

Project title: The time of infection of overwintered cauliflower and Brussels sprout by *Turnip yellows virus* (TuYV) and the potential of insecticides to control the virus.

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The results and conclusions in this report are based on an investigation conducted over a 21-month 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

This project has found ways for reducing aphid numbers in crops for better control of Turnip yellows virus.

Background

Turnip yellows virus (TuYV, formerly known as Beet western yellows virus) readily infects Brassicas, including oilseed rape and horticultural Brassicas e.g. Brussels sprouts, cauliflowers, cabbage, broccoli etc. It is transmitted by aphids and the peach-potato aphid, *Myzus persicae*, is considered to be the major vector.

Incidences of up to 55% and 60% were found in commercial Brussels sprout crops and commercial cauliflower crops respectively in the 2010-2011 season despite the relative lack of aphids during this period. Recent surveys have also shown up to 100% infection of plants in some oilseed rape fields in the UK.

Crop infection often goes undetected by growers as the symptoms are similar to those of stressed plants. However, the virus can have a big impact on crop quality and yield. It causes tipburn in cabbage. Project FV 365 demonstrated that yield loss caused by TuYV can be as high as 65% in some sprout varieties and 30% in some varieties of cauliflower and cabbage (FV 160b). The virus is especially common in areas where vegetable Brassicas are grown intensively (Lincolnshire, Kent, Cornwall) and also where they are grown alongside oilseed rape.

Oilseed rape represents a massive reservoir of virus which can infect horticultural Brassicas, putting crops with a long growing season (such as over-wintered cauliflower and Brussels sprouts) at particular risk of infection.

In order to determine the time of natural infection of cauliflower and Brussels sprout plants in the field by TuYV, experimental plots were grown within commercial crops in Lincolnshire and Kent. Plants in the plots were tested at regular intervals to determine when infection occurred during the growing season.

Fully replicated, controlled, glasshouse experiments were also carried out to determine the efficacy of various insecticide sprays, dummy seed-treatments and a pre-transplant drench in controlling TuYV infection.

Summary

- Turnip yellows virus (TuYV) causes significant reductions in yields of vegetable Brassicas, however growers are rarely aware of its presence, as infections are often symptomless.
- Levels of infection of Brassica crops with TuYV were strongly linked with peach-potato aphid numbers and infection is most likely to occur in June and July when numbers of aphids flying are particularly high.
- Results from insecticide trials in the glasshouse showed that some treatments, in particular those using dummy seed treated with Gaucho (Imidacloprid), or HDCI 043, were more effective than others in reducing TuYV infection. However, no treatments prevented plants from becoming infected. By combining such treatments with growing the least susceptible Brassica varieties (FV 160b and FV 365) and the best spray treatments (applied based on Rothamsted Insect Survey suction trap peach-potato aphid catches), growers can now start to optimise an integrated approach to TuYV control.
- Breeding crops with genetic resistance to the virus is likely to provide the best means of protection against TuYV and incorporating this in to an integrated control strategy will provide maximum protection against infection. In the meantime, the currently available components of an integrated control strategy need to be evaluated in the field.

Time of TuYV spread in the field

The amount of infection in experimental plots of Brassica plants growing in commercial fields in Lincolnshire and Kent was associated with the numbers of peach-potato aphids trapped in the Rothamsted Insect Survey suction trap closest to the experimental sites.

During the early summer months of June and July 2011, peach-potato aphid numbers were particularly high in Kirton, Lincolnshire, with over 1000 aphids trapped in the week beginning 4 July 2011. When sprout plants in the experimental plot were tested on 10 July 2011, 96% had become infected by TuYV. One month later all plants were infected.

Given the loss of marketable yield (up to 65%) and quality that TuYV infection causes in sprouts, this level of infection could significantly reduce profitability.

Later in the growing season (September, October and November 2011), peach-potato aphid numbers were much lower (maximum of 44 caught in any one week during this period), resulting in much lower TuYV infection. Only 36% of sprout plants (which had been protected from infection by gauze sheeting up to late August) became infected.

Cauliflower plots transplanted in to the fields in mid-August in Lincolnshire and Kent had 8% and 2% infection respectively (maximum of 44 and 29 peach-potato aphids caught in any one week in Lincolnshire and Kent suction traps respectively during this period).

These results clearly indicate that infection is most likely to occur when numbers of peach-potato aphids caught in the local suction trap are high. In 2011, numbers were high in June and July in Lincolnshire, resulting in all experimental plants becoming infected. Later in the year after cauliflowers had been transplanted (August), the numbers of peach-potato aphids caught in the local suction trap were much lower and infection of cauliflower plants was also much lower.

In some years, the numbers of peach-potato aphids caught in suction traps later in the year are much higher and in such years later planted crops, such as overwintered cauliflower, will be at greater risk of TuYV infection than those in these trials.

The results from the project provide growers with knowledge of when their crops are most at risk of infection by TuYV and hence when control measures against aphids (e.g. insecticide applications) are required.

Efficacy of insecticide treatments in controlling TuYV

A robust, reliable and reproducible glasshouse assay for determining the efficacy of insecticides in controlling TuYV was developed, evaluated and successfully deployed in the project.

The experiments demonstrated that some insecticide treatments were more effective than others at controlling peach-potato aphids and reducing TuYV infection in Brassica plants.

The most effective treatments appeared to be those using treated dummy seed (Gaucho [Imidacloprid] and coded product HDCI 043).

With the exception of Movento (Spirotetramat) after 1 week, all insecticide treatments significantly reduced (relative to numbers on untreated plants) the numbers of peach-potato aphids on treated plants, after 1, 2 and 4 weeks of exposure to the aphids.

Despite reducing aphid numbers, none of the insecticide treatments prevented plants from becoming infected by TuYV. However, most significantly reduced the amount of virus detected in plants after exposure to peach-potato aphids carrying TuYV.

The insecticides Plenum (Pymetrozine), Movento, Biscaya (Thiacloprid), HDCI 040 (spray), HDCI 041 (spray), HDCI 042 (spray) and HDCI 044 (drench) significantly reduced the amount of virus detected in plants after exposure to peach-potato aphids carrying TuYV, though to varying degrees.

Plants sprayed with Aphox (Pirimicarb) did not have significantly lower levels of virus than control plants that received no insecticide treatment.

By using one of the effective dummy seed treatments combined with one of the best spray treatments, growers can now optimise TuYV control. That said, these treatments may not prevent the plants becoming infected, but hopefully will reduce the amount of virus in plants. At this stage there is no data on whether this will reduce the negative effects of TuYV on crop yield and quality.

Breeding genetic resistance to TuYV into crops is likely to give the best protection against the virus in future and could be the main stay of an integrated approach to TuYV control. In the meantime, currently available components of an integrated control strategy (including growing the least susceptible Brassica varieties with a dummy seed treatment and timing of foliar sprays to coincide with peak suction trap catches of peach-potato aphids) need to be evaluated in the field.

Financial Benefits

Using dummy seed treatments may reduce the amount of virus accumulating in plants in the field and thereby increase the yield and quality of crops, improving profitability.

Using the most effective insecticide sprays and timing their application(s) to coincide with peak peach-potato aphid flights (as detected in suction traps) will save time and money by avoiding applications that would have little, or no benefit.

Combining a dummy seed treatment with timed sprays of the most effective insecticides and less susceptible Brassica varieties (identified in FV365), will have associated financial benefits due to improvements in crop quality and yield.

Action Points

In order to control TuYV and reduce aphid numbers in crops:

When planting in May, June, or July, it would be advisable to use a dummy seed treatment (Gaucho). We would also advise monitoring your local Rothamsted Insect Survey suction trap catches of peach-potato aphids (*Myzus persicae*) and applying an effective foliar aphicide promptly when they start to be caught / numbers increase. Aphid bulletins can be accessed via the Rothamsted Insect Survey website:

<http://www.rothamsted.ac.uk/insect-survey/STAphidBulletin.php> or on the HDC Pest Bulletin on the Syngenta Website.

SCIENCE SECTION

Introduction

The problem

Turnip yellows virus (TuYV, formerly known as Beet western yellows virus) readily infects Brassicas, including oilseed rape and horticultural Brassicas e.g. Brussels sprouts, cauliflowers, cabbage, broccoli etc. It is transmitted by aphids and the peach-potato aphid, *Myzus persicae* is considered to be the major vector.

Incidences of up to 55% and 60% were found in commercial Brussels sprout crops in the UK and commercial cauliflower crops respectively in the 2010-2011 season despite the relative lack of aphids during this period. Recent surveys have also shown up to 100% infection of plants in some oilseed rape fields in the UK.

Crop infection often goes undetected by growers as the symptoms are similar to those of stressed plants. However, the virus can have a big impact on crop quality and yield. It causes tipburn in cabbage. Project FV 365 demonstrated that yield loss caused by TuYV can be as high as 65% in some sprout varieties and 30% in some varieties of cauliflower and cabbage. The virus is especially common in areas where vegetable Brassicas are grown intensively (Lincolnshire, Kent, Cornwall) and also where they are grown alongside oilseed rape.

Oilseed rape represents a massive reservoir of virus which can infect horticultural Brassicas, putting those with a long growing season (such as over-wintered cauliflower and Brussels sprouts) at particular risk of infection.

The need for the project

Project FV 365 which investigated the effect of TuYV infection on vegetable Brassicas has shown the severe effect that the virus can have on crop quality and yield.

Crops with long growing seasons e.g. Brussels sprouts and overwintered cauliflower are at particular risk of these effects due to the extended periods over which they are exposed to aphids and infection. Monitoring the time of TuYV infection in a situation that reflects the commercial one will give information on when preventative measures will be of most use.

No information is currently available on the efficacy of various insecticides in preventing the spread of TuYV. This information will allow growers to choose the most effective insecticides during peak times of TuYV spread, in order to prevent infection.

Project outline

The project is made up of 2 distinct components.

Component 1 was designed to determine the time of natural infection of Brussels sprouts in Lincolnshire and overwintered cauliflowers in Lincolnshire and Kent by TuYV in the field. Due to their long growing season, they are exposed to the virus for prolonged periods, resulting in crop quality and yield being at greater risk than for crops with shorter growing seasons. To investigate the time of TuYV infection, experimental plants were transplanted into commercial crops in Kent and Lincolnshire. The plants were tested for the presence of TuYV at regular intervals to determine the progression of infection. Numbers of peach-potato aphids caught in local Rothamsted Insect Survey Suction Traps were compared with TuYV infection data.

Component 2 of the project involved developing a glasshouse assay for determining the efficacy of insecticides in controlling TuYV and using this to evaluate 10 insecticides for their ability to control the peach-potato aphid and TuYV in sprout plants over a 4 week period. The glasshouse experiment was fully replicated and included untreated control plants.

Materials and methods

Component 1

Component 1 of the project involved determining the time of natural TuYV infection of test plants growing in plots within commercial crops. One hundred Brussels sprout variety Doric seeds were sown 08 April 2011 in 345 modular trays in Levington's M2 compost. These were grown in a temperature-controlled glasshouse at 18°C with a 12h day length. Two lots of 100 cauliflower variety Jerome seeds were sown in the same manner on 27 June 2011. On 02 June 2011, the Brussels sprout seedlings were transplanted into a field of commercially grown sprouts in Lincolnshire. On 17 August 2011 and 18 August 2011, 100 cauliflower seedlings were transplanted into commercial cauliflower crops in Kent and Lincolnshire respectively. Individual test plants were numbered 1-100. After transplanting, plants 1-50 in each experimental plot were left uncovered (and therefore exposed to TuYV infection), whilst plants 51-100 were covered with aphid-proof gauze, as shown in Fig 1.



Figure 1. The trial plot of cauliflowers in Lincolnshire. Plants 1-50 uncovered, plants 51-100 covered in aphid-proof gauze.

Sampling plants 1-50 for TuYV testing

After transplanting, plants 1-50 were left for 4 -5 weeks to grow, before they had put on enough growth to allow virus testing. Young leaves were then picked from the uncovered plants at regular intervals (~ every two weeks) and tested for the presence of TuYV by ELISA. Sampling of sprouts plants 1-50 began on 10 July 2011 and ended on 10 August 2011. Sampling of Kent and Lincolnshire cauliflowers began on 18 September 2011 and 19 September 2011 respectively and ended on 17 November 2011.

Part way through each crop's growing season the aphid-proof gauze was removed from plants 51-100, exposing them to aphids and TuYV infection. It was removed from the sprouts, Kent cauliflowers and Lincolnshire cauliflowers on 26 August 2011, 03 December 2011 and 09 December 2011 respectively. Sampling and testing of the sprout plants 51-100 ran fortnightly between 19 September 2011 and 17 November 2011. Very few peach-potato aphids were flying by 17 November and no new infection was being detected. No peach-potato aphids were caught in the local Rothamsted Insect Survey suction traps in Lincolnshire and Kent during December, so cauliflower plants 51-100 were not sampled during this period.

Serological testing of plants for TuYV infection

The young plant leaves were macerated between electric rollers and sap collected for testing by triple antibody sandwich Enzyme-Linked Immunosorbent Assay (TAS-ELISA) as described by Hunter *et al.* (2002). Once plants were found to be infected they were no longer sampled.

Information on peach-potato aphid flights

Rothamsted Insect Survey suction trap data was monitored for traps at Kirton, Lincolnshire and Wye, Kent at the following website,

<http://www.rothamsted.ac.uk/insect-survey/STAphidBulletin.php> and compared with the progression of TuYV infection.

Component 2

Component 2 of the project involved developing a glasshouse assay for determining the persistence and efficacy of insecticides in controlling TuYV over a four week period and then testing a range of insecticidal treatments. All experiments in component 2 were at Wellesbourne, Warwickshire, UK.

Development of the assay

Experiments were carried out in January and February 2012 to determine the best Brussels sprout host and experimental method for the assay. Brussels sprouts varieties NIZ16-4391, Doric and Genius seeds were sown into 345 modular trays of Levington's M2 compost and grown in a glasshouse compartment (18°C). Supplementary lighting was set on a 12h cycle. Plants were watered every 2-3 days as necessary. After 4 weeks the seedlings were transplanted into 7cm pots of Levington's M2 compost. These untreated plants were arranged in two circles of 12 plants, each circle with 75cm diameter (Fig. 2). A peach-potato aphid-infested, TuYV-infected oilseed rape plant (variety Mikado) that had been infected with TuYV three weeks previously, was placed in the centre of each circle. An individual 90 x 90 x 60 cm gauze frame was placed over each circle, ensuring no aphid movement between treatments (Fig. 3). The plants were watered every 1-2 days as necessary. Colonisation was allowed to occur naturally for 4 weeks and then plants were tested for TuYV infection by ELISA as described above. The number of aphids on each plant of the different varieties was estimated.



Figure 2. The circular arrangement of 12 sprout plants around a central TuYV-infected oilseed rape plant colonised by peach-potato aphids.

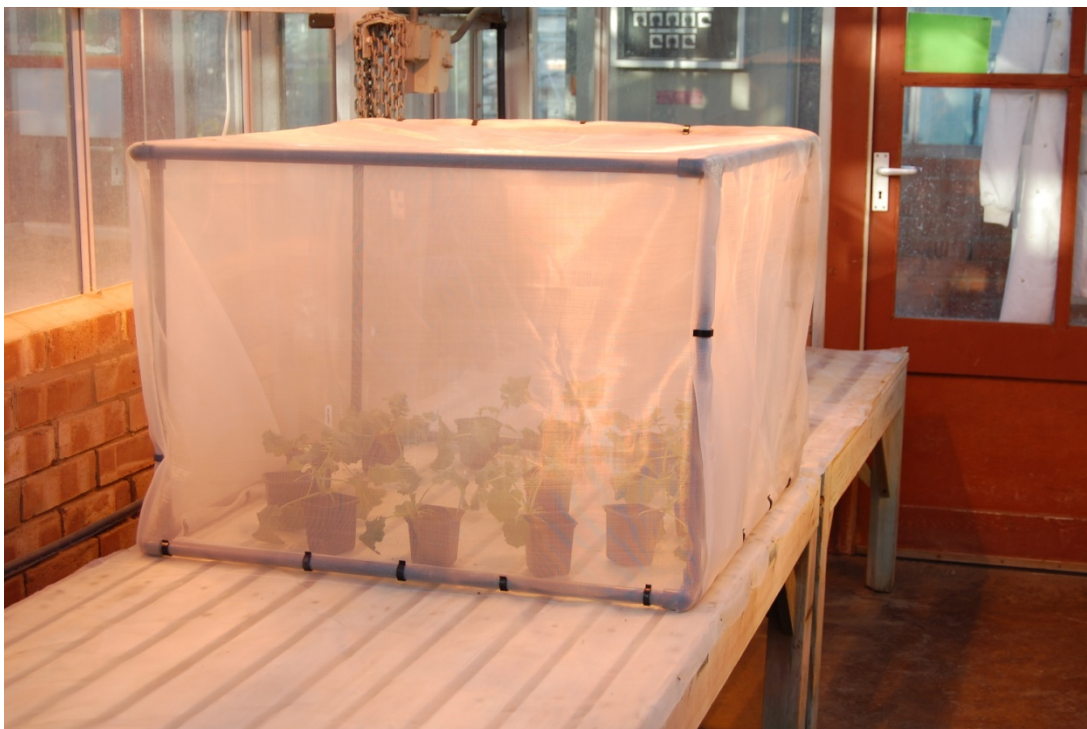


Figure 3. A circle of sprout plants and a TuYV-infected, peach-potato aphid-infested oilseed rape plant covered by an aphid-proof gauze frame.

Statistical analysis (ANOVA) was carried out and this showed that after 4 weeks, although sprout variety NIZ16-4391 was colonised by fewer aphids than Doric and Genius,

significantly more TuYV was detected in them than in Genius and slightly more than in Doric. Hence, based on these results, Brussels sprouts variety NIZ16-4391 was selected for subsequent experiments. The results showed the experimental assay was reproducible, effective and suitable for purpose.

Experiments to determine the efficacy of insecticides in controlling TuYV over a four week period

The insecticides selected for testing were applied as a pre-transplant drench, sprays, or dummy seed treatments. The products chosen for testing are listed in Table 1.

Table 1. Insecticides and active ingredients used in experiments and their application rates.

| Insecticide treatment and active ingredient | Application rate | Insecticide mode of action | Approval Status |
|--|--|-----------------------------------|------------------------------------|
| Plenum spray (50% w/w Pymetrozine) | 400g product / ha | Contact | On-label approval |
| Movento spray (150g/L Spirotetramat) | 500ml product / ha | Systemic | On-label approval |
| Aphox spray (50% w/w Pirimicarb) | 420g product / ha | Systemic | On-label approval |
| Biscaya spray (240g/L Thiacloprid) | 400ml product / ha | Systemic | On-label approval |
| HDCI 040 spray (50% w/w Coded Product) | 160g product / ha | Systemic | Not approved |
| HDCI 041 spray (120g/L Coded Product) | 200ml product / ha | Not known | Not approved |
| HDCI 042 spray (50g/L Coded Product) | 250ml product / ha | Not known | Not approved |
| Gaucha dummy seed (Imidacloprid) | 140g active ingredient / 100,000 seeds | Systemic | Specific off-label approval (SOLA) |
| HDCI 043 dummy seed (Coded Product) | 140g active ingredient / 100,000 seeds | Systemic | Not approved |
| HDCI 044 drench (200g/L Coded Product) | 15ml product / 1000 plants | Systemic | Not approved |
| Untreated plants | N/A | N/A | N/A |

Brussels sprouts variety NIZ16-4391 were sown into 345 modular trays of Levington's M2 compost and grown as above. Twelve seeds had a dummy seed treated with Gaucha sown alongside and another 12 had a dummy seed treated with coded product HDCI 043 sown

alongside (see Table 1 for application rates). After 4 weeks 12 transplants received the HDCI 044 drench (for concentration of drench and rate see Table 1). These were then transplanted into 7cm pots of Levington's M2 compost along with the other plantlets. The spray treatments were applied the following day (12 plants per treatment, see Table 1 for products, active ingredients and rates). Plants were then transferred to another glasshouse compartment (18°C) and the 12 plants from each insecticide treatment were arranged into circles with 75cm diameter. A peach-potato aphid-infested, TuYV-infected oilseed rape plant (variety Mikado) that had been infected with TuYV 3 weeks previously, was placed in the centre of each circle. Individual 90 x 90 x 60 cm gauze frames were placed above each circle, ensuring no aphid movement between treatments (Fig. 4). The plants were watered every 1-2 days as necessary.



Figure 4. Circles of sprout plants with TuYV-infected, peach-potato aphid-infested oilseed rape plants covered by aphid-proof gauze frames.

The insecticide treatments were applied on four separate occasions with 12 plants treated with each product, on each occasion.

Aphids were counted on test plants at 1, 2 and 4 weeks after exposure to peach-potato aphids. The number of aphids on each plant was estimated according to a semi-log scale (Table 2).

Table 2. Semi-log scale used for scoring aphid numbers on test plants.

| Number of aphids | Score |
|------------------|-------|
| 0 | 0 |
| 1 | 1 |
| 5 | 2 |
| 10 | 3 |
| 30 | 3.5 |
| 50 | 4 |
| 75 | 4.5 |
| 100 | 5 |
| 300 | 5.5 |
| 500 | 6 |
| 750 | 6.5 |
| 1000 | 7 |

After 4 weeks exposure to peach-potato aphids, all plants were transferred to another insect-free glasshouse compartment and sprayed with Movento (Spirotetramat) (1ml / L) to kill all the aphids and prevent any further TuYV transmission. The plants were kept for a further 4 weeks before testing for the virus, during which time they were regularly checked for aphids and if necessary, sprayed again with Dovetail (Lambda-Cyhalothrin / Pirimicarb) (2.5ml / L) and Plenum (Pymetrozine) (0.75g / L). Young leaves were then harvested from all plants and tested for the presence of TuYV by TAS-ELISA, as previously described above. The dates of activities for each rep are shown in Table 3 below.

Table 3. The dates of activities for each experimental replicate of Component 2.

| Activity | Rep 1 | Rep 2 | Rep 3 | Rep 4 |
|---|----------|----------|----------|----------|
| NIZ16-4391 seeds potted in 345 modules, 12 sown with dummy seed treated with Gaucho and 12 sown with dummy seed treated with HDCI 043 | 20/03/12 | 27/04/12 | 25/05/12 | 25/06/12 |
| Twelve seedlings drenched with HDCI 044 | 15/04/12 | 23/05/12 | 20/06/12 | 22/07/12 |
| NIZ16-4391 seedlings transplanted into FP7 pots | 16/04/12 | 24/05/12 | 21/06/12 | 23/07/12 |
| Insecticide sprays applied and plant circles set up. Aphid-infested, TuYV-infected plant introduced into the centre of the circles. | 17/04/12 | 25/05/12 | 22/06/12 | 24/07/12 |
| End of test period. Aphids killed using Movento spray and plants transferred to insect-free glasshouse area | 15/05/12 | 21/06/12 | 20/07/12 | 21/08/12 |
| Plant leaves tested for TuYV infection by ELISA | 12/06/12 | 19/07/12 | 16/08/12 | 18/09/12 |

The ELISA results were log-transformed and analysed using ANOVA to calculate means for each treatment and statistical significance. The aphid data was also analysed using an ANOVA test.

Results

Component 1

At first testing of the Brussels sprout plants 1-50 in Lincolnshire, just over 5 weeks after transplanting, 48 (96%) were infected by TuYV. Two weeks later one of the two remaining plants had become infected and the last plant was infected after a further ~ 2 weeks (Table 4).

Table 4. Infection of Lincolnshire Brussels sprout plants 1-50, transplanted into the field on 2 June 2011.

| Date plants sampled | Number of new infections | Cumulative number infected |
|---------------------|--------------------------|----------------------------|
| 10/07/11 | 48 | 48 |
| 25/07/11 | 1 | 49 |
| 10/08/11 | 1 | 50 |

At first testing of the Brussels sprout plants 51-100 that had been covered with aphid-proof gauze at transplanting, 7 plants (14%) were infected 3½ weeks after being uncovered on 26 August, 2011. Infection continued for a further 6 weeks, by which time 18 plants (36%) had become infected (Table 5).

Table 5. Infection of Lincolnshire Brussels sprout plants 51-100, transplanted into the field 02 June 2011 under aphid-proof gauze and uncovered 26 August 2011.

| Date plants sampled | Number of new infections | Cumulative number infected |
|---------------------|--------------------------|----------------------------|
| 19/09/11 | 7 | 7 |
| 04/10/11 | 4 | 11 |
| 17/10/11 | 6 | 17 |
| 31/10/11 | 1 | 18 |
| 17/11/11 | 0 | 18 |

Testing of cauliflower plants 1-50 in Lincolnshire detected first TuYV infection 7 weeks after transplanting, by which time only 2 plants (4%) were infected (Table 6). Just less than 2 weeks later, a further 2 plants were infected, after which time no further infection was detected (Table 6).

Table 6. Infection of Lincolnshire cauliflower plants 1-50, transplanted into the field 17 August 2011.

| Date plants sampled | Number of new infections | Cumulative number infected |
|---------------------|--------------------------|----------------------------|
| 19/09/11 | 0 | 0 |
| 4/10/11 | 2 | 2 |
| 17/10/11 | 2 | 4 |
| 31/10/11 | 0 | 4 |
| 17/11/11 | 0 | 4 |

As no peach-potato aphids were caught in the Rothamsted Insect Survey suction trap at Kirton, Lincolnshire during December, cauliflower plants 51-100 were not sampled during this period.

Testing of cauliflower plants 1-50 in Kent detected first TuYV infection 7 weeks after transplanting, by which time only 1 plant (2%) was infected (Table 7). No further plants became infected over the following 6½ weeks (Table 7).

Table 7. Infection of Kent cauliflower plants 1-50, transplanted into the field 18 August 2011.

| Date plants sampled | Number of new infections | Cumulative number infected |
|---------------------|--------------------------|----------------------------|
| 18/9/11 | 0 | 0 |
| 3/10/11 | 1 | 1 |
| 18/10/11 | 0 | 1 |
| 01/11/11 | 0 | 1 |
| 17/11/11 | 0 | 1 |

No peach-potato aphids were caught in the local Rothamsted Insect Survey suction trap at Wye, Kent during December, so cauliflower plants 51-100 were not sampled during this period.

The numbers of peach-potato aphids caught in Rothamsted Insect Survey suction traps in Kent and Lincolnshire throughout the growing season were obtained from the website and plotted against TuYV incidence (Figs. 5-7). This showed that the aphid flights in Lincolnshire started at the time the Brussels sprouts were transplanted in to the field on 2 June, 2011 and that the number trapped each week increased to over 1000 in the week beginning 4 July, by which time 96% of the exposed plants had become infected (Fig. 5). The remaining plants were infected when tested on 10 August.

When Brussels sprout plants 51-100 were uncovered on 26 August, 2011, no peach-potato aphids were being caught in the local suction trap. It wasn't until 23 days later that aphids were caught and this coincided with the first detection of TuYV infection in sprout plants 51-

100. Aphid numbers caught increased to a maximum of 44 and virus infection reached only 36%.

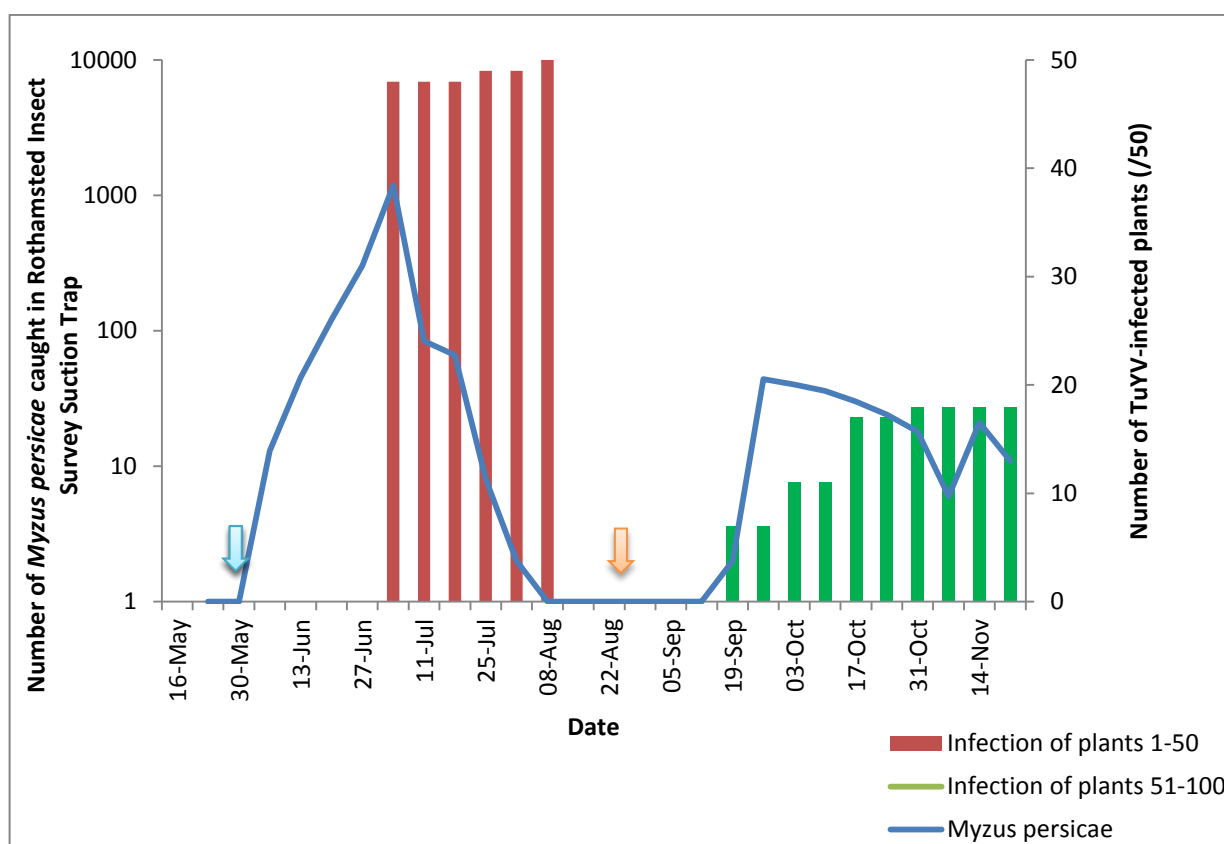


Figure 5. Number of peach-potato aphids (*Myzus persicae*) trapped in the Rothamsted Insect Survey suction trap at Kirton, Lincolnshire and the cumulative number of TuYV-infected Brussels sprout plants 1-50 (red) and 51-100 (green). Note the blue arrow indicating the time of transplanting and orange arrow indicating the date plants 51-100 were uncovered.

Later in the season when the cauliflowers were transplanted in Lincolnshire (18 August, 2011), no aphids were being caught in the local Rothamsted Insect Survey suction trap and TuYV infection of plants 1-50 wasn't observed until 4 October (10 days after trap catches peaked at 44 aphids in the week of 25 September) (Fig. 6). The low numbers of aphids caught corresponded with only 8% of cauliflower plants becoming infected. After cauliflower plants 51-100 were uncovered on 9 December, no aphids were caught in the local suction trap and no TuYV infection of these plants was detected.

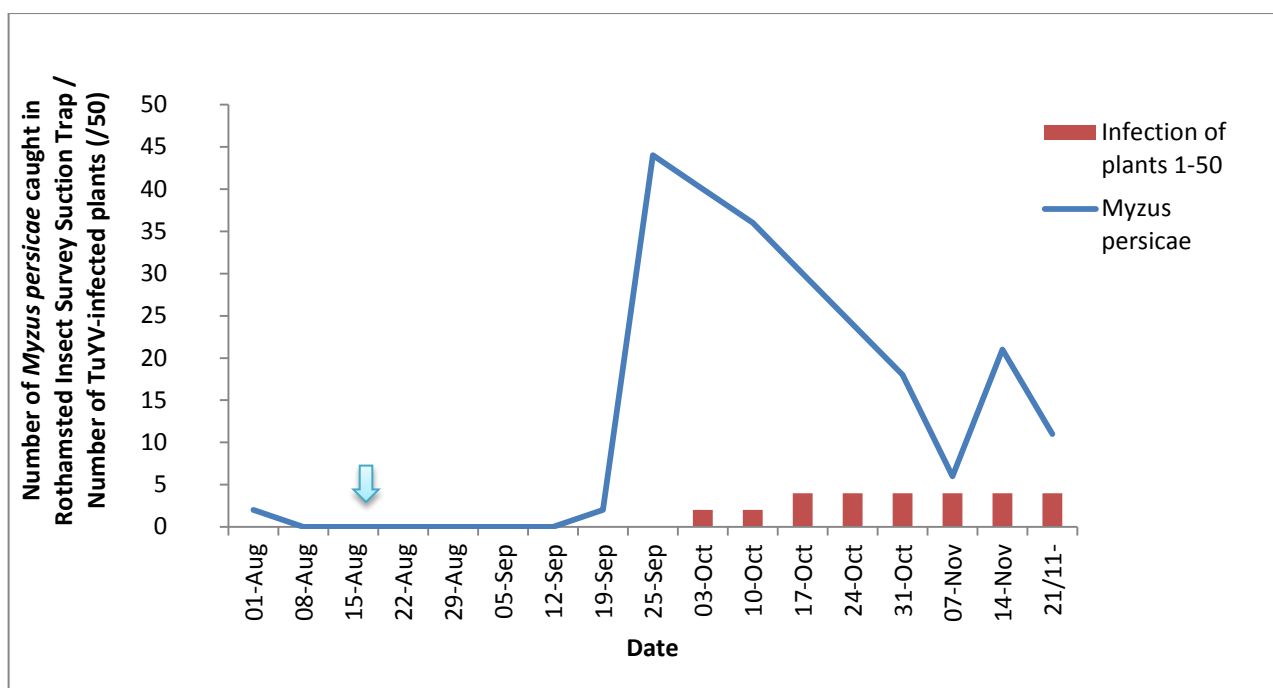


Figure 6. Number of peach-potato aphids (*Myzus persicae*) trapped in Rothamsted Insect Survey suction trap at Kirton, Lincolnshire and the cumulative number of TuYV-infected cauliflowers 1-50. Note the blue arrow indicating the time of transplanting.

Two peach-potato aphids were caught in the suction trap in Kent in the week of 29 August and then none were caught until the week of 25 September, after which aphids continued to be caught (maximum number in any one week, 29) until the trap was shut down after the week of 21 November (Fig. 7). The only TuYV-infected cauliflower was recorded on 3 October and again this low incidence is associated with low aphid numbers caught in the local trap.

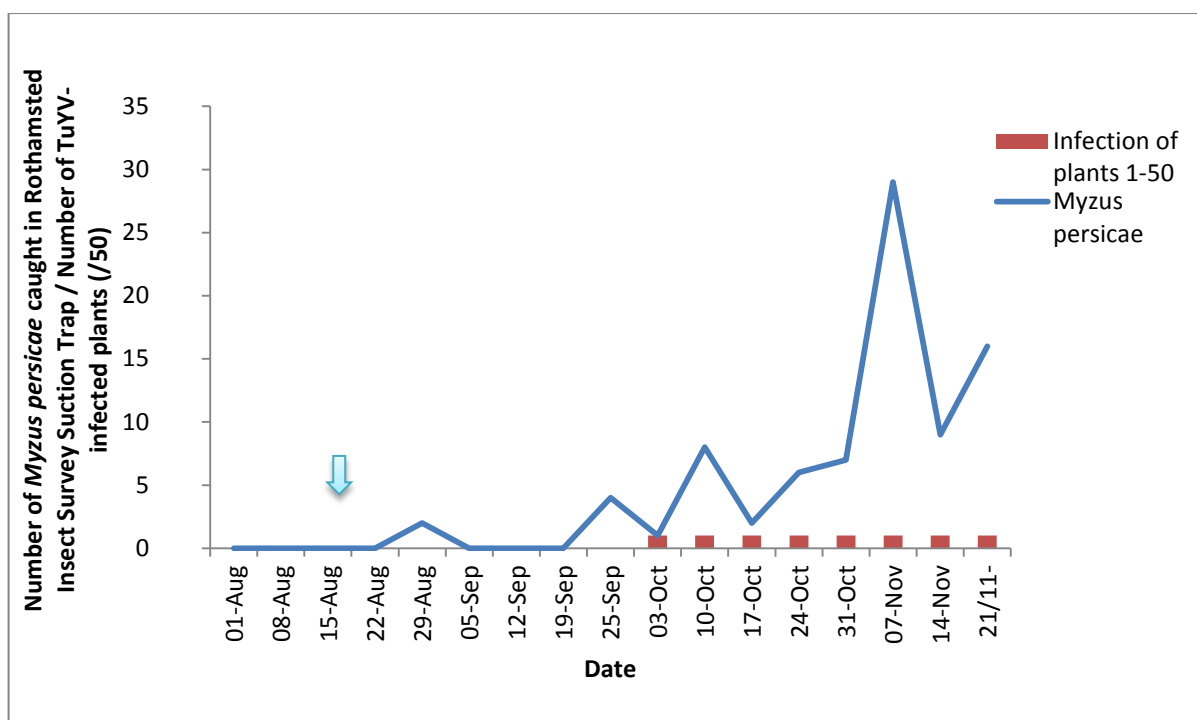


Figure 7. Number of peach-potato aphids (*Myzus persicae*) trapped in Rothamsted Insect Survey suction trap at Wye, Kent and the cumulative number of TuYV-infected cauliflowers 1-50. Note the blue arrow indicating the time of transplanting.

Component 2

The mean peach-potato aphid scores for each insecticide treatment and the control plants were calculated using data from all 4 experimental replicates for week 1, week 2 and week 4 by analysis of variance (ANOVA). Mean aphid scores were then back-transformed to give mean numbers of aphids per plant for each treatment. The results are shown in Table 8.

The mean virus content (ELISA values) of plants was calculated for each insecticide treatment and control plants, again using the data from all 4 experimental replicates. The higher the ELISA value, the greater the virus content of the plant. All ELISA data was log-transformed and normalised before ANOVA. This allowed statistical comparisons of different treatments and the control.

These results clearly demonstrated significant differences in the ability of the insecticide treatments to control TuYV infection. All insecticide treatments (except Movento at week 1) also significantly reduced the numbers of aphids on sprout plants at weeks 1, 2 and 4, relative to untreated plants. Untreated plants hosted an average of almost 1000 aphids per plant and had high levels of virus (mean transformed and normalised ELISA value of 1.17).

The most effective treatments appeared to be those involving dummy seeds (Gaucho and HDCI 043); aphid numbers on plants were consistently low over the four-week test period

(<1 aphid / plant on average after 4 weeks). The low aphid numbers were reflected in the low virus levels detected in these plants (mean ELISA values 0.33 and 0.32 respectively) (Table 8).

Aphox was the least effective insecticide at controlling aphids and TuYV over the four-week period. In week 1, aphid numbers were low, with an average of 8 per plant. By week 4 however, there was an average of 375 aphids per plant. The average ELISA value for the plants was the highest of any treatment (0.90) and not significantly different to the ELISA value of untreated plants (1.17). Although treating plants with Aphox reduced aphid numbers, it gave no significant protection against TuYV infection, relative to the untreated control (Table 8).

Other insecticide-treated plants had mean ELISA values that were significantly lower (and therefore contained less virus than their untreated counterparts), though no insecticide treatment was able to prevent plants becoming infected by TuYV. In comparison to untreated plants, all of the insecticide treatments were successful in reducing the numbers of aphids on plants, however this was not enough to prevent plants becoming infected by the virus to some degree (Table 8). Even the plants treated with dummy seed showed low levels of infection, despite the very low numbers of aphids found on plants over the four week test period.

Table 8. The mean aphid numbers per plant at 1, 2 and 4 weeks and the mean plant ELISA value.

| Insecticide treatment | Mean aphid numbers per plant | | | Transformed ELISA value (A_{405}) ^{1, 2} |
|-----------------------|------------------------------|--------|--------|---|
| | Week 1 | Week 2 | Week 4 | |
| Plenum (spray) | 10 | 40 | 91 | 0.52* |
| Movento (spray) | 33 | 60 | 94 | 0.68* |
| Aphox (spray) | 8 | 88 | 375 | 0.90 |
| Biscaya (spray) | 6 | 56 | 183 | 0.68* |
| HDCI 040 (spray) | 20 | 46 | 92 | 0.61* |
| HDCI 041 (spray) | 11 | 43 | 154 | 0.56* |
| HDCI 042 (spray) | 8 | 40 | 77 | 0.57* |
| Gaucho (dummy seed) | 2 | 0.8 | 0.1 | 0.33* |
| HDCI 043 (dummy seed) | 0.6 | 0.6 | 0.1 | 0.32* |
| HDCI 044 (drench) | 9 | 27 | 49 | 0.55* |
| Untreated Plants | 66 | 450 | 933 | 1.17 |

*The ELISA value is significantly lower than that of the untreated control plants.

¹ The higher the ELISA value, the greater the levels of virus in the plant.

² The least difference between two mean ELISA values for it to be significant at 5% is 0.29 (33 degrees of freedom).

A comparison of the mean numbers of aphids at week 4 for each treatment with the treatment ELISA means showed a strong correlation ($R^2 = 0.93$) (Fig. 8).

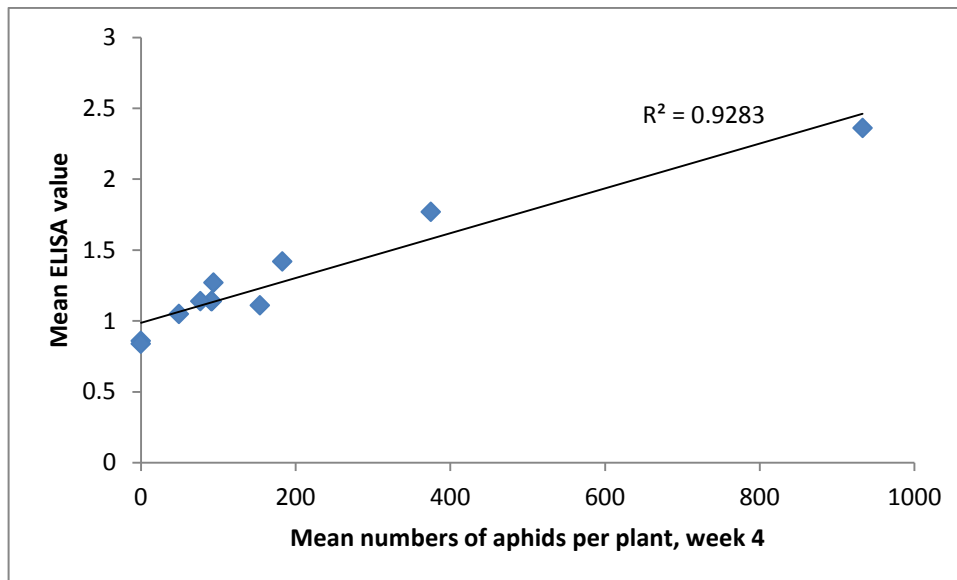


Figure 8. The mean numbers of aphids at week 4 for each treatment plotted against the mean ELISA value for each treatment.

The levels of virus detected by ELISA and / or numbers of aphids on plants seemed to be linked to the physical appearance of the test plants. Untreated plants hosted many aphids, had high levels of virus and appeared discoloured (red and yellow) and stunted in comparison to plants in many treatments (particularly those that had been treated with dummy seed; Fig. 9).



Figure 9. Plants treated with Gaucho dummy seed (left) and untreated plants (right) following exposure to aphids carrying TuYV, showing the difference in size and colour.

Discussion and conclusions

The experiments went very well and have provided clear and useful information.

Component 1

Monitoring Brussels sprout and cauliflower plots growing in commercial crops for TuYV infection provided clear information on the time of infection. It was clear that infection by TuYV coincided with peach-potato aphid flights and the greater the numbers of aphids flying, the greater the number of plants infected. In most years more aphids are caught in suction traps in June, July and August, so Brassica crops are at particular risk of infection during these months. This is compounded by the fact plants are particularly susceptible when young (up to 65% yield reduction of Brussels sprouts, project FV 365) and early infection of cabbage has been shown to reduce yield to a greater extent than later infection (project FV 160b). Later in the year, infection levels of the sprouts and cauliflower plants were lower, due to the lower number of aphids flying. However, in some years much higher numbers of aphids are caught in suction traps in September and October and this will result in later infections of Brussels sprouts and early infection of overwintered cauliflower. We have seen 100% infection of winter oilseed rape plants (usually sown ~September) in some crops. Consequently it is important to monitor peach-potato aphid numbers caught in your

local Rothamsted Insect Survey suction trap throughout the growing season in order to target control treatments most effectively (ideally to coincide with the start of peak aphid flights). Later transplanted crops are likely to be at less risk of TuYV infection in some years.

Component 2

Significant differences in the efficacy of insecticides in reducing peach-potato aphid numbers and the amount of TuYV in Brussels sprout plants were seen in the experiments.

Despite the various degrees of insecticide efficacy, none of the insecticides stopped the plants from becoming infected, it appears they may delay infection, or reduce the amount of virus transmitted to plants. However, almost all treatments gave some protection of plants, dummy seed treatments (Gaucho and HDCI 043) being the most effective; the majority of plants treated with dummy seeds were free of aphids by the 4th week. The active ingredients of the dummy seed treatments (added at sowing) had longer to move systemically in the sprout plants than the sprays (applied just prior to transplanting). This and / or the efficacy of the active ingredients might explain why they were more effective.

Only Aphox didn't significantly reduce the amount of virus detected in plants relative to untreated control plants and only Movento at week 1 didn't significantly reduce the number of aphids on plants relative to untreated plants. There were slight variations in the efficacy of the other spray treatments and the drench in terms of virus control, despite the fact there were a number of significant differences between them in terms of aphid control. Some treatments, e.g. Plenum controlled aphids (mean of 91 aphids / plant at end of experiment) better than other treatments, e.g. Biscaya (mean of 183 aphids / plant at the end of the experiment), but reduced TuYV levels to a similar degree (mean ELISA values of 0.52 and 0.68 respectively). These results and the fact the dummy seed treatments had an average of less than 1 aphid per plant for most of the time throughout the experiment suggests that very few aphids are needed to establish TuYV infection.

Conclusions

Field trials have clearly shown that TuYV infection of Brassica crops appears to be strongly linked to the time of aphid flights and the quantity of aphids in these flights.

The relative efficacy of a range of insecticides applied as dummy seeds, a drench and sprays has been determined. The dummy seed treatments were shown to be the most effective at controlling peach-potato aphids and TuYV. By combining such treatments with growing the least susceptible Brassica varieties (FV 160b and FV 365) and the best spray treatments, applied based on Rothamsted Insect Survey suction trap aphid catches,

growers can now start to optimise an integrated approach to TuYV control. That said, these treatments may not prevent the plants becoming infected, but hopefully will reduce the amount of virus in plants, and / or delay infection.

Breeding crops with genetic resistance to the virus is likely to provide the best means of protection against TuYV and incorporating this in to an integrated control strategy will provide maximum protection against infection. In the meantime the currently available components of an integrated control strategy need to be evaluated in the field.

Knowledge and Technology Transfer

Talks

John Walsh gave a talk on projects FV 365 and FV 365a to the Brassica Growers Association's R&D committee at Stockbridge House, Yorkshire on 2nd June, 2011, entitled 'The incidence of Turnip yellows virus (TuYV) in overwintered cauliflower and Brussels sprout and the effect of the virus in yield'.

John Walsh gave a talk on projects FV 365 and FV 365a at the Brassica Growers Association Conference and Trade Exhibition, Lincolnshire Show Ground, Lincoln on 17th January, 2012, entitled 'Turnip yellows virus (TuYV), the hidden problem, effects on Brassica yields and quality'.

John Walsh gave a talk on projects FV 365 and FV 365a at the Brassica Technical Seminar at Briars Hall Hotel, Lancashire on 3rd July, 2012, entitled 'Turnip yellows virus (TuYV) – the hidden problem affects Brassica yields and quality'.

Articles

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Glossary

TuYV – Turnip yellows virus (formerly known as Beet western yellows virus)

References

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