Involvement of *Beet western yellows virus*, *Cauliflower mosaic virus*, and *Turnip mosaic virus* in Internal Disorders of Stored White Cabbage

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Accepted for publication 22 March 2002.

ABSTRACT

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.

Experiments over two growing seasons clearly showed that *Turnip mosaic virus* (TuMV) infection was associated with internal necrosis (sunken necrotic spots 5 to 10 mm in diameter) and *Beet western yellows virus* (BWYV) infection was associated with collapse of leaf tissue at the margins (tipburn) in heads of stored white cabbage (*Brassica oleracea* var. *capitata*). Virtually no tipburn was seen in cv. Polinius, whereas cv. Impala was affected severely. Internal necrotic spots were seen in both cultivars. BWYV appeared to interact with TuMV. Plants infected with both viruses showed a lower incidence of external symptoms and had less

internal necrosis than plants infected with TuMV alone. *Cauliflower mosaic virus* (CaMV) did not induce significant amounts of internal necrosis or tipburn, but did, in most cases, exacerbate symptoms caused by TuMV and BWYV. BWYV-induced tipburn worsened significantly during storage. Post-transplanting inoculation with TuMV induced more internal necrosis than pre-transplant inoculation. There was a significant association between detection of TuMV just prior to harvest and subsequent development of internal necrotic spots. Individually, all three viruses significantly reduced the yield of cv. Polinius, whereas only BWYV and CaMV treatments reduced the yield of cv. Impala.

Additional keywords: cultivar reaction, dual infections, epidemiology, internal necrotic spots, time of infection, virus-virus interactions, yields.

Dutch white cabbage (*Brassica oleracea* var. *capitata*) is grown worldwide. In Europe, certain cultivars are grown specifically for processing; in the United Kingdom, most of the cabbage is processed for coleslaw. The crop is grown over approximately 8 months, after which time some heads are processed at harvest whilst the majority are placed into cold storage for a further period of up to 8 months. This represents a significant investment of capital, time, and resources by the grower over a period of 16 months.

One of the major problems facing growers of white cabbage is the development of internal disorders during storage of heads that appear healthy at harvest. The two most common forms of disorder currently facing the industry in the United Kingdom are the presence of necrotic spots 5 to 10 mm in diameter on leaves throughout the head (known as "cigar burn" by some growers) (36) and collapse or discoloration of tissue at the leaf margins ("tipburn") throughout the head. Due to the problems of identifying affected cabbage heads and the increased automation of processing, the most cost-effective quality control measure for cabbage processors is to reject entire batches of heads where disorders are found. This has caused considerable losses to growers and the industry. In the United Kingdom, average annual losses due to internal disorders are estimated to be 10% of stored material, with considerably higher losses recorded in some years (2). Severe losses due to internal necrotic disorders were experienced in the

United Kingdom in the 1970s (33), 1980s, and 1990s (36). Increased incidences of leaf tissue collapse or tipburn appears to be a recent phenomenon in cabbage in the United Kingdom. It is also reported to be a common problem in cabbage in the Netherlands (19) and parts of the United States (7).

The presence of internal necrotic spots in cabbage heads has been associated with *Turnip mosaic virus* (TuMV) (32,33,36) and *Cauliflower mosaic virus* (CaMV) (18,30,36). Both viruses are transmitted by aphids and were reported to have been isolated from necrotic spots in the outer leaves of field-grown cabbage (33). However, TuMV appeared to be associated specifically with the necrotic spots, whereas CaMV was distributed uniformly throughout the infected leaves (33). Development of internal necrotic spots in heads of stored cabbage was associated with TuMV infection rather than CaMV, although mixed infections involving both viruses did produce symptoms (33). These mixed infections produced the most severe external symptoms in the cabbages. In another study, CaMV was isolated from 53% of cabbage heads with internal necrotic spots, compared with isolation of TuMV from 4% of the heads, although the author warned that this may have been due to the relative efficiency of recovering these viruses from infected leaf material, rather than their relative importance in the disorder (36). There has been some confusion between the presence of small necrotic (≈ 1 mm) lesions on cabbage leaves and virus-induced necrotic spots. The smaller lesions are known as pepper spot (6) or black speck (10) and are considered to be physiological in origin and affected by growth rate.

Hitherto, tipburn was considered to be a physiological disorder with no suggestion of viral etiology. The appearance of symptoms is reported to alter as it progresses, beginning with the tissue at the leaf tip becoming thin and almost translucent, progressing through a gray or brown color to darker necrosis as the symptom develops (19). Tipburn is considered to be induced by many different sorts of stress (26) and may be due to insufficient calcium reaching the

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*The e-Xtra logo stands for "electronic extra" and indicates the HTML abstract available on-line contains supplemental material not included in the print edition. Diagrammatic representation of gauzehouse experimental designs for the 1998–1999 and 1999–2000 experiments available online.

Publication no. P-2002-0603-01R

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growing leaf tips inside the head because of the reduced transpiration capacity of the enclosed leaves (20). Sufficient calcium to prevent the development of tipburn symptoms can be delivered to the head when pressure gradients allow mass flow of water from roots and the base of the head (20,41). As a consequence, factors that affect water potential gradients, such as soil moisture, soil salt and oxygen content, and soil temperature, have been associated with tipburn (20). Nutritional factors, including levels of nitrogen (22,23) and potassium (7), also have been associated with the development of tipburn symptoms in cabbage. Boron levels have been associated with tipburn in Chinese cabbage (*B. rapa* subsp. *pekinensis*) (17). Similar physiological causes for tipburn have been demonstrated in other brassica crops (9,16,25) and lettuce (4).

Beet western yellows virus (BWYV) has not been shown to be associated with internal disorders of white cabbage. High incidences of BWYV were recorded in the United Kingdom in 1985 (38), 1992, and 1993 (11). BWYV was the most prevalent virus disease of oilseed rape, with approximately 4.5 times higher incidence than CaMV (the next most prevalent virus) in 1992 and 1.5 times higher incidence in 1993 (11). Alternative names of *Turnip yellows virus* (TuYV) (27), *Brassica yellows virus* (BrYV) (13), and *Brassica yellowing virus* (BrYV) (12) have recently been proposed for BWYV.

Following the severe outbreaks of internal necrosis in white cabbage in the United Kingdom between 1970 and 1980, quantitative partial resistance to TuMV was identified in some white cabbage cultivars (32) and introduced into commercial breeding programs (37). Since this earlier work, there have been no detailed tests on newer cultivars of white cabbage. The objective of this study was to evaluate the effect of TuMV, CaMV, and BWYV on white cabbage in single and dual infections and to clarify their relative importance in the etiology of internal disorders of white cabbage.

MATERIALS AND METHODS

Virus maintenance and experimental inoculations. Cultures of the TuMV isolate UK 2 (33,35) and CaMV isolate UK 4 (isolated from white cabbage growing in Lincolnshire, UK in 1993) were maintained in plants of *B. juncea* (cv. Tendergreen). The culture of BWYV (21) was maintained in oilseed rape (*B. napus* subsp. *oleifera*) plants (cv. Mikado).

TuMV and CaMV were inoculated according to the method of Walsh and Tomlinson (40) except that inocula were not filtered. Cabbage plants were inoculated pre-transplanting at the 3- to 4-true-leaf stage. Plants also were infected pre-transplanting at the 2- to 3-true-leaf stage with BWYV using viruliferous aphids (*Myzus persicae*). Aphids were cultured on BWYV-infected rape plants for a minimum of 3 weeks to allow virus acquisition. Detached rape leaves with aphids were then placed on the young cabbage plants. The aphids were left to feed for 5 days on the cabbage plants to allow virus transmission. They were then killed by an application of insecticide (Decis at 0.7 ml liter⁻¹ [Aventis Crop Science UK Ltd., Ongar, UK] in 1998 or Dovetail at 1 ml liter⁻¹ [Zeneca Crop Protection, Haslemere, UK] in 1999). BWYV inoculations were performed in separate small metal-framed tunnels covered with gauze (gauzehouses) and separation maintained between BWYV, BWYV + TuMV, BWYV + CaMV, and all the other virus treatments and uninfected control plants until transplanting into the main gauzehouses. In the 1998–99 experiment, all plants were infected at the 3- to 4-true-leaf stage on 6 to 14 May 1998 prior to transplanting. In the 1999–2000 experiment, plants were infected prior to transplanting (4 to 12 May 1999) with all viruses. Some plants were infected later (23 June 1999) with TuMV and CaMV only, while growing in the main gauzehouses. In both years, the uninoculated control plants were mock inoculated with inoculation buffer.

Experimental design. Experiments were carried out in four identical, insect-proof gauzehouses designed to exclude aphids and located at Horticulture Research International (Wellesbourne, Warwick, UK). Prior to the start of each experiment, the gauzehouses were steam sterilized and then fertilized with ammonium nitrate at the rate of 171 kg ha⁻¹ in houses 2 and 3 and at the rate of 85 kg ha⁻¹ in houses 1 and 4 in 1998. In 1999, applications were made at 90 kg ha⁻¹ in house 1 and 181 kg ha⁻¹ in houses 2, 3, and 4. The fertilization (based on soil analysis) brought soil nitrogen levels up to 180 kg ha⁻¹ (1) in all houses. A top dressing of ammonium nitrate at 215 kg ha⁻¹ was applied to all houses in June of both years.

Each year, seeds of two F₁ hybrid cultivars of Dutch white cabbage, Impala and Polinius (Bejo Zaden B.V., Warmenhuizen, Netherlands), bred for processing, were sown in March in Levington F2 compost plugs in 14-by-22-cell modular trays and germinated under glass. Seedlings were moved into small gauzehouses at the first true-leaf stage and acclimatized to outdoor conditions for approximately 6 weeks, during which period pre-transplanting inoculations of viruses were performed. Seedlings then were transplanted into the gauzehouses with a 60-cm spacing within and between rows. In each house there were seven rows of 20 plants (140 plants per house, four houses, 560 plants in total) in both years. Houses were irrigated at regular intervals depending upon prevailing conditions using a seep-hose system.

In the 1998–99 experiment, there were four plots per house, each of 35 heads (seven rows with five plants per row). Each plot was planted with uninoculated cabbage or cabbage that had previously been inoculated with BWYV, CaMV, TuMV, BWYV + CaMV, BWYV + TuMV, or TuMV + CaMV. The experiment contained two replicate plots of each virus treatment and four replicate plots of the uninoculated control treatment (one in each gauzehouse). Each plot was planted with alternate rows of the two cultivars. The most easterly row of each plot was not assigned a storage regime and was assessed for internal disorders at harvest. Equal numbers of each cultivar and treatment were planted in the easterly rows. Each row of the remaining six rows was assigned to a different storage regime (long or short term [7 or 4 months, respectively] in a sealed store or in an unsealed store [5 months]). The positions of the cultivars and storage regime assignments within each plot and the position of the treatments within each of the four gauzehouses were randomized.

The 1998–99 experiment confirmed earlier work (32), showing that TuMV induced internal necrotic spots in cv. Polinius; therefore, only cv. Impala was used in the 1999–2000 experiment (a single row of 20 plants of Polinius was included as a positive control for TuMV-induced necrosis). The row of Polinius divided the gauzehouse into two sections, one of four rows and one of two rows. Each section was divided into two blocks (either four rows by 10 plants or two rows by 10 plants). Different treatments were allocated to plots of five adjacent plants within a row (a total of eight different plots in the larger blocks and four in the smaller blocks). Plants for the larger blocks were inoculated pre-transplanting with the same treatments as in the 1998–99 experiment (six virus treatments and two uninoculated control replicates). Plants in the smaller blocks were uninoculated at transplanting; subsequently, one-quarter were left as controls and the remainder were inoculated 5 weeks after transplanting (23 June) on the three youngest fully expanded leaves, with CaMV, TuMV, or TuMV + CaMV.

Each row of five plants (one treatment replicate) was assigned to either short- or long-term storage. The position of the blocks, the positions of the plots (treatments) within each block, and the storage assignments were randomized.

Pesticide applications. Insecticides were applied to the plants from the commencement of acclimatization until harvest. The treatments comprised alternate weekly applications of Ambush at 62 ml liter⁻¹ and Aphox at 0.5 g liter⁻¹ (Zeneca Crop Protection,

Haslemere, UK) in the 1998–99 experiment, and Dovetail at 2.5 ml liter⁻¹ and Toppel 10 at 0.62 ml liter⁻¹ (United Phosphorus Ltd., Warrington, UK) in the 1999–2000 experiment. Sprays of Nuvan (Novartis Crop Protection UK Ltd., Cambridge, UK) were applied at intervals of 4 weeks to control thrips. In addition, a pre-planting drench of Dursban at 4 ml liter⁻¹ (Dow AgroSciences, Hitchin, UK) and Spannit granules (pbi Agrochemicals Ltd., SumiAgro [UK] Ltd., Epping, UK) at transplanting (0.2 g per plant) were applied to control any cabbage root fly. The fungicide Fubol 58W (Novartis Crop Protection) was applied at 2 g liter⁻¹ in June to prevent white blister.

Recording of external symptoms. External symptoms on cabbage plants were recorded at weekly intervals throughout the growing season from transplanting until harvest. The dates on which plants first developed external symptoms were recorded.

Harvesting and storage. Heads were harvested in early November in both years. Following industry protocols, heads were cut to leave 2.5 cm of stalk and the outer leaves trimmed. The heads were weighed and placed in mesh bags (five heads per bag labeled with the cultivar, virus treatment, and storage treatment). Any heads that were in very poor condition at harvest due to virus infection or secondary fungal (*Botrytis* spp.) infection were discarded and not stored. Discarded heads were recorded as zero head weight because they would have been commercially unacceptable. Bagged heads were sprayed to run-off with fungicides (Ridomil MBC 60WP [Novartis Crop Protection] and Rovral WP [Aventis Crop Science UK Ltd.] at 1 g liter⁻¹ for each compound) following commercial protocols. Cabbage heads were stored in two commercial cold stores near Kirton, Lincolnshire, UK, one atmospherically sealed and the other not. The latter, unsealed store was used only in the 1998–99 experiment. Heads were monitored occasionally throughout the storage period to check for excessive deterioration.

Virus testing. Samples for virus detection were taken from plants 1 week prior to harvest and after storage. Enzyme-linked immunosorbent assay (ELISA) tests were carried out for BWYV and TuMV on fresh leaves from growing cabbage and leaves from stored heads. Double-antibody sandwich (DAS)-ELISA (3) failed to detect CaMV from inoculated cabbage, but did detect CaMV in the infected propagation host (*Brassica juncea*). Consequently, this virus was detected by inoculation to the susceptible host plant (*B. juncea*, cv. Tendergreen) followed by ELISA testing of the *B. juncea* 28 days postinoculation.

Detection of BWYV was performed using a triple-antibody sandwich (TAS)-ELISA protocol (8). The coating globulin was as described previously (38). The secondary antibodies used were monoclonal antibodies (MAbs) BWYV-510H (8) in the 1998–99 experiment and MAFF 24 (28) in the 1999–2000 experiment. The alkaline phosphatase conjugates (goat anti-mouse in 1998–99 and

goat anti-rat in 1999–2000) and substrate were from a commercial source (Sigma-Aldrich Company Ltd., Poole, UK). Detection of TuMV in the 1998–99 experiment was by the plate-trapped antigen (PTA) method as described by Jenner and Walsh (15), except that sap was extracted by grinding tissue under liquid nitrogen and was diluted 1:1 rather than 1:4. In the 1999–2000 experiment, TAS-ELISA was used. Plates were coated with a rabbit polyclonal antiserum to TuMV, samples were macerated between two steel rollers (Polhane, Germany), the secondary antiserum was the MAb EMA 67 (14), and the tertiary antibody (goat anti-mouse)-alkaline phosphatase conjugate and substrate were from a commercial source (Sigma-Aldrich Ltd.).

Assessment for internal disorders. Heads from the most easterly row of each plot in the 1998–99 experiment were cut open and assessed for internal disorders at harvest. Heads were removed from the atmospherically sealed store after 4 and 7 months in the 1998–99 and 1999–2000 experiments. Heads were removed from the unsealed store after 5 months rather than the intended 7 months due to deterioration caused by secondary fungal (*Botrytis* spp.) infection in the 1998–99 experiment.

To assess the incidence and severity of internal necrosis and tipburn symptoms, heads were cut into quarters longitudinally. The internal leaves of two diagonally opposite quarters were peeled off one by one, and assessed for the presence and severity of either symptom on scales of 0 to 3 (Table 1).

Statistical analyses. Mean scores for severity of internal necrosis and tipburn reflected both the incidence and severity of the symptoms. A_{405} values from ELISA tests for levels of TuMV and BWYV in cabbage plants and stored heads were transformed using the formula $\ln[100(A_{405} + 0.065)]$ to eliminate negative values. The data were analyzed using analysis of variance where possible. Where unequal sample sizes were compared (e.g., due to loss of cabbage heads through rotting) residual maximum likelihood (REML) or regression analyses were used. For the symptom score data from the 1998–99 experiment, the large number of 0 scores skewed the distribution of the data, preventing analysis of variance or REML; hence, Mann-Whitney U tests were performed. Analyses were done using the Genstat 5 release 3 1993 program (Committee of the Statistics Department Rothamsted Experimental Station).

RESULTS

Leaf symptoms. Two types of symptom were observed during the growth of the plants: vein clearing (which progressed to interveinal chlorosis as plants matured) and necrotic spotting. Vein clearing or chlorotic symptoms were clearly associated with CaMV inoculation, while necrotic spotting was clearly associated with TuMV inoculation. Some plants inoculated with both CaMV and TuMV showed both symptoms. BWYV-inoculated plants showed few symptoms (vein-clearing type) that were very mild.

Combined BWYV + TuMV inoculations (Fig. 1B and D) resulted in lower incidences of leaf symptoms than with TuMV alone (Fig. 1A and C) in both experiments. Combined BWYV + CaMV and TuMV + CaMV inoculations (Fig. 1B and D) induced more rapid appearance of leaf symptoms, compared with the respective single virus treatments (Fig. 1A and C) in both experiments. No leaf symptoms were observed in control plants prior to harvest.

Head yield. In the 1998–99 experiment, mean trimmed head weight yields of cv. Polinius at harvest (Table 2) showed significant reductions (16 to 63%) for all virus treatments compared with the uninoculated control; the yield reductions were significantly greater in the BWYV + CaMV and the TuMV + CaMV treatments than in the other virus treatments. Mean trimmed head weight yields of cv. Impala at harvest were not significantly affected by inoculation with TuMV alone or in combination with BWYV in both years. BWYV alone, CaMV alone, and dual inoculation of

TABLE 1. Symptom severity score descriptions for internal necrosis and internal tipburn symptoms in cabbage heads

Score	Symptom severity
Internal necrotic spots	
0	No symptoms
1	>One spot on one leaf to a few spots on <25% of leaves
2	Spots on >25% of leaves, or severe spots on >10% of leaves
3	Spots on >50% of leaves, or severe spots on >25% of leaves
Internal tipburn	
0	No symptoms
1	>0 to 5% of leaves with symptoms
2	5 to 10% of leaves with symptoms or >0 to 5% of leaves with severe symptoms
3	>10% of leaves with symptoms or 5 to 10% of leaves with severe symptoms

Fig. 1. Percentage incidences of external symptoms recorded for A, single virus inoculations and B, mixed virus inoculations in the 1998–1999 experiment and C, single virus inoculations and D, mixed virus inoculations in the 1999–2000 experiment.

these two viruses significantly reduced the yield of heads of both cultivars in both years.

In the 1999–2000 experiment (cv. Impala only), there were significant effects of both treatment and inoculation time. Following pre-transplanting inoculation, all CaMV treatments (CaMV alone and in combination with TuMV or BWYV) and BWYV alone induced significant yield losses (22 to 41%). Post-transplanting inoculations of CaMV and CaMV with TuMV significantly reduced yield, while the TuMV single virus treatment did not. Yield losses recorded for the CaMV and TuMV + CaMV treatments were significantly greater for plants inoculated later (63 to 76%) than for those inoculated pre-transplanting (23 to 34%).

Internal disorders. In the 1998–99 experiment, of the plants assessed at harvest, 27% of those inoculated with TuMV showed internal necrotic spots (5 to 10 mm in diameter) (Fig. 2), while only 2% inoculated with virus other than TuMV had such symptoms. If the TuMV + BWYV dual treatment (which showed no symptoms) is excluded, 40% of plants inoculated with TuMV showed internal necrosis. Necrotic spots appeared to be more frequent in cv. Polinius than in cv. Impala.

Examination of the mean symptom scores in the 1998–99 experiment revealed some consistent trends. The Mann-Whitney U test showed significantly ($P < 0.001$) higher symptom scores for internal necrotic spots in treatments including TuMV than in treatments without TuMV. Scores for internal necrotic spots (Table 3) were higher in cv. Polinius than cv. Impala ($P = 0.006$, Mann-Whitney U test). Much less internal necrosis was seen in plants inoculated with BWYV + TuMV. Single infections of CaMV

or BWYV induced no internal necrotic spots in cv. Polinius and very few in cv. Impala.

There was a marked difference for internal tipburn (collapse of leaf tissue at the apex) (Fig. 3) between cv. Impala and cv. Polinius in the 1998–99 experiment, with virtually no symptoms in cv. Polinius (Table 3). The highest mean symptom scores for tipburn in cv. Impala were associated with treatments involving BWYV after both sealed and unsealed storage. After 4 months in the sealed store, the highest mean score for tipburn was recorded in the BWYV + CaMV treatment. After 7 months in the sealed store, the highest mean score for tipburn symptoms in cv. Impala was recorded for the BWYV + TuMV treatment, followed by BWYV by itself and then BWYV + CaMV. In the sealed store, mean tipburn symptoms were higher at 7 than at 4 months (Table 3).

In the 1999–2000 experiment, there were significant effects of both virus treatment and inoculation time on severity of internal necrotic spots in cv. Impala. All significant scores for internal necrotic spots were associated with treatments involving TuMV (Table 4). There were no significant differences between any of the virus treatments for the plants inoculated in May after 4 months in storage. After 7 months in storage, a significant level of internal necrotic spots was detected in the TuMV single virus treatment. In the plants inoculated in June and stored for 4 months, significant levels of internal necrotic spots were recorded in the TuMV and TuMV + CaMV treatments. The mean symptom score for the TuMV + CaMV treatment was significantly higher than that for TuMV alone. For plants inoculated in June and stored for 7 months, significant levels of internal necrotic spots were seen only in those inoculated with TuMV + CaMV. Heads from plants inoculated in June with TuMV + CaMV (after 4 and 7 months in storage) and with TuMV alone (after 4 but not after 7 months in storage) had more severe symptoms (internal necrotic spots) than heads from plants that were inoculated in May with these virus combinations. After 7 months in store, heads from plants that had been inoculated in May with TuMV alone showed more severe internal necrotic spots than those inoculated in June.

Without exception in the 1999–2000 experiment, all significant levels of tipburn were associated with BWYV treatments (Table 4); analysis for cv. Impala indicated significant effects of treatment on the severity of internal tipburn symptoms compared with the uninoculated control. Analysis of the data also showed a significant difference between the treatments for internal tipburn symptoms. The severity of tipburn symptoms in the BWYV + CaMV treatment after heads had been stored for 4 months was significantly higher than in the controls. After 7 months in storage, all of the treatments involving BWYV had significant levels of tipburn. Significantly higher levels of tipburn (after 4 and 7 months in storage) were seen in the combined BWYV + CaMV inoculation than in other BWYV treatments. A significant increase ($P < 0.01$) in tipburn between 4 and 7 months in storage was detected for the BWYV single virus treatment (data not shown).

Levels of virus detected in plants prior to harvest and in heads after storage. In ELISA tests carried out 1 week prior to harvest, TuMV was detected in 67% (1998–99 experiment, PTA ELISA) and 75% (1999–2000 experiment, TAS-ELISA) of the cabbage heads that developed internal necrotic spots. In contrast, TuMV was detected in only 5% (1998–99) and 24% (1999–2000) of cabbage plants that did not develop internal necrotic spots. There was a significant association (χ^2 tests were significant at $P < 0.001$) between TuMV detection prior to harvest and the development of internal necrotic spots during storage. When tested 1 week prior to harvest, there were significantly higher levels of TuMV in cv. Polinius than in cv. Impala for all TuMV treatments (Table 5) (data not shown). No TuMV was detected in uninoculated control plants prior to harvest or in heads from these plants after storage.

BWYV was detected by ELISA 1 week prior to harvest in 95% of BWYV-inoculated cv. Impala plants that subsequently develop-

TABLE 2. Yield data (mean trimmed head weight at harvest) for the 1998–1999 and 1999–2000 experiments with *Beet western yellows virus* (BWYV), *Cauliflower mosaic virus* (CaMV), and *Turnip mosaic virus* (TuMV)

Cultivar, date ^a , treatment	Mean head weight, no. of reps			
	1998–1999 ^b		1999–2000 ^c	
	Weight (kg) ^d	Reps	Weight (kg) ^d	Reps
Polinius, May				
BWYV	3.423*	6	...	...
BWYV + CaMV	1.525***	6	...	...
BWYV + TuMV	3.143**	6	...	...
CaMV	3.215**	6	...	...
TuMV + CaMV	1.627***	6	...	...
TuMV	3.382*	6	...	...
Uninoculated	4.069	12	...	...
Control vs. treatment ^e	0.510	...	...	...
Treatment vs. treatment ^e	0.588	...	...	...
Impala, May				
BWYV	2.763*	6	2.907*	8
BWYV + CaMV	2.767*	6	2.177***	8
BWYV + TuMV	2.925	6	3.229	8
CaMV	2.753*	6	2.430***	8
TuMV + CaMV	2.897	6	2.852*	8
TuMV	3.477	6	3.354	8
Uninoculated	3.532	12	3.702	16
Control vs. treatment ^e	0.641	...	0.651	...
Treatment vs. treatment ^e	0.741	...	0.752	...
Impala, June				
CaMV	...	...	0.867***	8
TuMV + CaMV	...	...	1.379***	8
TuMV	...	...	3.582	8
Uninoculated	...	...	3.677	8
Control vs. treatment ^e	...	...	0.752	...
Treatment vs. treatment ^e	...	...	0.752	...

^a Date of inoculation: May inoculations were carried out pre-transplanting and June inoculations were carried out post-transplanting.

^b Significance tested using analysis of variance.

^c Significance tested using residual maximum likelihood and regression analyses.

^d *, **, and *** indicate significantly different at $P = 5$, 1, and 0.1%, respectively, from appropriate uninoculated control.

^e Least significant difference at $P \leq 0.05$.

ed tipburn symptoms in both years and 86% of cv. Impala plants inoculated with BWYV that did not develop tipburn. There was no clear association between BWYV levels detected and the severity of tipburn symptoms. No consistent differences between the mean levels of BWYV were detected between the two cultivars (Table 6). No BWYV was detected in uninoculated control plants prior to harvest or in heads from these plants after storage. The levels of BWYV detected increased significantly ($P < 0.01$) between 4 and 7 months in storage for all treatments involving BWYV in both cultivars in the 1998–99 experiment, and for the BWYV + TuMV treatment ($P < 0.001$) in the 1999–2000 experiment (data not shown).

CaMV was detected from 55% of heads co-inoculated with TuMV that developed internal necrotic spots and 46% of heads co-inoculated with TuMV that did not develop necrotic spots. It was detected from 62% of heads co-inoculated with BWYV that developed tipburn symptoms and 56% of heads co-inoculated with BWYV that did not develop tipburn. Detection levels of CaMV prior to harvest in plants inoculated with this virus ranged from 50 to 100%; whereas, after storage, detection rates ranged from 0 to 100% (Table 7).

DISCUSSION

There was a very clear association between BWYV inoculation and the presence of tipburn symptoms in heads of cv. Impala but not cv. Polinius. The experiments are the first to investigate the effect of BWYV infections on internal symptoms in white cabbage and provide the first evidence of a virus being associated with tipburn, which was previously believed to be physiological in nature (26). There was a clear effect of duration of storage on tipburn symptoms in cv. Impala. In both experiments, symptom severity was higher after 7 months storage than after 4 months, indicating that the symptoms continued to develop during the stor-

age period. CaMV appeared to exacerbate BWYV-induced tipburn. We chose BWYV and CaMV for these experiments because they have been found to be the most common viruses infecting oilseed rape in the United Kingdom (11). Oilseed rape is now grown widely in the United Kingdom and the most common type, winter rape (planted in August to September and harvested in July to August of the following year) provides a vast overwintering reservoir for brassica viruses and their aphid vectors (34,40). The similarity between BWYV-induced tipburn and what is reported to be purely physiologically induced tipburn suggests that BWYV may act in a manner that induces the same physiological changes in the plant tissue (i.e., possibly affecting calcium movement). Calcium movement in plants is restricted to the xylem and is dependent on water flux. BWYV is known only to be phloem restricted, therefore, infection may affect nutrient flow in cabbage heads. The mechanism by which this could occur is at present unclear. Although tipburn is generally considered to be a calcium-related disorder, it has been claimed that other stresses may be important (26).

Although losses caused by tipburn vary dramatically from year to year, the past 6 to 8 years have seen increased losses due to tipburn in a number of white cabbage cultivars in the United Kingdom compared with earlier times. This coincided with the introduction of cultivars of cabbage, such as Impala, with decreased susceptibility to TuMV. There are no records of severe losses from tipburn in the 1970s when there were very severe outbreaks of most aphid-borne brassica viruses. Also, the older cabbage cultivar (Polinius) shows no tipburn symptoms when infected by BWYV; conceivably, selection for resistance to TuMV may have inadvertently resulted in white cabbage cultivars with increased susceptibility to BWYV-induced tipburn. Similar scenarios have been experienced previously. In the 1970s, release of an F_1 hybrid Brussels sprout cultivar selected for high yield and uniformity resulted in very severe losses when it

Fig. 2. White cabbage cv. Impala that had been inoculated with *Turnip mosaic virus* (TuMV) pre-transplanting. The outer leaves have been removed to reveal the internal necrotic spots.

became apparent that the cultivar was extremely susceptible to TuMV (29).

There was a very clear association between TuMV infection and the presence of internal necrotic spots in both cultivars of cabbage investigated. The lack of necrosis in any control plants supports this association. The lack of detection of any of the viruses in any of the uninoculated control cabbage plants confirmed the integrity of the treatments and lack of cross-contamination. The incidences of internal necrotic spots (both years, both cultivars) (data not shown) for the TuMV (29%) and TuMV + CaMV (38%) treatments were much higher than for BWYV (1%), CaMV (1%), and BWYV + CaMV (13%), and the symptom scores for internal necrotic spots were higher in treatments including TuMV than in treatments without TuMV. These results confirm earlier work for the cv. Polinius (32).

Following the severe epidemics of TuMV in brassica crops in the United Kingdom in the 1970s (33), lines of white cabbage with resistance to TuMV-induced internal necrosis were identified (24). Some of these lines were incorporated into European breeding programs (37). Impala is a relatively new cultivar of white cabbage originating from such a resistance-breeding program. Results obtained in our 1998–99 experiment suggest that, although

Impala is susceptible to TuMV-induced internal necrosis, it is less susceptible than cv. Polinius. Results from ELISA testing of plants and heads (data not shown) suggest that TuMV accumulation is less in Impala than in Polinius.

Our data on the severity of internal necrotic symptoms have shown interactions between TuMV and CaMV and between TuMV and BWYV. CaMV appears to exacerbate TuMV-induced internal necrosis in cv. Polinius and possibly also in cv. Impala. Combined TuMV + CaMV inoculations induced a more rapid appearance of leaf symptoms during growth of the cabbage, compared with the respective single virus treatments in both experiments. The results from ELISA tests for TuMV in Polinius also may help to explain this effect, in that a significantly higher mean level was detected in plants inoculated with TuMV + CaMV than in plants inoculated with TuMV alone (data not shown). BWYV appears to have the opposite effect compared with CaMV regarding internal necrosis, in that lower levels occurred in plants inoculated with BWYV + TuMV than in plants inoculated with TuMV alone, in both cultivars. Combined BWYV + TuMV inoculations also resulted in lower incidences of leaf symptoms in the growing cabbage than with the TuMV single inoculation. BWYV by itself did not induce significant internal necrosis.

TABLE 3. Mean scores for the severity of internal necrotic spots and tipburn 1998–1999 following inoculation with *Beet western yellows virus* (BWYV), *Cauliflower mosaic virus* (CaMV), and *Turnip mosaic virus* (TuMV)

Cultivar, treatment ^a	Mean severity score, months in storage					
	Internal necrotic spots			Internal tipburn		
	4 months (sealed)	7 months (sealed)	5 months (unsealed)	4 months (sealed)	7 months (sealed)	5 months (unsealed)
Polinius						
BWYV	0	0	0	0	0	0
BWYV + CaMV	0.33	0.13	0.43	0	0.08	0
BWYV + TuMV	0.15	0	0.30	0.01	0	0
CaMV	0	0	0	0	0	0
TuMV + CaMV	1.42	1.08	1.43	0	0	0
TuMV	0.80	0.70	0.85	0	0	0
Uninoculated	0.03	0	0	0	0	0
Impala						
BWYV	0.05	0	0	0.20	1.80	0.61
BWYV + CaMV	0.15	0.10	0	0.60	1.45	0
BWYV + TuMV	0.20	0	0	0.10	2.55	0.15
CaMV	0	0.07	0	0.13	0.57	0.05
TuMV + CaMV	0.08	0.17	0	0.33	0.44	0.06
TuMV	0.50	0.45	0.50	0.20	0	0.15
Uninoculated	0.03	0	0	0.34	0.28	0.05

^a All virus inoculations were carried out pre-transplanting (May).

Fig. 3. White cabbage cv. Impala that had been inoculated with *Beet western yellows virus* (BWYV) pre-transplanting. The outer leaves have been removed to show the internal tipburn symptoms.

We were unable to confirm earlier work (18,30) showing that CaMV induced black necrotic spots in white cabbage. CaMV single virus infections were not associated with significant levels of internal necrosis, in agreement with other work in the United Kingdom (31). Raised pale brown lesions (3 to 8 mm in diameter) were observed at low incidence (6%) in the 1999–2000 experiment in plants from treatments involving CaMV. These were distinct from the sunken black necrotic spots associated with TuMV infection, but may be similar to those symptoms described and attributed to CaMV by van Hoof (30) and Natti (18).

There appeared to be little change in the severity of internal necrosis in cabbage heads between 4 and 7 months in storage. This indicates that the development of TuMV-induced internal necrosis in cabbage may be largely complete within the heads by 4 months post-harvest. Internal necrotic symptoms were seen at harvest in TuMV-inoculated heads of both cultivars (taken from the most easterly rows) in the 1998–99 experiment; therefore, it is clear that some internal symptoms developed prior to harvest. Although there was no adverse effect of using an unsealed store compared with a sealed store in terms of the severity of internal

TABLE 4. Mean scores for the severity of internal necrotic spots and tipburn 1999–2000 following inoculation of cv. Impala with *Beet western yellows virus* (BWYV), *Cauliflower mosaic virus* (CaMV), and *Turnip mosaic virus* (TuMV)

Inoculation time ^a , treatment	Mean severity score, months in storage ^b							
	Internal necrotic spots				Internal tipburn			
	4 months (sealed) ^c		7 months (sealed) ^d		4 months (sealed) ^c		7 months (sealed) ^d	
	Mean score	SEM	Mean score	SEM	Mean score	SEM	Mean score	SEM
May								
BWYV	0	±0.10	0.01	±0.21	0.04	±0.19	0.94***	±0.13
BWYV + CaMV	0.12	±0.10	0.01	±0.21	1.10***	±0.19	1.41***	±0.13
BWYV + TuMV	0.13	±0.10	0.16	±0.21	0.03	±0.19	0.48**	±0.13
CaMV	0	±0.10	0	±0.25	0.29	±0.17	0.14	±0.15
TuMV + CaMV	0.17	±0.13	0.15	±0.22	0.04	±0.19	0.15	±0.12
TuMV	0.18	±0.10	0.98***	±0.21	0	±0.19	0.06	±0.13
Uninoculated	0	±0.07	0.01	±0.15	0.02	±0.13	0.02	±0.09
June								
CaMV	0	±0.14	0.06	±0.22	0.15	±0.26	0.09	±0.13
TuMV + CaMV	1.51***	±0.10	0.83*	±0.25	0.22	±0.19	0	±0.15
TuMV	0.49***	±0.08	0	±0.25	0	±0.17	0.09	±0.15
Uninoculated	0	±0.10	0.01	±0.21	0	±0.19	0.06	±0.15

^a May inoculations were carried out pre-transplanting and June inoculations were post-transplanting.

^b *, **, and *** indicate significantly different at $P = 5$, 1, and 0.1%, respectively, from appropriate uninoculated control. SEM = standard error of the mean.

^c Significance tested using regression analysis.

^d Significance tested using residual maximum likelihood and regression analyses.

TABLE 5. Mean absorbance values (A_{405}) for *Turnip mosaic virus* (TuMV) enzyme-linked immunosorbent assay (ELISA) of leaves from cabbage plants and heads following inoculation with *Beet western yellows virus* (BWYV), *Cauliflower mosaic virus* (CaMV), and TuMV^a

Cultivar, date ^b , treatment	In storage							
	Prior to harvest		4 months (sealed)		7 months (sealed)		5 months (unsealed)	
	A_{405}	SEM	A_{405}	SEM	A_{405}	SEM	A_{405}	SEM
1998–1999 ^c								
Polinius, May								
TuMV + BWYV	3.58*	±0.21	1.54*	±0.08	1.81**	±0.15	0.33*	±0.16
TuMV + CaMV	4.16**	±0.21	1.77*	±0.17	2.89***	±0.39	0.67**	±0.34
TuMV	3.25*	±0.21	1.61*	±0.17	2.27**	±0.38	0.35*	±0.23
Uninoculated	2.19	±0.21	0.99	±0.23	0.58	±0.28	0.14	±0.23
Impala, May								
TuMV + BWYV	2.72	±0.21	1.50	±0.04	1.65**	±0.08	0.29**	±0.07
TuMV + CaMV	2.62	±0.21	1.51	±0.04	1.05*	±0.25	0.27**	±0.03
TuMV	3.14	±0.21	1.49	±0.09	1.04*	±0.29	0.28**	±0.04
Uninoculated	2.23	±0.21	1.25	±0.19	0.47	±0.19	0.12	±0
1999–2000 ^d								
Impala, May								
TuMV + BWYV	0.49	±0.21	0.31	±0.23	0.31	±0.30	...	...
TuMV + CaMV	0.57	±0.22	0.31	±0.26	0.29	±0.30	...	...
TuMV	0.79**	±0.21	0.42	±0.23	0.55*	±0.30	...	...
Uninoculated	0.37	±0.18	0.29	±0.14	0.23	±0.30	...	...
Impala, June								
TuMV + CaMV	1.07***	±0.22	1.50***	±0.24	1.09***	±0.27	...	...
TuMV	0.64*	±0.21	0.58*	±0.24	0.58	±0.27	...	...
Uninoculated	0.33	±0.22	0.29	±0.14	0.22	±0.23	...	...

^a A_{405} values were transformed using the formula $\ln[100(A_{405} + 0.065)]$ to eliminate negative values. *, **, and *** = significantly different at 5, 1, and 0.1% levels, respectively, from appropriate uninoculated control. SEM = standard error of the mean.

^b Date of inoculation: May inoculations were carried out pre-transplanting and June inoculations were carried out post-transplanting.

^c A_{405} values (plate-trapped antigen ELISA) for tests prior to harvest were analyzed using residual maximum likelihood and regression analyses, A_{405} values for tests following storage were analyzed by regression analysis.

^d A_{405} values (triple-antibody sandwich ELISA) for tests prior to harvest were analyzed using regression analysis, A_{405} values for tests following storage were analyzed by residual maximum likelihood and regression analysis.

symptoms recorded, the heads were deteriorating rapidly in the unsealed store due to secondary pathogens. In some TuMV treatments, losses of heads at harvest due to secondary infection by *Botrytis* spp. were high; in the 1999–2000 experiment (cv. Impala only), 15% of heads from plants inoculated with TuMV were lost and 38% of heads from plants inoculated with TuMV + CaMV were lost. Severe secondary infection by *Botrytis cinerea* has previously been correlated with severe TuMV infection of white cabbage (31).

BWYV produced minor levels of external symptoms and CaMV induced extensive external symptoms, therefore, the appearance of such symptoms prior to harvest does not correlate with the development of internal disorders during storage. Consequently, the

lack of external symptoms at harvest is not indicative of long-term storage potential (as previously noted by growers). Although it has been reported that TuMV-infected heads with no external necrotic symptoms at harvest tended not to develop internal necrotic symptoms during storage (24), there was a lack of correlation between external and internal symptoms (32). This highlights the immediate need to develop nondestructive pre-harvest tests that will predict the likelihood of development of internal disorders during storage.

The significant association between TuMV detection prior to harvest and the development of internal necrotic spots during storage suggests that ELISA tests may be useful in predicting storage potential of heads in terms of internal necrosis. CaMV is known to

TABLE 6. Mean absorbance values (A_{405}) for *Beet western yellows virus* (BWYV) enzyme-linked immunosorbent assay (ELISA) of leaves from cabbage plants and heads following inoculation with BWYV, *Cauliflower mosaic virus* (CaMV), and *Turnip mosaic virus* (TuMV)^a

Cultivar, treatment ^b	Prior to harvest		In storage					
	A_{405}	SEM	4 months (sealed)		7 months (sealed)		5 months (unsealed)	
	A_{405}	SEM	A_{405}	SEM	A_{405}	SEM	A_{405}	SEM
1998–1999 ^c								
Polinius								
BWYV	3.76***	±0.07	2.10**	±0.18	8.01***	±0.21	4.81**	±0.20
BWYV + CaMV	4.75***	±0.07	1.73**	±0.36	9.38***	±0.18	5.51**	±0.25
BWYV + TuMV	3.87***	±0.07	1.85**	±0.18	6.24***	±0.17	4.13**	±0.19
Uninoculated	1.54	±0.07	0.22	±0.19	0.22	±0.15	0.07	±0.24
LSD at $P \leq 0.05$	0.22	...	...	...	...	...	...	...
Impala								
BWYV	4.04**	±0.07	3.30***	±0.11	8.78***	±0.21	6.37***	±0.17
BWYV + CaMV	3.90***	±0.07	4.02***	±0.19	10.25***	±0.16	7.09***	±0.18
BWYV + TuMV	4.09***	±0.07	3.58***	±0.11	10.28***	±0.14	6.47***	±0.17
Uninoculated	1.48	±0.07	0.17	±0.10	0.09	±0.22	0.08	±0.23
LSD at $P \leq 0.05$	0.22	...	...	...	...	...	...	...
1999–2000 ^d								
Impala								
BWYV	1.46***	±0.17	2.19**	±0.27	2.31***	±0.19	...	...
BWYV + CaMV	3.85***	±0.15	3.09**	±0.27	3.49***	±0.19	...	...
BWYV + TuMV	1.76***	±0.15	1.19*	±0.27	3.05***	±0.19	...	...
Uninoculated	0.25	±0.16	0.39	±0.27	0.36	±0.19	...	...

^a A_{405} values were transformed using the formula $\ln[100(A_{405} + 0.065)]$, to eliminate negative values. *, **, and *** = significantly different at $P = 5$, 1, and 0.1%, respectively, from appropriate uninoculated control. LSD = least significant difference, SEM = standard error of the mean.

^b All virus inoculations were carried out pre-transplanting (May).

^c A_{405} values for tests prior to harvest were analyzed by analysis of variance, A_{405} values for tests following storage were analyzed using regression analysis.

^d A_{405} values analyzed by regression analysis.

TABLE 7. Percentage detection of *Cauliflower mosaic virus* (CaMV) by inoculation to susceptible host plants (*Brassica juncea*) and subsequent enzyme-linked immunosorbent assay confirmation following inoculation of cabbage plants with *Beet western yellows virus* (BWYV), CaMV, and *Turnip mosaic virus* (TuMV)

Cultivar, date ^a , treatment	Detection (%)			
	Prior to harvest	4 months in storage (sealed)	7 months in storage (sealed)	5 months in storage (unsealed)
1998–1999				
Polinius, May				
CaMV + BWYV	83	100	75	29
CaMV	83	0	0	13
TuMV + CaMV	70	0	50	29
Uninoculated	0	0	0	0
Impala, May				
CaMV + BWYV	60	100	20	55
CaMV	50	100	63	13
TuMV + CaMV	93	100	33	50
Uninoculated	0	0	0	0
1999–2000				
Impala, May				
CaMV + BWYV	100	82	76	...
CaMV	100	100	85	...
TuMV + CaMV	100	40	100	...
Uninoculated	0	0	0	...
Impala, June				
CaMV	100	87	100	...
TuMV + CaMV	100	54	83	...
Uninoculated	0	0	0	...

^a Date of inoculation: May inoculations were carried out pre-transplanting and June inoculations were post-transplanting.

form intracellular inclusion bodies composed of a protein matrix in which the virus particles are embedded (5). This may account for the lack of detection in cabbage leaves by ELISA. In the light of the exacerbation of internal disorders by CaMV, there is a need for a cheap detection method for this virus which is reliable, quantifiable, and more rapid than the 3 to 4 weeks required for detection by inoculation to indicator plants. Development of such tests for the pre-harvest detection of TuMV and BWYV, as well as CaMV, would allow programmed removal of heads from cold storage to reduce losses. Those heads least likely to develop internal disorders could be stored for the longest period of time and those heads most likely to develop such disorders could be removed early and processed before the symptoms develop. Detection of BWYV prior to harvest in cv. Impala heads that did not develop symptoms was high (86%) and similar to that for heads that did subsequently develop symptoms (95%); this shows that infection by BWYV alone is not always sufficient to induce tipburn symptoms. Symptom development may be associated with tissue-specific location of the virus or other unknown interactions.

All virus treatments were found to reduce head yield (in terms of trimmed head weight) in cv. Polinius. The effects of TuMV with CaMV and of BWYV with CaMV were particularly severe. TuMV had little effect on the yield of cv. Impala. This provides evidence for tolerance to TuMV in Impala with respect to yield. Particularly high yield reductions were seen in Impala following post-transplanting inoculations with CaMV and TuMV + CaMV. This, coupled with the exacerbating effect of CaMV co-infection on both BWYV-induced tipburn and TuMV-induced internal necrosis, means that CaMV is likely to remain a major concern to growers despite its lack of direct involvement in internal disorders.

From this study, we have identified what appears to be extreme resistance to tipburn in the white cabbage cv. Polinius. It should be possible to incorporate this resistance into new cabbage cultivars. However, care will have to be taken not to introduce increased susceptibility to TuMV-induced necrotic spots or any other disorder. The resistance to TuMV identified in white cabbage (24) and other *Brassica oleracea* (*Brassica C* genome) types (J. A. Walsh and C. E. Jenner, *personal communication*) to date has been quantitative. However, in a range of *Brassica A* genome types (J. A. Walsh, *unpublished data*), we have identified a number of extreme sources of resistance to TuMV where we can detect no virus replication following inoculation. We have mapped one such TuMV resistance gene (39) and are in the process of mapping more. We hope to incorporate such genes into the *B. oleracea C* genome by marker-assisted interspecific transfer and deploy them in a range of *B. oleracea* types.

ACKNOWLEDGMENTS

This work was funded under the HortLINK initiative (project Hort 15) with funding from the Department for Environment, Food and Rural Affairs, U.K. (HL0114LFV), the Horticultural Development Council (project FV160a), United Vegetables, Solway Foods, Tinsley Foods Ltd., Fisher Foods Ltd., Smedley's Foods Ltd., Geest Ltd., Elsoms Seeds Ltd., Nickerson Zwaan Ltd., and Novartis Seeds Ltd. We thank P. E. Thomas (USDA, Prosser, WA) for the BWYV polyclonal antiserum; L. Torrance (SCRI, Dundee) for the mouse BWYV MAb (510H); M. Stevens (IACR Brooms Barn, Suffolk) for the rat BWYV MAb (MAFF 24) and the BWYV isolate; United Vegetables (Kirton, Lincolnshire) for the organization of the cold storage and use of the facilities; J. Bambridge and C. Jenner for expert technical assistance; C. Clay and C. Evered for electron microscopy; B. Ellis and N. Kift for aphid cultures and facilities for aphid transmission work; C. Hole and D. Gray for their involvement in the project; and R. Collier for the review of this manuscript.

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