Project Title:	White cabbage: reducing losses from internal disorders and improving supply - the role of timing of virus infection.
Project number:	HL0114LFV and FV160a
Project Leader:	J Walsh, HRI, Wellesbourne
Report:	Final Report November 2002
Previous Reports:	Annual Reports September 1998, 1999, 2000, Final Report, September 2001 (White cabbage: reducing losses from internal disorders and improving supply)
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Consortium partners:	Tinsleys Prepared Foods Solway Foods Smedleys Foods Ltd Del Monte Fresh Produce (formerly Fisher Foods Ltd) United Vegetables Nickerson Zwaan Elsoms Seeds Syngenta (formerly Novartis Seeds Ltd) Horticultural Development Council Horticulture Research International
Government sponsor:	DEFRA (formerly MAFF)

The results and conclusions of the extension to the project in this report are based on experiments conducted over a single growing season. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use except where the crop or situation is the subject of an off-label extension of use (The UK pesticide Guide 1999).

Before using all pesticides and herbicides check the approval status and conditions of use.

Read label before use: use pesticides safely.

The following are members of the Consortium LINK project HL0114LFV:

Tinsleys Prepared Foods
Solway Foods
Smedleys Foods Ltd
Del Monte Fresh Produce (formerly Fisher Foods Ltd)
United Vegetables
Nickerson Zwaan
Elsoms Seeds
Syngenta (formerly Novartis Seeds Ltd)
Horticultural Development Council
Horticulture Research International

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GROWER SUMMARY (including results from original project and extension)

Headline

- Turnip mosaic virus has been shown to be the cause of internal necrosis (cigar burn) in white cabbage
- Beet western yellows virus has been shown to cause tip burn in white cabbage
- In order to fully exploit the findings of the project and extract maximum value from the research, it is essential to:
  - screen cabbage lines and germplasm for their relative susceptibilities to TuMV-induced cigar burn and BWYV-induced tip burn using the specific tests devised in this project
  - to obtain a better understanding of host and / or environmental factors involved in the appearance of cigar burn and tip burn symptoms
  - to evaluate the ability of field tests to predict cigar burn and tip burn incidence and severity
- Until cultivars resistant to both disorders are available, it is essential to control aphids as much as possible, use 'high grade' sealed stores as much as possible and lobby for the further research necessary to fully exploit the results from the project

Background and expected deliverables

Losses from various storage disorders of white cabbage, which are only infrequently evident at harvest, vary from year-to-year. In most years DEFRA (MAFF) record losses of 10% of the crop but on occasions much higher losses occur. Several years ago, a number of major co-operatives and growers recorded complete loss of stored material (up to 600 tons in one store) with others recording substantial losses in the range of 20-80%. These losses are often compounded by substantial buying of cabbage from abroad at short notice at prices of 2 to 3 times higher than offered for the contracted UK crop. For commercial reasons the extent of this is not revealed but clearly unreliability of supply is a major problem.

The presence of internal necrotic spots along with the presence in the internal tissues of necrotic areas usually at the margins of leaves (so-called tip burn - Fig. 1.) are the more commonly occurring disorders of stored white cabbage. The symptoms are not usually evident in cabbage heads at harvest or at store loading and are often only revealed at processing. Until recently, tip burn had been attributed to deficiency in the local supply of calcium in the plant, associated with inadequate transport and with calcium metabolism (Palzkill *et al.,* 1976).

The expected deliverables from this project are:

- Identification of the causes of 'cigar burn' and tip burn internal disorders of white cabbage
- Information on effect of different storage conditions on the disorders
- An evaluation of whether time of infection by viruses affects the severity of internal disorders
- An indication of whether tests capable of predicting the risk of internal disorders could be developed
- A clear estimate of the ability of Gaucho and Aztec pesticides to control tip burn

Summary of the project and main conclusions

The earlier part of this HortLINK project (Final Report; Gray *et al*., 2001; Hunter *et al*., 2002) showed that:

- Cigar burn is caused by turnip mosaic virus (TuMV) infection.
- Beet western yellows virus (BWYV) infection causes tipburn, however, not all cultivars are equally susceptible.
- Both TuMV-induced cigar burn and BWYV-induced tipburn symptoms are exacerbated by cauliflower mosaic virus (CaMV) infection.
- TuMV infection in June produced greater levels of cigar burn than infection in April (pre-transplanting).
- BWYV-induced tip burn symptoms may increase over time in store
- BWYV is probably not the only cause of tip burn
- It is important to minimise virus infection and have good aphid control
- TuMV, BWYV and CaMV caused significant reductions in harvested head weight.
- The 'high grade' (sealed) store significantly reduced secondary infections such as *Botrytis* relative to the 'low grade' (unsealed) store.
- It is unlikely that the Industry Grower Protocols as they define spacing, fertiliser application and irrigation will induce tipburn by disrupting calcium supplies to the head. Calcium concentrations in the heads of field grown plants were in the range 0.4 to 0.8% of Dry Matter and significantly higher than those concentrations (<0.2%) inducing tipburn in hydroponic systems.
- There appear to be cultivar differences in calcium-induced susceptibility to tipburn.

The objectives of the extension to the project requested by the consortium were as follows:

1. Compare the effect of early and late infection by BWYV on the severity of tip burn symptoms developing during storage of cabbage heads.
2. Determine if serological testing for BWYV prior to harvest can distinguish early and late infected plants.
3. Determine the relationship between severity of tip burn symptoms that develop during storage and the amount of BWYV detected serologically prior to harvest.
4. Determine whether the insecticide treatments Gaucho (seed treatment) and Aztec (as 3 spray treatments) are capable of reducing the incidence of BWYV and hence of reducing the incidence and/or severity of tip burn in cabbage heads.

The results from the experiments carried out during the extension on cultivar Impala only showed that:

1. The time of infection of cabbage by BWYV under field conditions had no apparent effect on severity of tip burn that subsequently developed during storage.

2. Serological testing for BWYV by enzyme-linked immunosorbent assay (ELISA) prior to harvest was unable to distinguish early and late infected plants.

3. When BWYV was detected in cabbage heads by the serological method (ELISA) it did not follow that heads always developed tip burn subsequently in store. Levels of BWYV detected prior to harvest in those heads that went on to develop tip burn symptoms did not give a reliable indication of the severity of the tip burn symptoms. The method used for detecting BWYV is not currently effective as a predictive test for tip burn. There appears to be an environmental or plant genetic component to tip burn.

4. Neither Gaucho (imidocloprid) (seed treatment) nor Aztec (triazamate) (3 sprays) were effective in reducing the incidence of BWYV infection, or the severity of subsequent tip burn at the disease pressures seen in the field experiment. There may be a reduction in yield of Dutch white cabbage cultivar Impala associated with the use of the insecticide Aztec.

5. Aphids with resistance (modified acetylcholinesterase positive) to Aztec were found on Aztec-treated heads in the field.

6. BWYV infections close to harvest may not be detectable by any serological tests because of the lag between infection and the build up of detectable levels of virus.

The experiments carried out during the extension confirm earlier findings that BWYV causes tip burn in white cabbage. As the time of BWYV infection appears to have no effect on the severity of tip burn in cabbage it is important to develop measures that avoid tip burn (e.g. resistant cultivars) or control BWYV for the whole growing season.

If growers wish to schedule cabbage removals from store so that BWYV infected heads (likely to develop tip burn symptoms during storage) are processed early before tip burn symptoms develop, there is a need to understand some of the plant and/or environmental factors involved in tip burn development. Current detection of the virus in heads although highly sensitive, is not enough to predict whether tip burn will develop. Information on the other host or environmental factors involved in tip burn may allow tests to be developed that will not only predict whether cabbage will develop tip burn, but also the severity of tip burn.
If cabbage are tested prior to harvest, approximately 50% of those in which BWYV is detected may develop symptoms. Removing such cabbage from store early will reduce the risk of tip burn. These indications are based on experiments with cultivar Impala.

It appears that the use of Gaucho treated seed and/or Aztec sprays may not provide protection against BWYV and hence tip burn.

The quickest and most cost-effective measure to reduce losses from internal disorders (tip burn and cigar burn) is likely to be rigorous testing of current white cabbage cultivars and breeding lines for resistance to BWYV-induced tip burn and TuMV-induced cigar burn.

Further information on the involvement of BWYV in internal tip burn and and TuMV in cigar burn is given in the Final Report of the earlier part of this project (Gray *et al*., 2002) and the scientific paper of Hunter *et al*. (2002).

Financial benefits

- By storing virus infected cabbage in high grade sealed stores growers should be able to reduce losses from secondary pathogens, particularly *Botrytis*
- In years when aphids and viruses are prevalent, testing cabbage for TuMV will give a good indication of the likelihood of cigar burn developing in store and hence, TuMV-infected crops could be processed early to reduce losses
- Neither Gaucho nor Aztec pesticides reduced the incidence of BWYV and hence the incidence of tip burn

Action points for growers

- Controlling aphids and TuMV will reduce 'cigar burn'; infections by TuMV later in the growing season may induce greater levels of cigar burn than infection earlier in the season
- Controlling aphids and BWYV will reduce tip burn
- BWYV is probably not the only cause of tip burn
- It is unlikely that the Industry Grower Protocols as they define spacing, fertiliser application and irrigation will induce tip burn by disrupting calcium supplies to the head. Calcium concentrations in the heads of field grown plants were in the range 0.4 to 0.8% of Dry Matter and significantly higher than those concentrations (<0.2%) inducing tip burn in hydroponic systems.
- Controlling aphids and cauliflower mosaic virus will reduce the severity of 'cigar burn' and tip burn in TuMV and BWYV infected cabbage heads
- 'High grade' (sealed) stores may significantly reduce secondary infections such as *Botrytis* relative to the 'low grade' (unsealed) stores
- In years when aphids and TuMV are abundant, testing cabbage heads for the presence of TuMV will give a reasonable indication of the risk of cigar burn and allow infected crops to be processed early in the hope of avoiding losses from cigar burn
- In years when aphids and BWYV are abundant, if cabbage heads are tested for the presence of BWYV, approximately 50% of heads in which BWYV is detected are likely to develop tip burn
- Growers should consult seed companies for comparative information on the susceptibilities of different cabbage cultivars to TuMV-induced 'cigar burn' and BWYV-induced tip burn
- Now that the causes of cigar burn and tip burn have been identified there is the need for research to be commissioned on controlling TuMV and BWYV.
- Aztec reduced the yield of cabbage cultivar Impala in our experiment over one growing season, growers should contact the manufacturers (BASF) and the seed companies about potential effects of this insecticide on cabbage yields

MILESTONES

Monitoring and Evaluation

Milestone		**Completed** Yes No
		☐ ☐
Objective 1		
1.1	Infect some plants with BWYV pre-transplanting and establish all plants in gauzehouses	☐
1.2	Infect further plants post-transplanting with BWYV	☐
1.3	Determine whether timing of infection by BWYV has any effect on the severity of tip burn symptoms	☐
Objective 2		
2.1	ELISA testing of all cabbage plants complete prior to harvest	☐
2.2	Analysis of whether serological testing for BWYV prior to harvest can discriminate between early and late infected plants	☐
Objective 3		
3.1	Full analysis of whether amount of virus detected in heads prior to Harvest is an indicator of the severity of tip burn symptoms post-storage	☐
Objective 4		
4.1	Establish field experiment at Kirton	☐
4.2	Determine whether the insecticide treatments Gaucho and Aztec reduce the incidence of BWYV and hence incidence and/or severity of tip burn in cabbage heads	☐

SCIENCE SECTION

SUMMARY OF RESULTS (results from extension only)

1. No statistically significant effect of the time of infection of Beet western yellows virus (BWYV) on levels of virus (detected by ELISA 1 week prior to harvest) or on the severity of tip burn symptoms assessed in heads after cold storage was detected. It should be noted, however that a lag of 3-4 weeks was observed between BWYV inoculation in the gauzehouse experiment and BWYV detection in ELISA tests, consequently, infections very close to the harvest date might go undetected.

2. Considerable numbers of aphids were seen on Aztec (triazamate) treated plants. This may have been due to the presence of a proportion (approximately 37%) of insecticide resistant aphids within the population. Neither of the insecticide treatments significantly reduced the incidence of BWYV compared to the untreated plots. Levels of BWYV detected in cabbage plants 1 week prior to harvest were significantly lower in both the Aztec and Gaucho (imidocloprid) treated plots compared to the untreated controls. However, these reductions in virus content of cabbage were not reflected in the severity of tip burn seen following storage; in other words none of the insecticide treatments reduced the incidence or severity of tip burn. No statistically significant differences between treatments were detected.

3. There were no apparent phytotoxic effects of the Gaucho seed treatment on cultivar Impala in our experiment, although a significant reduction in head weight in the Aztec treated plants compared to the other treatments was seen.

4. Of the cabbage that tested positive for BWYV in the serological tests (ELISA) using diluted sap from plants sampled 1 week prior to harvest 27% of those from the gauzehouse developed tip burn and 73% did not. From the field experiment 55% of cabbage that tested positive for BWYV developed tip burn and 45% did not. In other words when BWYV was detected in cabbage heads by ELISA it did not follow that such heads always developed tip burn subsequently in store. This suggests that there is a plant genetic, or environmental component to the disorder. Levels of BWYV detected by ELISA prior to harvest in those heads that went on to develop tip burn symptoms did not give a reliable indication of the severity of the tip burn symptoms. The method used for detecting BWYV is not currently effective as a predictive test for tip burn. BWYV infections close to harvest may not be detectable by any serological tests because of the lag between infection and the build up of detectable levels of virus.

INTRODUCTION

Two thousand seven hundred ha. of white cabbage, valued at approximately £20m p.a. are grown for storage each year in the UK. The crop represents a significant investment and risk over a period of 18 months of growing and storage before it is sold. Losses from various storage disorders, which are only infrequently evident at harvest, vary from year-to-year. In most years DEFRA (MAFF) record losses of 10% of the crop but on occasions much higher losses occur. Several years ago, a number of major co-operatives and growers recorded complete loss of stored material (up to 600 tons in one store) with others recording substantial losses in the range of 20-80%. These losses are often compounded by substantial buying of cabbage from abroad at short notice at prices of 2 to 3 times higher than offered for the contracted UK crop. For commercial reasons the extent of this is not revealed but clearly unreliability of supply is a major problem. Crop loss is incurred when the extent of the disorders evident in the head on inspection would make it uneconomic to process. Crops are accepted by processors having a certain proportion of heads or areas of affected tissue within a head provided this does not i) incur excessive handling costs on the line, ii) substantially reduce product recovery on shredding or iii) reduce shred length, an important quality attribute with retail customers. The unreliability of product supply also makes it difficult to match product output and labour requirement with retail customer demand so influencing the processors' profitability. High incidences of disorders also increase the costs of waste removal to the processors.

The presence in the internal tissues of necrotic areas usually at the margins of leaves (so-called tip burn - Fig. 1) is one of the more commonly occurring disorders of stored white cabbage. The symptoms are not usually evident in cabbage heads at harvest or at store loading and are often only revealed at processing. Until recently, tip burn had been attributed to deficiency in the local supply of calcium in the cabbage head associated with inadequate transport and with calcium metabolism (Palzkill *et al.,* 1976). Factors reported to induce calcium deficiency include: a) high leaf expansion rates due to high temperature, b) high transpiration rates, (Adams and Holder, 1993), c) calcium metabolism or re-distribution in the leaf during storage (Tao *et al*., 1986; Berard *et al.,* 1986). Ca^{2+} supply and transport has also been clearly linked to internal browning in Brussels sprout buttons, though the effects are often so severe that the disorder is clearly evident early in growth and at harvest (reviewed by Coleman, 1982).

The precursor LINK project to this current extension (HORT15 - White cabbage: reducing losses from internal disorders and improving supply) investigated the role of three viruses, turnip mosaic virus (TuMV), cauliflower mosaic virus (CaMV) and beet western yellows virus (BWYV) in the development of internal disorders in white cabbage. The project clearly demonstrated that:

- Cigar burn is caused by TuMV infection.

- **BWYV infection causes tip burn symptoms indistinguishable from those typically associated with calcium deficiency, however, not all cultivars are equally susceptible.**

- Both TuMV-induced cigar burn and BWYV-induced tip burn symptoms are exacerbated by CaMV infection.

- BWYV-induced tip burn increased in severity during storage

- **TuMV infection in June produced greater levels of cigar burn than infection in April (pre-transplanting).**

- TuMV, BWYV and CaMV caused significant reductions in harvested head weight.

- The 'high grade' (sealed) store significantly reduced secondary infections such as *Botrytis* relative to the 'low grade' (unsealed) store.

BWYV-induced tip burn increased in severity during storage. BWYV is by far the most common virus in arable brassicas. Incidences of up to 100% have been observed in crops (Smith and Hinckes, 1985) and it is very widespread (Hardwick *et al*., 1994), consequently the involvement of BWYV in internal disorders of cabbage is a major concern for production and storage of white cabbage in the UK. Although the timing of infection of cabbage plants by TuMV had a serious impact on the severity of cigar burn symptoms that developed, prior to this extension, nothing was known about the effect of the timing of BWYV infection on the severity of tip burn symptoms.

This extension project was requested by the Consortium of HortLINK Project HORT15, to study the effects of the time of infection by BWYV on the severity of tip burn infection in stored Dutch white cabbage. Aphids transmit BWYV in a persistent manner requiring relatively long feeding times for efficient virus transmission. As a consequence, unlike TuMV (where transmission is rapid [seconds]), certain insecticides that kill aphids rapidly and/or have anti-feedant activity are capable of controlling BWYV (Walsh *et al*., 1989). This extension project also included a study of the efficacy of two insecticide treatments (one seed treatment and one spray) in preventing infection of cabbage with BWYV and the effect on the associated development of tip burn during storage. In addition, infections with TuMV, CaMV and Turnip yellow mosaic virus (TYMV) were also monitored. The data generated were also used to investigate the potential of serological tests (ELISA) for the prediction of storage potential of cabbage heads in terms of BWYV infection and tip burn development.

Prior to the precursor project it was not known how to produce a crop free from internal disorders which would store reliably. Furthermore there were no reliable methods of pre-storage assessment of storage potential in relation to occurrence of such disorders. It has been suggested that if more knowledge on the interaction between different viruses (and their pathotypes) and current cabbage varieties can be obtained, and more sensitive detection methods developed, disease avoidance strategies can be devised (Walsh, 1994).

Fig. 1. Typical BWYV-induced tip burn symptoms in heads of Dutch white cabbage cultivar Impala.

EXPERIMENTAL WORK

Materials and Methods

Two separate experiments were performed in parallel, both experiments were carried out on cultivar Impala only. One experiment was carried out in insect-proof gauzehouses at Horticulture Research International (HRI), Wellesbourne and involved deliberate infection of cabbages with BWYV at different times during the growing period to investigate the effect of time of infection on severity of tip burn. The second experiment was carried out in the field at HRI, Kirton. This experiment was designed for three purposes, firstly to monitor incidences and levels of naturally occurring infection by 4 different viruses (BWYV, CaMV, TuMV and TYMV) over a single growing season, secondly to investigate the efficacy of two different insecticides (Gaucho: a systemic seed treatment and Aztec: a foliar spray) in controlling BWYV and associated tip burn and thirdly to investigate the effect of time of infection on severity of tip burn. In both experiments, the incidence of BWYV infection was monitored throughout, and the relative levels of BWYV determined in individual plants 1 week prior to harvest by enzyme-linked immunosorbent assay (ELISA). In addition incidences of TuMV, CaMV and TYMV were monitored throughout the field experiment. The subsequent development of tip burn (and any other internal disorders) was assessed after storage of cabbage heads.

Seed treatment with Gaucho, sowing and pre-transplanting infection with BWYV

A sub-sample of 10,000 seeds of white cabbage cultivar Impala were treated with a 24% (w/v) solution of Gaucho in 1.8% (w/v) PVA in a fluidised bed seed treatment system at a flow rate of 1.5ml min^{-1}. Both untreated and Gaucho treated seed were subsequently sown in modular trays. The Gaucho treated sub-sample was sown in a separate tray. Seedling emergence was recorded for both treated and untreated seed.

Seedlings were kept under glass until they had reached the two true-leaf stage after which they were removed to an insect proof gauzehouse. One quarter of the plants for the HRI, Wellesbourne gauzehouse experiment were infected with BWYV using viruliferous aphids prior to transplanting. Aphids were left on the seedlings for 7 days to allow virus transmission before being killed with an application of the insecticide Dovetail (lambda-cyhalothrin). These plants were kept separate from the other plants until transplanting. Following initial BWYV infection and treatment with Dovetail, all plants were drenched pre-transplanting with Dursban (chlorpyrifos) against cabbage root fly infestation.

Field experiment

Soil was prepared according to standard commercial practice and ammonium nitrate applied at 290kg ha^{-1}. The cabbage plants were transplanted into 18 replicate plots of 20 plants each. The plots were arranged in 6 rows of 3 plots with a single row of guard plants around each plot. Cabbages were planted 0.6m apart in each direction within plots and plots were spaced 2.4m apart in each direction (Fig. 2). The experiment was divided into 2 blocks consisting of 3 rows of three plots each. Within each block, the three insecticide treatments (Gaucho, Aztec and the untreated control) were assigned to plots in a randomised Latin square format with one plot of each treatment in each plot row. The Aztec treatment was applied as foliar sprays on June 11th, July 4th and September 25th. These dates were selected by monitoring the numbers of aphid infested plants in the Aztec treated plots throughout the experiment (Fig. 3)

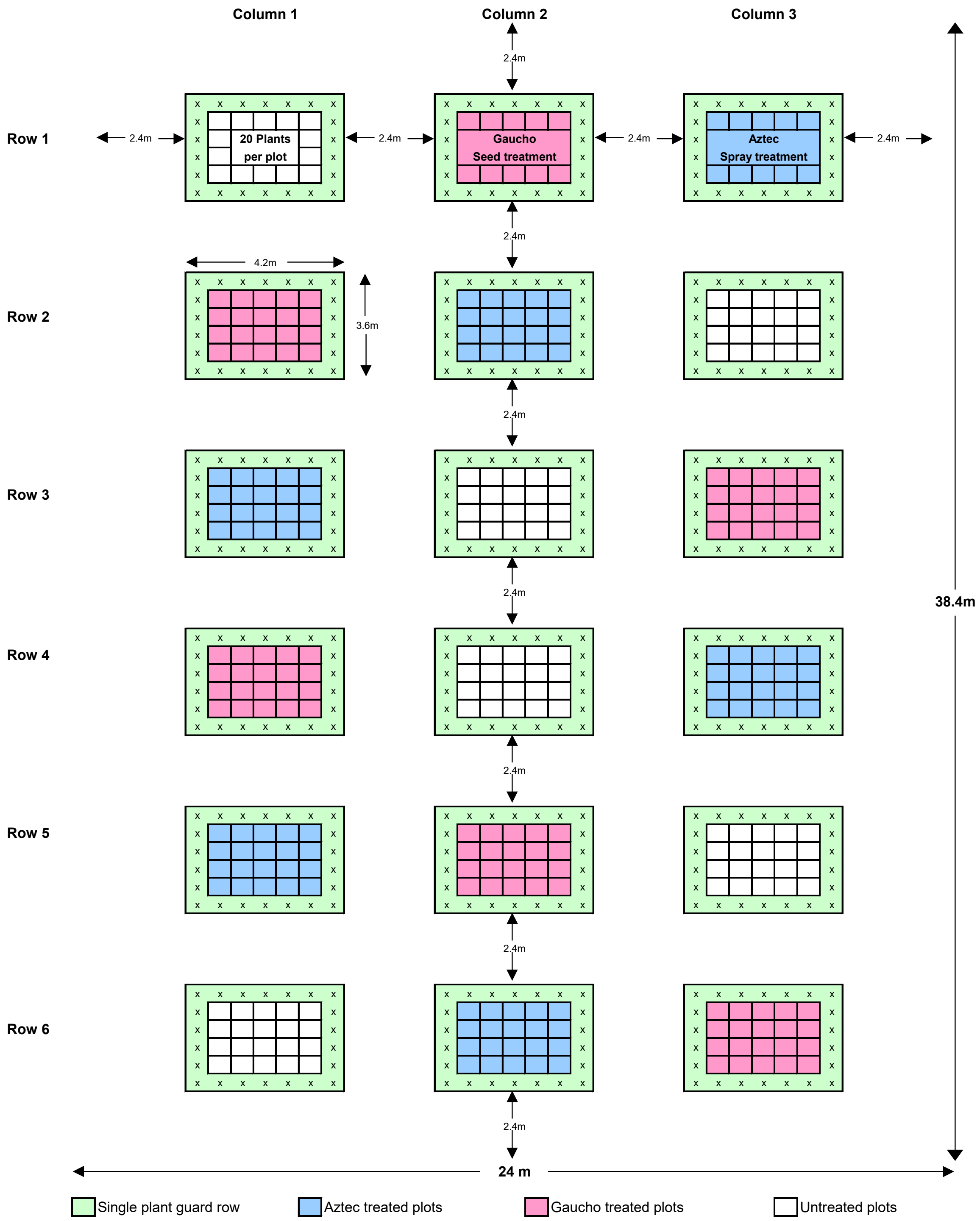

Fig. 2. Layout and design of the field experiment conducted at HRI- Kirton.

The experiment was top-dressed with ammonium nitrate at 435kg ha^{-1} in June. The fungicide Fubol Gold (mancozeb) was applied in July and September to prevent white blister and *Alternaria*. Spannit (chlorpyrifos) granules were applied in July to control cabbage root fly. The insecticides Agree (*Bacillus thuringensis*) and Dipel (*Bacillus thuringensis*) were applied in August and September to control caterpillars noted on the crop. Weeds were controlled by application of Propachlor (propachlor) and Dacthal (chlorthal-dimethyl) in June supplemented by hand weeding in July. All chemical applications were made as per commercial practice.

Leaf samples were taken for virus detection from one quarter of the plants in each of the months June, July, August and September. The plants were sampled at random, with no plant being sampled more than once. All plants were then sampled (a second sampling) in October 1 week prior to harvest. Leaf samples were tested for the presence of BWYV, TuMV and TYMV. Only half of the samples taken on each of the four sampling dates between June and September were tested for the presence of CaMV. Those chosen were selected at random from within the sample. The remaining 50% of plants that had not been tested for CaMV were tested at the pre-harvest sampling in October.

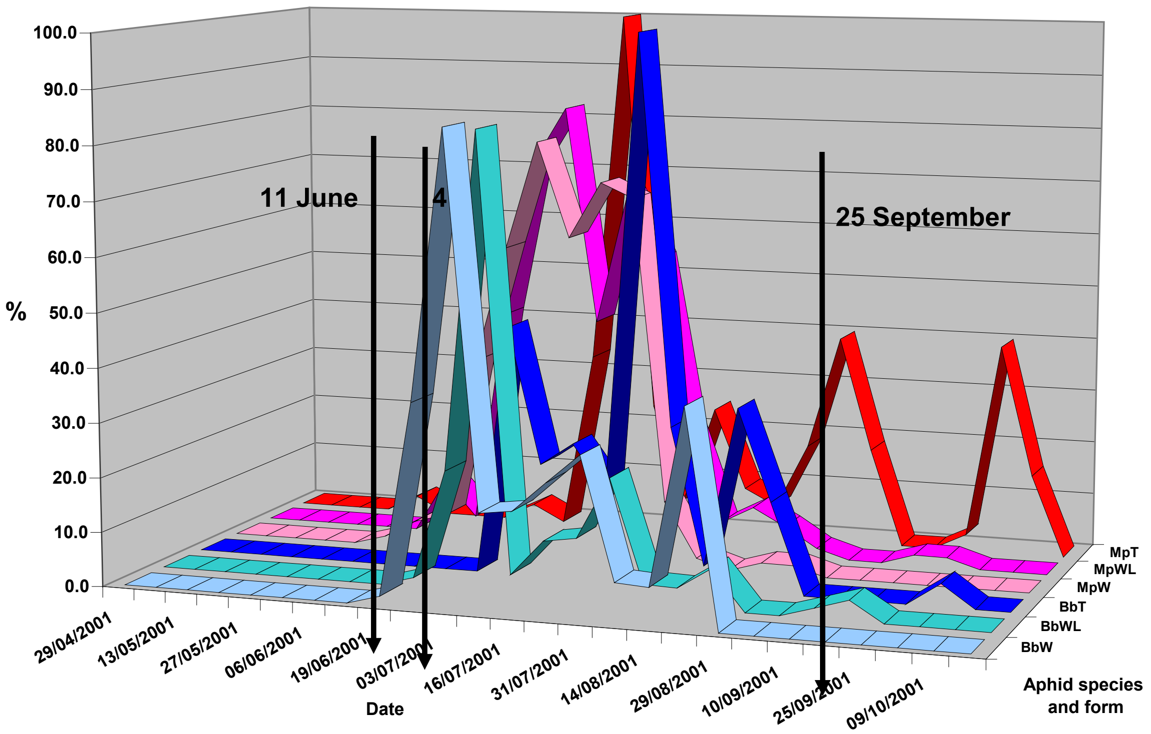

Fig 3. Number of aphid infested plants in the Aztec treated plots of the HRI-Kirton field experiment. Two aphid species were monitored, *Brevicoryne brassicae* (Bb) and *Myzus persicae* (Mp) and both the winged (W) and wingless (WL) forms were counted. The traces marked T indicate the numbers of aphids of each species (winged forms) detected in suction traps. The traces represent either the percentage of plants infested with aphids in all the plots to be sprayed with Aztec or the numbers of aphids trapped in suction traps, expressed as a percentage of the total number trapped in the course of the experiment. Arrows indicate the times of Aztec application.

Gauzehouse experiment

Soil in 4 identical gauzehouses was steam sterilised prior to the commencement of the experiment and ammonium nitrate applied at 130kg ha^{-1} in all houses. Each gauzehouse contained 7 rows of 20 plants with a 0.6m spacing both within and between rows (Fig. 4). Outside rows (with the exception of the northern ends of gauzehouses) were used as guards and guards were also used to divide the gauzehouses into 4 plots of 20 plants (5 rows x 4 plants per row). The following treatments were assigned to one plot in each gauzehouse: uninfected plants, plants infected with BWYV prior to transplanting, plants infected with BWYV in June and plants infected with BWYV in August. The relative positions of the different plots were randomised across the 4 houses, with the restriction that the uninoculated control plots and August inoculated plots were at opposite ends of the houses to reduce the possibility of contamination of the uninfected plots (Fig 4). Spannit granules were applied immediately after transplanting to control any cabbage root fly. The gauzehouses were irrigated at regular intervals using a seep-hose system. The experiment was top-dressed with ammonium nitrate at 250kg ha^{-1} in June. The fungicide Fubol 58W (mancozeb) was applied in July to prevent white blister. Insecticide treatments were all performed as per industry protocols. Alternate fortnightly applications of the insecticides Dovetail and Toppel (cypermethrin) 10 were commenced from the date of transplanting, they were suspended 3 weeks prior to the June aphid inoculations and terminated 4 weeks before the August aphid introductions.

The post-transplanting infections were performed using clip cages (small Perspex cages with gauze tops) to enclose small, aphid bearing, pieces of leaf from a BWYV infected plant to leaves of the experimental plants. Two clip cages were applied to each plant on the youngest available leaves. For the June inoculations, the leaves holding the clip cages were also covered in perforated thin polythene (bread) bags as an added protection against aphid escape. The June inoculated plants were treated with the insecticide Dovetail 7 days after inoculation. By August, the leaves being inoculated were too big to be enclosed. The final insecticide treatment for aphid control (August) was by application of a drench of the systemic insecticide Intercept (imidocloprid) to the control (uninoculated) plots only. The infections were verified by testing leaf samples for the presence of BWYV seven weeks after aphid inoculation. Leaf samples were taken from all plants in October, 1 week prior to harvest and tested for the presence of BWYV following 1:1 dilution of the plant sap.

Virus detection
Serological enzyme linked immunosorbent assay (ELISA) tests were carried out on cabbage leaves to determine the presence of BWYV, TuMV and TYMV. Detection of BWYV and TuMV was by triple antibody sandwich (TAS) format ELISA, whilst plate trapped antigen (PTA) format ELISA was used for TYMV detection. For determination of BWYV levels 1 week prior to harvest, a sample dilution of 1:1 was used in ELISA. At all other times (for infection-monitoring purposes during the course of the experiment), undiluted sap was used in BWYV ELISA. For detection of CaMV, cabbage leaf material was used to inoculate mustard plants (cv. Tendergreen) (TGM). This method also acted as a further test for the presence of TuMV and TYMV. Electron microscope examination of all TGM plants that subsequently developed symptoms was used to confirm virus identity.

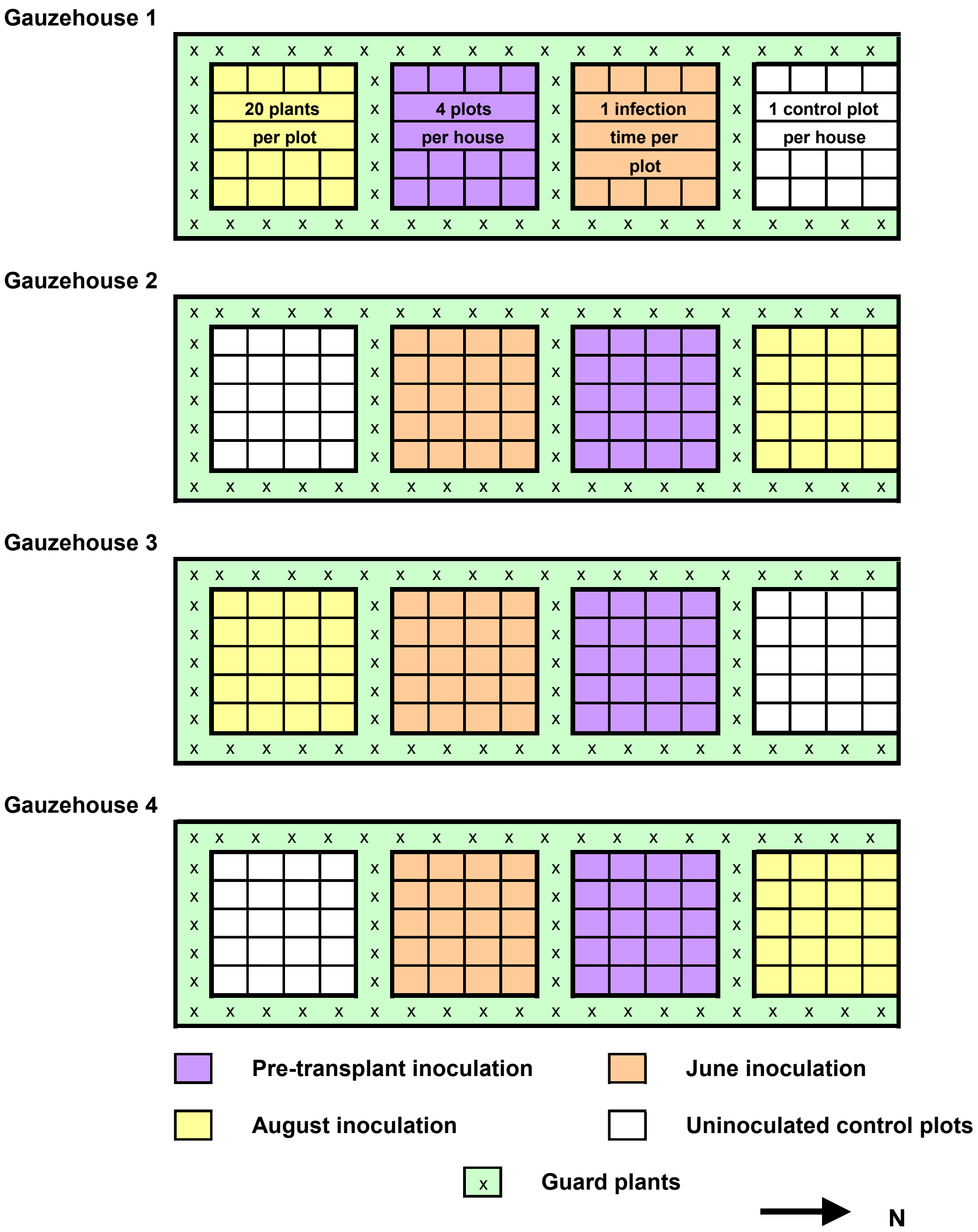

Fig. 4. Layout and design of the gauzehouse experiment at HRI, Wellesbourne.

Harvesting and storage

Heads from the field experiment were harvested in late October, heads from the gauzehouse experiment were harvested one week later, in early November. Following industry protocols, heads were cut to leave 2.5cm of stalk and the outer leaves were trimmed. The heads were weighed and placed in mesh bags (5 heads per bag). Any heads that were regarded as unlikely to store (usually due to secondary fungal infection) were discarded. Discarded heads were recorded as zero head weight. Bagged heads were sprayed to run-off with the fungicides Ridomil MBC 60WP (carbendazim + metalaxyl) and Rovral WP (iprodione) according to commercial protocols. Cabbage heads were stored in a United Vegetables commercial cold store until assessment in June 2002.

Post-storage assessment for tip burn and other disorders
Heads from both experiments were assessed after approximately 7 months in cold storage. Heads were cut into quarters longitudinally and the severity of tip burn in internal leaves of 2 diagonally opposite quarters assigned a score of 0-3 according to the scoring regime in Table 1. The presence of any other internal disorders was also noted. Samples were taken from each head for detection of BWYV in ELISA tests. Additionally, samples from the field experiment were also tested for the presence of CaMV, TuMV and TYMV by inoculation to susceptible host plants (TGM).

Table 1. Criteria used to score cabbage heads for severity of tip burn symptoms

Tip burn Symptom Score	Criteria
0	No symptoms
1	0 – 5% of leaves with tip burn
2	5 – 10% of leaves with tip burn or 0 – 5% of leaves with severe tip burn
3	>10% of leaves with tip burn or 5 – 10% of leaves with severe tip burn

RESULTS AND DISCUSSION

Objectives 1 and 2: Effects of early and late infections with BWYV on the severity of tip burn and ELISA based discrimination of early and late infected plants.

Results from ELISA detection of BWYV 1 week prior to harvest (using plant sap at a dilution of 1:1) was compared with tip burn scores after storage. The data are shown in Table 2. Of the cabbage that tested positive for BWYV, 27% of those from the gauzehouse developed tip burn and 73% did not. From the field experiment 55% of cabbage that tested positive for BWYV developed tip burn and 45% did not. Consideration of data on BWYV detection after harvest revealed that 12 of the plants that tested negative for BWYV prior to harvest but subsequently developed tip burn, tested positive for BWYV at assessment. These plants may have been infected too close to harvest for virus to have increased to detectable levels by the time of testing one week prior to harvest. The presence of plants that tested positive for BWYV but did not develop symptoms may be attributable to plant to plant variation within the cultivar or environmental effects. Considerable amounts of virus were detected in the cabbage cultivar Polinius which showed almost no tip burn symptoms (earlier Hort 15 project report, Gray *et al*., 2001).

Table 2. Comparison of BWYV detection by ELISA 1 week prior to harvest using a sap dilution of 1:1 and subsequent development of tip burn symptoms

		Number of plants	
BWYV	**Tip burn**	**Field experiment**	**Gauzehouse experiment**
Detected	Present	183 (55%)	27 (27%)
Detected	Absent	150 (45%)	74 (73%)
Not detected	Present	11	7
Not detected	Absent	5	209

Statistical analyses of the data for both detection of BWYV by ELISA and severity of tip burn symptoms detected after storage are shown in Table 3. Due to the sampling method employed in the field experiment and the fact that virus infection was by natural incursion of the aphid vector, precise timings of infection for this experiment could not be determined. By comparing results from those plants tested for BWYV at different times throughout the experiment with results obtained from the same plants 1 week prior to harvest, a time period during which any infection probably occurred was determined. The analyses showed no statistically significant effect of infection timing on either levels of BWYV detected by ELISA 1 week prior to harvest (Table 3; $P= 0.077$ for the field data and $P= 0.180$ for the gauzehouse data) or the severity of tip burn symptoms assessed in heads after cold storage (Table 3; $P= 0.434$ for the field data and $P= 0.8$ for the gauzehouse data). As some heads tested negative for BWYV by ELISA at harvest, whereas following storage they had tip burn symptoms and tested positive for BWYV, it seems likely that BWYV may not have built up to detectable levels by the time of harvest. Such late virus multiplication is obviously still capable of inducing tip burn. It should be noted that the apparently large differences in mean transformed ELISA values (Table 3; ranges 0.074 to 0.827 and 0.670 to 1.107) are the products of the data transformation and correspond to relatively small shifts in observed ELISA values (0.080 and 0.027 respectively).

Table 3. Comparison of levels of BWYV detected by ELISA in diluted plant sap 1 week prior to harvest, time of virus infection and subsequent severity of tip burn after storage

Experiment	Mean transformed ELISA values at harvest	Mean tip burn score after storage
Field Experiment Inferred time of infection based on ELISA detection of BWYV		
May – July	0.826	1.03
May – August	0.809	0.66
May – September	0.866	0.84
June – October	1.068	0.72
July – October	1.107	0.70
August – October	0.820	0.55
September – October	0.670	0.74
Gauzehouse experiment Time of aphid transmission treatment of BWYV		
May	0.568	0.08
June	0.827	0.53
August	-0.074	0.00
September	0.794	0.47
October	0.273	0.00

Regression analyses indicated no statistically significant differences within any data set. ELISA data transformed according to the formula $\log_{10}[100 \times (\text{data} + 0.05)]$

The results show that no matter when BWYV infection occurs in the field, it can lead to the development of tip burn symptoms during storage of the cut heads. In the field experiment, BWYV was detected in some cabbage 1 week prior to harvest from infections assumed to have occurred as late in the growing period as September – October and a number of these heads subsequently developed tip burn. A number of heads that tested negative for BWYV subsequently developed symptoms and tested positive for BWYV after storage; this implies that the efficacy of any pre-harvest predictive test for tip burn based on serological detection of BWYV may be reduced in cases where infection occurs very late in the growing season.

Objective 3: The relationship between the severity of tip burn symptoms and levels of BWYV detected.

The sensitivity of the ELISA protocol for the detection of BWYV in the previous part of the project (earlier Hort 15 project report), using undiluted plant sap was so high as to give little discrimination between those cabbage with very low levels of virus and those with high levels.

To investigate any relationships between ELISA value (A_{405}) and severity of tip burn, a protocol was developed using diluted plant sap (1:1). The results from this analysis are shown in Table 4. There was no significant correlation between the ELISA values at harvest and subsequent tip burn severity. There is a considerable difference in the mean virus levels observed in plants that did not subsequently develop tip burn, between the field and gauzehouse experiments with higher levels in the field. This difference may be accounted for by the presence of the uninoculated control treatment in the gauzehouse experiment (which was rigorously kept free of BWYV) and the absence of an uninfected control treatment in the field. Including such a treatment in the field experiment would have caused significant problems and affected yield etc. Uninoculated control plants in the gauzehouse experiment did not develop tip burn. In any test developed, a lack of detection of infection very close to harvest (which can still lead to tip burn in store) may remain a problem.

Table 4. Comparison of mean observed (untransformed) ELISA values for each mean tip burn score category.

Mean tip burn score following storage	Mean untransformed ELISA A_{405} values at harvest	
	Field experiment	**Gauzehouse experiment**
Tip burn absent		
0	0.186	0.037
Tip burn present		
0.5	0.104	0.166
1.0	0.160	0.205
1.5	0.350	0.147
2.0	0.229	0.415
2.5	0.217	0.017
3.0	0.355	0.518

Objective 4: Efficacy of insecticide treatments in reducing BWYV and tip burn

The insecticide Gaucho was applied as a seed treatment. Low levels of phytotoxicity have previously been indicated for imidoclоprid (the active ingredient of Gaucho), consequently seedling emergence was monitored for the Gaucho treated seed and compared with the untreated seed. (Table 5). The results showed no apparent effect of Gaucho seed treatment on seedling emergence.

Table 5. Emergence counts for Gaucho treated and untreated seed of white cabbage cv. Impala.

Treatment	Number of seed sown	Number of seedlings	Emergence (%)
Gaucho	308	292	95%
Untreated	1848	1773	96%

The insecticide Aztec was applied as a spray at times indicated by monitoring of the numbers of plants infested with aphids in the Aztec treated plots. The first application (11 June) was made at the first appearance of aphids in the monitored plots (Fig 3). The second application was made three weeks later (4 July), since aphid numbers in the monitored plots were still increasing following the first application. A small sample of aphids were taken from the treated plots following the first application and tested for insecticide resistance. Three of the 8 aphids tested (37%) were found to be MACE (modified acetylcholinesterase) positive, a phenotype correlated with resistance to certain insecticides (Moores *et al.,* 1994). Following the second Aztec treatment, the populations of both species monitored (*Brevicoryne brassicae* and *Myzus persicae*) did fall off, this was most notable in the case of the *B. brassicae.* Around the same time, the numbers of aphids caught in the suction traps dropped off dramatically, indicating a reduction in numbers of flying aphids and hence migration into the plots. The final application (25 September) was made at the first re-appearance of aphids following the late summer population crash. In this case, Aztec application may have prevented the subsequent migration of aphids noted in the trap data from establishing in the crop.

Analysis of the head weight data collected at harvest for the field experiment indicated significantly smaller heads in the Aztec treatment, than in either the Gaucho treatments or the untreated controls (Table 6). There was no significant difference in head weight between the Gaucho treatment and the untreated heads. This could indicate a potential phytotoxic effect of Aztec (triazamate) toward cultivar Impala.

Table 6. Analysis of variance of head weight data recorded at harvest in the field experiment

Treatment	Mean head weight at harvest (kg)
Aztec	3.778 ***
Gaucho	4.419
Untreated	4.379
l.s.d. (5%)	0.142

*** indicates difference from untreated control at 0.1% level of significance

Incidence of BWYV (detected by ELISA) was not significantly different between the untreated plots and the two insecticide treatments (Table 7). Statistical analysis of the ELISA values from infected plants 1 week prior to harvest however, showed significantly lower levels of BWYV in both the Aztec and Gaucho treatments compared to the untreated controls. There was no significant difference between the levels of virus observed in the two insecticide treatments.

Table 7. Statistical analyses of data on the incidence of BWYV and the levels of virus detected by ELISA at harvest in cabbage heads from the field experiment

Treatment	Mean percentage of BWYV infected plants per plot	Mean transformed ELISA values at harvest
Aztec	94.5	1.236 *
Gaucho	94.7	1.228 *
Untreated	96.7	1.333
l.s.d. (5%)	6.3	0.095

* indicates difference from untreated control at 5% level of significance. The incidence data was analysed using regression analysis, the ELISA data was analysed by ANOVA. The formula $\log_{10}$ (100[data]) was used to transform the ELISA data for statistical analysis

Despite differences observed between virus levels (as determined by ELISA) in insecticide treated cabbage and untreated cabbage (Table 7) there were no differences in subsequent tip burn incidence or severity (Table 8). Regression analyses for data on the incidence and severity of tip burn in the field experiment (Table 8) showed no statistically significant differences either between insecticide treatments or between the treatments and the untreated control. If a threshold level of virus infection is required to induce tip burn symptoms, these results indicate that such a threshold was reached in a similar proportion of all plants irrespective of insecticide treatment. This experiment indicates that the insecticide treatments Aztec and Gaucho are not effective at controlling BWYV and hence, BWYV-induced tip burn despite some apparent effect on virus levels within plants. Some significant reductions of BWYV incidence in winter oilseed rape crops with the pyrethroid insecticide lambda-cyhalothrin have been reported previously Walsh *et al.* (1989).

Table 8. Regression analyses of data on the incidence and severity of tip burn detected after storage of cabbage heads from the field experiment

Treatment	Mean percentage of plants per plot showing tip burn	Mean tip burn score
Aztec	59.1	0.752
Gaucho	50.4	0.724
Untreated	59.1	0.737
l.s.d. (5%)	18.1	0.229

Of the three other viruses being monitored, the overall incidence of CaMV was 19.7%. Neither Aztec or Gaucho treatments significantly reduced the incidence of CaMV (Table 9). TuMV and TYMV were not detected. Inoculation to TGM plants for CaMV detection also revealed two plants infected with broccoli necrotic yellows virus.

Table 9. Comparison of mean percentage incidence of CaMV in treated and untreated plots from the field experiment

Treatment	Mean percentage of CaMV infected plants per plot
Aztec	13.5
Gaucho	20.0
Untreated	25.8
l.s.d. (5%)	18.9

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TECHNOLOGY TRANSFER

The following TT actions were agreed by the Consortium.

Events

Dr D Gray outlined the project objectives and background information on cabbage disorders at a Grower walk at Kirton in September 1999.

Dr C Hole made a presentation of the project on the HDC MAFF stand exhibiting HORTLINK Projects at Vegex on 13 September 2000.
Dr J Walsh was invited to make a presentation on the project at the *Brassica* Conference on 16 January 2002 organised by HDC/HRA/BGA.

Talk by Dr J Walsh at the Brassica Growers Association/HDC/HRI sponsored meeting (2001) HRI-Wellebourne

Publications

The project was featured in Agriculture Link July 1999.

Hunter P. J., Jones J. E. and Walsh J. A. (2002). Involvement of *Beet western yellows virus, Cauliflower mosaic virus* and *Turnip mosaic virus* in internal disorders of stored white cabbage. Phytopathology 92: 816-826 (this paper was featured on the front cover of this major international journal).

A HortLINK leaflet on the project has been produced

Laminated photographs of the disorders in cabbage heads were provided to all members of the Consortium early in the project at their request. The Processers used this to inform process staff and the others used them to provide information to their grower clients and as a reference source.

A fully illustrated article summarising the results of the project was published in issue No. 93 of HDC News in June 2003 and was also featured on the cover.

An article for the popular press entitled 'Sunken and Disorderly' will be written by John Walsh and Paul Hunter this year.

A HDC Factsheet entitled 'White cabbage: reducing losses from internal disorders' will be produced by John Walsh, Paul Hunter and Ross Newham this year.

A summary of the Final Report will be linked to the HDC website

Other

The UK branches / agencies of seed companies will communicate the findings of the project to the plant breeders and raise awareness of 'cigar burn' and tip burn in the companies and breeding programmes.

HRI has provided breeders with virus isolates and diagnostic materials (antisera) for resistance work.

FUTURE RESEARCH REQUIREMENTS

From the research carried out during the original project and this extension a number of important future research priorities have been identified relating to virus infections of white cabbage:

- **Examine the relative resistance / susceptibility of different white cabbage cultivars and breeding lines to BWYV-induced tip burn and TuMV-induced cigar burn**

- **Further investigations to develop pre-harvest predictive tests for tip burn and cigar burn**

- **The need to evaluate predictive tests for tip burn and cigar burn in commercial crops including an evaluation of pre-harvest detection of TuMV by PTA ELISA to determine its potential to predict subsequent levels of cigar burn**

- **Development of integrated control strategies for BWYV, TuMV and CaMV**

- **Develop pre-harvest detection methods for CaMV which in combination with BWYV and TuMV detection give accurate prediction of the subsequent exacerbation of cigar burn**

EXPLOITATION PLAN

Having identified the causes of tip burn and cigar burn, the results of the project will be exploited over a period of time and the benefits will be incremental.

- The UK branches / agencies of seed companies will communicate the findings of the project to the plant breeders and raise awareness of 'cigar burn' and tip burn in the companies and breeding programmes.
- Now the 'cigar burn' and tip burn symptoms can be reproduced reliably at HRI, HRI will be able to offer resistance testing services to the industry. HRI will enter in to a dialogue with breeders about the evaluation of breeding lines and exploitation of the resistance test.
- Similar services are available at HRI for testing the relative susceptibilities of cabbage cultivars to 'cigar burn' and tip burn. HRI will enter in to a dialogue with the industry about evaluating current cultivars.
- A diagnostic test for TuMV is available that gave an approximately 70% reliable prediction of the possibility of cigar burn symptoms developing in infected cabbage heads. This needs evaluating / examining under field conditions.
- An integrated control programme for BWYV and tip burn is needed, currently the most likely components of such a programme would be natural plant resistance and aphid monitoring with testing of aphids for BWYV in order to inform when insecticide(s) could be used most effectively. Funding from DEFRA and HDC will needed for these aspects of BWYV control; HRI will submit concept notes to DEFRA and HDC.

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