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| **Project title:** | Brassicas: Integrated management of whitefly, *Aleyrodes proletella* |
| **Project number:** | FV 406 |
| **Project leader:** | Simon Springate / John Colvin, University of Greenwich |
| **Report:** | Final Report, March 2013 |
| **Previous report:** | N/A |
| **Key staff:** | Simon Jackson, Allium & Brassica Agronomy Ltd. |
| **Location of project:** | Elsoms Seeds, Natural Resources Institute, Allium & Brassica Agronomy Ltd |
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| **Date project commenced:** | 01 April 2012 |
| **Date project completed (or expected completion date):** | 31 March 2013 |

## DISCLAIMER



*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.

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# GROWER SUMMARY

## Headline

Early insecticide applications based on monitoring proved as effective as periodic applications in controlling whiteflies on kale. Two applications of a coded product were comparable to existing systemic products. Releases of parasitoid wasps provided control levels equivalent to insecticides at the point of release.

## Background

The Brassica whitefly, *Aleyrodes proletella*, has become an increasing problem in Brassica horticulture, particularly on Brussels sprouts and kale. The reasons for this are likely to be complex, with climate and weather being significant factors. Loss of insecticides, pyrethroid resistance and difficulty in targeting the pest may also play roles. Over-reliance on registered systemic products may lead to further resistance development. Natural enemies of the native whitefly such as parasitoid wasps may prove useful as a component of pest management systems.

## Summary of the project and main conclusions

The aim of the project was to field test the impact of releasing parasitoid wasps (*Encarsia tricolor*) on whitefly-infested kale and to explore the effect of early insecticide applications, including a novel coded product, based on monitoring of whitefly populations. The work was undertaken by staff of the Natural Resources Institute (NRI; University of Greenwich), Allium & Brassica Agronomy Ltd. and Elsoms Seeds.

An experimental field trial was carried out in 2012 on 9 x 9 plant kale plots in Lincolnshire. Each treatment (Table 1) was applied to four plots. Netting was applied after whitefly had begun to infest the crop, to assess the effect of restricting parasitoid dispersal. Early applications of Movento (Spirotetramat) and a coded product (HDCI 039) based on monitoring of whiteflies were compared with a spray regime similar to that used in the industry for control of heavy whitefly infestations. Due to production difficulties, parasitoids were released at lower numbers over a more prolonged period than planned. To partially compensate for this, measures of whitefly numbers were carried out from the centre of plots (the point of parasitoid release) to the edge.

**Table 1.** Experimental treatments applied in the field trial

| | **Treatments** | |
|---|---|---|
| A. | **Control** | (no insecticide/biocontrol) |
| B. | **Netting Control** | |
| C. | **Encarsia** | (early *Encarsia tricolor* release) |
| D. | **Encarsia + Movento** | (early *Encarsia tricolor* release + late Movento) |
| E. | **Net + Encarsia** | (Netting with early *Encarsia tricolor* release) |
| F. | **Movento (early)** | |
| G. | **HDCI 039 (early)** | (coded product; 2 applications, 10 days apart) |
| H. | **'Industry'** | (Movento, Biscaya, Movento. approx. 1 month apart) |

Whitefly levels on the trial site were higher than on crops in the region in 2012. All insecticide applications had a significant impact on pest infestations. The coded product had an immediate impact on adult numbers and thereby egg-laying, whereas the registered treatments showed a lag in mortality, resulting in slightly higher egg numbers. There was no difference between the two registered treatments (F and H). These early effects were reflected in later assessments of larval density and leaf quality (Fig. 1). There was little evidence that late 'Movento' applications had an influence in either the combined parasitoid treatment or the 'Industry' rotation, though rainfall after application may have impaired activity.

The netting treatments reduced levels of adult whiteflies compared to uncovered control plots, suggesting that immigration of insects onto crops took place over a period of at least a month. Parasitoid treatments had a limited impact on whitefly levels and contamination overall but there was evidence of reductions to levels approaching those found in insecticide treatments close to the point of release (Fig. 2). Levels of parasitism outside of release plots were negligible, with two other parasitoid species found at low levels in samples.

In 2012 whitefly levels were low on commercial crops in the region and whilst this trial suggests pest management components and strategies that are clearly effective, the trial took place at a single location in one year and results may differ under different conditions.

Further work should explore the optimal combination and timing of insecticide products and other control measures, driven by monitoring and/or prediction of whitefly infestations. Understanding annual movements of whitefly adults in the agricultural landscape and the period of immigration into the crop would also have value as would the opportunities for integrating biological, cultural and chemical controls.

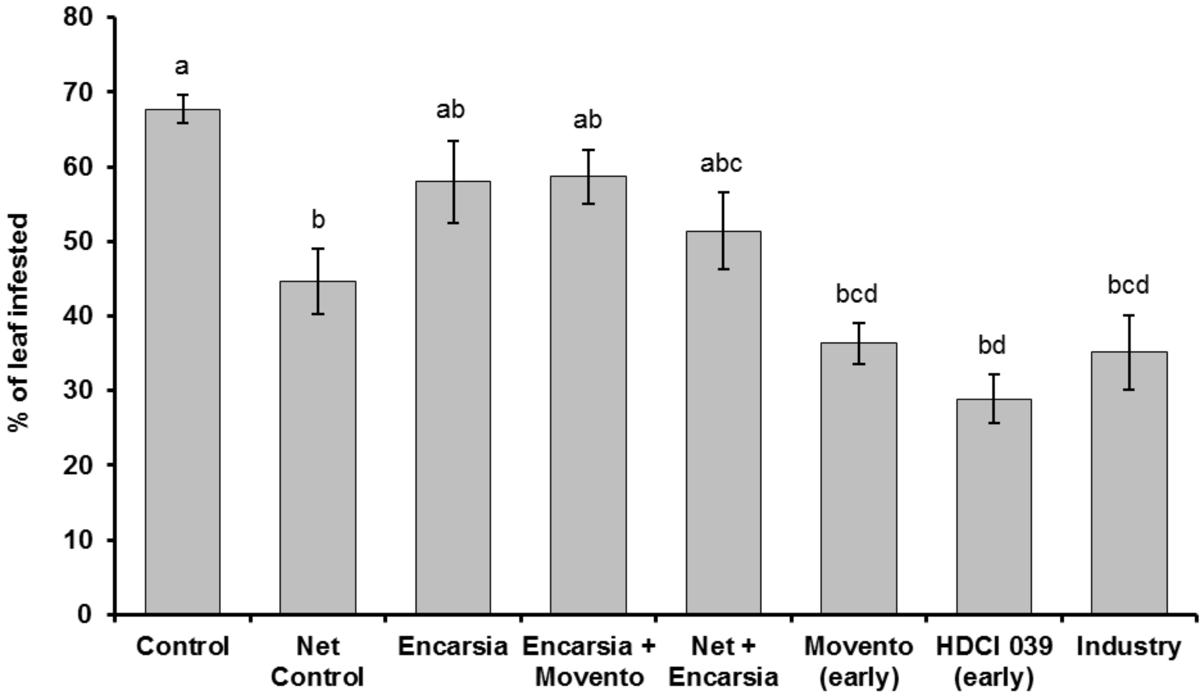


**Figure 1.** Mean percentage contamination of leaves in each treatment at harvest and in a crop in the same region.

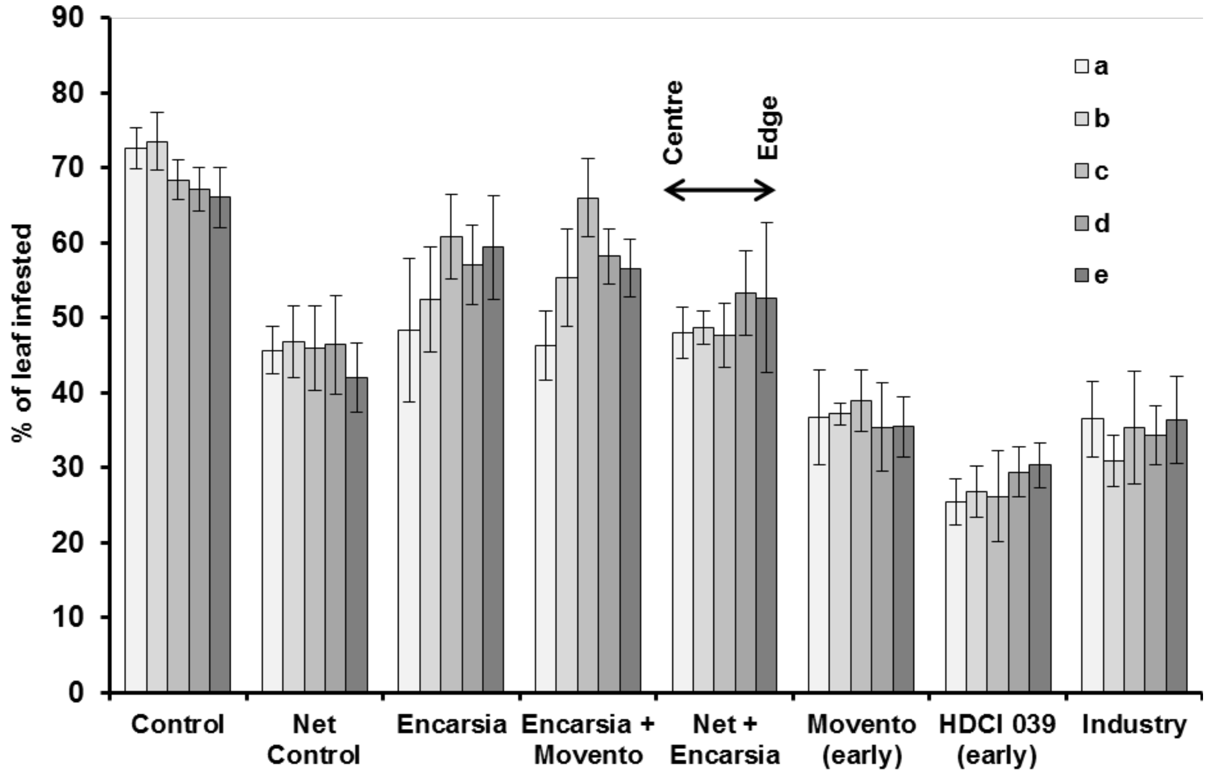


**Figure 2.** Mean percentage contamination of leaves in each treatment at different positions in plots.

## Financial Benefits

Brassica crops occupy more than 32,000ha and have an annual market value of about £160 million (HDC, 2010). While a limited proportion of this total consists of crops economically damaged by whiteflies in practice, other Brassicas may act as pest reservoirs for nearby or following susceptible crops.

Losses due to whiteflies are difficult to quantify, as the impact on yield is through rejection of produce within the supply chain. Also, whitefly pest pressure varies from year to year and so the annual impact is variable. The Brassica crops most affected by the presence of whitefly and associated contamination/quality issues are Brussels sprouts and particularly kale. The research carried out by this project has shown that a novel coded product can provide effective control and that early insecticide applications produced a similar impact to regularly spaced applications throughout the period of crop growth. Reduction in number of applications would reduce costs, but this may be offset by staff time for monitoring, even if an efficient system was devised.

Estimating unit costs of such parasitoids to the grower is difficult as (a) mass rearing systems for this agent do not already exist, (b) release rates for field scale control have not been determined and would ideally depend on whitefly levels in a particular year and (c) prices per customer would depend on area to be treated, the size of the market and negotiation with suppliers.

## Action Points

- Early interventions provided considerable and long-lasting reductions in whitefly populations. This can be done by ensuring that insecticides are applied while the infestation is still in its early stages and preferably when plants are small, to reduce the population at harvest.
- All of the insecticide treatments significantly improved harvest quality of leaves over the control, but the best performance was achieved by the coded product. When the coded product is registered for use in the UK, ensure that it can be applied to kale and Brussels sprouts as part of insecticide rotations.
- At the centres of the plots, the effect of the beneficial insects was equivalent by some measures to that achieved by the insecticides. Substantial releases targeted at an early stage of whitefly infestation may provide control.
- Adult whitefly immigration is clearly a contributing factor to the problem and may take place over a prolonged period. Where possible, site susceptible crops away from older, infested and overwintering crops to limit adult whitefly migration. Also

identifying whitefly overwintering may provide the most efficient target for intervention with cultural, chemical or biological controls.

 

# SCIENCE SECTION

## Introduction

Brassica whitefly, *Aleyrodes proletella*, has become an increasing problem in certain sectors of Brassica horticulture in Europe including the U.K., particularly on Brussels sprouts and kale (Trdan *et al*, 2003; Van Rijn *et al*, 2008; Muniz & Nebreda, 2003; Schultz *et al*, 2010). The reasons for this are likely to be complex, with climate and weather being significant factors; dry winters/springs will reduce early season mortality while high temperatures will lead to more rapid development and more generations per year. Withdrawal of organophosphate products (Garthwaite *et al*, 2000, 2004, 2008) and resistance development to pyrethroid insecticides (Springate & Colvin, 2012) will also have played a role, as will the ability of spray equipment to hit the target pest within the crop canopy (FV 399).

New systemic products such as neonicotinoids (Gaucho, Biscaya) and tetramic acids (Movento) have been approved for field use in recent years and appear to be providing a level of control, which had been lost. However, over reliance on these products may engender insecticide resistance development in the whitefly population. Identifying additional products for use in rotations and non-chemical control methods will aid in resistance management.

Whitefly natural enemies had previously been identified by S. Springate in the U.K. on both Wild Cabbage and on crops (PhD thesis). It was hoped that native species would be able to operate as candidate biological control agents under current environmental conditions. Two of these, the parasitoid *Encarsia tricolor* and the ladybird *Clitostethus arcuatus*, were successfully cultured and tested in outdoor cage trials at NRI. In these tests, the parasitoid proved most effective when introduced during the first generation of whiteflies. *E. tricolor* has also been found in crops and in margins and is likely to be cold-hardy.

In studies in organic Brassica production in Germany, Schultz *et al* (2010) combined netting covers with the release of *E. tricolor* and *C. arcuatus*. Whiteflies were released in June, with natural enemies introduced in July and August. Poor weather conditions limited the effectiveness of the trial, with no evidence of *C. arcuatus* reproduction. *Encarsia tricolor*, however, did establish successfully, with all natural enemy treatments leading to reduced whitefly levels by late October, though these were not always significant. It was found that netting alone could reduce whitefly infestations by 77% (though there are practical, plant quality and growth issues associated with this technology). The addition of natural enemies in such a case would likely reduce the whitefly populations even further. Inundative releases of parasitoids during the first whitefly generation were recommended.

The aim of the project was to field test the impact of parasitoid releases on whitefly infestations on kale and to explore the effect of early insecticide applications, including a novel coded product, based on monitoring of whitefly populations. The work was undertaken by staff of the Natural Resources Institute (University of Greenwich), Allium & Brassica Agronomy Ltd. and Elsoms Seeds.

## Materials and Methods

### *Parasitoid rearing*

Whitefly and parasitoid cultures were maintained at NRI in controlled environment facilities separate from quarantine insect facilities, as per DEFRA instructions. Kale plants grown in NRI glasshouses were housed in plastic gauze cages (Rothamsted design) (Fig. 1a). A whitefly stock culture was established using pyrethroid susceptible strains from Lancashire and Kent.

Fresh plants were placed into this cage for 1 to 2 days for oviposition, the adults were then blown off and the plant removed and placed in a perforated sandwich bag while larvae developed. In a separate room, cages were set up into which these plants were placed when larvae of a suitable age were observed in significant numbers (12-15 days). Adult parasitoids were added to these cages to parasitise the whitefly larvae over a period of approximately a week. At the end of this period, parasitoids were blown off into the cage and the plant bagged again for parasitoid development. New whitefly-infested plants were then placed into the cages and additional parasitoids were added. Five parasitoid cages were eventually in operation, containing plants infested with whiteflies on different days.

*Encarsia tricolor* females develop from fertilised eggs laid inside whitefly nymphs. Male eggs, however, are unfertilised and must be laid in a previously parasitised whitefly, where they consume the parasitoid larva within; they are hyperparasitoids. They prefer other parasitoid species but can use *E. tricolor* female larvae.

After ~5 days parasitoid development, plants were placed back in the cages for 24 hours to permit a low level of hyperparasitism to produce males for both the cages and for field release. The female:male ratio in samples from the releases did not exceed 1:0.12, suggesting that this method permitted sufficient mating without impairing productivity through excess hyperparasitism (though it is possible that males were less likely to migrate upwards into collecting tubes).


**Figure 1.** Parasitoid rearing (a) insect cages (b) collecting jar apparatus

After 7-10 days further development, when black parasitised whitefly pupae were visible, infested leaves were removed from plants and placed into jars with mesh lids, topped with a funnel and tube apparatus (Fig. 1b). The jar was wrapped in thick coloured paper to block light from the sides. This permitted emerging parasitoids to travel upwards towards a light source, collecting in the tube. This tube could then be removed regularly and parasitoids from all jars on that date consolidated into one or more tubes with a smear of honey for nutriment. Further honey was added to these tubes every few days, if necessary. A proportion of parasitoids collected were returned to the cages to maintain production.

Twenty-four hours prior to field release, adult parasitoids collected in the previous two weeks were anaesthetised with $CO_2$ and divided into 12 equal groups. This enabled checks of individual insects to ensure no contamination with whiteflies or other insects and to estimate sex ratios from subsamples. Parasitoids were transported as adults in 30ml sterilin tubes with a smear of honey provided as food. Almost no mortality during transport or release was observed during the field trial, with tubes usually being empty the same day or by the next release, and those insects which did die may have been damaged during counting.

### *Field Trial*

Kale plants (cv. 'Reflex') were planted at the Elsoms Seeds Ltd. research site outside Spalding, Lincs. on 30th May. The trial was initially a block of 40 x 91 plants with ~60cm spacing between plants. This was rearranged by removal of paths and replanting around the edge, providing 32 plots of 81 plants (9 x 9) in a 4 x 8 grid (Fig. 2). Lengthwise paths were 1.8m wide, widthwise paths were 2.5m wide.

Around 10th June, all plants were effectively defoliated by pigeons (Fig. 3). However, they recovered well with some undamaged leaves on most plants by 28th June, when the first adult whiteflies and eggs were observed, though not in all plots. By 5th July, whiteflies were visible in flight arriving at plots and monitoring of ten plants per plot showed adult whitefly to be present with an average of at least one per plant and ≥50% of plants on all plots, despite weeding of some plots having been disrupted by inclement weather. This was taken as a threshold to initiate spray applications, though rainfall again caused delays. As no significant differences between lengthwise rows were evident despite weeding differences, treatments were assigned fully randomly rather than in blocks.

**Figure 2.** Established kale plots

**Figure 3.** Plant defoliated by pigeons

Field trial treatments are listed in Tables 1 and 2. Each treatment was applied to four plots, assigned randomly. The primary function of the trial was to compare the control provided by timed release of parasitoid wasps alone (C) and in combination with later pesticide application (D) with pesticide application alone (F-H). The efficacy of covering crops with plastic meshes to exclude pests has been shown previously (see above) but such an approach may not be practical for large growers and may have impacts on yield and quality. In this context, netting treatments were used not to exclude whiteflies but to compare the

**Table 1.** Experimental treatments applied in the field trial

| | Treatments | |
|---|---|---|
| A. | **Control** | (no insecticide/biocontrol) |
| B. | **Netting Control** | |
| C. | **Encarsia** | (early *Encarsia tricolor* release) |
| D. | **Encarsia + Movento** | (early *Encarsia tricolor* release + late Movento) |
| E. | **Net + Encarsia** | (Netting with early *Encarsia tricolor* release) |
| F. | **Movento (early)** | |
| G. | **HDCI 039 (early)** | (coded product; 2 applications, 10 days apart) |
| H. | **'Industry'** | (Movento, Biscaya, Movento. approx. 1 month apart) |

**Table 2.** Insecticide rates used in the field trial

| Product | Active ingredient | Application rate | Water volume |
|---|---|---|---|
| Movento | Spirotetramat | 0.50 l/ha | 300 l/ha |
| HDCI 039 | Coded product | 0.75 l/ha (with Codacide at 2.5 l/ha) | |
| Biscaya | Thiacloprid | 0.40 l/ha | |

**Table 3.** Timing of events during the field trial

| Date | Action | Monitoring |
|---|---|---|
| 30/5 | Field Trial planted | |
| 07/6 | Replanting and Plots organised | |
| ~10/6 | Plants defoliated by pigeons | |
| 28/6 | | First whiteflies observed |
| 05/7 | | >50% of plants infested |
| 12/7 | | Adult + egg monitoring |
| 16/7 | *Encarsia* released | |
| 20/7 | First pesticide application | |
| | Cages erected | |
| 26/7 | *Encarsia* released | Adult + egg monitoring |
| | Follow up HDCI 039 application | |
| 09/8 | *Encarsia* released | Adult + egg monitoring |
| 22/8 | Second pesticide application | Adult + egg monitoring |
| | *Encarsia* released | |
| 06/9 | *Encarsia* released | Adult + egg monitoring |
| 20/9 | Third pesticide application | Adult + egg monitoring/ Larval counts |
| | *Encarsia* released | |
| 4/10+ | | Harvest quality estimate |

effect of containing parasitoids on the plots as opposed to leaving them to migrate away. The schedule of events on the field trial is summarised in Table 3 and described below.

Early applications of Movento (Spirotetramat) (one application) and a coded product (HDCI 039) (two applications ~10 days apart) based on monitoring of whiteflies were compared with a spray regime similar to that used in the industry for control of heavy whitefly infestations; Movento, followed by Biscaya (Thiacloprid), then Movento, with about a month between applications. It should be noted that the first application of this treatment would be concurrent with the other spray treatments, which would be at a lower level of infestation than growers would normally consider spraying.

An AZO knapsack sprayer powered by compressed air with VP02F conventional nozzles was used for spray application, operated by trained personnel. Insecticide was applied under dry conditions but subsequent precipitation may have limited the effectiveness of the third application (late Movento). Such effects were unavoidable given the conditions in summer 2012.

A few plants were marked at the time of first oviposition and monitored on each weekly visit to determine the state of whitefly development, in order to identify the optimal time of parasitoid release (3$^{rd}$–4$^{th}$ instar). To help support this, a simple degree-day model was devised and run using temperature data from a nearby weather station on the Met Office Weather Observation Website (WOW), updated daily. However, this was found to overestimate the rate of development, either due to faults in the model (though this was designed to be fairly conservative), to structural differences between the sites, or to lower temperatures at the level of the crop compared to the equipment at the weather station.

The first parasitoids were released on 16$^{th}$ July using an apparatus composed of a cane support, from which was suspended by wires a release tube, open end upwards, covered by an inverted dish (Fig. 4). This prevented the ingress of rain into the tubes and the tube could be easily exchanged for a fresh tube at each release. It was observed that, during wet or windy conditions, parasitoids did not leave the tube for several hours, whereas under sunny conditions, they would all spread into the crop within an hour.

**Figure 4.** Parasitoid release apparatus

As a consequence of unforeseen problems with the parasitoid production system, parasitoids were released continuously (6 releases) throughout the trial treatment period at lower numbers rather than at high numbers at the earliest stage. Whitefly numbers on the trial were also higher than was expected, given the experience of growers in the preceding year and a wet late spring. To gain as much useful data as possible given these restrictions, all parasitoids were released at the centre of the plots (multiple release points had been planned) to provide information on dispersal and impact. Total numbers released per plot were 310 adults (equivalent to 3.8 per plant).

Caged treatments consisted of two rectangular metal frames 3.7m long by 1.8m high, erected on opposite sides of a plot, two rows in. These were held up using rope lines and the tops of the vertical poles capped with tennis balls. Over the frames was placed a single piece of plastic insect-proof netting (0.77mm holes), which would trap/exclude whiteflies and most parasitoids, and the edges secured with soil so as to hold the net taut between the two frames (Fig. 5). This arrangement permitted easy access and enabled monitoring to be carried out relatively easily. Weather conditions delayed the erection of cages slightly but this was carried out on 20$^{th}$ July.

**Figure 5**. ABA field cage covering experimental kale plot

 

### *Monitoring*

Once a sufficient level of whitefly infestation had occurred, ten plants per plot were randomly selected and monitored every two weeks. The number of adult whiteflies and eggs were counted on the top five leaves of each plant as an indicator of the effect of treatments and of the future pest pressure and contamination. Position in the plot was recorded but not controlled for initially. As it became evident that there were strong differences between the middle and edge of plots in certain treatments, five strata in the plots were established and sampled, from the centre to the edge in four concentric circles, selected in the formula 1:2:2:2:3 from centre to edge, the edge stratum having more samples as it contained the largest number of plants per plot (Fig. 6). These strata were utilised in subsequent monitoring though only one plant from each stratum in Larval and Harvest assessments. As the infestation developed further it became impractical to count individual eggs and an estimate was used based on the number of egg circles multiplied by a factor derived from counting of both total eggs and egg circles on the same leaves (6.84). During September, migration to upper leaves and oviposition gradually ceased, presumably due to the shift to the diapausing winter morph, and this monitoring was abandoned.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| e | e | e | e | e | e | e | e | e |
| e | d | d | d | d | d | d | d | e |
| e | d | c | c | c | c | c | d | e |
| e | d | c | b | b | b | c | d | e |
| e | d | c | b | a | b | c | d | e |
| e | d | c | b | b | b | c | d | e |
| e | d | c | c | c | c | c | d | e |
| e | d | d | d | d | d | d | d | e |
| e | e | e | e | e | e | e | e | e |

**Figure 6.** Plan showing position of strata a - e (squares) in an idealised plot

On 20th September, in addition to monitoring the upper leaves, the number of whitefly larvae was estimated on the 15th leaf from the base of the plant and every 5th leaf above this up to leaf 35. This was carried out on a subset of five plants, one from each stratum.

On three occasions (23/8, 20/9, 18/10), lower leaves carrying 4th instar larvae and parasitism were sampled from each plot and brought back to NRI to determine % parasitism. The total number of pupae on each leaf was recorded, as well as any parasitism evident. Leaf sections carrying pupae that had not produced adult whiteflies by the time of processing were excised and placed in an incubator at $20^{o}C$, 16:8 h light:dark for 10 days to allow parasitoid development or adult emergence. The number of parasitised pupae was then recorded. These samples were kept until emergence to determine the identity of the parasitoid species present and, in the case of a subset of 20/9 samples, to check the sex ratios of *E. tricolor* emerging.

A harvest quality assessment was made in early October. Every 2nd leaf from the 16th leaf from the base was removed until ten leaves were gathered, on each of five plants per plot. In practice the uppermost leaves were lightly infested with eggs. One plant from each stratum was sampled per plot. In discussions with the industry representative, two options for harvest quality assessment were proposed – either based on the number of egg circles or the percentage cover of leaf with whitefly bodies or wastes. In practice, the second option was appropriate for this harvest date. In order to carry out a relatively rapid assessment, a laminated plastic grid (20 x 28cm) was pressed down onto the underside of leaves against a solid surface, limiting folding of leaf edges as much as possible. The number of squares (2 x 2cm) containing leaf surface was recorded, then the number of these squares contaminated by whiteflies, permitting a percentage of area contaminated to be calculated. This method was found to be more reliable than simple visual estimation, which tended to focus on the conditions at the centre of the leaf.

### *Analysis*

Data for adult, egg and larval numbers were analysed using Generalised Linear Models with quasipoisson distributions, analysis of variance and General Linear Hypothesis tests with Tukey contrasts. Harvest data was analysed using Analysis of Variance and Tukey HSD. Larval and Harvest means for treatments were calculated after proportional weighting of plant values by the size of the relevant stratum. All analyses were carried out using R 2.14.1 (R Foundation for Statistical Computing, Vienna, Austria).

## Results

### *Adults & Eggs*

In terms of adult and egg numbers on the upper leaves of plants, the insecticide treatments produced the greatest reductions in numbers (Tables 4 & 5, Figs. 7 & 8). As female whiteflies have a tendency to migrate to the youngest leaves to oviposit, activity here should predict the relative density of larvae on leaves as they grow. As mentioned above, numbers observed by this method dropped substantially in all treatments by late September, so little attention should perhaps be paid to patterns in this time period.

The initial application of HDCI 039 produced a greater reduction in adult numbers than other treatments. The second application appears not to have caused an additional reduction in numbers, though this may have eliminated further immigrant whiteflies (see below) and some of the first adults from eggs laid on the crop. The first Movento application in the 'Industry' treatment seemed not to have the same impact as that in the Movento-only (09/08, $P<0.05$). However, time or the Biscaya 2nd application appears to have corrected this (06/09, $P=0.999$) (Fig. 7, Table 4), though other treatments also show a reduction in numbers at this time. A rebound in adult and egg numbers is evident in all insecticide treatments as the first generation of nymphs produced adults, though this was not sufficient to eliminate their initial impact.

The reduction in egg numbers in insecticide treatments lagged behind that seen in the adult data. The same divergence seen between the registered treatments for adults was not evident in egg numbers on the upper leaves, with identical trends in both early Movento and 'Industry' treatments (Table 5), in part due to the reduction in fecundity caused by Movento. The greater effect of HDCI 039 throughout presumably reflects the higher initial kill of adults.

The net treatments reduced adult and egg numbers relative to the Control and Encarsia treatments, with no difference between net treatments (aside from lower adult numbers in the net control during the first immigration of whiteflies). For unknown reasons, the Net Control seemed to have substantially lower adult whitefly levels than the other treatments prior to cage erection. Given that numbers came to closely match those of the Net + Encarsia treatment, this may have been an artefact of sampling or plot position. The significantly reduced infestation in the net treatments relative to the control in the first generation suggests that migration of adult whiteflies onto the field trial continued into early August. This reduction did not persist to the same degree into the second generation. This may reflect better survival of whiteflies beneath the cages or a limitation of this monitoring method.

In terms of adults and eggs on the upper leaves, the Encarsia treatments showed no significant difference from the Control; the total numbers in fact exceeded those in the control at times. During the first influx of whiteflies, a lack of response on adult numbers is to be expected, because the parasitoids act on the developing nymphs. However, until the last assessment, no effect was seen on these metrics by the parasitoids.

**Table 4.** Mean adult whitefly counts on the upper five leaves in each treatment on different dates.

| Treatment | 12-July | 26-July | 09-Aug | 22-Aug | 06-Sept | 20-Sept |
|---|---|---|---|---|---|---|
| Control | 7.4 | 5.2 | 12.9 | 44.2 | 42.2 | 18.0 |
| Net Control | 2.6*** | 3.0** | 5.0*** | 51.5 | 29.7 | 7.7*** |
| Encarsia | 7.5 | 5.1 | 12.8 | 57.3 | 42.9 | 7.4*** |
| Encarsia + Movento | 7.4 | 5.5 | 14.1 | 54.3 | 43.9 | 11.9 |
| Net + Encarsia | 4.4* | 3.9 | 4.9*** | 55.5 | 29.0 | 9.3** |
| Movento | 5.8 | 5.7 | 7.6*** | 20.9*** | 21.2*** | 4.6*** |
| HDCI 039 | 6.3 | 2.3*** | 3.0*** | 17.4*** | 8.9*** | 1.4*** |
| Industry | 5.6 | 5.2 | 10.9 | 31.4 | 19.5*** | 3.4*** |
| Leaves | 6-10 | 12-16 | 15-19 | 25-29 | 31-35 | 35-39 |

* indicate significant differences from Control; *P<0.05, **P<0.01, ***P<0.001

**Table 5.** Mean whitefly egg counts on the upper five leaves in each treatment on different dates.

| Treatment | 12-July | 26-July | 09-Aug | 22-Aug | 06-Sept | 20-Sept |
|---|---|---|---|---|---|---|
| Control | 59.2 | 98.6 | 77.6 | 187.2 | 409.9 | 141.0 |
| Net Control | 26.0** | 66.3* | 43.6*** | 139.8 | 342.8 | 62.5*** |
| Encarsia | 60.0 | 98.3 | 74.5 | 228.1 | 423.5 | 65.0*** |
| Encarsia + Movento | 58.1 | 106.8 | 67.7 | 191.1 | 423.3 | 93.3* |
| Net + Encarsia | 43.7 | 84.0 | 39.0 | 162.9 | 322.2 | 76.9** |
| Movento | 47.0 | 89.5 | 24.6*** | 68.2*** | 183.9*** | 29.6*** |
| HDCI 039 | 70.4 | 81.2 | 10.1*** | 64.4*** | 72.4*** | 12.1*** |
| Industry | 48.4 | 96.9 | 24.4*** | 68.9*** | 166.1*** | 26.1*** |
| Leaves | 6-10 | 12-16 | 15-19 | 25-29 | 31-35 | 35-39 |

* indicate significant differences from Control; *P<0.05, **P<0.01, ***P<0.001

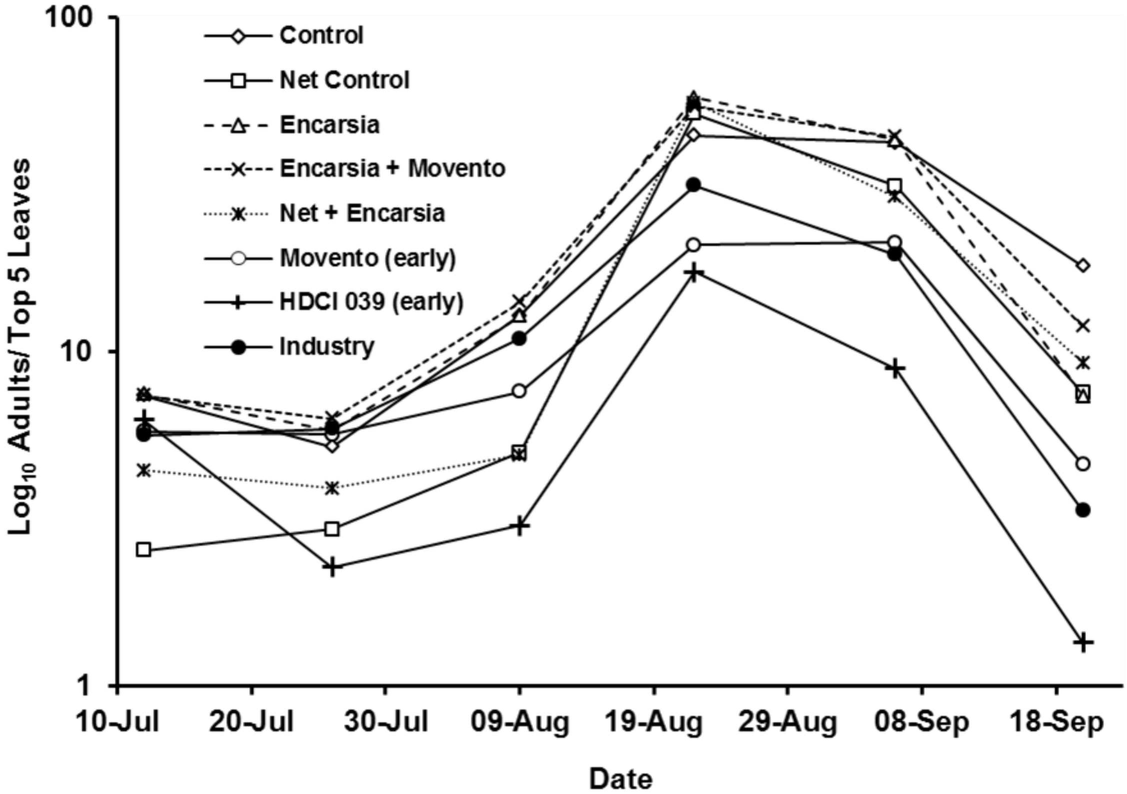


**Figure 7.** Mean adult whitefly numbers in each treatment on top five leaves (log scale)

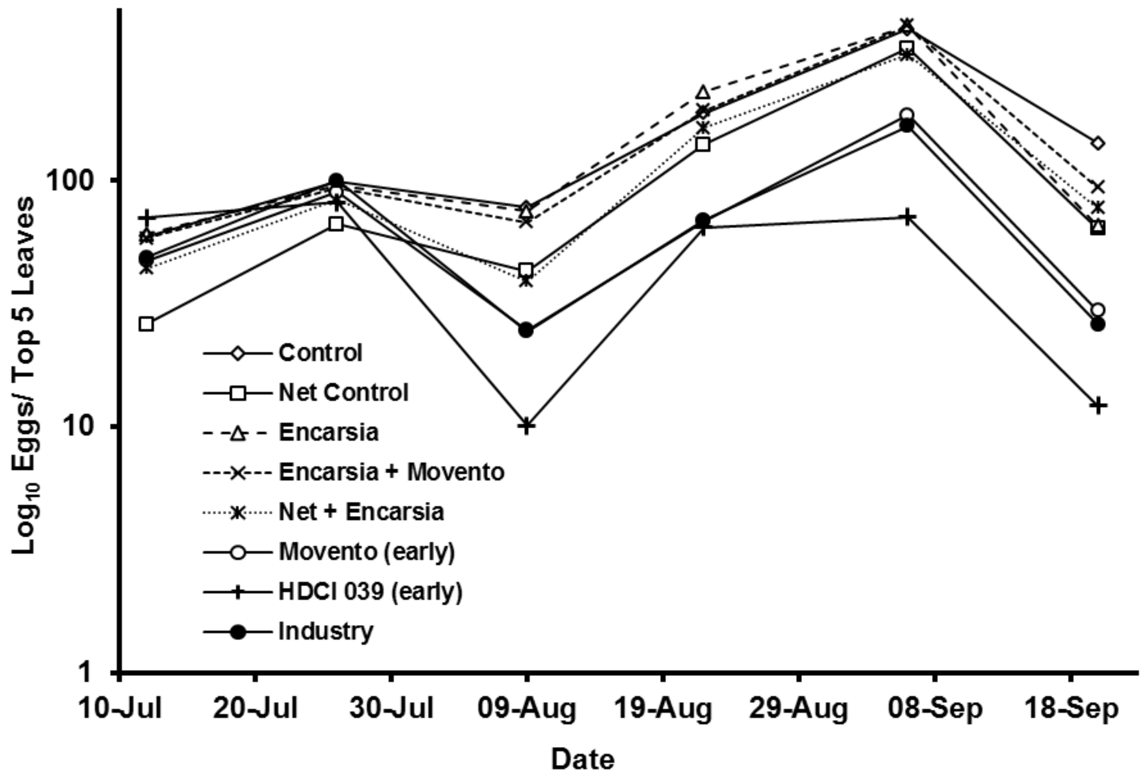


**Figure 8.** Mean whitefly egg numbers in each treatment on top five leaves (log scale)

### *Larvae*

The estimate of larval populations on different plants unsurprisingly reflected the monitoring of adults and eggs to some extent. The insecticide treatments produced the greatest reductions, particularly HDCI 039. The Net and Encarsia treatments all produced a similar limited decrease relative to the control (Fig. 9).

While the response appears poor in the Encarsia treatments, an impact of parasitoid release in the centre of the plots is apparent, being similar to the level of control found in the insecticide treatments, with no similar spatial pattern in the control plots (Fig. 10). An almost linear trend from the centre to edge of plots is visible in the Encarsia + Movento treatment, though there is no such clear progression in the other parasitoid treatments and identical patterns exist for strata a and b in this treatment, Net + Encarsia and early Movento.

However, the high variability in the data prevents significant differences being detected; while a significant effect of strata was present overall ($F_4$ = 5.698, P<0.001) only the HDCI 039 strata were different from any in the control and then only at P<0.05. In several treatments, reduced whitefly levels appear in stratum 4 relative to strata 3 and 5; the former may represent adult dispersal over time through the crop till a preferred level of protection is achieved, while the latter is likely to be a product of migration between plots. While this represents a property of the plot size, such edge effects may have implications for guiding the location of monitoring of whitefly numbers in crops.

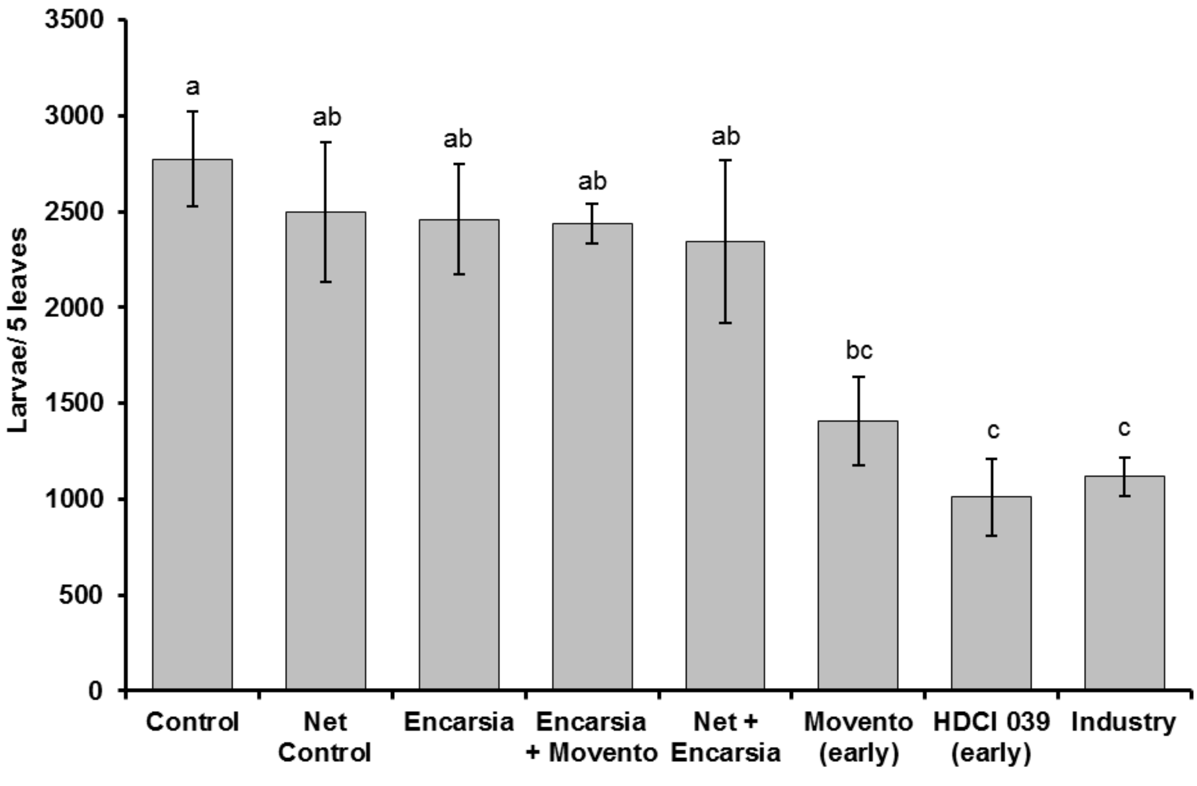


**Figure 9.** Mean total number of whitefly juveniles on five leaves per plant (weighted).

 

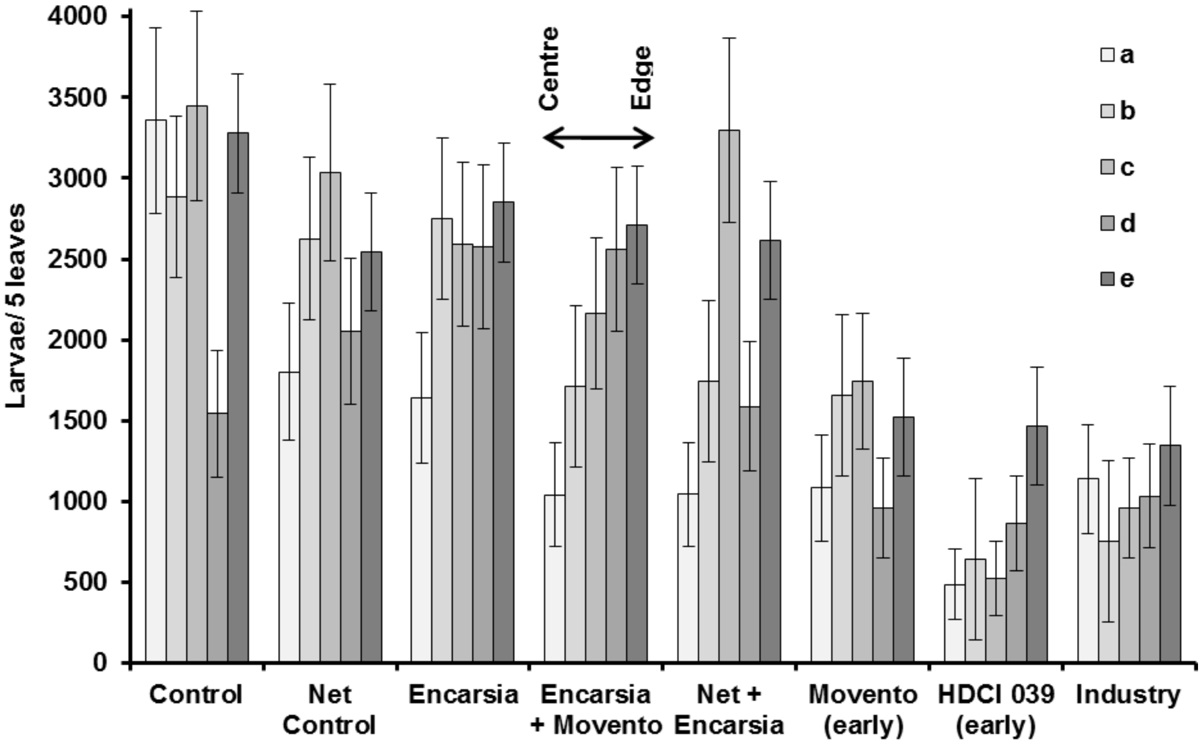


**Figure 10.** Mean total number of whitefly juveniles on five leaves per plant in each stratum.

### *Parasitism*

Levels of parasitism from monitoring were low in all treatments, but were highest in the Encarsia treatments (Fig. 11). In these three treatments, parasitoids were recovered from plants in all strata (a-e), though they were more common and numerous towards the centre. In terms of both percentage parasitism and mean actual number of parasitised pupae per leaf, the Encarsia + Movento treatment had higher levels than the other two parasitoid treatments. The limited parasitism observed in the other treatments was in all cases due to parasitism from one sample in a single plot. Whether this was due to migration from release plots or from local populations (see below) is unknown. Sex ratios from 20/09 were strongly female biased (1:0.07 F:M) implying that released females were overwhelmingly fertilised. However, such a low ratio may lead to reduced fertilisation, and therefore females, in the subsequent generation, should multiple generations of parasitism be required in any one season.

In addition to parasitism from *E. tricolor*, two other species of whitefly parasitoid were recovered from the field trial. In the August sample, *Euderomphale chelidonii* was found from one leaf at the edge of a control plot. A small number of putative *Encarsia inaron* (confirmation by specialist pending) emerged from black scales on an ‘Industry’ plot in

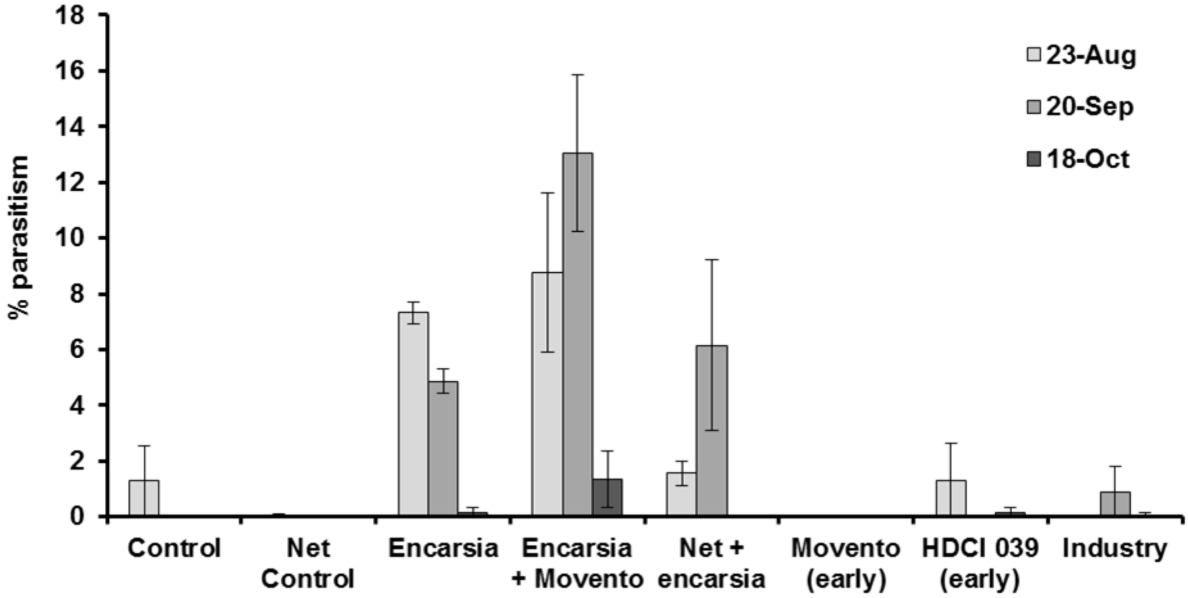


**Figure 11.** Mean percentage parasitism on lower leaves in each treatment on three dates.

October. In both cases, the plants were on the outside of the plot and both plots were at the periphery of the trial.

Both *E. chelidonii* and *E. tricolor* were recovered from a population of honeysuckle whitefly, *Aleyrodes lonicerae*, on honeysuckle in the north of Spalding in late August. No honeysuckle was found in hedgerows edging the trial site, nor evidence of other whiteflies on known hosts. Regular planting of Brassicas, some unsprayed, on the trial site may be sufficient to maintain these background parasitoids. Observation and sampling of a control plot on an adjacent trial with low levels of whitefly infestation produced no evidence of parasitism. Given the low numbers released relative to whitefly density, it may not be surprising that parasitoids did not have need to spread from the HDC trial onto this.

Leaf samples of whitefly pupae were taken at two conventional Brassica fields in the region on two occasions (late August and mid-October), ten leaves from each bearing a number of clusters of whitefly juveniles. No parasitoids emerged from any of these samples and nor was any parasitism observed on other leaves at these sites.

### *Harvest*

Leaves harvested in October were of generally poor quality though some almost clean leaves occurred in insecticide treatments (Fig. 12). The wet conditions during the summer did, however, limit the growth of sooty moulds on the upper surfaces of leaves, despite the high whitefly numbers. For comparison with the whitefly levels in the field trial, a kale crop in the area received one Movento application in late August in response to adult numbers similar to those seen at the start of the trial. Limited sampling at this site in October (n=10) produced a mean value around a third of that in the most successful treatment ('Crop' in Fig. 12).

As in other measures, the insecticide treatments had the greatest effect, being significantly different from the other treatments but not from each other. The two Net treatments were not significantly different from each other, nor were the three Encarsia treatments, though only the Net Control was significantly different from the Control when using weighted means. In contrast to the larval assessment, position of plant in plot had no significant effect on harvest quality both overall ($F_4$ = 0.612, P = 0.655) and when comparing strata within treatments, though an effect in the centre is suggested in the Encarsia treatments (Fig. 13). Comparing the strata for each treatment with their equivalent in the Control (e.g. Aa vs Ba, Ab vs Bb, etc.), all strata in the insecticide treatments were significantly different, but none in the other treatments.

Appendix A shows examples of harvested leaves at various representative percentage covers of whitefly contamination. A limitation of the method as developed is that it makes no distinction for the density or nature (wax, eggs, pupae) of contamination within a particular square, which may affect the difficulty of cleaning leaves, independent of percentage cover.

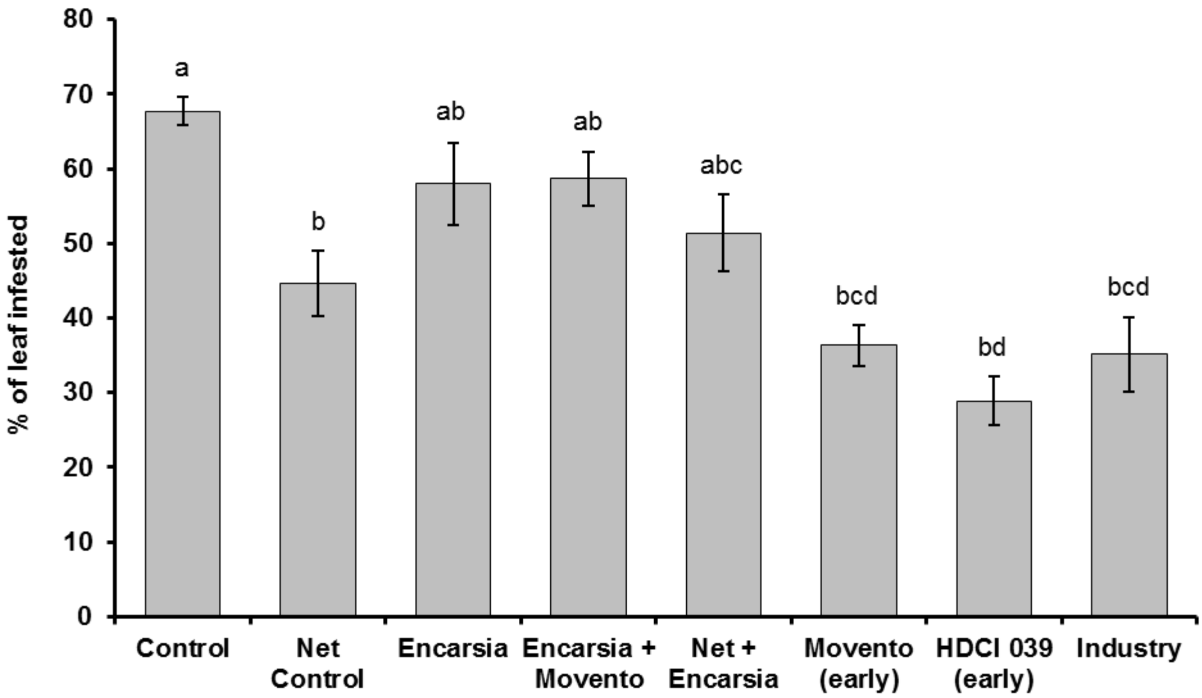


**Figure 12.** Mean percentage contamination of leaves in each treatment (weighted by strata). Columns sharing a letter are not significantly different.

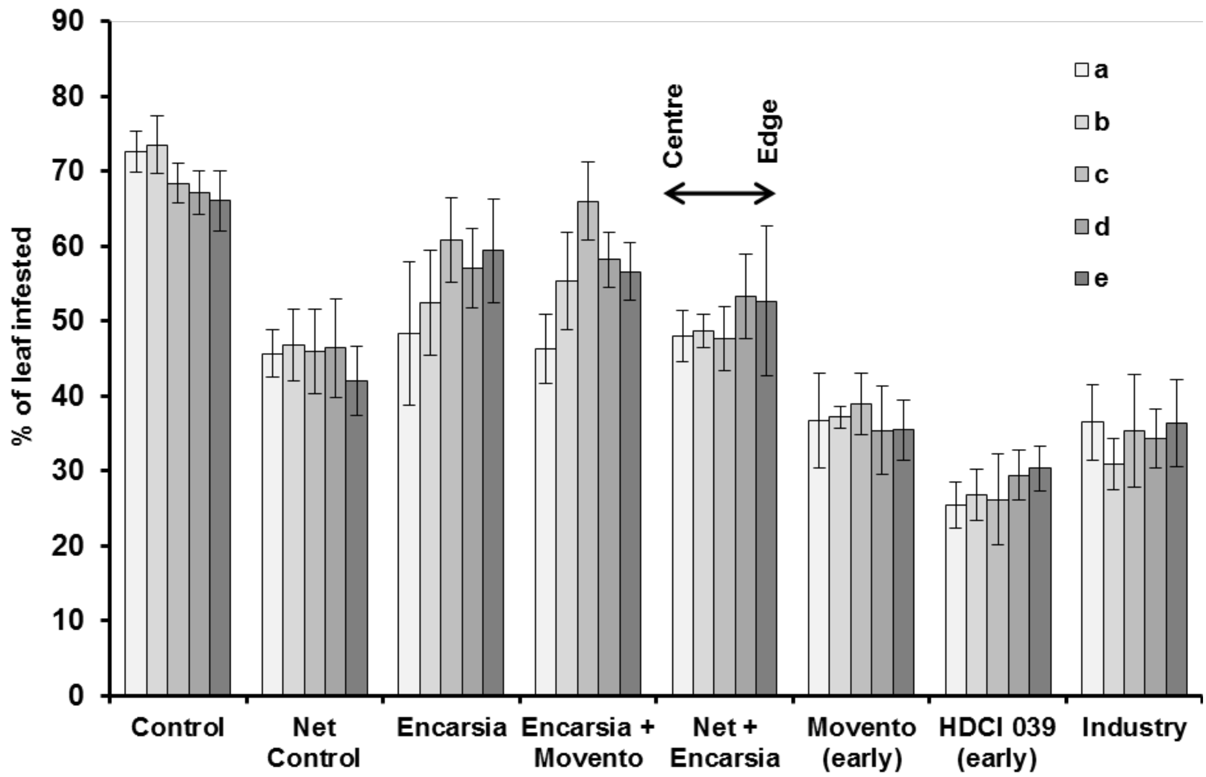


**Figure 13.** Mean percentage contamination of leaves in each treatment in different strata.

## Discussion

Early application of HDCI 039 gave effective short-term control with an apparent knock-on effect on populations which carried through in all measures to harvest. The second application of this product may admittedly have assisted this by eliminating later migrant adult whitefly, the presence of which is evidenced by the impact of adding cages after the time of the first application. The effectiveness of this treatment may in part be due to the small size of the plants at this time, enabling good coverage. Whether similar results would be achieved with later application on larger plants with a more closed canopy cannot be determined from this study.

While the early Movento application achieved identical effects to the industry rotation, the value of later treatments is illustrated by the ability of the middle treatment (Biscaya) to correct for poor control in the initial application. Reduced impact of Movento and 'Industry' treatments may reflect a sub-optimal early start to applications, permitting further colonisation after maximum efficacy had been lost. However, a previous HDC trial tested the effect of time on efficacy of systemic products under controlled conditions and showed no such reduction for Movento and mixed results for Biscaya (FV 399).

Late Movento application in addition to parasitoids had no discernible effect on harvest quality beyond that of *E. tricolor* release alone. This also seems to be the case when comparing the Movento treatment with the 'Industry'. Aside from reductions in overwintering adult numbers, such late applications may have limited impact on quality when pest pressure is high as in this trial.

Parasitoid release, even at lower than intended levels with high whitefly populations, did possibly show a strong impact close to the point of release. However, the inadequate numbers, high pest pressure and general variability in all treatments led to no significant impact at the plot scale and limits the ability to draw conclusions about this technology, as intended.

The value of netting in excluding adults has again been illustrated, even though these were applied after whiteflies were present. There were no significant differences between the net treatments, aside from a lower initial whitefly level on the control before cages were added. Any impacts of *E. tricolor* release at the centre of netted plots were obscured by levels elsewhere in these plots. This suggests that migration of parasitoids did not limit effectiveness though numbers released were too low for competition and displacement to become an issue.

As might be expected, the significant patterns seen in the adult and egg assessments prior to 20/09, an early impact of netting with effects of insecticides building over time, are reflected in the data from the larval and harvest estimates. Positional patterns evident in parasitoid treatments from the larval assessment were not reflected strongly in the stratified harvest quality data suggesting an effect at the monitored height or lower but swamped by numbers of whitefly/lack of parasitism.

It should be noted that these results are on a dedicated trial site in one year under certain (somewhat adverse) environmental conditions. Migration patterns on the trial site may not reflect that in the field; whiteflies were not a major problem on crops in the region this year, potentially due to early warmth followed by a wet spring causing adult mortality and/or to cumulative impact of Movento applications in successive years. Similarly, the presence of other parasitoid species on this site may be unusual, being due to proximity to urban areas, the presence of unsprayed areas in trials, landscape heterogeneity with areas of grassland and hedgerows or other undetermined factors.

The release of native natural enemies in the U.K. is currently unregulated, unlike non-natives. However, it may be worth considering the potential impacts of mass release of this parasitoid on the natural whitefly parasitoid populations in the vicinity, as evidenced by the other species found in the trial and in surrounding areas. The distribution, magnitude and pest suppression value of these species is almost completely unknown (to the best of our knowledge, these are the first records of any whitefly parasitoids for Lincolnshire). For this species in particular, risks would be limited to competition with the natural fauna and hyperparasitism of other beneficial insect species.

One of the clear lessons highlighted by this research is the benefit achieved by early application of the treatments. The data also suggest the potential benefits that could be achieved by parasitoid releases. In the current study, the numbers released were only sufficient to provide partial protection to the centre of the plots and so higher numbers would be needed to achieve better protection.

A number of future research questions are suggested by this work. Further exploration of the optimal combination and timing of these insecticide products and other control measures, driven by monitoring and/or prediction of whitefly infestations, is recommended. Understanding annual movements of whitefly adults in the agricultural landscape and the period of immigration into the crop would also have value as would the opportunities for integrating biological, cultural and chemical controls. In light of the above, information on the effect of planting times on speed of whitefly infestation and the relative value of pre-planting systemic treatments with different plantings may be useful. Determining any interaction effects between the parasitoids and the insecticide products would be advisable if they are to be incorporated in IPM programs. Biological control may be a more acceptable intervention where the source is outside grower's control, e.g. Oil Seed Rape, and may have value in organic systems or when initial pest pressure is low, providing year-on-year reductions in background pest populations.

## Conclusions

- Early insecticide spray applications based on monitoring may have value, producing similar results to rotations. However, a strategy reliant on this will be susceptible to factors which disrupt the initial application and to later immigration of pests.
- The coded product HDCI 039, applied twice once whiteflies have begun to arrive at a crop, produced comparable control to Movento and a Movento/Biscaya/Movento rotation.
- Late applications of Movento to heavy whitefly infestations may be of limited benefit.
- Limited releases of the parasitoid *Encarsia tricolor* were unable to produce comparable control to insecticides throughout plots but were still able to cause suppression and quality improvements close to the point of release. This suggests potential for control with sufficient numbers released, providing optimal timing can be achieved.
- Netting covers applied after whitefly had begun to arrive on plots led to a significant reduction in whitefly populations and a limited improvement in leaf quality,

suggesting that whitefly immigration or intra-crop movement occurs over a prolonged period (>1 month).

- Monitoring of adult and egg numbers at the top of the plant after insecticide treatment gave a relative indication of eventual harvest quality and may be useful in determining thresholds for action.
- The conditions in the trial may not be representative of normal conditions in the field with heavy precipitation throughout the summer and higher whitefly pressure than had been seen in crops in the region in 2012.

## Knowledge and Technology Transfer

- Presentation of preliminary data to BGA/HDC Technical meeting 11/10/12
- Advised on content of BGA/HDC Technical Update
- Planned publication in HDC News

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## Appendices

**Appendix A.** Examples of percentage whitefly contamination of harvested leaves**.**

(a) (b)



(c) (d)


(e) (f)



(g)