Project title	Reducing losses from virus-induced storage disorders of processing cabbage
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Project leader:	John A. Walsh, Warwick HRI
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Project coordinator:	Dick Evenden, H.L. Hutchinson Ltd., Boston, Lincolnshire.
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Signed on behalf of: Warwick HRI

Simon Bright 10 November 2008

Signature:…….…… ***Date***: …….
Name: Professor Simon Bright
Director and Head of Department

The results and conclusions in this report are based on an investigation conducted over a two and a half 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.

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Headline

Fifteen different cultivars of cabbage tested over two growing seasons for susceptibility to:

1. Cigar burn (caused by *Turnip mosaic virus* [TuMV]);
2. Tipburn (caused by *Beet western yellows virus* [BWYV] also known as *Turnip yellows virus* [TuYV]), showed significant differences in susceptibilities. Both viruses are transmitted by aphids.

Background and expected deliverables

Cabbage growers have experienced significant losses due to the internal disorders caused by viruses. In most years, losses of at least 3-5% are recorded, with much higher losses in certain years where some co-operatives and growers have recorded complete losses of stored material (up to 1200 tonnes in one store) and others suffering substantial losses in the range of 15-20%. In 2005, one grower alone recorded losses of £200,000.

The HortLink project carried out by John Walsh's group at Warwick HRI, Wellesbourne showed that *Beet western yellows virus* (BWYV) caused internal tipburn in processing cabbage and *Turnip mosaic virus* (TuMV) caused cigar burn. Both these viruses also significantly reduced the head weight of cabbage.

There were very big differences in the susceptibility of the 2 cabbage cultivars studied to tipburn and cigar burn. With the exception of these two cultivars, there is no reliable information on the relative susceptibilities of most current cabbage cultivars to these disorders

The HortLink project showed that later infection by TuMV (in June) increased the severity of cigar burn symptoms relative to earlier infection (during propagation). There is no information on the effect of time of infection by BWYV on the severity of tipburn symptoms.

In trials conducted by Warwick HRI, neither Gaucho (seed treatment) nor Aztec (3 sprays) were effective in controlling BWYV in cabbage, consequently alternative control measures are needed urgently. By identifying the cabbage cultivars that are most tolerant to tipburn, cigar burn, TuMV and BWYV, growers will be able to minimise losses caused by these disorders and viruses. In the longer term, effective insecticides need to be identified and good genetic resistance to both these viruses needs to be incorporated in to commercial Brassica cultivars.

The expected deliverables from this project are:

- A database of relative susceptibilities of different processing cabbage to virus-induced tipburn and virus-induced cigar burn
- A database of the yield reduction of different processing cabbage cultivars due to infection by BWYV and TuMV
- These databases would allow growers to reduce losses from the internal disorders by selecting varieties that are less susceptible to such problems
- The time of infection of cabbage by BWYV will be determined in Lincolnshire in natural crop situations. This information will allow growers to target their control strategies more effectively. It will also allow future experiments on the efficacy of insecticide trials to be carried out in a more rational and informed way, targeting experimental treatments at the most important times.
- Any differences in the effect of infection timing by BWYV on tipburn severity will inform growers of the more important stages in crop growth for controlling BWYV and hence tipburn.

Summary of the project and main conclusions

Gauzehouse experiments at Warwick HRI to determine the relative susceptibilities of different processing cabbage cultivars to cigar burn and tipburn

A selection of 15 different cultivars of cabbage were grown in gauzehouses at Warwick HRI for two growing seasons and infected with *Turnip mosaic virus* (TuMV; known to cause 'cigar burn'), *Beet western yellows virus* (BWYV; now known by the scientific community as *Turnip yellows virus* [TuYV] and known to cause tipburn) or were uninfected.

Plants of the 15 cultivars of processing cabbage growing in gauzehouses at Wellesbourne were successfully infected with TuMV, or BWYV and the uninfected control plots remained uninfected for the duration of both growing seasons (2006 and 2007). No cultivars were completely resistant to either of the viruses.

The heads were stored in a CA store at ~0^0C for ~8 months and then cut open and assessed for internal disorders.

The effect of Turnip Mosaic Virus (TuMV)

Visual assessment of the severity of external leaf symptoms induced by TuMV prior to harvest revealed that some cultivars showed less severe symptoms than others. Four cultivars showed no significant levels of symptoms in year 1 and one cultivar showed no significant level of symptoms in year 2. The different cultivars behaved quite consistently in both years in terms of their relative susceptibility to external symptoms caused by TuMV (see science section for tables and detailed results).

Weighing heads after storage showed that the yield of some cultivars was significantly reduced by TuMV infection. In year 1, TuMV didn't significantly reduce the weight yield of 9 cultivars, but reduced the weight yield of the 6 others by up to 26% relative to uninfected heads of the same cultivar. In year 2, it significantly reduced the weight yields of 9 cultivars by up to 36%, but didn't significantly affect the weight yields of the other 6 cultivars. There were small differences in the relative yields of the different cultivars following infection with TuMV between the two years, but most cultivars behaved reasonably consistently over the two growing seasons.

Table 1: Mean head weights (kg) for TuMV-infected and uninfected cabbage after storage, data from the year 1 and 2 experiments combined.

Cultivar	TuMV infected	Uninfected	% yield reduction due to TuMV infection	Yield of TuMV-infected plants significantly different from uninfected plants
Brigadier	5.210	5.169	-0.79	No
Kingston	3.686	3.705	0.51	No
Impala	3.174	3.194	0.63	No
Colmar	2.669	2.821	5.39	No
Polinius	2.643	2.832	6.67	No
Kronas	4.196	4.725	11.20	Yes
Counter	3.881	4.611	15.83	Yes
Cilion	2.906	3.553	18.21	Yes
Zerlina	3.049	3.782	19.38	Yes
Lennox	2.899	3.654	20.66	Yes
Kilaton	3.159	4.004	21.10	Yes
Lion	2.836	3.629	21.85	Yes
Shelton	3.024	3.953	23.50	Yes
Caid	2.973	3.962	24.96	Yes
Bartolo	2.155	2.907	25.87	Yes

The least difference between two means for the difference to be significant is 0.428.

Examination of internal symptoms after storage of the year 1 crop revealed that following infection with TuMV, different varieties showed differing degrees of cigar burn. The cultivars Impala, Kilaton, Shelton, Kronas and Brigadier showed very little cigar burn, Impala, Kilaton and Shelton heads infected by TuMV didn't have significant levels of cigar burn and Kronas and Brigadier heads infected by TuMV had only marginal levels of cigar burn.

The cigar burn symptoms in the year 2 crop were more severe than in the year 1 crop. Cultivar Brigadier was the only cultivar not to show significant levels of cigar burn following TuMV infection, although Shelton heads infected by TuMV had only marginal levels of cigar burn.

When the year 1 and year 2 data was combined, Shelton, Impala, Brigadier, Kilaton and Kronas had the least cigar burn following TuMV infection and storage, whereas Counter, Colmar, Zerlina, Polinius, Lennox, Caid, Kingston, Lion and Bartolo all had particularly severe cigar burn symptoms following TuMV infection and storage.

Table 2: The severity of cigar burn symptoms in cabbage cultivars after storage, year 1 and year 2 crop data combined.

Cultivar	Mean symptom score*, TuMV infected	Mean symptom score, not infected
Shelton	0.5592	0.0067
Impala	0.5691	0.0506
Brigadier	0.6061	0.0009
Kilaton	0.6638	0.0921
Kronas	0.9390	0.0425
Cilion	1.0679	0.0222
Counter	1.7192	0
Colmar	1.7522	0
Zerlina	2.0155	0.0105
Polinius	2.0884	0
Lennox	2.4360	0.0025
Caid	2.7488	0.0108
Kingston	3.2956	0.0111
Lion	3.3004	0.0317
Bartolo	3.8656	0

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant is 0.4434

Analysing all the cultivars together, there was a clear correlation between the severity of TuMV symptoms prior to harvest and the severity of cigar burn symptoms after storage; the more severe the TuMV symptoms prior to harvest, the more severe the cigar burn symptoms after storage. However, looking at individual cultivars, there was no evidence for a correlation between pre-harvest and post-storage symptoms for some of the cultivars. Assessment of TuMV symptoms in the field will give growers an indication of which cabbages / crops are likely to develop cigar burn during storage.

The effect of Beet Western Yellows Virus (BWYV)

Prior to harvest, there were no external symptoms in BWYV-infected cabbages, however it was clear for some varieties, that BWYV-infected cabbages were noticeably smaller than uninfected controls.

Weighing heads after storage showed that the yield of some cultivars was significantly reduced by BWYV infection. In year 1, BWYV didn't significantly reduce the weight yield of 9 cultivars, but reduced the weight yield of the 6 others by up to 32% relative to uninfected heads of the same cultivar. In year 2, it significantly reduced the weight yields of all cultivars except 2, by up to 36%. There were small differences in the relative yields of the different cultivars following infection with BWYV between the two crops, but most cultivars behaved reasonably consistently over the two growing seasons.

Table 3: Mean head weights (kg) for BWYV-infected and uninfected cabbage after storage, data from the year 1 and 2 experiments combined.

Cultivar	BWYV infected	Uninfected	% yield reduction due to BWYV infection	Yield of BWYV-infected plants significantly different from uninfected plants
Zerlina	3.413	3.782	9.76	No
Impala	2.755	3.194	13.75	Yes
Counter	3.887	4.611	15.70	Yes
Lion	3.058	3.629	15.73	Yes
Kingston	3.073	3.705	17.06	Yes
Bartolo	2.407	2.907	17.20	Yes
Shelton	3.195	3.953	19.18	Yes
Brigadier	4.044	5.169	21.76	Yes
Colmar	2.190	2.821	22.37	Yes
Polinius	2.175	2.832	23.20	Yes
Cilion	2.667	3.553	24.94	Yes
Kronas	3.450	4.725	26.98	Yes
Caid	2.886	3.962	27.16	Yes
Kilaton	2.896	4.004	27.67	Yes
Lennox	2.623	3.654	28.22	Yes

The least difference between two means for the difference to be significant is 0.428.

Examination of internal symptoms after storage of the year 1 crop revealed that following infection with BWYV, different varieties showed differing degrees of tipburn. The cultivars Polinius, Counter, Kilaton, Bartolo, Cilion and Colmar showed very little tipburn, Caid and Shelton heads infected by BWYV had only marginal levels of tipburn, whereas Kingston, Lennox, Zerlina, Impala and Brigadier had particularly severe tipburn symptoms.

The tipburn symptoms in the year 2 crop were less severe than in the year 1 crop. Cultivars Polinius, Counter, Kilaton, Bartolo, Cilion, Colmar, Caid, Shelton, Kronas, Kingston and Lennox showed little or no tipburn following BWYV infection, whereas Brigadier had particularly severe tipburn symptoms.

When the year 1 and year 2 data was combined, cultivars Polinius, Counter, Kilaton, Bartolo, Cilion, Colmar, Shelton, Lion and Caid had no significant tipburn following BWYV infection and storage and Kronas had very little tipburn, whereas Brigadier and Impala had particularly severe tipburn symptoms .

Table 4: The severity of tipburn symptoms in cabbage cultivars after storage, year 1 and year 2 crop data combined.

Cultivar	Mean symptom score*, BWYV infected	Mean symptom score, not infected
Polinius	0.0021	0.0046
Counter	0.0993	0.0941
Kilaton	0.1657	0.0188
Bartolo	0.2074	0.0259
Cilion	0.2258	0.0112
Colmar	0.4195	0.0387
Shelton	0.5562	0.1158
Lion	0.6673	0.0983
Caid	0.6859	0.0158
Kronas	0.7511	0.0066
Kingston	1.0049	0.1051
Lennox	1.2310	0
Zerlina	1.3543	0.1077
Impala	1.9972	0
Brigadier	3.8266	0.0528

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant is 0.6887

Serological testing of plants showed that some varieties contained more BWYV than other varieties. There was no correlation between the amount of virus detected in cabbage varieties and the severity of tipburn symptoms or the weight yield of cabbages in either year. In terms of the ranking of the lines for the amount of BWYV detected, most lines behaved consistently over the two years (three varieties had quite different rankings in the two years).

Serological testing of cabbages for BWYV in the field will not give growers an indication of the likely severity of tipburn symptoms that might develop during storage, but would give an indication of the possibility of tipburn symptoms occurring during storage.

Field experiment in Lincolnshire to determine the time of natural infection of cabbage crops by *Beet western yellows virus* (BWYV)

Monitoring of natural infection of cabbage growing within commercial crops at three sites in Lincolnshire showed that BWYV infection was first detected on 24th July, 2006, with incidences ranging from 2% at one site (A), to 8% at the other two sites (B & C). At site C, infection progressed rapidly with 88% of plants infected by 7th August and 100% infection by 24th August 2006. At site A, infection progressed much slower, with only 8% infection by 7th August, but then increasing rapidly to 96% infection by 24th August, 2006. The rate of infection at site B was intermediate to that of sites A and C.

The spread of BWYV in to the plots appeared to be related to the numbers of winged aphids (peach potato aphid [*M. persicae*] and woolly cabbage aphid [*Brevicoryne brassicae*])

caught in the Rothamsted suction trap in Kirton, Lincolnshire. The first aphids were caught on 12th July, 2006.

Unfortunately we have been unable to obtain spray records for site B. Spray records for sites A and C show that the insecticide spray regimes at these sites might explain why infection of the crop at site A progressed more slowly than at site C. At site A, insecticides for aphid control were sprayed on 12 occasions, whereas at site C, they were sprayed on 8 occasions. During the time aphids were migrating between mid-July and mid-August, insecticides were sprayed on 4 occasions at site A and 2 occasions at site C.

Half of the cabbages in the plots had been covered with aphid-proof mesh immediately after transplanting and then uncovered on 24th August, 2006. Observations on the uncovered half of the plots revealed that very little BWYV infection occurred after 24th August, 2006 within plots at two of the sites (only 2% of cabbages at site A), whereas at the third site (C), 25.5% of plants in the plot became infected. During this period, insecticides were sprayed on four occasions at site A for aphid control and two occasions at site C.

The results demonstrate that although insecticides sprayed early in the season did not stop BWYV infecting cabbage, they can delay BWYV infection and that later in the season when infection pressure is likely to be less, insecticide sprays may be able to prevent infection.

The effect of different times of infection of cabbage by *Beet western yellows virus* (BWYV) on the severity of tipburn symptoms

We successfully infected covered plots of cabbage plants in the field in Lincolnshire with BWYV in May, August, or October 2006 (there was an uninfected treatment too which remained uninfected). There was a clear relationship between the time of infection of cabbage by BWYV and the severity of tip burn symptoms after storage. The earlier the infection, the more severe the tipburn symptoms. There was also a clear relationship between the time of infection of cabbage by BWYV and the weight yield of cabbages after storage. The earlier the infection, the more severe the yield reduction.

Table 5: The effect of time of infection by Beet western yellows virus (BWYV) on the severity of tipburn symptoms and the weight yield of the cabbage heads.

Treatment	Mean weight of cabbage heads (kg)	Mean symptom score
BWYV infection in May	2.870	2.502
BWYV infection in August	3.108	1.038
BWYV infection in October	3.390	0.283
No BWYV infection	3.438	0.273
Least significant difference	0.163	0.333

Our results demonstrate that further research on using partially resistant cabbage cultivars identified in this study and different spray regimes and / or seed treatments is warranted and could provide good protection of cabbage from tipburn. In the longer term, it is important to develop cabbage cultivars with good levels of natural resistance to both tipburn and cigar burn.

Financial benefits

Choice of cultivars to grow

The results from the project will allow growers to choose varieties to grow based on their susceptibilities to internal tipburn and cigar burn, providing the opportunity to reduce losses due to heads being rejected by processors because of these internal disorders. Also the information on the effect of TuMV and BWYV on the weight yield of cabbage cultivars will allow growers to reduce losses from yield reductions. In 2006, commercial cabbage crops tested for BWYV were 100% infected showing that this virus is particularly widespread and controlling it will result in significant yield increases in many cultivars.

Timing of insecticide sprays

Information obtained on the time of BWYV infection of cabbage in the field will allow growers to target the timings of their insecticide sprays for aphid control better, thereby delaying BWYV infection.

The discovery that the time of infection of cabbage by BWYV has a dramatic effect on the severity of tipburn symptoms and the yield reduction caused by this virus will allow growers to target the timings of their insecticide sprays more effectively, thereby reducing losses from tipburn and increasing the weight yield of crops.

Results from monitoring natural infections of BWYV suggest that growers will be able to delay BWYV infection and hence reduce tipburn and increase yields by timing their sprays more carefully and applying enough sprays during important periods.

Action points for growers

- Growers can check from the list of relative susceptibilities of different cabbage cultivars to tipburn and cigar burn which are going to be less prone to both disorders.
- The list of percentage weight yield losses due to BWYV or TuMV infection can be consulted to reduce yield losses from these viruses.
- Inspecting crops in the field for TuMV symptoms will allow growers to identify crops that will be prone to cigar burn and get these processed early before cigar burn symptoms develop during storage.
- Testing leaves from cabbage plants growing in the field by ELISA for the presence of BWYV will allow growers to identify crops most at risk from tipburn and get these processed early before tipburn symptoms develop in store.
- By obtaining information from Rothamsted suction traps on when aphids are flying locally, particularly the peach potato aphid (*Myzus persicae*) and the cabbage aphid (*Brevicoryne brassicae*), insecticide sprays can be targeted more accurately to reduce BWYV infection and subsequent tipburn and weight yield losses.
- Spraying insecticides earlier in the season when aphids are moving is likely to give greater benefits than later sprays, as later BWYV infection does not induce as severe tipburn symptoms, or reduce weight yield as much as earlier infections.

Science Section

Introduction

Cabbage growers have experienced significant losses due to the internal disorders caused by viruses. In most years, losses of at least 3-5% are recorded, with much higher losses in some years, where some co-operatives and growers have recorded complete losses of stored material (up to 1200 tons in one store), with others suffering substantial losses in the range of 15-20%. In 2005, one grower alone recorded losses of £200,000.

The HortLink project carried out by John Walsh's group at HRI, Wellesbourne showed that *Beet western yellows virus* (BWYV) caused internal tipburn in processing cabbage and *Turnip mosaic virus* (TuMV) caused cigar burn. Both these viruses also significantly reduced the head weight of cabbage.

There were very big differences in the susceptibility of the 2 cabbage cultivars studied to tipburn and cigar burn. With the exception of these two cultivars, there is no reliable information on the relative susceptibilities of most current cabbage cultivars to these disorders.

The HortLink project showed that later infection by TuMV (in June) increased the severity of cigar burn symptoms relative to earlier infection (during propagation). There is no information on the effect of time of infection by BWYV on the severity of tipburn symptoms.

In trials conducted by HRI, neither Gaucho (seed treatment) nor Aztec (3 sprays) were effective in controlling BWYV in cabbage, consequently alternative control measures are needed urgently. By identifying the cabbage cultivars that are most tolerant to tipburn and cigar burn, growers will be able to minimise losses caused by BWYV and TuMV. In the longer term, effective insecticides need to be identified and genetic resistance to both these viruses needs to be incorporated in to commercial brassica cultivars.

Prior to this project there was no reliable comparative data on the relative susceptibilities of different cabbage cultivars to tipburn and cigar burn. If the industry has this information, they will be able to make informed choices of cultivars in order to reduce or minimise risk from tipburn and cigar burn.

Although information has been obtained from the earlier HortLINK project on the effect of time of TuMV infection on the severity of cigar burn symptoms, nothing is known about the effect of time of infection by BWYV on the severity of tipburn symptoms. Providing this information will allow aphid control treatments to be targeted/timed more effectively.

Information on cultivar susceptibility will provide options for growers to reduce losses from tipburn and cigar burn and minimise the chances of rejection of produce by processors

Materials and methods

Virus isolates used in experiments at Warwick HRI and in Lincolnshire

The UK isolate of *Beet western yellows virus* (BWYV) found to cause tipburn by Hunter *et al*. (2002) was used in those experiments where we deliberately infected plants for experimental purposes. BWYV is not mechanically transmissible, so aphids have to be used to infect plants with this virus. The UK 2 isolate of TuMV (Walsh, 1989) shown to cause cigar burn of cabbage (Hunter *et al.* 2002) was also used in these experiments. TuMV is mechanically transmissible so can be inoculated to plants by rubbing inoculum on to leaves and aphids don't have to be used.

Cabbage cultivars used in experiments at Warwick HRI and in Lincolnshire

The 15 cultivars of cabbage that were tested for susceptibility to BWYV (tipburn) and TuMV (cigar burn) in gauzehouses at Warwick HRI, Wellesbourne were as follows:

Bartolo (Elsoms)
Brigadier (Clause)
Caid (Clause)
Cilion (Syngenta)
Colmar (Elsoms)
Counter (Elsoms)
Impala (Elsoms, known to be susceptible to tipburn from previous experiments)
Kilaton (Syngenta)
Kingston (Syngenta)
Kronas (Seminis)
Lennox (Elsoms)
Lion (Nickersons)
Polinius (Elsoms, known to be susceptible to cigar burn from previous experiments)
Shelton (Elsoms)
Zerlina (Elsoms)

For experiments carried out in Lincolnshire only cabbage cultivar Impala was grown.

Gauzehouse experiments at Warwick HRI in 2006 and 2007

The 15 cabbage cultivars were planted into Hassey 308 trays on 20th April, 2006 and 14th March 2007. Trays containing all cultivars were infected with BWYV on 23rd May, 2006 and 8th May, 2007. This was done by transferring aphids (*Myzus persicae*) from virus-infected oilseed rape plants to the plants growing in the trays. The plants were subsequently sprayed with Decis to kill the aphids on 30th May, 2006 and 22nd May, 2007 and this was

followed by a Dursban drench on 9th June, 2006. Further trays containing all cultivars were infected (mechanical inoculation) with TuMV on 5th June, 2006 and 14th May, 2007 by rubbing the leaves with macerated mustard leaves infected with the TuMV isolate. Some trays containing all cultivars were also kept virus free. The soil in the gauzehouses was fertilised prior to planting and top-dressed in August, 2006 and July, 2007 in line with the Cabbage (crop ID-58), Crop Specific Protocol produced by Assured Produce Ltd. The cabbages were transplanted in to four gauzehouses with a spacing of 60cm on 12th June, 2006 and 23rd May, 2007. Three plants of each cultivar per treatment (BWYV, TuMV and uninfected) were transplanted in to each of the four gauzehouses in plots of 3 plants following an alpha randomised design for each gauzehouse; a total of 135 cabbage per gauzehouse and a total of 540 cabbage for the experiment. One of the gauzehouses is shown in Fig. 1.

Figure 1. *Gauzehouse no. 1 with cabbages growing in photographed on 1st August, 2006*

Following transplanting, the gauzehouses were sprayed with the fungicide Bravo. Every two weeks they were also given insecticide sprays alternating between Dovetail and Toppel in order to ensure no aphids infested the cabbage and compromised the different treatments. The plants previously inoculated with TuMV received a second mechanical inoculation with TuMV on 5th September, 2006 and in 2007, the two inoculations were carried out on the same day prior to transplanting (14th May, 2007). The plants were monitored regularly for

any problems. Following the discovery of one plant infected by *Botrytis*, the cabbage in all gauzehouses were sprayed with Rovral on 11th and 13th October, 2006 and 29th May and 24th August, 2007. Bravo was also sprayed on 17th August, 2007. On 16th November, 2006 and 12th November, 2007 all cabbages were visually assessed for virus symptoms and scored for the severity of any symptoms seen on a scale of 0-5, where 0 was no symptoms and 5 was very severe symptoms. On 21st November, 2006 and 14th November, 2007 the cabbage plants in all four gauzehouses were harvested, with the cutting knives sterilised in alcohol between cutting each head. Each cabbage head was weighed, recorded and numbered in order to be able identify them when they are assessed following storage and the three heads from each plot were put in onion bags and the bags labeled. The heads from each gauzehouse were put in to separate wooden bins, transported to Lincolnshire, drenched with SL567a (Metalaxyl-m; 104ml per 1000L) and Rovral WP (Iprodione; 1kg per 1000L) at a rate of 20L spray mixture per tonne on 22nd November and 15th November, 2007 and stored in a CA store running at ~0°C.

Serological testing of plants for virus infection

On 27th June, 2006 and 12th June and 10th October, 2007, leaf samples were taken from those cabbage plants infected with BWYV. The leaves were macerated between electric rollers and the sap collected for testing for the presence of BWYV by Enzyme-linked Immunosorbent Assay (ELISA). A triple antibody sandwich (TAS)-ELISA format was used for the BWYV testing as described by Walsh *et al.* (1989) using the same antisera as in the experiments carried out in 1999-2000 and described by Hunter *et al*. (2002).

On 11th July, 2006, leaf samples were collected from cabbage plants inoculated with TuMV, macerated as described above and tested for the presence of TuMV by TAS-ELISA as described by Walsh *et al.* (2002).

Assessing cabbage heads for tipburn and cigar burn symptoms

The 2006 cabbage heads were removed from CA storage on 1st August, 2007 and the 2007 heads on 7th July, 2008. All heads were weighed and then cut in to quarters and opposite quarters assessed for the presence of tipburn and cigar burn symptoms. The severity of any symptoms seen, were scored on a scale of 0-5 (where 0 was no symptoms and 5 was very severe symptoms) and recorded.

Determining the time of infection by BWYV and the effect of this on tipburn symptom severity

Cabbage cultivar Impala seed was planted into Hassey 308 trays on 31st March, 2006 at Warwick HRI. On 21st April, the trays containing the young plants were moved to an aphid proof screenhouse to harden off. The plants were transported to Lincolnshire on 1st June, 2006 and transplanted into the middle of commercial cabbage crops the following day

(2nd June, 2006) at three different locations. One hundred Impala plants were transplanted at each site and half of the plants at each site (50 plants per site) were covered with mesh, to exclude aphids and hence virus infection. The plants at one of the sites (C) were eaten by pigeons and hence this site was replanted on 20th June, 2006. The cabbage plots received the same treatments as the surrounding crop.

Leaves from each of the 50 uncovered plants at each site were sampled every two weeks from 26th June to 24th August, 2006 and sent to Warwick HRI where they were tested for BWYV by ELISA as described above.

On 24th August, 2006, the plants that had been covered with mesh at each of the three sites were uncovered and the mesh was used to cover the 50 plants that had not been covered. From then on, the newly uncovered 50 plants were sampled and tested for BWYV infection by ELISA every two weeks from 11th September to 6th November, 2006.

The cabbage from two sites (B and C) were harvested on 14th November, 2006 and the third site (A) on 15th November. Each head was labeled, placed in wooden bins, drenched with SL567a (Metalaxyl-m; 104ml per 1000L) and Rovral WP (Iprodione; 1kg per 1000L) at a rate of 20L spray mixture per tonne on and stored in a CA store running at ~0°C in Lincolnshire.

Determining the effect of the time of infection by BWYV on tipburn symptom severity

Cabbage cultivar Impala seed was planted into Hassey 308 trays on 31st March, 2006 at Warwick HRI. On 21st April, the trays containing the young plants were moved to an aphid proof screenhouse to harden off. On 9th May, some of the cabbage seedlings (>54) were infected with BWYV by transferring aphids (*M. persicae*) from virus-infected oilseed rape plants to the cabbage plants. Further aphids from BWYV-infected oilseed rape were put on to these plants in May. The plants were then sprayed on 31st May with Decis to kill the aphids.

The plants were transported to Lincolnshire on 1st June, 2006 and transplanted into the middle of a commercial cabbage crop at site A on 2nd June, 2006. The plants were planted in 20 plots of 18 plants (3 rows of 6). Each of the four treatments (infected with BWYV pre-transplanting in May, infected in August, infected in late September and uninfected) was replicated five times. The plants were covered with mesh stretched over plastic hoops (Fig. 2). The plants did not receive any insecticide sprays applied to the surrounding commercial crop, but otherwise received the same treatments as the surrounding crop. BWYV-infected oilseed rape plants with aphids (*M. persicae*) feeding on them were taken to Lincolnshire on 2nd August and 2nd October and on each occasion five plots were partially uncovered, BWYV-infected leaves with aphids feeding on them put on to each of the 18 plants in each plot and then the plots were covered with the mesh again. On 14th August, those plots infected with BWYV on 2nd August were sprayed with Aphox and

Hallmark (420g Aphox + 75ml Hallmark in 300L/ha using a knapsack sprayer) to kill the aphids and on 9th October, the plots infected on 2nd October were sprayed with Aphox and Hallmark at the same rate as above to kill the aphids. The cabbage were harvested on 15th November, 2006, each head labeled, placed in wooden bins, drenched with SL567a (Metalaxyl-m; 104ml per 1000L) and Rovral WP (Iprodione; 1kg per 1000L) on the day of harvest at a rate of 20L spray mixture per tonne and stored in a CA store running at ~0°C.

Figure 2. *Covered plots used to investigate the effect of the time of infection by BWYV on tipburn symptom severity at site A in Lincolnshire*

The cabbage heads were removed from CA storage on 1st August, 2007. All heads were weighed and then cut in to quarters and opposite quarters assessed for the presence of tipburn symptoms. The severity of any symptoms seen, were scored on a scale of 0-5 (where 0 was no symptoms and 5 was very severe symptoms) and recorded.

Statistical analyses

The design for the gauzehouse trials was an alpha design, which was applied across all houses within a year, with separate randomisations for each year.

The ELISA data on the amount of BWYV detected in the cabbage plants, the symptom scores and yield data for the Warwick HRI experiment on the 15 cabbage cultivars

was analysed using Residual Maximum Likelihood (REML). The ELISA data was transformed as described previously by Hunter *et al.* (2002), (except 0.065 was not added to each value) prior to analysis.

Yield data and symptom scores were also analysed using Residual Maximum Likelihood (REML) without transformation of data.

Regression analyses were carried out to investigate relationships between ELISA data and tipburn symptoms and weight yields and between pre-harvest TuMV symptom scores and post-storage cigar burn symptoms.

The experiment on the effect of time of BWYV infection on cabbage yields and tipburn severity was designed as a row-and-column design (extended Latin Square) and analysed using analysis of variance (ANOVA).

Results

Gauzehouse experiments at Warwick HRI

The mean ELISA values (optical densities [ODs] following transformation) for each cultivar and BWYV infection are given below. Data from the year 1 experiment (Table 1) and the year 2 (Table 2) experiment are given separately. The analysis of the results showed that there were significant differences in the amount of BWYV detected in the different cultivars.

Table 1: ***The mean OD from the ELISA carried out on 27th June, 2006 to investigate the amounts of BWYV in the different cabbage cultivars***

Cultivar	Mean ELISA value[1]
Kingston	4.862
Lennox	4.896
Lion	4.896
Impala	4.920
Zerlina	4.966
Counter	4.977
Caid	4.986
Kronas	4.986
Polinius	4.987
Shelton	5.015
Kilaton	5.074
Bartolo	5.205
Brigadier	5.233
Cilion	5.267
Colmar	5.401

[1] This value indicates the quantity of BWYV detected in the cabbage, the higher the value, the greater the amount of BWYV present.
The least difference between two means for the difference to be significant at 5% is 0.217 (339 degrees of freedom).

Table 2: The mean OD from the ELISA carried out on 12th October, 2007 to investigate the amounts of BWYV in the different cabbage cultivars

Cultivar	Mean ELISA value[1]
Zerlina	4.005
Bartolo	4.015
Kingston	4.074
Shelton	4.238
Impala	4.485
Cilion	4.498
Counter	4.556
Kilaton	4.589
Lion	4.600
Brigadier	4.689
Caid	4.848
Kronas	4.862
Colmar	4.902
Polinius	4.941
Lennox	5.019

[1] This value indicates the quantity of BWYV detected in the cabbage, the higher the value, the greater the amount of BWYV present.
The least difference between two means for the difference to be significant at 5% is 0.438 (41 degrees of freedom).

None of the uninfected cabbage plants showed any symptoms of virus infection.

None of the cabbage plants infected by BWYV showed any external leaf symptoms, consistent with previous experiments (Hunter *et al.* 2002). However, where uninfected plants and BWYV-infected plants were growing close, or adjacent to each other, it was clear that in many instances, the BWYV-infected plants were visibly smaller than the uninfected plants.

Many plants infected by TuMV showed necrotic symptoms in the outer leaves, mostly necrotic spots and ringspots typical of infection of cabbage by this virus (Fig. 3).

Figure 3. *Typical necrotic symptoms seen in some TuMV-infected cabbage cultivars growing in the gauzehouses*

Visual assessment of the severity of external leaf symptoms induced by TuMV prior to harvest revealed that some cultivars showed less severe symptoms than others. Four cultivars (Impala, Kronas, Brigadier, Kilaton) showed no significant levels of symptoms in year 1 (Table 3) and one cultivar (Kronas) showed no significant level of symptoms in year 2 (Table 4).

Table 3: Mean symptom severity scores for the external leaves of TuMV-infected and uninfected cabbage plants prior to harvest on 16th November, 2006 for the different cultivars in the year 1 experiment

Cultivar	Mean symptom score*, TuMV infected	Mean symptom score, not infected
Impala	0.0833	0
Kronas	0.4167	0
Brigadier	0.7314	0
Kilaton	0.8333	0
Cilion	1.1667	0
Polinius	1.3333	0
Counter	1.5000	0
Zerlina	1.6667	0
Lion	2.0000	0
Colmar	2.2500	0
Kingston	2.2500	0
Shelton	2.2500	0
Lennox	2.3333	0
Bartolo	3.9167	0
Caid	4.3333	0

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.9267 (326 degrees of freedom).

Table 4: Mean symptom severity scores for the external leaves of TuMV-infected and uninfected cabbage plants prior to harvest on 12th November, 2007 for the different cultivars in the year 2 experiment

Cultivar	Mean symptom score* TuMV infected	Mean symptom score not infected
Kronas	0.331	0.003
Impala	0.745	0.001
Counter	0.914	0.003
Kilaton	1.007	0
Brigadier	1.167	0.005
Cilion	1.242	0
Kingston	1.821	0
Shelton	1.830	0.002
Lion	3.086	0.009
Zerlina	3.326	0
Colmar	3.412	0
Caid	3.670	0.009
Lennox	4.089	0
Polinius	4.673	0
Bartolo	4.930	0

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.691 (177 degrees of freedom).

The different cultivars behaved quite consistently in both years in terms of their relative susceptibility to external symptoms caused by TuMV; the combined year 1 and year 2 data is provided in Table 5.

Table 5: Mean symptom severity scores for the external leaves of TuMV-infected and uninfected cabbage plants prior to harvest for the different cultivars, data for the year 1 and year 2 experiments combined

Cultivar	Mean symptom score* TuMV infected	Mean symptom score not infected
Kronas	0.386	0.005
Impala	0.414	0.006
Kilaton	0.920	0
Brigadier	0.958	0.010
Counter	1.206	0
Cilion	1.210	0
Kingston	2.026	0
Shelton	2.031	0
Zerlina	2.492	0
Lion	2.536	0
Colmar	2.829	0
Polinius	3.012	0.006
Lennox	3.134	0.003
Caid	4.005	0.013
Bartolo	4.416	0

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.491 (177 degrees of freedom).

As can be seen from the least significant differences, there are clear and significant differences in the severity of symptoms seen in different cultivars (a significant interaction between TuMV and cultivar).

Weighing heads after storage showed that the yield of some cultivars was significantly reduced by TuMV infection. In year 1, TuMV didn't significantly reduce the mean head weight yield of 9 cultivars (Kingston, Impala, Polinius, Lennox, Brigadier, Bartolo, Kronas, Zerlina and Colmar), but reduced the weight yield of the 6 others by up to 26% relative to uninfected heads of the same cultivar (Table 6).

Table 6: Mean head weights (kg) for TuMV-infected and uninfected cabbage after storage from the year 1 experiment

Cultivar	TuMV infected	Uninfected	% yield reduction due to TuMV infection	Yield of TuMV-infected plants significantly different from uninfected plants
Kingston	3.510	3.243	-8.23	No
Impala	2.876	2.769	-3.86	No
Polinius	2.474	2.520	1.83	No
Lennox	2.787	2.845	2.04	No
Brigadier	4.610	4.725	2.43	No
Bartolo	2.073	2.142	3.22	No
Kronas	3.814	4.126	7.56	No
Zerlina	2.827	3.229	12.45	No
Colmar	2.381	2.741	13.13	No
Caid	2.928	3.528	17.00	Yes
Counter	3.233	3.968	18.52	Yes
Kilaton	2.726	3.479	21.64	Yes
Shelton	2.727	3.486	21.77	Yes
Lion	2.343	3.154	25.71	Yes
Cilion	2.599	3.528	26.33	Yes

The least difference between two means for the difference to be significant at 5% is 0.557 (132 degrees of freedom).

In year 2, TuMV significantly reduced the mean head weight yields of 9 cultivars by up to 36%, but didn't significantly affect the weight yields of the other 6 cultivars (Brigadier, Colmar, Impala, Polinius, Cilion and Kingston) (Table 7).

Table 7: Mean head weights (kg) for TuMV-infected and uninfected cabbage after storage from the year 2 experiment

Cultivar	TuMV infected	Uninfected	% yield reduction due to TuMV infection	Yield of TuMVV-infected plants significantly different from uninfected plants
Brigadier	5.861	5.709	-2.66	No
Colmar	2.985	2.921	-2.19	No
Impala	3.453	3.655	5.53	No
Polinius	2.798	3.071	8.89	No
Cilion	3.194	3.555	10.16	No
Kingston	3.593	4.186	14.17	No
Kronas	4.590	5.377	14.64	Yes
Counter	4.487	5.270	14.86	Yes
Lion	3.365	4.036	16.63	Yes
Kilaton	3.585	4.494	20.23	Yes
Zerlina	3.310	4.261	22.32	Yes
Shelton	3.371	4.433	23.96	Yes
Caid	3.022	4.363	30.74	Yes
Lennox	3.068	4.457	31.16	Yes
Bartolo	2.368	3.676	35.58	Yes

The least difference between two means for the difference to be significant at 5% is 0.622 (132 degrees of freedom).

There were small differences in the relative yields of the different cultivars following infection with TuMV between the two crop seasons, but most cultivars behaved reasonably consistently over the two growing seasons; the combined year 1 and year 2 data is provided in Table 8.

Table 8: Mean head weights (kg) for TuMV-infected and uninfected cabbage after storage, data from the year 1 and 2 experiments combined

Cultivar	TuMV infected	Uninfected	% yield reduction due to TuMV infection	Yield of TuMV-infected plants significantly different from uninfected plants
Brigadier	5.210	5.169	-0.79	No
Kingston	3.686	3.705	0.51	No
Impala	3.174	3.194	0.63	No
Colmar	2.669	2.821	5.39	No
Polinius	2.643	2.832	6.67	No
Kronas	4.196	4.725	11.20	Yes
Counter	3.881	4.611	15.83	Yes
Cilion	2.906	3.553	18.21	Yes
Zerlina	3.049	3.782	19.38	Yes
Lennox	2.899	3.654	20.66	Yes
Kilaton	3.159	4.004	21.10	Yes
Lion	2.836	3.629	21.85	Yes
Shelton	3.024	3.953	23.50	Yes
Caid	2.973	3.962	24.96	Yes
Bartolo	2.155	2.907	25.87	Yes

The least difference between two means for the difference to be significant at 5% is 0.428 (266 degrees of freedom).

The analyses of the data also showed that there were significant weight yield differences between cultivars, as can be seen from the least significant differences.

Weighing heads after storage showed that the yield of some cultivars was also significantly reduced by BWYV infection. In year 1, BWYV didn't significantly reduce the mean head weight yield of 9 cultivars (Kingston, Bartolo, Counter, Shelton, Zerlina, Impala, Lennox, Polinius and Lion), but reduced the weight yield of the 6 others by up to 32% relative to uninfected heads of the same cultivar (Table 9).

Table 9: Mean head weights (kg) for BWYV-infected and uninfected cabbage after storage from the year 1 experiment

Cultivar	BWYV infected	Uninfected	% yield reduction due to BWYV infection	Yield of BWYV-infected plants significantly different from uninfected plants
Kingston	3.230	3.243	0.40	No
Bartolo	2.049	2.142	4.34	No
Counter	3.746	3.968	5.60	No
Shelton	3.183	3.486	8.69	No
Zerlina	2.929	3.229	9.29	No
Impala	2.466	2.769	10.94	No
Lennox	2.414	2.845	15.15	No
Polinius	2.122	2.520	15.79	No
Lion	2.613	3.154	17.15	No
Colmar	2.180	2.741	20.47	Yes
Caid	2.758	3.528	21.83	Yes
Brigadier	3.650	4.725	22.75	Yes
Cilion	2.628	3.528	25.51	Yes
Kronas	3.052	4.126	26.03	Yes
Kilaton	2.370	3.479	31.88	Yes

The least difference between two means for the difference to be significant at 5% is 0.557 (132 degrees of freedom).

In year 2, BWYV significantly reduced the mean head weight yields of all cultivars by up to 36% except for two (Zerlina and Lion) where the mean head weight was not significantly affected (Table 10).

Table 10: Mean head weights (kg) for BWYV-infected and uninfected cabbage after storage from the year 2 experiment

Cultivar	BWYV infected	Uninfected	% yield reduction due to BWYV infection	Yield of BWYV-infected plants significantly different from uninfected plants
Zerlina	3.921	4.261	7.98	No
Lion	3.552	4.036	11.99	No
Impala	2.993	3.655	18.11	Yes
Brigadier	4.405	5.709	22.84	Yes
Counter	4.040	5.270	23.30	Yes
Bartolo	2.819	3.676	23.31	Yes
Cilion	2.686	3.555	24.44	Yes
Kilaton	3.381	4.494	24.77	Yes
Colmar	2.170	2.921	25.71	Yes
Kronas	3.911	5.377	27.26	Yes
Polinius	2.209	3.071	28.07	Yes
Shelton	3.187	4.433	28.11	Yes
Kingston	2.970	4.186	29.05	Yes
Caid	3.050	4.363	30.09	Yes
Lennox	2.855	4.457	35.94	Yes

The least difference between two means for the difference to be significant at 5% is 0.622 (132 degrees of freedom).

There were small differences in the relative yields of the different cultivars following infection with BWYV between the two crops, but most cultivars behaved reasonably consistently over the two growing seasons; the combined year 1 and year 2 data is provided in Table 11.

Table 11: Mean head weights (kg) for BWYV-infected and uninfected cabbage after storage, data from the year 1 and 2 experiments combined

Cultivar	BWYV infected	Uninfected	% yield reduction due to BWYV infection	Yield of BWYV-infected plants significantly different from uninfected plants
Zerlina	3.413	3.782	9.76	No
Impala	2.755	3.194	13.75	Yes
Counter	3.887	4.611	15.70	Yes
Lion	3.058	3.629	15.73	Yes
Kingston	3.073	3.705	17.06	Yes
Bartolo	2.407	2.907	17.20	Yes
Shelton	3.195	3.953	19.18	Yes
Brigadier	4.044	5.169	21.76	Yes
Colmar	2.190	2.821	22.37	Yes
Polinius	2.175	2.832	23.20	Yes
Cilion	2.667	3.553	24.94	Yes
Kronas	3.450	4.725	26.98	Yes
Caid	2.886	3.962	27.16	Yes
Kilaton	2.896	4.004	27.67	Yes
Lennox	2.623	3.654	28.22	Yes

The least difference between two means for the difference to be significant at 5% is 0.428 (266 degrees of freedom).

The analyses of the data also showed that there were significant weight yield differences between cultivars, as can be seen from the least significant differences.

Internal symptoms after storage of the year 1 crop revealed that following infection with TuMV, different varieties showed differing degrees of cigar burn (Table 12). The cultivars Impala, Kilaton, Shelton, Kronas and Brigadier showed very little cigar burn, Impala, Kilaton and Shelton heads infected by TuMV didn't have significant levels of cigar burn and Kronas and Brigadier heads infected by TuMV had only marginal levels of cigar burn (Table 12). Cultivars Caid, Lion, Kingston and Bartolo had particularly severe cigar burn symptoms (Table 12 and Fig. 4).

Table 12: The severity of cigar burn symptoms in cabbage cultivars after storage, year 1 crop

Cultivar	Mean symptom score*, TuMV infected	Mean symptom score, not infected
Impala	0.1095	0.0412
Kilaton	0.2803	0.2179
Shelton	0.5086	0
Kronas	0.7074	0.0700
Brigadier	0.8457	0
Polinius	0.9480	0
Cilion	1.0555	0
Colmar	1.2494	0
Zerlina	1.2500	0
Lennox	1.5811	0
Counter	1.6435	0.0410
Caid	2.5147	0.0027
Lion	2.6535	0.1061
Kingston	3.1036	0.0280
Bartolo	3.7303	0

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.6277 (87 degrees of freedom).

Figure 4. *Severe cigar burn symptoms seen in some TuMV-infected cabbage heads following storage*

The cigar burn symptoms in the year 2 crop were more severe than in the year 1 crop. Cultivar Brigadier was the only cultivar not to show significant levels of cigar burn following TuMV infection, although Shelton heads infected by TuMV had only marginal levels of cigar burn. Cultivars Counter, Colmar, Zerlina, Caid, Lennox, Kingston, Polinius, Bartolo and Lion had particularly severe cigar burn symptoms (Table 13 and Fig. 4).

Table 13: The severity of cigar burn symptoms in cabbage cvs after storage, year 2 crop

Cultivar	Mean symptom score*, TuMV infected	Mean symptom score, not infected
Brigadier	0.357	0
Shelton	0.678	0
Kilaton	1.042	0
Cilion	1.055	0
Impala	1.160	0.070
Kronas	1.171	0
Counter	1.783	0.047
Colmar	2.167	0.020
Zerlina	2.690	0.073
Caid	2.970	0.205
Lennox	3.363	0
Kingston	3.453	0.003
Polinius	3.495	0.036
Bartolo	3.978	0
Lion	4.031	0.040

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.594 (87 degrees of freedom).

When the year 1 and year 2 data was combined, Shelton, Impala, Brigadier, Kilaton and Kronas had the least cigar burn following TuMV infection and storage (Table 14) and cultivars Counter, Colmar, Zerlina, Polinius, Lennox, Caid, Kingston, Lion and Bartolo all had particularly severe cigar burn symptoms (Table 14 and Fig. 4).

Analysing all the cultivars together, there was a clear correlation between the severity of TuMV symptoms prior to harvest and the severity of cigar burn symptoms after storage; the more severe the TuMV symptoms prior to harvest, the more severe the cigar burn symptoms after storage. However, looking at individual cultivars, there was no evidence for a correlation between pre-harvest and post-storage symptoms for some of the cultivars. Assessment of TuMV symptoms in the field will give growers an indication of which cabbages / crops are likely to develop cigar burn during storage.

Table 14: The severity of cigar burn symptoms in cabbage cultivars after storage, year 1 and year 2 crop data combined

Cultivar	Mean symptom score*, TuMV infected	Mean symptom score, not infected
Shelton	0.5592	0.0067
Impala	0.5691	0.0506
Brigadier	0.6061	0.0009
Kilaton	0.6638	0.0921
Kronas	0.9390	0.0425
Cilion	1.0679	0.0222
Counter	1.7192	0
Colmar	1.7522	0
Zerlina	2.0155	0.0105
Polinius	2.0884	0
Lennox	2.4360	0.0025
Caid	2.7488	0.0108
Kingston	3.2956	0.0111
Lion	3.3004	0.0317
Bartolo	3.8656	0

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.4434 (177 degrees of freedom).

Examination of internal symptoms after storage of the year 1 crop revealed that following infection with BWYV, different varieties showed differing degrees of tipburn; the cultivars Polinius, Counter, Kilaton, Bartolo, Cilion and Colmar showed very little tipburn and Caid and Shelton heads infected by BWYV had only marginal levels of tipburn (Table 15). Kingston, Lennox, Zerlina, Impala and Brigadier had particularly severe tipburn symptoms (Table 15 and Fig. 5).

Table 15. The severity of tipburn symptoms in cabbage cultivars after storage, year 1 crop

Cultivar	Mean symptom score*, BWYV infected	Mean symptom score, not infected
Polinius	0.0039	0.0185
Counter	0.2452	0.1434
Kilaton	0.3619	0.0402
Bartolo	0.3881	0.0899
Cilion	0.4358	0.0661
Colmar	0.8679	0.0723
Caid	1.0150	0.0610
Shelton	1.1324	0.2010
Lion	1.3049	0.0862
Kronas	1.4949	0.0108
Kingston	1.6598	0.2170
Lennox	1.8744	0
Zerlina	1.9235	0.1262
Impala	2.7456	0
Brigadier	4.4791	0.1017

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.8475 (87 degrees of freedom).

Figure 5. *Severe tipburn symptoms seen in some BWYV-infected cabbage heads following storage*

The tipburn symptoms in the year 2 crop were less severe than in the year 1 crop. Cultivars Counter, Kilaton, Colmar, Shelton, Polinius, Kronas, Cilion, Bartolo, Lion, Kingston, Caid, Lennox and Zerlina showed little or no tipburn following BWYV infection (Table 16). Brigadier had particularly severe tipburn symptoms (Table 16 and Fig. 5).

Table 16: The severity of tipburn symptoms in cabbage cultivars after storage, year 2 crop

Cultivar	Mean symptom score*, BWYV infected	Mean symptom score, not infected
Counter	0	0.0434
Kilaton	0	0
Colmar	0	0.0089
Shelton	0	0.0326
Polinius	0	0
Kronas	0.0023	0.0031
Cilion	0.0167	0
Bartolo	0.0253	0
Lion	0.0276	0.1145
Kingston	0.3449	0
Caid	0.3570	0
Lennox	0.5833	0
Zerlina	0.7804	0.0918
Impala	1.2491	0
Brigadier	3.1772	0.0050

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.6887 (177 degrees of freedom).

When the year 1 and year 2 data was combined, cultivars Polinius, Counter, Kilaton, Bartolo, Cilion, Colmar, Shelton, Lion and Caid had no significant tipburn following BWYV infection and storage and Kronas had very little tipburn. Brigadier and Impala had particularly severe tipburn symptoms (Table 17 and Fig. 5).

Table 17: The severity of tipburn symptoms in cabbage cultivars after storage, year 1 and year 2 crop data combined

Cultivar	Mean symptom score*, BWYV infected	Mean symptom score, not infected
Polinius	0.0021	0.0046
Counter	0.0993	0.0941
Kilaton	0.1657	0.0188
Bartolo	0.2074	0.0259
Cilion	0.2258	0.0112
Colmar	0.4195	0.0387
Shelton	0.5562	0.1158
Lion	0.6673	0.0983
Caid	0.6859	0.0158
Kronas	0.7511	0.0066
Kingston	1.0049	0.1051
Lennox	1.2310	0
Zerlina	1.3543	0.1077
Impala	1.9972	0
Brigadier	3.8266	0.0528

*The higher the symptom score, the more severe the symptoms.
The least difference between two means for the difference to be significant at 5% is 0.6887 (177 degrees of freedom).

Serological testing of plants showed that some varieties contained more BWYV than other varieties. There was no correlation between the amount of virus detected in cabbage varieties and the severity of tipburn symptoms or the weight yield of cabbages in either year. In terms of the ranking of the lines for the amount of BWYV detected, most lines behaved consistently over the two years (three varieties had quite different rankings in the two years).

Serological testing of cabbages for BWYV in the field will not give growers an indication of the likely severity of tipburn symptoms that might develop during storage, but would give an indication of the possibility of tipburn symptoms occurring during storage.

Determining the time of natural virus infection of cabbage in the field

Following the transplanting of the cabbage in to the commercial crops in Lincolnshire on 2nd June, 2006, it was a few weeks before infection was first detected (almost 8 weeks, first infection detected 24th July, 2006). The incidence of virus infection detected at the different sampling points in the three plots of 50 plants left uncovered following transplanting is shown below.

Table 18: The incidence of BWYV detected in cabbage plots growing in commercial crops at three sites in Lincolnshire between 2nd June and 24th August 2006

Event	Percentage incidence of BWYV detected by ELISA		
	Site A	**Site B**	**Site C**
Transplanting date	2.6.06	2.6.06	20.6.06
Covered	24.8.06	24.8.06	24.8.06
Sampling 1, 26.6.06	0	0	-
Sampling 2, 10.7.06	0	0	-
Sampling 3, 24.7.06	2	8	8
Sampling 4, 7.8.06	8	55	88
Sampling 5, 24.8.06	96	94	100

BWYV infection was first detected on 24th July, 2006, with incidences ranging from 2% at one site, to 8% at the other two sites. At site C, infection progressed rapidly with 88% of plants infected by 7th August and 100% infection by 24th August 2006. At site A, infection progressed slower, with only 8% infection by 7th August, but then increasing rapidly to 96% infection by 24th August, 2006. The rate of infection at site B was intermediate to that of sites A and C (Fig. 6).

The spread of BWYV in to the plots appeared to be related to the numbers of winged aphids (peach potato aphid [*M. persicae*] and woolly cabbage aphid [*Brevicoryne brassicae*]) caught in the Rothamsted suction trap in Kirton, Lincolnshire (Fig. 6).

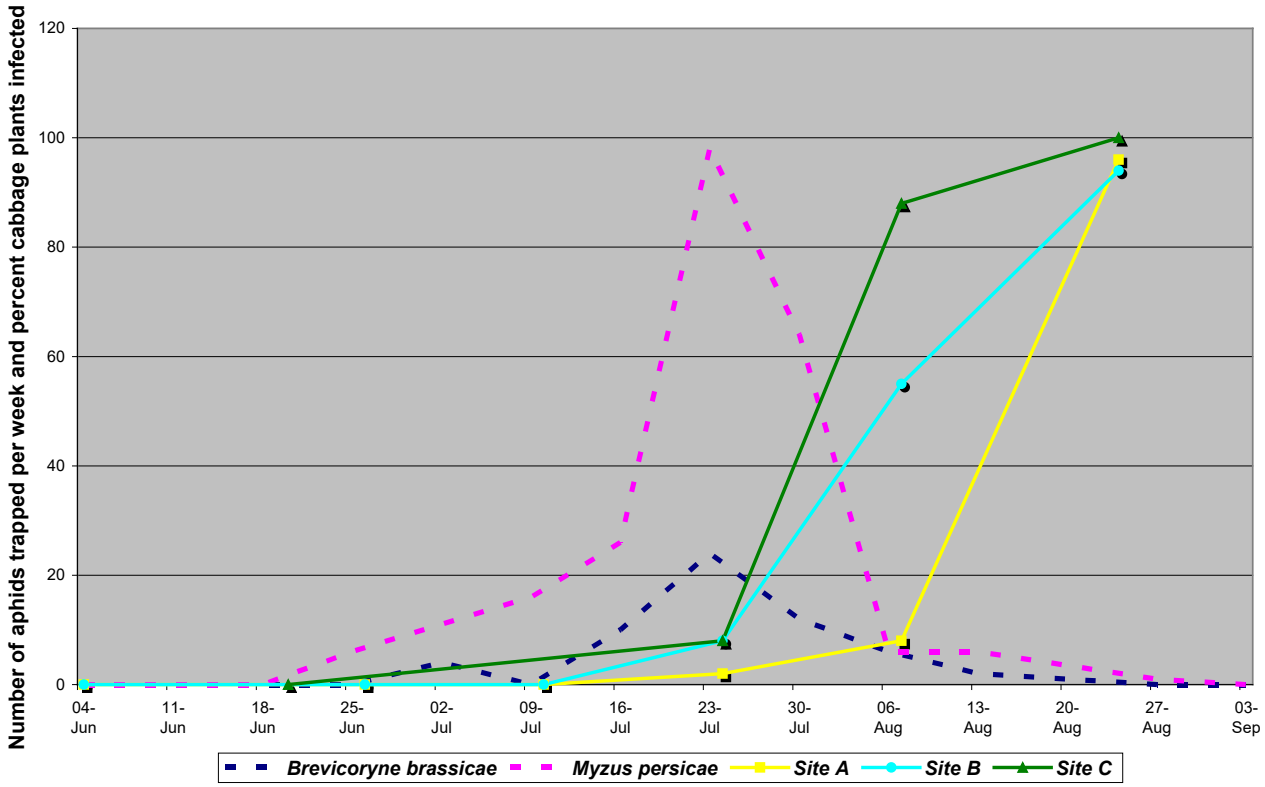

Figure 6. *The numbers of aphids (peach potato aphid* [M. persicae] *and woolly cabbage aphid* [B. brassicae]*) caught in the Rothamsted Insect Survey suction trap at Kirton and the incidence of* Beet western yellows virus *(BWYV) at the three sites*

The incidence of virus infection detected at the different sampling times in the plots of 50 cabbage plants that were uncovered half way through the growing season (24th August, 2006) at the three sites is shown below.

Table 19: The incidence of BWYV detected in cabbage plots growing in commercial crops at three sites in Lincolnshire between 24th August and 6th November 2006

Event	Percentage incidence of BWYV detected by ELISA		
	Site A	**Site B**	**Site C**
Transplanting date	2.6.06	2.6.06	20.6.06
Uncovered	24.8.06	24.8.06	24.8.06
Sampling 6, 11.9.06	0	0	4
Sampling 7, 21.9.06	2	0	14
Sampling 8, 9.10.06	2	0	14.2
Sampling 9, 23.10.06	2	0	22.4
Sampling 10, 6.11.06	2	0	25.5

Following the uncovering of the plots of 50 plants at two of the sites (A and B), very little infection occurred (2% at site A and no infection at site B). However, at site C, where infection had previously occurred most rapidly, infection levels rose steadily, reaching 25.5% by the 6th of November, 2006.

Unfortunately we have been unable to obtain spray records for site B. Spray records for sites A and C show that the insecticide spray regimes at these sites probably explain why infection of the crop at site A progressed more slowly than at site C (Table 20). At site A, insecticides for aphid control were sprayed on 12 occasions, whereas at site C, they were sprayed on 8 occasions. During the time aphids were migrating between mid-July and mid-August, insecticides were sprayed on 4 occasions at site A and 2 occasions at site C (Table 20).

Table 20: The incidence of BWYV detected in cabbage plots growing in commercial crops at three sites in Lincolnshire between 2nd June and 24th August 2006 and the dates that insecticide sprays were applied

Event	Site A		Site C	
	Percentage BWYV incidence	**Dates of insecticide sprays**	**Percentage BWYV incidence**	**Dates of insecticide sprays**
Transplanting date	2.6.06	6.6.06 12.6.06	20.6.06	7.6.06 29.6.06
Covered	24.8.06		24.8.06	
Sampling 1, 26.6.06	0	26.6.06 4.7.06	-	 5.7.06
Sampling 2, 10.7.06	0	11.7.06	-	 20.7.06
Sampling 3, 24.7.06	2	29.7.06	8	 4.8.06
Sampling 4, 7.8.06	8	8.8.06 16.8.06	88	 22.8.06
Sampling 5, 24.8.06	96	24.8.06	100	

In the second half of the season (after 24th August, 2006), when covers were removed from a second set of cabbages growing in the commercial crops, only 2% of cabbage at site A became infected with BWYV, none at site B and 25.5% at site C. During

this period, insecticides were sprayed on four occasions at site A for aphid control and two occasions at site C (Table 21).

Table 21: The incidence of BWYV detected in cabbage plots growing in commercial crops at three sites in Lincolnshire between 24th August and 6th November 2006 and the dates that insecticide sprays were applied

Event	Site A		Site C	
	Percentage BWYV incidence	**Dates of insecticide sprays**	**Percentage BWYV incidence**	**Dates of insecticide sprays**
Transplanting date	2.6.06		20.6.06	
Uncovered	24.8.06	24.8.06	24.8.06	
Sampling 6, 11.9.06	0	5.9.06	4	6.9.06
Sampling 7, 21.9.06	2	26.9.06	14	28.9.06
Sampling 8, 9.10.06	2		14.2	
Sampling 9, 23.10.06	2	16.10.06	22.4	
Sampling 10, 6.11.06	2		25.5	

Determining the effect of the time of infection by BWYV on tipburn symptom severity.

ELISA testing of leaf samples from the covered plots of 18 cabbage plants that we infected with BWYV prior to transplanting, on the 2nd of August, 2006 and on 2nd of October, 2006 confirmed that most plants were infected. All of the plants in most plots were infected; the lowest incidence detected in any plot was 16 out of 18 plants. There was an uninfected treatment too, which remained uninfected.

There was a clear relationship between the time of infection of cabbage by BWYV and the severity of tip burn symptoms after storage. The earlier the infection, the more severe the tipburn symptoms (Table 22).

There was also a clear relationship between the time of infection of cabbage by BWYV and the weight yield of cabbages after storage. The earlier the infection, the more severe the yield reduction (Table 22).

Table 22: The effect of time of infection by Beet western yellows virus (BWYV) on the severity of tipburn symptoms and the weight yield of the cabbage heads

Treatment	Mean weight of cabbage heads (kg)	Mean symptom score
BWYV infection in May	2.870	2.502
BWYV infection in August	3.108	1.038
BWYV infection in October	3.390	0.283
No BWYV infection	3.438	0.273
Least significant difference at 5% (9 degrees of freedom)	0.163	0.333

Discussion and Conclusions

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

Clear differences in the amount of BWYV detected in the different cabbage cultivars were found. These differences were confirmed by the statistical analysis of the ELISA data for the different cultivars. Unfortunately there was no clear relationship between the ELISA value and the severity of internal tipburn symptoms following storage. Although ELISA will not be useful for indicating the severity of tipburn symptoms, it will still be useful for detecting BWYV in symptomless crops and hence identifying crops at risk of developing tipburn in store.

No clear symptoms of BWYV infection were seen in the leaves of any of the cabbage cultivars, however, where uninfected plants and BWYV-infected plants were growing close, or adjacent to each other, it was clear that in many instances, the BWYV-infected plants were visibly smaller than the uninfected plants. It is unlikely that growers will see this in the field as crops can be 100% infected.

Big differences in the severity of external TuMV symptoms in different cabbage cultivars were seen. These differences are reflected in the mean symptom scores for the different cultivars. Plants of cultivars Bartolo, Caid, Lennox and Polinius infected by TuMV showed particularly severe external necrotic symptoms and plants of cultivars Kronas and Impala infected by TuMV showed very little in the way of symptoms. There was a good correlation between the severity of TuMV symptoms prior to harvest and the severity of cigar burn symptoms after storage which is not surprising bearing in mind the severity of the external symptoms.

Both viruses caused big reductions in the weight yield of some cabbage cultivars at harvest. The weight yield of cultivars Bartolo, Lennox and Caid were all depressed by over 30% when infected by TuMV in one year. There was no weight yield reduction of Brigadier, Colmar, Impala, Polinius or Kingston following TuMV infection in either year. The weight

yield of cultivars Lennox and Cilion were reduced by over 30% in one year. Only two cultivars, Zerlina and Lion showed no yield loss in both years when infected by BWYV. The lack of effect of the viruses on the weight yield does not appear to be a good indicator of susceptibility to virus infection or storage disorders. For example, although the weight yields of cultivars Polinius and Kingston were not affected by TuMV infection, both cultivars developed severe cigar burn symptoms in both years. Impala's weight yield wasn't affected by TuMV, but we know from previous studies (Hunter *et al*. 2002) and this project that it is very susceptible to tipburn. Also although the yield of Lion wasn't greatly affected by BWYV infection, it is very susceptible to cigar burn.

Some cultivars appeared to be very resistant to tipburn, e.g. Polinius and Bartolo but very susceptible to cigar burn. Others e.g. Impala and Brigadier appeared to be very resistant to cigar burn, but very susceptible to tipburn. Cultivars that appeared to be reasonably resistant to both tipburn and cigar burn were Shelton and Kilaton, although both BWYV and TuMV had big effects on the weight yields of these two cultivars.

Testing our cabbage plants growing in the commercial crops of cabbage in Lincolnshire showed that there is high infection pressure from BWYV in cabbage fields in Lincolnshire; infection levels reached 94%, 96% and 100% at the three sites by 24th August. After the 24th August, very little BWYV infection occurred at two of the sites, whereas at the third site 25.5% of plants became infected between 24th August and 6th November, 2006. This result along with differences between the sites in terms of how quickly the cabbage became infected in July / August show that it is be possible to delay infection by BWYV with good crop husbandry / well timed insecticidal sprays. The date on which we first detected BWYV infection in the field (24th July, 2006), was just over 4 weeks after the first aphids were caught in the Rothamsted suction trap at Kirton. This might suggest that there is a window of opportunity to control aphids in order to prevent BWYV infection of brassicas, however, we do not know at this time, how long it takes from a cabbage plant being infected in the field by BWYV, to the time when BWYV reaches detectable levels in the cabbage (i.e. the latent period between infection and detectability). The spray information for two of the sites suggests spraying with insecticides at the right time and more frequently might delay BWYV infection, however, it is also possible that differences in BWYV infection levels at the two sites could be due to different infection pressures rather than the different spray regimes. It seems clear that although insecticide spray might delay infection, ultimately most plants become infected by BWYV.

The very clear effect of the time of BWYV infection on the severity of tipburn symptoms following storage and the weight yield of cabbage might suggest that delaying BWYV infection will be enough to stop significant losses from tipburn and BWYV-induced weight yield losses. However, we know from earlier studies (Hunter *et al*., 2002) that this

isn't the case for TuMV and cigar burn, where latter infections result in more severe cigar burn than earlier infections.

The fact that delaying BWYV infection reduces the incidence of tipburn and reduces weight yield losses and evidence that certain insecticide spray regimes seem to delay BWYV infection, demonstrates that further research on using partially resistant cabbage cultivars identified in this study and different spray regimes and / or seed treatments is warranted and could provide good protection of cabbage from tipburn. In the longer term, it is important to develop cabbage cultivars with good levels of natural resistance to both tipburn and cigar burn.

Technology transfer

- John Walsh gave a presentation at the Brassica Growers' Association meeting, White Hart Hotel, Boston, Lincs., 9th January 2007 on the progress of the project.
- John Walsh gave a presentation to the Vegetable Consultants Association, Charlecote Pheasant, Charlecote, Warks., on 31st July, 2007 on the progress of the project. The attendees were also given a guided tour of part of the year 2 experiment at Warwick HRI, Wellesbourne.
- John Walsh gave a presentation at the Brassica Grower's Association's Brassica Conference at Warwick HRI on 9th January, 2008 on the progress of the project.
- An article in HDC News, December 2008.

Glossary

CA; controlled atmosphere
BWYV; *Beet western yellows virus*
DAS-ELISA; double antibody sandwich, enzyme-linked immunosorbent assay
ELISA; enzyme-linked immunosorbent assay
TuMV; *Turnip mosaic virus*
TuYV; *Turnip yellows virus*

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