. Populations of whitefly at each field edge also differed significantly from each other.
- Whiteflies are caught most effectively on yellow sticky traps at ground level, suggesting they are dispersing near to the ground. Increasing the height to 60cm led to, on average, a ten-fold reduction in catch rate.
- Activity of overwintering female whitefly increased during February-March showing potential for these females to move into new crops. Peak activity of adult whitefly was in May-June when first generation of adults were emerging. It is likely to be the time of greatest colonisation of new crops.
- Female cabbage whiteflies undertake longer-range dispersal flights at higher altitudes in the autumn once they enter ovarian diapause (captures in suction traps and on blue sticky traps).

- It was estimated that 455 day-degrees above a threshold of 6.3°C is required to complete development from egg to adult. This was shown to be accurate in predicting the emergence of the first generation in 2013 and 2014; subsequent generations in 2014 were predicted with less accuracy.
- No parasitoids and very few natural predators were seen feeding on cabbage whitefly in the field. An entomopathogenic fungus was seen to cause an epizootic often killing >90% of the adult whiteflies.

Knowledge and Technology Transfer

- October 2012. Attended Brassica Growers' Association R&D Committee meeting.
- March 2013. Abstract for University of Warwick student symposium.
- August 2013. Poster presented at HDC student conference.
- September 2013. Oral presentation at IOBC-WPRS Working group 'Integrated protection in field vegetables. Bergerac, France.
- October 2013. Attended Brassica Growers' Association R&D Committee meeting, Lincolnshire.
- November 2013. Presented at Brassica Technical Seminar, Lincolnshire.
- January, 2014. Attended Brassica Growers' Association Conference, Lincolnshire.
- March 2014. Presented poster and abstract at University of Warwick student symposium.
- September 2014. Presentation given as part of Tomato Growers' Association student conference. Poster also presented, Warwickshire.
- March 2015. Presented at the University of Warwick student symposium.
- September 2015. Presented at the AHDB Horticulture Studentship Conference.

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