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(Year 2)

White cabbage: reducing losses from internal disorders and improving supply

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I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

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PRACTICAL SECTION FOR GROWERS

Objectives and background

Two thousand seven hundred ha of white cabbage, valued at approximately £20m pa, 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 MAFF record losses of 10% of the crop but on occasions much higher losses occur. In the last few years several major co-operatives and growers have recorded complete loss of stored material (up to 600 tons in one store) with others recording substantial losses in the range of 20-80%. This is 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 it. Crops are accepted by processors having a certain proportion of heads or areas of tissue within a head affected 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 spots (so called cigar burn), necrotic areas on specific leaves in the phyllotaxis and usually at the margins (so-called tipburn) and pepper spot are commonly occurring disorders. These problems are not usually evident in cabbage heads at harvest and at store loading .

Cigar burn has been associated with the infection of plants by turnip mosaic virus (TuMV) and cauliflower mosaic virus (CaMV). Tipburn and pepper spot have been attributed to deficiency in the local supply of calcium associated with inadequate transport and with calcium metabolism although other cation deficiencies are implicated in pepper spot. There appear to be interactions between calcium supply and virus infection recorded, but not verified, in other crops in relation to tipburn and we have recently recovered CaMV from stored cabbage heads displaying tipburn symptoms but no cigar burn symptoms.

Factors inducing calcium deficiency include: a) high leaf expansion rates due to high temperature causing high demand for calcium and to high N and K levels; b) high transpiration may increase calcium import by the outer at the expense of inner leaves. Conditions in the leaf during storage may affect calcium re-distribution or calcium metabolism.

It is not known currently how to produce a crop free from these disorders which will store reliably and there are no reliable methods of pre-storage assessment of storage potential in relation to occurrence of disorders. Pictures of these disorders are given in Appendix Figs 5, 6 and 7.

The aim of the project is to reduce the impact on the efficiency and profitability of production and preparation of storage white cabbage used for processing from the unpredictable occurrence of internal disorders by:

- identifying the causes
- developing agronomic techniques and breeding selection procedures
- developing and introducing physiological and virological tests of produce before storage to inform decision making strategies

The scientific objectives are:

1) to identify the role of the three most prevalent viruses (TuMV, CaMV and beet western yellows (BWYV) and mixtures of these in internal disorders during storage;

2) identify the role of calcium supply, transport and metabolism on internal tipburn and relate this to factors which influence growth rate and to storage environment and its duration;

3) determine if Ca^{2+} transport and Ca^{2+} status of leaves is affected by virus infection;

4) devise growing techniques to reduce the incidence of disorders; develop improved field-based selection techniques to screen germplasm for resistance to Ca^{2+} and virus induced disorders;

5) develop diagnostic tests, including RT-PCR or immuno-capture RT-PCR-based methods to detect viruses for predicting storage potential at the time of harvest.

SUMMARY OF RESULTS

1. Cigar burn is associated predominantly with TuMV. There was no indication of a solely physiological cause of this symptom. Cultivar *Polinius* appeared to be more susceptible to cigar burn than cultivar *Impala*.

2. Tipburn was predominantly associated with BWYV although there was also the possibility of an association with CaMV. TuMV did not appear to be associated with this symptom. There was evidence that the incidence of tipburn symptoms can increase over time in store. Cultivar *Impala* appeared to be more susceptible to tipburn than cultivar *Polinius*.

3. Whilst all virus treatments significantly reduced yield of cultivar *Polinius* heads, only treatments involving BWYV and CaMV significantly reduced yields of cultivar *Impala*.

4. Cabbage heads lost weight progressively during storage. In the case of tipburn there was the possibility that a physiological response might be involved in the development of some symptoms. Virus(es) associated with tipburn (CaMV and BWYV) might exacerbate any such physiological effects.

5. Application of different levels of nitrogen and water input to a crop of cabbages over a prolonged period did not result in symptoms of internal browning/necrosis of stored heads.

6. Maintenance of a low concentration of calcium in a hydroponic system prior to and during head formation caused severe external necrosis.

7. Calcium withdrawal in a hydroponic system after head formation resulted in an increased incidence of internal browning and necrosis in discrete layers, typical of tipburn.

8. Calcium concentrations in old mature leaves are much higher than in younger tissue. This would be expected because calcium moves in the transpiration stream only. Transpiration would have occurred over a longer period in older leaves than in younger exposed leaves and would be greater in all exposed leaves than in those enclosed in the head.

9. Concentrations of calcium in soil-grown heads showing no internal browning was estimated at about 0.4%. For plants grown in hydroponics head tissue contained about 0.2%. Tissue from necrotic/brown areas of heads produced from calcium withdrawal treatments contained about 0.6% calcium. Reasons for this are discussed. There were no comparable changes in concentration of the other nutrients analysed.

10. Although heads produced from plants in hydroponics were not as large as those from plants grown in soil, the method provides a suitable means of reproducing "tipburn"-like symptoms seen in commercially grown material.

ACTION POINTS FOR GROWERS:

- Different cultivars appear to have different susceptibilities to cigar burn and tipburn; *Polinius* is more susceptible to cigar burn than *Impala* and *Impala* is more susceptible to tipburn than *Polinius*.

- Cigar burn appears to be due to turnip mosaic virus infection.

- Tipburn appears to be due to deficiency of calcium but it is also associated with beet western yellows and /or cauliflower mosaic virus.

SUMMARY OF PROGRESS

Milestones

Physiological studies, Objective 2

Year 2 crop, April 1998	-	Establish plants in hydroponic systems to study influence of factors on Ca^{2+} supply and transport and complete storage assessments	Completed August 1998 with re-run completed March 1999
Year 2 Crop, April 1998	-	Establish 'field' experiment to examine effect of nitrogen and water supply on internal disorders and complete storage	Completed May/June 1999

Agronomic and screening Studies; Objective 4

Year 1,2,3,4 crops from Industry	Collect crop protocol/disorder information from companies	Ongoing

Virological studies; Objectives 1 and 5

Year 2 crop; April 1998.	Inoculate plants grown in gauze houses with the three major viruses and complete storage	Completed March/June 1999

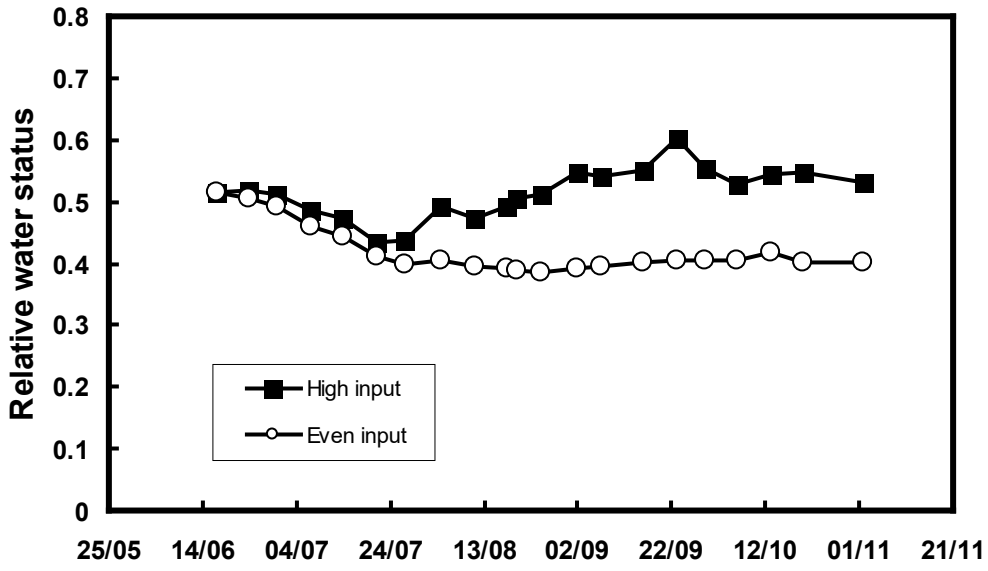
EXPERIMENTAL SECTION

Physiological Studies
Objective 2

Material & Methods

Polythene tunnel experiment 1998.
Cultivation of these plants was based on industry protocol.
Dutch White cabbage plants (cv. *Polinius*) were planted into plugs on 18 March and raised in a polythene tunnel before planting out on 28 May into soil (a sandy loam of the Wick Series) in two fan-ventilated polytunnels. Plants were spaced at 60cm within and between rows. There were 42 rows of 11 plants. Allowing for guard areas, this provided 45 plants per plot. The crop in each tunnel was split into three blocks along the length of the tunnel to offset effects of end-to-end temperature gradient. Within each block the treatments were randomly allocated to one of two plots.

Fig. 1. Relative soil moisture at 30 cm below the surface, for plots in polytunnel experiment 1998



Irrigation was applied by seep hose laid along each row of 11 plants. All plots were provided with the same quantity of water before hearting. After this time, the HIGH input treatment was watered more frequently (from 2 August). Soil water status was measured using a neutron probe and demonstrated clear differences between the treatments (Fig. 1). Calibrations of soil moisture content to soil water potential are in progress.

HIGH input plots also received a top dressing of 216 kg ha^{-1} nitrogen on 28 July. EVEN input plots received the same amount of nitrogen as top dressing, but this was applied in two lots of 108 kg ha^{-1} on 9 September and 7 October. All plots received a base dressing of 170 kg ha^{-1} nitrogen before planting.

Plants were sampled on three occasions to monitor progress of growth in the two treatments. Samples of cabbage leaf and heart were taken for analysis of calcium content.

On 3 November 40 heads were cut from each plot and sprayed with a mixture of Rovral and Ridomil MBC as per commercial specification. The heads were placed in nets for future handling and then into commercial bins. The bins were transported to storage at 1°C in a commercial sealed store in which oxygen concentration was at about 17% and carbon dioxide concentration at 3% for much of the storage period. (Records of store environment are given in Appendix Figs 2,3 and 4).

On 1 March and 14 June 1999, 20 heads from each plot were removed from store and assessed for the presence of internal browning/necrosis disorders.

Glasshouse hydroponic experiments 1998

All experiments in hydroponics were done in a specially-constructed solution culture system and experimental unit system (Fig 2).
This was located in a single glasshouse compartment with automatic venting and sun screening. The system comprised:

i) 4 nutrient supply tanks to enable the provision of different nutrient regimes,
ii) 128 plant containers each plumbed with fittings to connect to inlet and drain pipes and each provided with a structure to support large plants
iii) “supply-side” pipework and fittings to carry nutrient to each plant container
iv) drainage pipework to return nutrient to tanks
v) a pure water supply for refilling tanks
vi) 8 benches supporting 16 plants each
vii) shading structures that can be mounted over 4 benches.

For statistical purposes, the eight benches were used as blocks within which there were four replicates connected to each tank. These were randomised within sub-blocks of 8 containers at the north and south ends of the bench. Because the pipework is fixed, the layout for tank connections is always the same. Nutrient treatments can be allocated to different tanks in different experiments, but the randomisation is fixed. Treatments such as variety can be randomised anew each time. Shading treatments were applied to whole benches (blocks). As a result, blocks were considered in pairs (superblocks) to enable the shading treatment to be evaluated in the appropriate stratum of an analysis of variance.

Fig. 2 Diagrammatic layout of system for growing cabbage in hydroponics.

Target concentrations of minerals in full strength solution are shown in Table 1. For young plants this was usually provided at quarter strength and increased to half-strength before the full strength solution was supplied. pH was maintained between 5 and 7 (target 5.8) by addition of molar hydrochloric acid or sodium hydroxide as appropriate.

Table 1. Glasshouse experiment. Target concentrations (in mg l^{-1} or ppm) for full strength nutrient solutions.

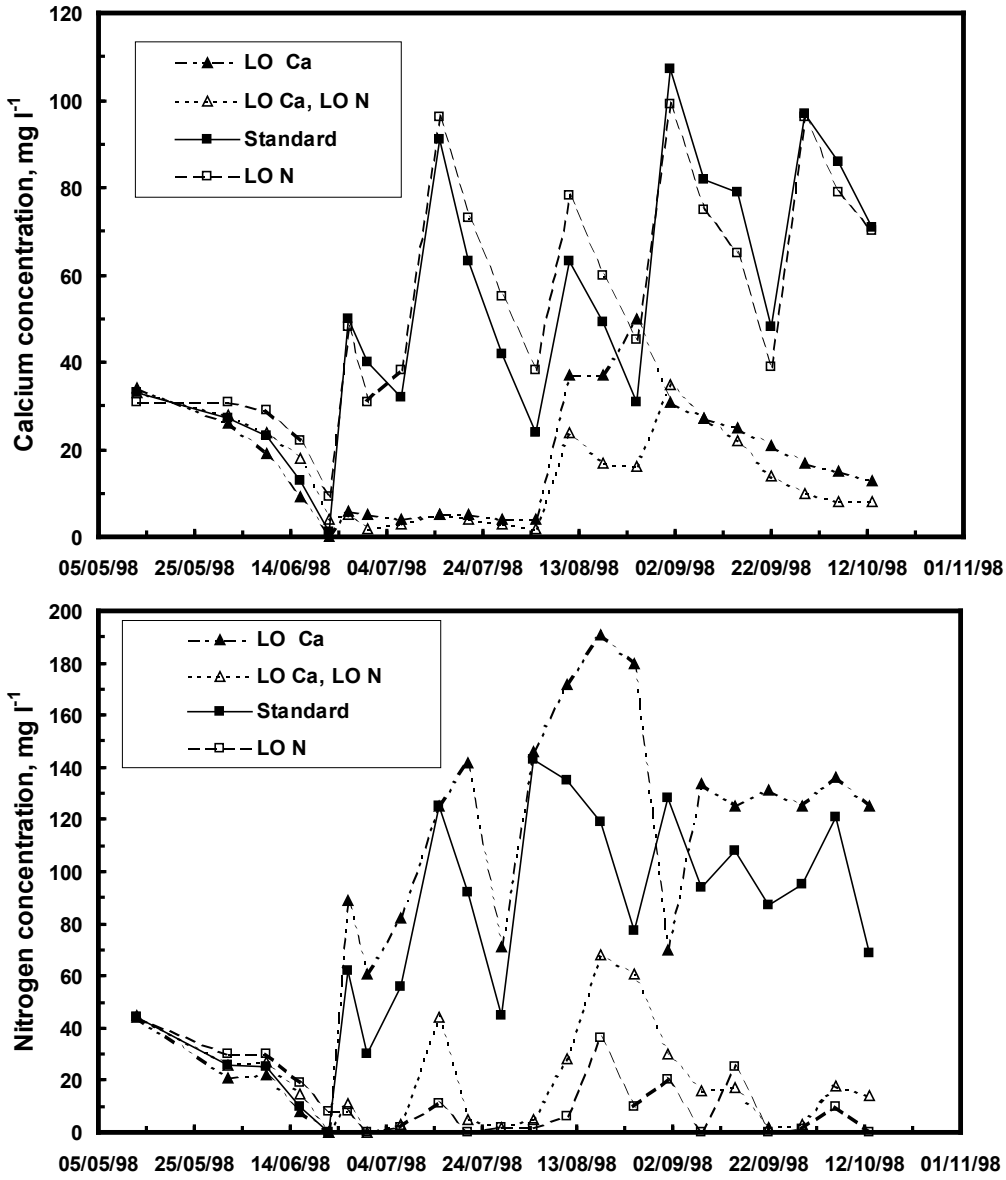
Nutrient	Low Calcium	Low Nitrogen Low Calcium	Full nutrient	Low nitrogen
Nitrogen	169	***21***	169	***21***
Phosphorus	40	40	40	40
Potassium	**460**	230	230	230
Calcium	***6.1***	***6.1***	122	122
Sodium	36	36	36	36
Magnesium	36	36	36	36
Iron	6.2	6.2	6.2	6.2
Sulphur	48	48	48	48
Chlorine	12	**174**	12	**297**
Manganese	0.55	0.55	0.55	0.55
Boron	0.32	0.32	0.32	0.32
Zinc	0.065	0.065	0.065	0.065
Copper	0.059	0.059	0.059	0.059
Molybdenum	0.039	0.039	0.039	0.039

Concentrations presented in ***bold italic*** are deliberately low. Those presented in **bold** only are unavoidably high because of the need to use other substances to provide ingredients normally given with the deficient nutrient. For example, in low calcium solution potassium concentration is high because nitrogen, some of which is normally added as calcium nitrate, is provided as additional potassium nitrate so that calcium concentration may be maintained at a low level.

Two hydroponic experiments were begun in 1998.

In the first experiment, plants were sown into pots containing Perlite and irrigated regularly with water and nutrient as appropriate. Plants were transplanted from these pots into the hydroponic system on 18 May when they weighed about 60g, had 15 visible leaves and 11 leaf primordia. Treatments comprised a two by two factorial combination of high and low nitrogen and calcium inputs plus shading imposed on plants of two cultivars, *Polinius* and *Impala*. Nutrient treatments were imposed on 24 June before the plants had begun to form heads. The concentrations of calcium and nitrogen applied to achieve the treatments are shown in Fig. 3. Because calcium withdrawal resulted in severe effects on the plants, calcium stress was

Fig. 3. Changes in concentration of nitrogen and calcium in solutions used in first hydroponic experiment.



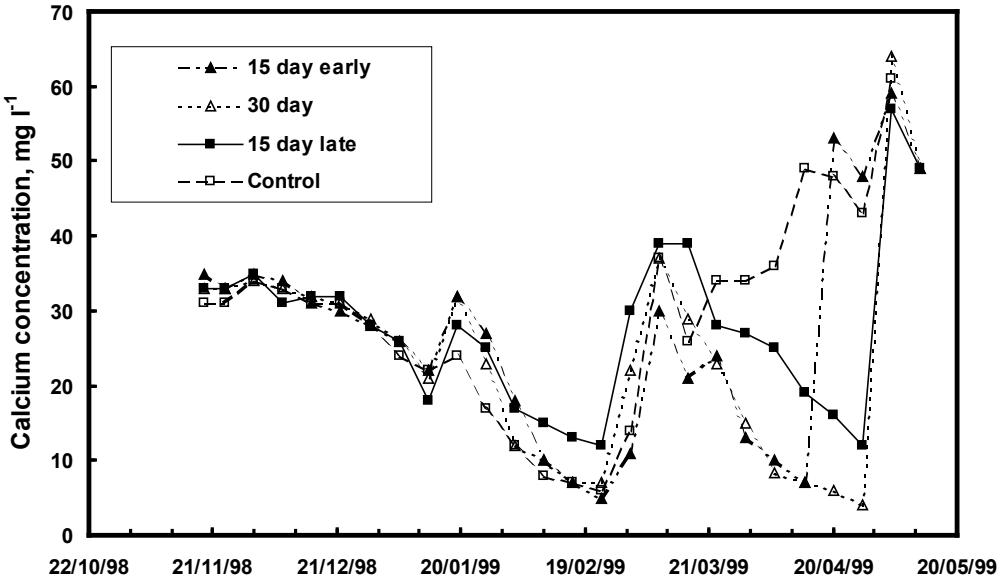
temporarily relieved on 4 August. Shading, which decreased incident photosynthetic radiation to 40% of daylight, was placed around four of the eight blocks. Plants were photographed and assessed for internal browning/necrosis between 12 and 15 October. Samples of head tissue were taken for calcium analysis.

Water stress, which occurred soon after transplanting, resulted in loss of some plants. As a result, surviving plants were re-arranged into six of the eight blocks. Infection of some plants in the system by *Pythium* also affected numbers. Zoospores in the nutrient solution were suppressed by routinely adding a wetter, “Agral”, to maintain a concentration of 15 mg l^{-1} from the beginning of the experiment.

A second experiment began on 19 November 1998. Three seeds of Dutch White cabbage were sown directly into each plant container by supporting them on paper

wicks that enabled contact with the nutrient solution. When the seedlings' cotyledons were fully expanded, they were thinned to one plant per container. Daylight was supplemented with light from 16 high pressure sodium lamps from 1 December 1998 to 7 May 1999 in order to achieve adequate growth during the winter period. Treatments in this experiment comprised only calcium withdrawal from plants of cvs *Polinius* and *Impala*. All plants were grown in quarter strength solution until 23 February 1999 when, with the exception of calcium, the concentration of nutrients was increased to full strength. Calcium concentration was replenished to quarter strength and allowed to deplete in two of the tanks ("A" and "B") from 9 March (Fig. 4), after hearting had begun (Fig. 5). In one of the remaining tanks it was increased to half strength over a period of six weeks ("D") and in the other tank ("C") calcium concentration was allowed to deplete from 16 March 1999. Calcium concentration in

Fig. 4. Changes in concentration of calcium in solutions used in second hydroponic experiment.



A was restored to half strength on 13 April 1999 and in B and C on 27 April (Fig 4). Agral was added to the solution from 26 February as described above. Plants were photographed and assessed for internal browning/necrosis at harvest on 11 and 12 May and on 9 August after storage at 3°C. Samples of head tissue were taken for calcium analysis.

Calcium analysis

A sample of approximately 4g fresh weight of tissue was taken and shredded, oven-dried and weighed again. It was then ashed at 550°C and the residue digested until dry in 2ml of 50% HCl on a hotplate at 160°C. This was then dissolved in 10ml of 50% HCl and sent to HRI analytical laboratory for analysis of calcium concentration by Inductively Coupled Plasma (ICP). Concentrations of magnesium, phosphorus, iron, zinc, manganese, copper, boron and sulphur were also measured because the same

instrument readily supplies estimates of these at the same time. Concentrations of total mineral ash and of nutrients in plant tissue were expressed on a dry matter basis.

Statistical analyses

The data have been analysed using residual maximum likelihood (REML) and binomial regression.

REML permits the partition of variance components and determines the association of these with treatment factors (e.g. “calcium”) and random factors (e.g. “blocks”) so that the effects of these factors can be determined. In essence the outcome is similar to that for analysis of variance (ANOVA). However, REML is preferred to ANOVA in situations where the experimental design is either intentionally unbalanced or has become so because plants have been lost during the experiment. Insertion of large numbers of missing values in the data in order to make ANOVA possible would not be acceptable.

Regression is traditionally used to quantify associations between variables (e.g. the slope of a simple straight-line relationship). This applies to data that are continuously variable and conform to a normal distribution. Data that contain only two possible states (e.g. “presence” or “absence” of necrosis) conform to a binomial distribution. Under these circumstances the effects of factors on the frequency of one of these states can be determined from a generalised regression analysis by using a binomial distribution.

Fig. 5. Plants growing in hydroponics on 9 March 1999 after having been sown directly into system on 19 November 1998.

Results

Polytunnel experiment 1998

The purpose of this experiment was to examine how variation in growth rate of cabbage plants might influence the incidence of internal browning and necrosis (Fig.6) in cabbage heads after storage. The experiment was based on the hypothesis that when growth rate is rapid, calcium supply to young developing organs is restricted, leading to poor cell wall formation and ultimately tissue deterioration. Thus half the plots ("EVEN input" treatment) were provided with nitrate fertiliser as a base dressing and two split top dressings and irrigated less frequently than the remaining plots which received nitrate as a base dressing and a single top dressing and were watered more frequently ("HIGH" input). Heads of plants from HIGH input plots were heavier than those of plants from EVEN input plots (Table 2) and had lower dry matter content (9.9% compared with 10.8%, LSD at p=0.05, 0.25). At planting, plants had six visible leaves and seven leaf primordia. Prior to hearting this had increased to 33 visible leaves with a further 35 leaves and primordia within the apex. After hearting, the number of open leaves remained at 33, but the number comprising the head increased enormously (to greater than 62). Around 40 of these would have formed after 2 July. Leaf development was affected little by treatment. There was no sign of internal browning/necrosis in 12 heads sampled at lifting and no development of any symptoms in any of the 480 heads examined after 17 and 32 weeks in cold store.

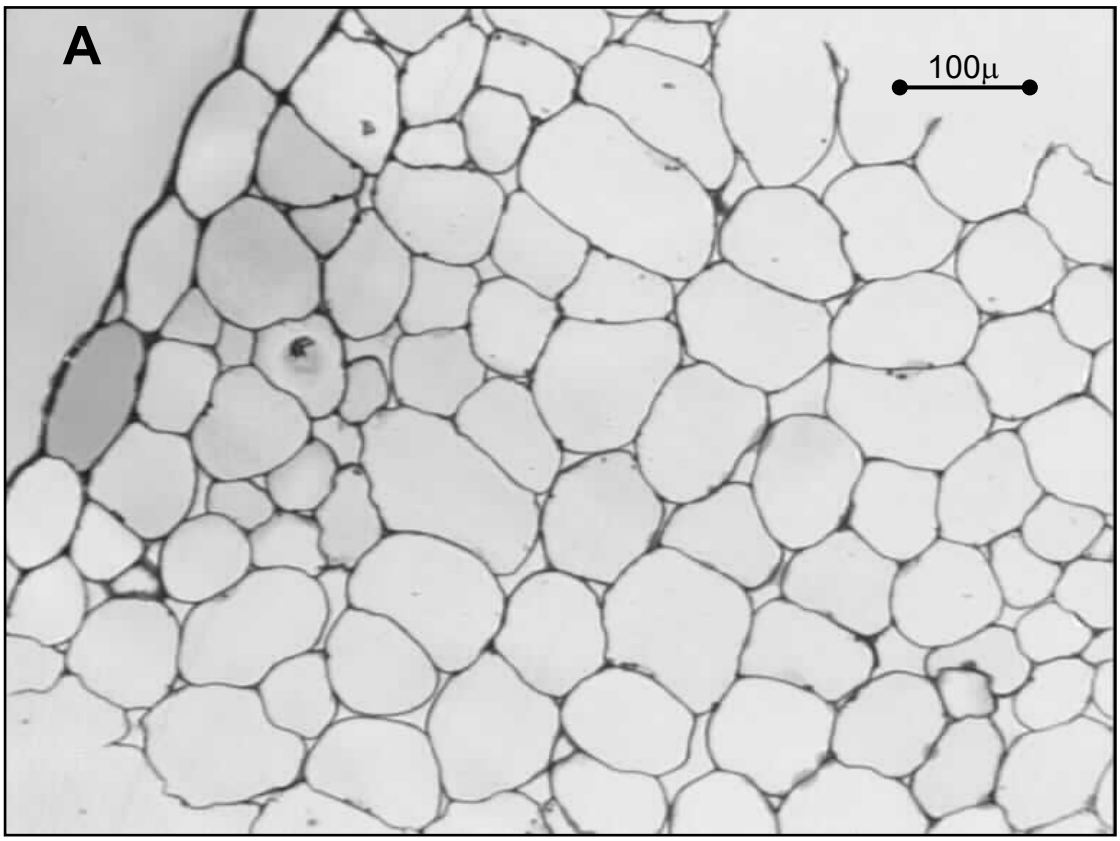
Table 2. Weights of plants and heads and numbers of leaves formed during growth of cabbage in Polytunnel experiment 1998.

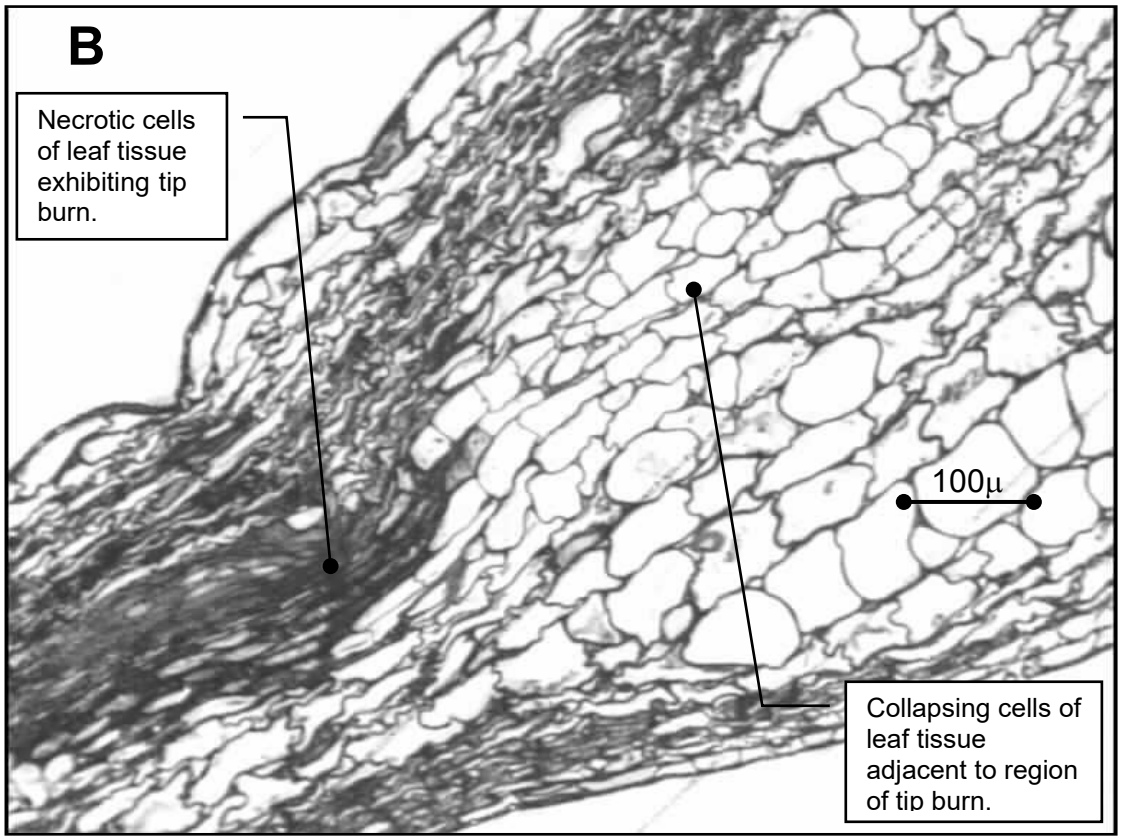
Date	Top fresh weight, g		Last expanded leaf		Leaves and primordia	
	Even input	High input	Even input	High input	Even input	High input
28 May	7.7		6		13	
2 July	701	673	20	19	55	54
(LSD p=0.05)	(300)					
28 July	3139	3222	33	34	95	96
LSD (p=0.05)	(794)					
28 August	4466	5098	33	33		
	(1025)					
	[1555]	[1935]				
LSD (p=0.05)	(393)					
4 November	[2473]	[3093]				
LSD (p=0.05)	(411)					

Weights in square brackets are for heads only.

Many heads exhibited symptoms of intumescence – white/brown crusty outcrops on leaf surfaces within the head. Incidence was greatest in plots nearest the end where air enters the Polytunnels and decreased towards the outlet. This profile

Fig. 6. Transverse section of leaf from inside a cabbage head showing normal tissue (A) and tissue from a necrotic region with typical symptoms of tip burn (B).
(Micrographs courtesy of Carol Evered and Colin Clay, HRI Electron Microscopy.)





would be consistent with the hypothesis that intumescence results from damage caused by thrips.
Calcium concentration per unit dry matter was least in head tissue and less in younger leaves than older leaves (Table 3). Although these trends were also evident in mineral ash content, differences in calcium content were much greater. For example, the concentration of calcium in the youngest open leaf was 2.4 times that in the head, while the concentrations of total mineral ash and of magnesium were respectively 1.3 and 1.2 times those in head tissue. No significant effects of HIGH and EVEN input treatments on the concentration of calcium were detected during growth or after storage and there were no changes associated with storage duration.

Table 3. Changes in mineral ash, calcium and magnesium concentrations of cabbage tissue during growth and storage. Polytunnel experiment 1998.

Date	2/7/98	28/7/98	26/8/98	4/11/98	1/3/99	14/6/99
Calcium concentration, % dry matter						
Head tissue			**0.44**±0.065	**0.36**±0.043	**0.43**±0.055	**0.37**±0.036
Youngest open leaf	Leaf 18 **1.4**±0.20	Leaf 33 **0.91**±0.100	Leaf 33 **1.1**±0.91			
Mature leaf (6 nodes below)	Leaf 12 **4.8**±0.25	Leaf 27 **2.2**±0.45				
Mineral ash, % dry matter						
Head tissue			**7.3**±0.22	**7.6**±0.19	**7.8**±1.17	**7.2**±0.11
Youngest open leaf	**11.4**±0.49	**8.7**±0.41	**9.8**±0.47			
Mature leaf (6 nodes below)	**19.8**±0.62	**12.6**±1.15				
Magnesium concentration, % dry matter						
Head tissue			**0.13**±0.006	**0.09**±0.004	**0.11**±0.066	**0.10**±0.065
Youngest open leaf	**0.23**±0.008	**0.15**±0.008	**0.15**±0.008			
Mature leaf (6 nodes below)	**0.40**±0.025	**0.22**±0.027				

In stored cabbage head tissue, the different cultivation protocols significantly affected the concentrations of magnesium, manganese and iron only (Table 4). The HIGH input regime increased the concentration of each of these elements. There were no effects of cultivation on the concentrations of boron, phosphorus, sulphur, zinc and copper. Concentrations of some elements (Fe, Mg, Mn, P and S) were less after longer storage, while the concentration of boron increased markedly. No changes

were observed for measured elements (see Materials and Methods) not included in Table 4.

Table 4. Effects of cultivation treatment and storage on mean concentrations of some mineral nutrients in cabbage heads. Polytunnel experiment 1998.

Nutrient	INPUT		STORAGE UNTIL	
	EVEN	HIGH	1/3/99	14/6/99
Iron	0.055	0.072	0.071	0.056
($mg\ g^{-1}$) LSD	0.0161		0.0125	
Magnesium	0.094	0.109	0.107	0.096
% LSD	0.0076		0.0054	
Manganese	11.9	19.0	19.5	11.3
($\mu g\ g^{-1}$) LSD	1.58		1.71	
Boron			0.095	0.231
($mg\ g^{-1}$) LSD			0.028	
Phosphorus			3.08	2.88
($mg\ g^{-1}$) LSD			0.171	
Sulphur			0.32	0.45
% LSD			0.038	

LSDs are quoted for p=0.05. Comparisons for significant effects only are shown

Mineral ash concentration for tissue taken from areas of tipburn in virus experiments were greater than for control (unaffected tissue) from similar locations (10.9 % vs 8.8%, LSD p=0.05, 0.34). Many of the nutrient concentrations were thus similarly greater in affected tissue. For example, calcium concentrations were 0.57% for areas of tipburn and 0.40% for unaffected tissue (LSD p=0.05, 0.075). It might be expected that affected tissue would contain less calcium. However, the issue is complicated by the fact that browning and necrotic tissue is usually in a state of breakdown, which may involve a relative reduction in the non-mineral component.

Hydroponic experiment 1

The purpose of this experiment was to determine whether an association between calcium availability, rate of plant growth and symptoms of internal browning disorders in cabbage heads exists. Low calcium concentration in the nutrient was intended to cause calcium stress. Low nitrogen availability and shading were intended to decrease growth rate. Two varieties were used because it was believed that *cv. Polinius* may be more sensitive than *cv. Impala*.

Calcium concentration was maintained at a low level before heads had started to form. As a result, many of the symptoms were external and heads did not form on many treated plants. If they did form then they soon became necrotic and died. For this reason, we examined the association of the incidence of external necrosis with treatment. The severity of external necrosis on older leaves and outer leaves of heads was significantly greater ($p<0.001$) for plants subjected to low levels of

calcium availability (Table 5). Significant, but small, interactions were also observed for calcium treatment with cultivar ($p<0.05$) and with nitrogen treatment ($p<0.05$). These suggested that the effect of calcium was greater at low nitrogen and for *cv. Impala*. No significant effects of shading were evident.

Table 5. Incidence of plants with no symptoms of external necrosis on mature leaves and heads; predicted from binomial regression analysis.

Cultivar	Nitrogen	Calcium: Control		Calcium: Low	
Polinius					
	Control	68±17.3	(12)	29±18.1	(12)
	Low	100± 9.9	(11)	36±13.3	(10)
cv. mean		83±10.3	(23)	32±12.1	(22)
Impala					
	Control	100± 9.9	(12)	40±21.0	(10)
	Low	100±12.7	(10)	18±23.9	(10)
cv mean		100± 8.0	(22)	29±17.6	(20)
Nitrogen					
	Control	84±10.2	(24)	34±15.2	(22)
	Low	100± 8.0	(23)	27±14.3	(20)
Calcium		91± 6.6	(47)	31±12.2	(42)

Mean incidence was estimated across levels of shading because no significant effect of this treatment was detected.
Number of plants contributing to each mean is shown in brackets. Errors are 95% confidence limits.

Low calcium supply also resulted in the survival of fewer heads ($p<0.001$) and a reduced head fresh weight ($p<0.001$) for those that did survive (Table 6). Mean survival, **predicted** from binomial regression analysis, detected no significant effects of any treatments other than calcium and no interactions. Analysis of head fresh weight revealed a significant interaction between shading and calcium treatments ($p<0.001$). There was a bigger difference between head fresh weight of the low calcium treatment and control for unshaded plants than for shaded plants. This was the overriding treatment effect observed, but other significant interactions were detected; calcium, shading plus nitrogen and calcium plus cultivar.

Table 6. Effects of shading, calcium, nitrogen and variety on head survival and fresh weight. Hydroponic experiment 1.

Shading	Nitrogen / Cultivar	Head fresh weight, g — Calcium: Control	Head fresh weight, g — Calcium: Low	Head survival, % — Calcium: Control	Head survival, % — Calcium: Low
Unshaded	Control				
	Polinius	1421	*	88±18.3	11±29.0
	Impala	1997	500	89±26.0	55±35.1
	Low				
	Polinius	1211	-5	89±26.0	10±27.8
	Impala	1133	785	71±33.7	56±35.1
	LSD (p=0.05)	464			
	Unshaded mean	1440	186	84±15.6	33±19.6
	LSD (p=0.05)	446			
Shaded	Control				
	Polinius	589	193	94±18.5	60±40.7
	Impala	674	394	93±19.5	42±39.6
	Low				
	Polinius	701	243	93±19.5	61±40.5
	Impala	524	588	94±18.5	78±34.7
	LSD (p=0.05)	464			
	Shaded mean	622	355	93±11.2	60±24.3
	LSD (p=0.05)	214			
Calcium mean		1031	270	89±9.1	47±11.9
	LSD (p=0.05)	238			

Mean head fresh weights and LSDs have been predicted from analysis of variance components by residual maximum likelihood method (REML).
Head survival is reported as mean ±95% confidence limits **predicted** from regression analysis.
* No heads.

Calcium concentrations in heads from this hydroponic experiment were decreased by low calcium treatment (Table 7). Generally the concentrations in these heads were less than those from soil-grown plants (see Table 3 for comparison). Shading, nitrogen and variety had no effect on calcium concentration. Dry matter content was greater in tissue from the low calcium treatment as was the concentration of many mineral nutrients other than calcium (Table 7). The concentrations of some of these nutrients were affected by treatments other than calcium availability (data not shown). Total mineral ash content was not affected by calcium treatment.

Table 7. Dry matter, mineral ash and nutrient concentrations in tissue of heads from hydroponic experiment 1.

	Calcium treatment		LSD (p=0.05)
	High	Low	
Dry matter %	8.6	9.4	0.76
Mineral ash %	9.2	9.4	0.47
Calcium %	0.19	0.14	0.029
Boron mg g^{-1}	0.046	0.056	0.0056
Copper μg g^{-1}	2.5	3.7	0.32
Iron mg g^{-1}	0.050	0.056	0.0113
Magnesium %	0.15	0.25	0.015
Manganese μg g^{-1}	29	43	3.9
Phosphorus mg g^{-1}	3.7	4.6	0.25
Sulphur %	0.23	0.26	0.016
Zinc μg g^{-1}	29	52	5.5

Hydroponic experiment 2

The purpose of this experiment was to determine whether an association between calcium availability and symptoms of internal browning disorders in cabbage heads could be demonstrated by a period of calcium withdrawal after visible heads had begun to form. This differs from the previous experiment in that no attempt was made to maintain calcium concentration at a low level during the starvation period and calcium stress was introduced at a later stage of development and for a shorter period of time.

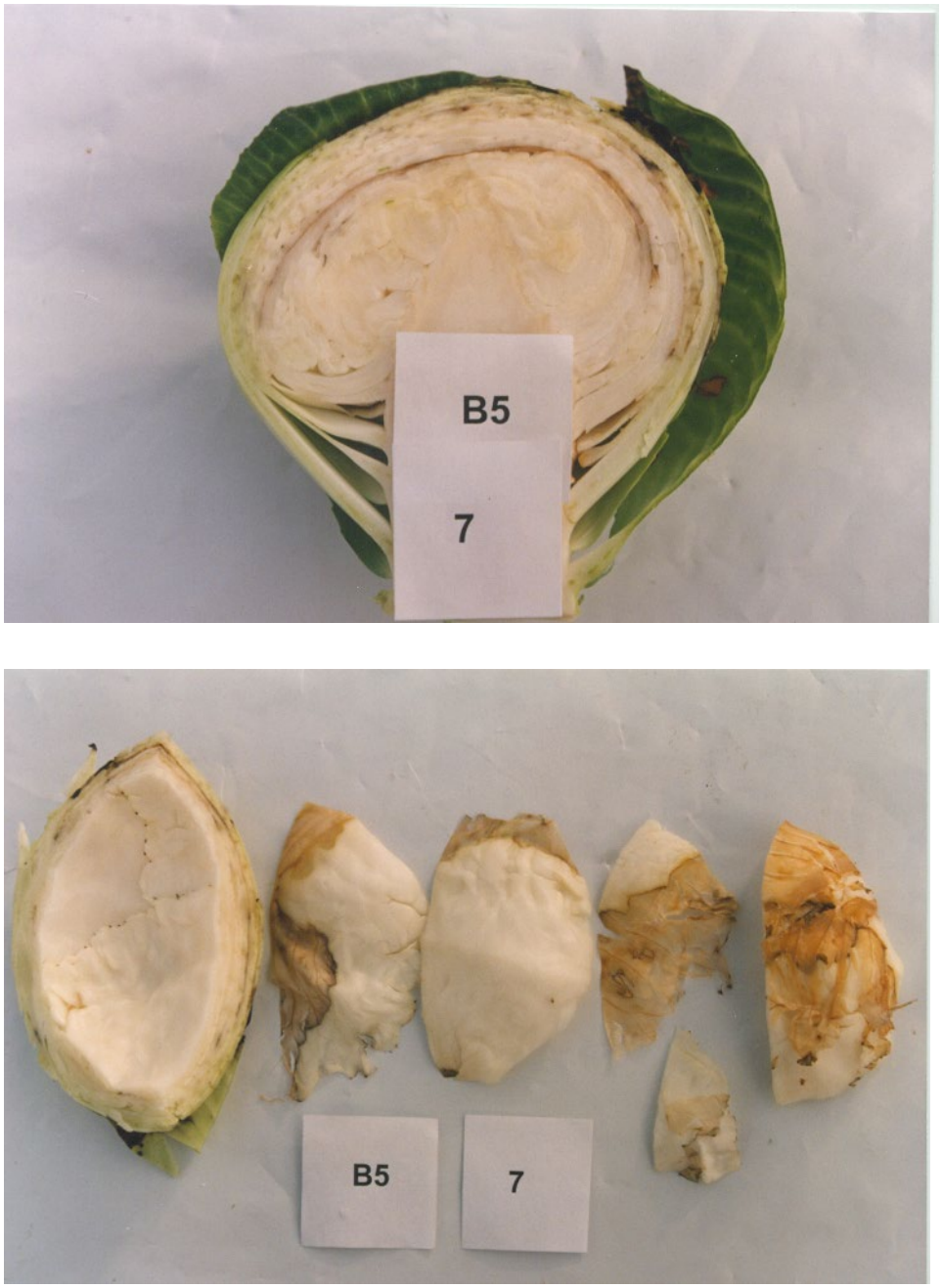
Some measurements from this experiment have yet to be completed and the results fully analysed. However, preliminary examination of data from material assessed at harvest in May indicates an association between the incidence of external necrosis, internal browning of the heads and the most severe calcium withdrawal treatment (Table 8). Calcium concentrations of head tissue from this treatment were much less than in tissue from untreated and less severely calcium-stressed plants. Concentrations of other nutrients did not appear to be affected. Magnesium concentrations are shown to illustrate this.

Table 8. Effect of calcium withdrawal on incidence of plants showing no symptoms of internal and external browning or necrosis, dry matter content, mineral ash content, calcium and magnesium concentrations. Hydroponic experiment 2.

	Control	15 day early depletion	15 day late depletion	30 day depletion
Absence of internal browning %	80	73	75	36
Absence of external necrosis %	40	47	38	7
Dry matter %	10.2	12.7	12.1	11.2
Mineral ash %	8.6	8.7	8.6	8.8
Calcium %	0.15	0.11	0.10	0.06
Magnesium %	0.17	0.16	0.19	0.18

Symptoms of internal browning/necrosis tended to occur in layers within the head. Affected leaves were particularly thin and papery at their margins, though browning of fresh tissue spread well into some of the affected leaves (Fig. 7).

Fig. 7. Cabbage head from the first hydroponic experiment exhibiting symptoms typical of internal necrosis, usually described as tipburn and attributed to local calcium deficiency.



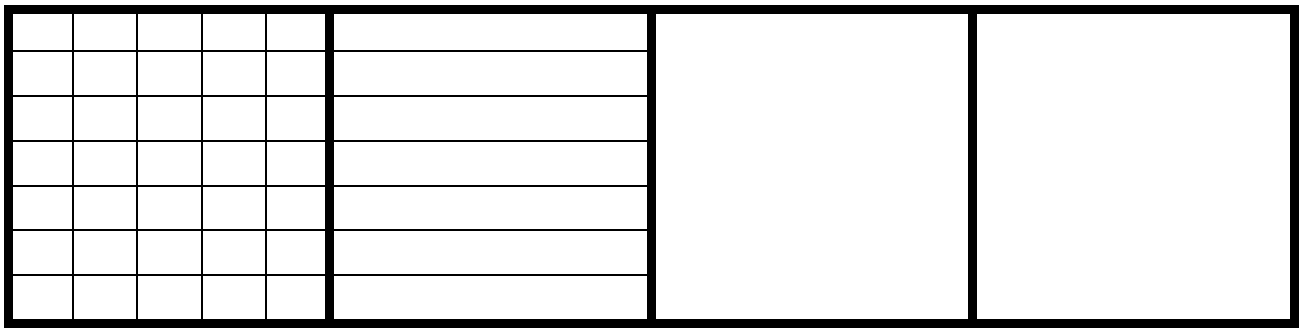
Virological studies

Materials & Methods

Seedlings of two cultivars (*Polinius & Impala)* of white cabbage were sown in modules on the 17 March 1998 and grown initially under glass then transferred to insect-free gauze houses on the 27 March. The seedlings were inoculated with three viruses; beet western yellows virus (BWYV), turnip mosaic virus (TuMV) and cauliflower mosaic virus (CaMV), both individually and in dual combinations (i.e. BWYV; CaMV; TuMV; BWYV & CaMV; BWYV & TuMV; TuMV & CaMV). BWYV is not mechanically transmissible; therefore plants were infected with this virus using aphids on the 7 May and the aphids subsequently killed by treatment with Decis on the 11 May. The seedlings were then mechanically inoculated with CaMV on the 14 May and with TuMV on the 15 May. Uninoculated control plants were also included in the experiment. Dursban was applied to the seedlings as a pre-planting drench to prevent cabbage root fly infestation.
A nutrient analysis of the soils in the Tygan houses used for growing the transplants was made. The soils were fertilised with nitrogen at either 85 kg ha^{-1} (houses 1 and 4) or 170 kg ha^{-1} (houses 2 and 3) (based on the analysis) on the 5 May, raising concentrations of nitrogen in the soil to commercially acceptable levels. No additional potassium or phosphate was required. The seedlings were transplanted into the insect-proof Tygan houses on the 21 May. Plants were spaced 60 cm apart in plots of 35 plants, with four plots to a house (Fig 8). Each plot represented one of the virus treatments or uninoculated controls and contained both cultivars. The layout of the plots within the houses and the cultivars within each plot were randomised. Each house contained one uninoculated control plot, and each virus treatment was replicated twice within the experimental layout (Fig. 9). Applications of Aphox (pirimicarb) and Ambush C (cypermethrin) to prevent aphids were applied alternately at fortnightly intervals, Bravo 500 (chlorothalonil) was applied monthly to prevent *Alternaria* and *Botrytis* and three treatments of Fubol 75 WP (mancozeb + metalaxyl) were applied during the season to prevent white blister. A top dressing of nitrogen at 215kg ha^{-1} was applied to all houses on the 23 June.

Fig. 8. Cabbages in Tygan houses showing virus infection.

Fig. 9. Planting Layout



5 x 7 = 35 plants / Plot

1 cultivar / row
1 storage treatment / row

1 virus treatment / plot

4 plots / house
Control plots in each house
2 x each treatment
4 x control plots
Randomised layout

House 1

BWYV	Uninoculated	TuMV	CaMV

House 2

CaMV	BWYV	BWYV & CaMV	Uninoculated

House 3

Uninoculated	TuMV	TuMV & CaMV	TuMV & BWYV

House 4

TuMV & BWYV	TuMV & CaMV	Uninoculated	BWYV

The growth of plants and appearance of symptoms were recorded at weekly intervals. The plants were assayed for the presence of the viruses prior to harvest. BWYV was readily detectable from cabbage leaves by conventional ELISA. A modification of the extraction protocol involving grinding leaf samples under liquid nitrogen also allowed the detection of TuMV from cabbage leaves by ELISA. No modifications to the CaMV ELISA protocol attempted resulted in the detection of CaMV from cabbage leaves. Consequently this virus was assayed for by inoculation to susceptible host plants (Mustard cv. Tendergreen). The presence or absence of the virus in the mustard plants was then determined by ELISA.

The cabbage heads were harvested on 3 November following commercial guidelines, weighed and placed in nets, each net containing heads from a single row (a maximum of 5 heads per net). Heads were then drenched to run-off with Rovral and Ridomil, again according to commercial practice, prior to placement in two cold storage facilities, one atmospherically sealed the other not. Records of store environment are given in Appendix Figs 1,2, 3 and 4. The heads were separated into three batches for assessment after approximately 4 months and 6 months in the sealed store and 6 months in the unsealed store. In addition, five heads comprising a

single row of each plot were assessed for the presence of internal symptoms and tested for the presence of the three viruses at harvest.

The first heads were removed from the sealed cold store and assessed on 1 March 1999 (17 weeks post harvest). Head weight was recorded, the external fungal growth on the head noted and assessments of internal symptoms from diagonally opposite quarters of the heads were made. It should be noted that the levels of fungal growth observed were most likely exacerbated by secondary infection following abrasion to the heads caused by the storage nets during transport and handling. The external fungal growth, internal tipburn symptoms and internal cigar burn symptoms were recorded on four point scales (Table 8). Samples were taken from each head for virus testing. Two further assessments were made in a similar manner on 19 April (unsealed store; 24 weeks post harvest) and 14 June (sealed store; 32 weeks post harvest). The material in the unsealed store deteriorated faster than anticipated, requiring that it be assessed earlier than the intended 32 weeks post harvest.

Table 8. Scoring regime

Cigar burn
0 No symptoms
1 One spot to a few spots on ≤25% of leaves
2 Spots on >25-50% leaves or severe spots on >10% of leaves
3 Spots on >50% of leaves, or severe spots on >25% of leaves

Tipburn
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

External fungus
0 No external fungus
1 Slight amount external fungus over part of head
2 Light fungus infection over all of head or heavy infection with tissue damage over part of head.
3 Heavy fungus infection over most of head with significant tissue damage

Results and Discussion

Symptoms during growth

Two main types of symptom were observed during the growth of the plants: vein clearing (which progressed to inter-veinal chlorosis as plants matured) and necrotic spotting. The latter symptom appeared to be associated with TuMV treatments whilst the vein clearing symptoms were more prevalent in CaMV-inoculated plants. Some plants inoculated with a combination of CaMV and TuMV showed both vein-clearing and necrotic spotting symptoms in the same plant. The level of symptoms recorded

is shown graphically in Figs 10-15. No distinction has been made between the vein–clearing / chlorosis and the necrotic spotting symptoms in these figures.

BWYV-infected plots showed little evidence of symptoms (Fig. 10). The few symptoms that were seen were of the vein-clearing type. Symptoms in the TuMV-inoculated plots increased dramatically in late August (approximately 4 months post transplanting) and reached maximum levels of approximately 30% (*Impala*) and 60% (*Polinius*) of the plants inoculated by harvest (Fig. 11). Symptoms in CaMV-inoculated plots started to appear earlier (early June, approximately 1 month after transplanting) and reached the highest incidence for any of the single virus treatments, with approximately 55% (*Impala*) and 60% (*Polinius*) of plants affected (Fig. 12). BWYV in combination with TuMV did not appear to increase the incidence of symptoms when compared with TuMV alone (Figs 11 and 13). BWYV in combination with CaMV, however, induced a more rapid appearance of symptoms and a slight increase in incidence in *Polinius* compared to CaMV alone (Fig. 12 and 14). The combination of CaMV with TuMV caused a rapid increase in symptoms (Fig. 15) similar to the BWYV / CaMV combination with a higher incidence than any other treatment (approximately 85% (*Impala*) and 95% (*Polinius*) of inoculated plants). The appearance of the necrotic spotting occurred late (late August) in this treatment as in the other TuMV treatments.

Fig. 10. Percentage of BWYV-inoculated plants with symptoms

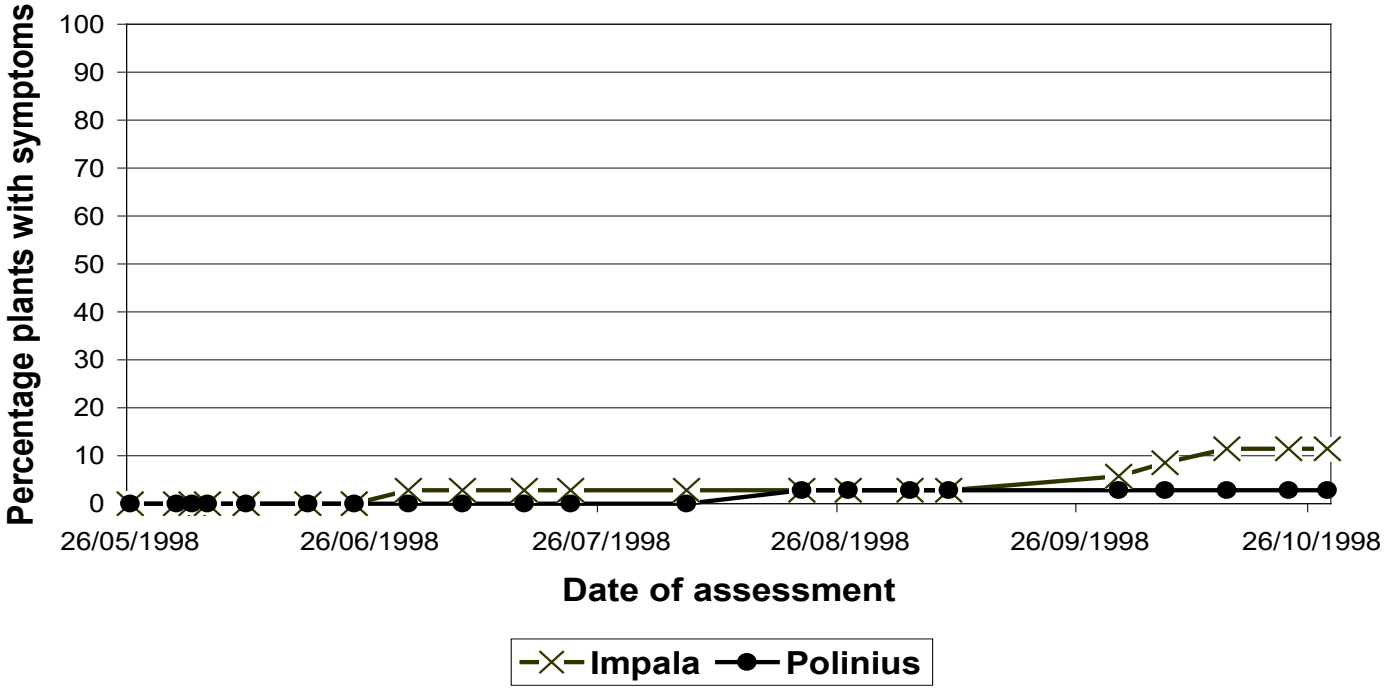


Fig. 11. Percentage of TuMV-inoculated plants with symptoms.

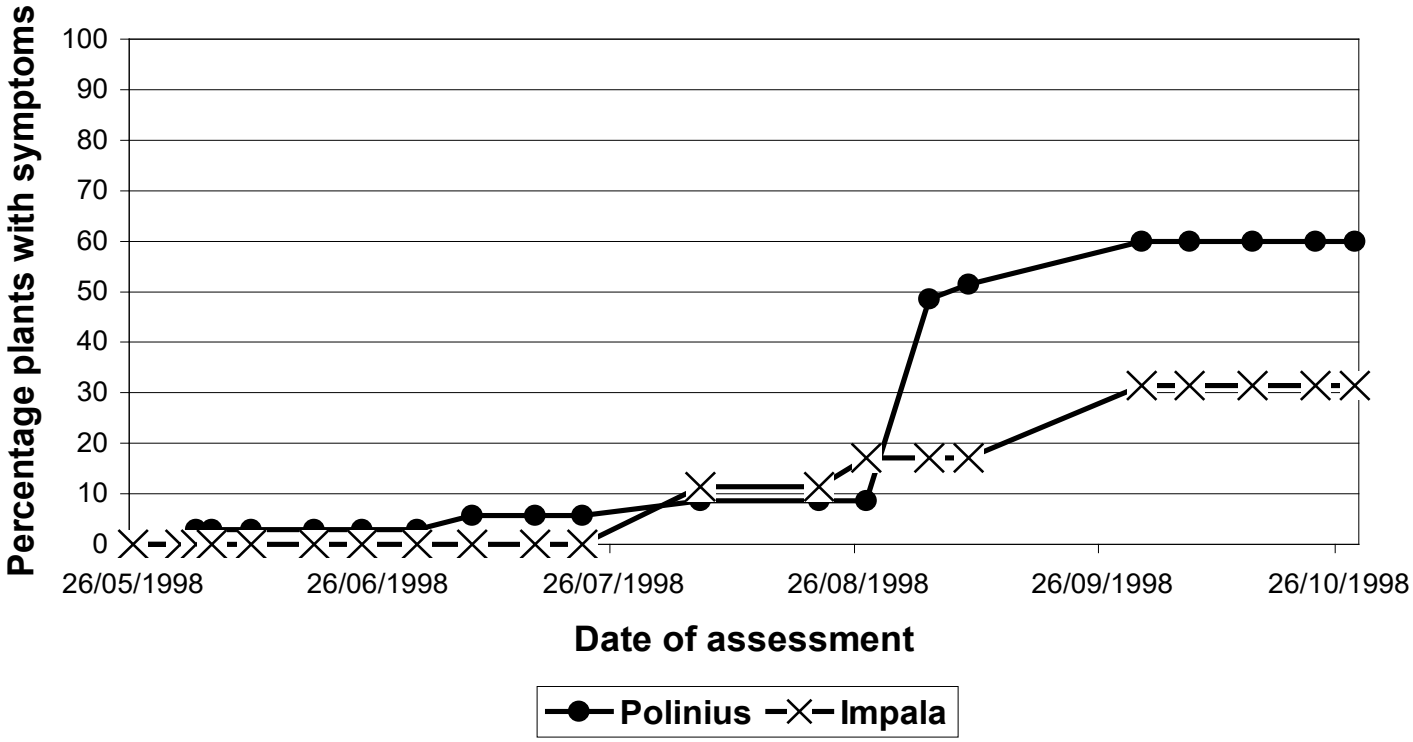


Fig. 12. Percentage of CaMV-inoculated plants with symptoms

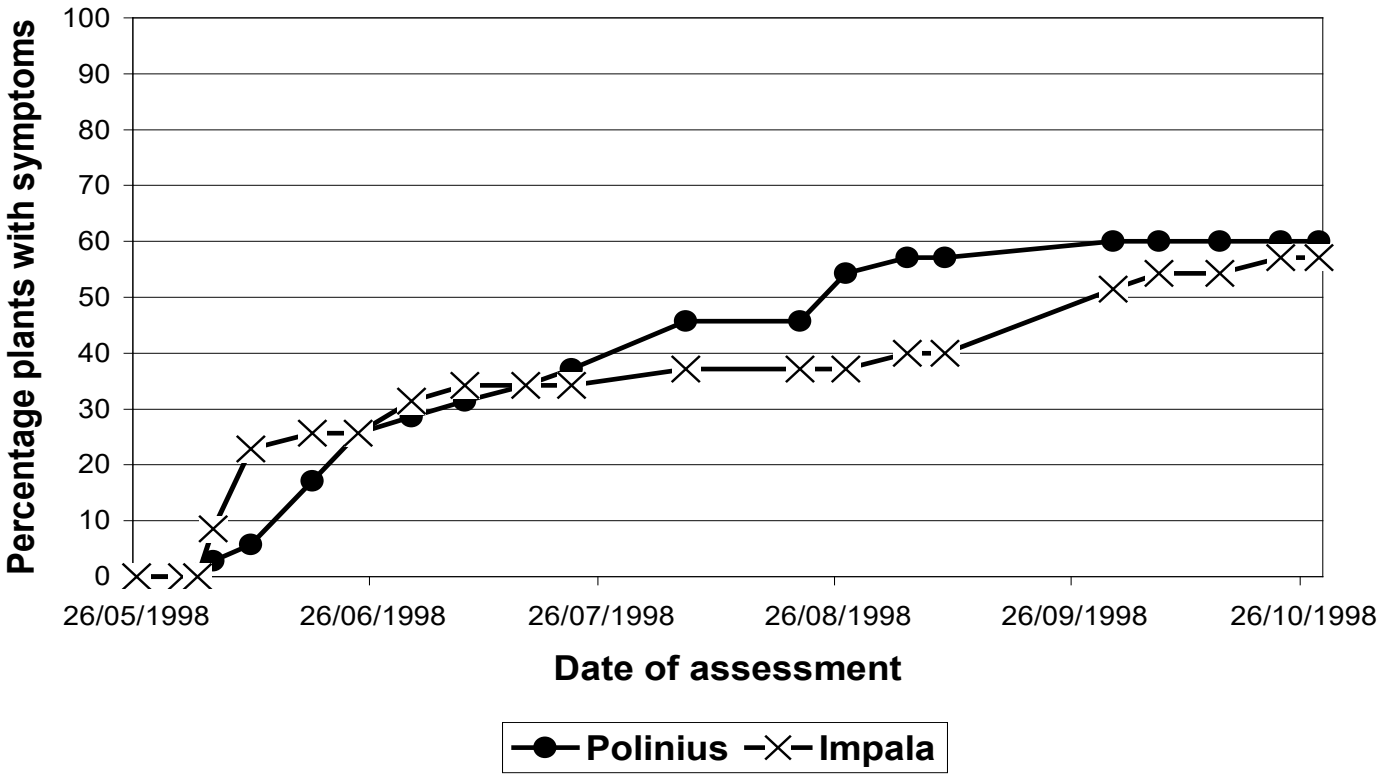


Fig. 13. Percentage of plants inoculated with the combination of BWYV and TuMV showing symptoms

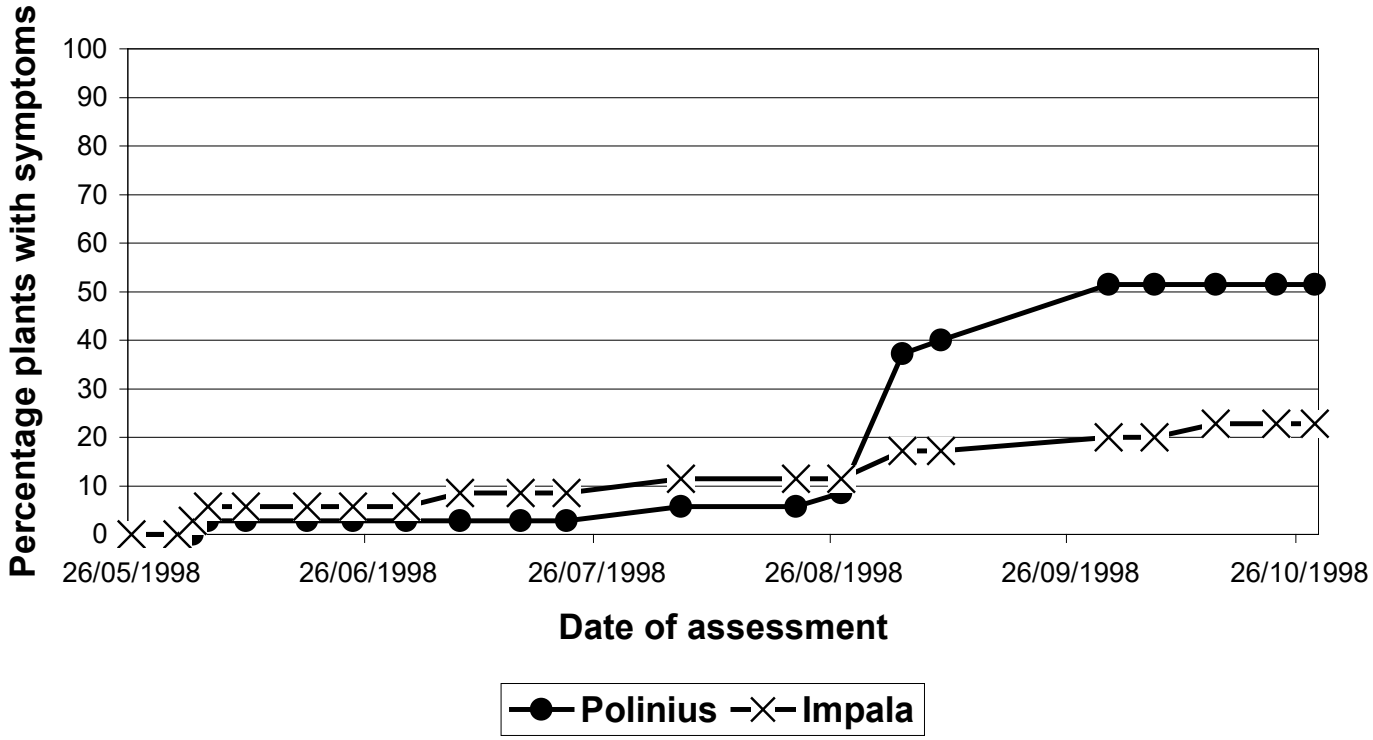


Fig. 14. Percentage of plants inoculated with the combination of BWYV and CaMV showing symptoms

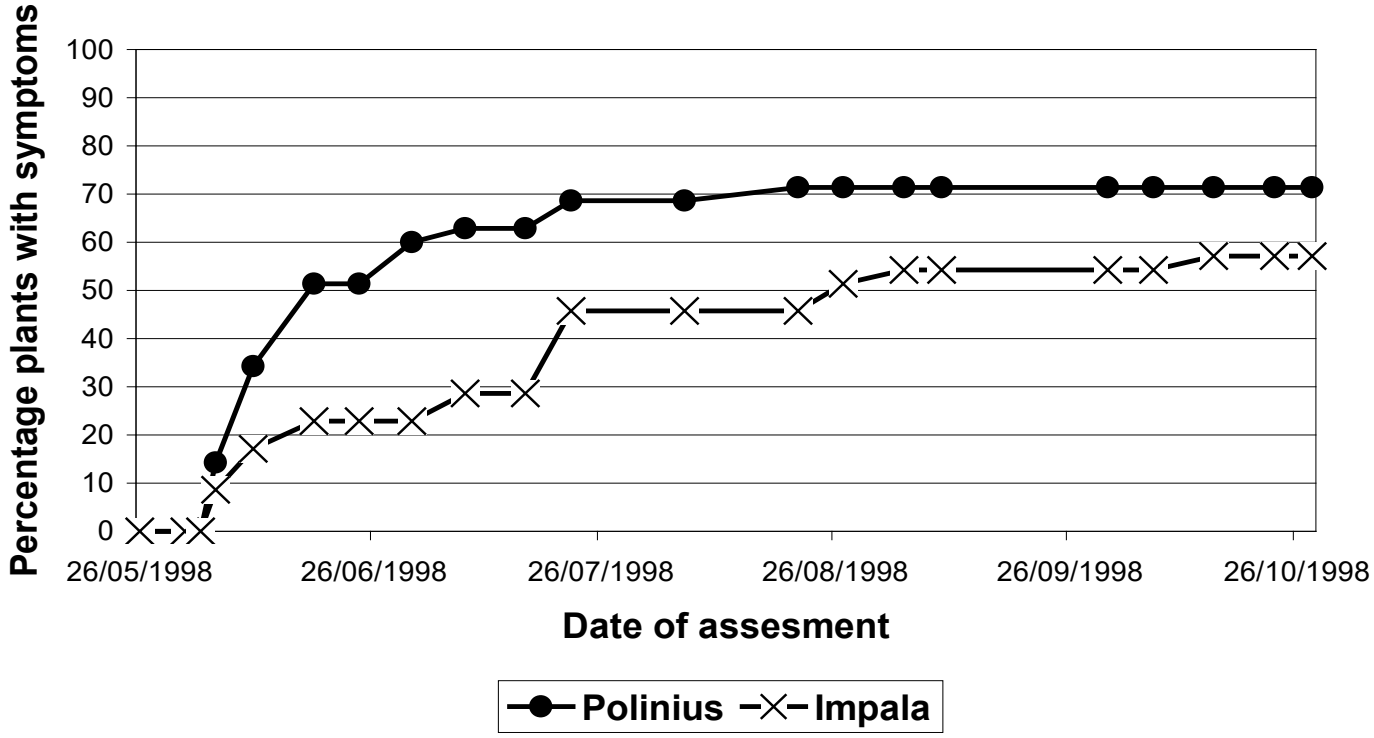
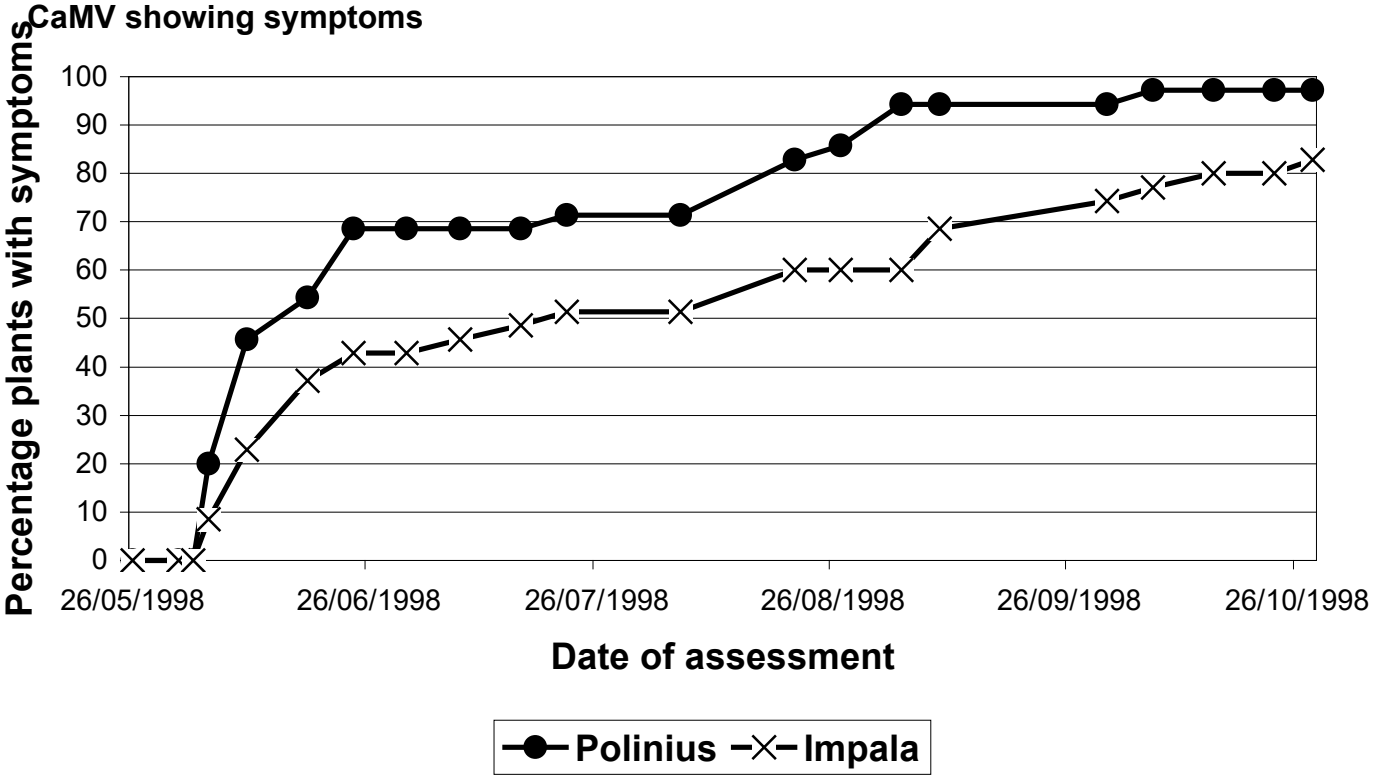


Fig. 15. Percentage of plants inoculated with the combination of TuMV and CaMV showing symptoms



Concentration and incidence of virus detected in plants

Analysis of variance of ELISA data for BWYV in plants tested prior to harvest is shown in Table 9. In cultivar *Polinius*, the data indicated a significant increase in the level of BWYV in plants inoculated with a combination of BWYV and CaMV compared to plants inoculated with BWYV and TuMV in combination or with BWYV alone. No significant differences were observed in levels of BWYV between virus treatments in cultivar *Impala*. Both cultivars showed a significant difference between all BWYV treatments and uninoculated controls.

Table 9. Analysis of BWYV ELISA data at pre-harvest testing

	Mean Absorbance Values at 405nm (A_{405})	
	Impala	**Polinius**
BWYV	4.009	3.722
BWYV & TuMV	4.058	3.838
BWYV & CaMV	3.862	4.732
Uninoculated	0.835	0.990

lsd for comparison between cultivars = 0.208
lsd for comparison between treatments = 0.279

A REML analysis of the ELISA data from plants inoculated with TuMV, sampled prior to harvest is shown in Table 10. The analysis indicated a significant difference in level of virus detected between cultivars in all TuMV treatments, with *Polinius* showing higher levels of TuMV than *Impala*. Differences in TuMV levels between treatments including TuMV were not significantly different for either cultivar.

Table 10. Analysis of TuMV ELISA data at pre-harvest testing

	Mean A_{405} Values	
	Polinius	*Impala*
TuMV	2.320	2.099
TuMV & BWYV	2.604	1.637
TuMV & CaMV	3.468	1.385
Uninoculated	1.549	1.470

	Wald Statistic	**d.f.**	**Wald statistic at 5% significance**	**Significance level**
Uninoculated	13.5	1	3.84	0.1%
Treatment	0.4	2	5.99	Not significant
Cultivar	5.4	1	3.84	5%
Uninoculated . cultivar	2.1	1	3.84	Not significant
Treatment . cultivar	3.4	2	5.99	Not significant

Statistical analysis of levels of CaMV in the cabbage was not possible due to the necessity of using inoculation to a susceptible host as a detection method. Incidence of the CaMV in cultivar *Impala* (Table 11) detected by this method, was highest in the treatment CaMV in combination with TuMV (80% of plants infected), lowest in the CaMV only treatment (50% of plants infected) and intermediate in the treatment

CaMV in combination with BWYV (63% of plants infected). The incidence of CaMV appeared to be the same for all CaMV treatments in cultivar *Polinius* however, with 80-83% of plants infected. CaMV was not detected in any uninoculated control plants.

Table 11. Detection of CaMV pre-harvest by inoculation to Tendergreen mustard followed by confirmation by ELISA

	Impala			Polinius		
	Plants assessed	Plants with CaMV	Percentage infection	Plants assessed	Plants with CaMV	Percentage infection
CaMV	30	15	50%	30	25	83%
CaMV & BWYV	30	19	63%	30	25	83%
CaMV & TuMV	30	24	80%	30	24	80%
Uninoculated	60	0	0%	60	0	0%

Head weight data

REML analysis of the head weight data recorded at harvest (Table 12) showed a significant reduction in mean head weight in virus-inoculated plants compared to uninoculated controls in both cultivars, however, the data for the two cultivars showed different patterns. An analysis of variance within each cultivar showed that in cultivar *Impala* the weight loss was less for treatments including TuMV inoculation than for those not inoculated with TuMV. In cultivar *Polinius*, the greatest weight loss was detected in the treatments with CaMV in combination with BWYV and CaMV with TuMV.

Table 12. Analysis of weight data at harvest

	Wald statistic	d.f.	Wald statistic at 5% significance	Significance level
Uninoculated	29.7	1	3.84	0.1%
Treatment	22.5	5	11.07	0.1%
Cultivar	0.0	1	3.84	Not significant
Uninoculated . cultivar	8.7	1	3.84	1.0%
Treatment . cultivar	37.2	5	11.07	0.1%

	Polinius Mean Head Weight (kg)	Impala Mean Head Weight (kg)
BWYV	3.423	2.763
BWYV & CaMV	1.525	2.767
BWYV & TuMV	3.143	2.925
CaMV	3.215	2.753
TuMV	3.328	3.477
TuMV & CaMV	1.627	2.897
Uninoculated	4.069	3.532

lsd for comparisons between treatments in cv. Polinius = 0.509
lsd for comparisons between treatments in cv. Impala = 0.642

Recording of the weight of heads after storage enabled an analysis of the weight loss during storage to be carried out. This analysis (Table 13) showed that both treatment and storage regime had significant effects on weight loss, however, since there were no significant differences detected between the uninoculated controls and the virus inoculated treatments, the differences are unlikely to be due to virus infection. The analysis indicated a significantly greater weight loss after 32 weeks in

the sealed store than after 17 weeks. Since the temperature was relatively constant within stores (Appendix Figs 1 to 2), with the majority of any variation being in the early weeks of storage, the difference is probably due to the effect of storage time. Since the assessment of the heads in the unsealed store was made at a different time to either of the assessments of the heads in the sealed store, no direct comparison of the effects of storage temperature and atmospheric conditions was possible.

Table 13. Analysis of weight loss during storage

	Wald statistic	d.f.	Wald statistic at 5% significance	Significance level
Uninoculated	0.5	1	3.84	Not significant
Treatment	16.8	5	11.07	1.0%
Cultivar	2.9	1	3.84	Not significant
Storage regime	167.1	2	5.99	0.1%
Uninoculated. Cultivar	0.1	1	3.84	Not significant
Treatment . cultivar	10.6	5	11.07	Not significant
Uninoculated . storage regime	0.7	2	5.99	Not significant
Treatment . storage regime	7.5	10	18.31	Not significant
Cultivar . storage regime	4.7	2	5.99	Not significant
Uninoculated . cultivar . storage regime	0.2	2	5.99	Not significant
Treatment . cultivar . storage regime	10.2	10	18.31	Not significant

	Mean weight losses (kg) during storage		
	Sealed store		Unsealed store
	17 weeks	**32 weeks**	**24 weeks**
BWYV	0.2031	0.4523	0.2606
BWYV & CaMV	0.0998	0.3448	0.1866
BWYV & TuMV	0.1415	0.4365	0.2395
CaMV	0.1775	0.5281	0.2857
TuMV	0.1467	0.3692	0.2622
TuMV & CaMV	0.1523	0.5598	0.3014
Uninoculated	0.1846	0.4496	0.2781

lsd. for comparison between virus treatments is 0.0911
lsd. for comparison between storage regimes is 0.0674

	Time in store	Mean weight loss	Lsd
Sealed	17 weeks	0.1579	
Sealed	32 weeks	0.4487	0.0492
Unsealed	24 weeks	0.2592	

Internal symptoms

Assessment of symptoms at harvest (Table 14) suggested an association between the presence of internal cigar burn symptoms and TuMV inoculation. There were insufficient plants for a statistical analysis, however, 27% of plants inoculated with TuMV in various combinations showed cigar burn symptoms whilst only 1 plant not inoculated with TuMV from the remaining 50 (2%) showed cigar burn symptoms. Furthermore the symptoms appeared to be more prevalent and severe in cultivar *Polinius* than in cultivar *Impala*. In the case of tipburn, symptoms appeared to be associated with CaMV, with 20% of heads inoculated with CaMV showing symptoms.

Table 14. Scoring of heads at harvest for symptoms

	Impala		Polinius		Overall	
	Plants assessed	Plants with symptoms	Plants assessed	Plants with symptoms	Plants assessed	Plants with symptoms
Cigar burn						
BWYV	5	0 (0%)	5	0 (0%)	10	0 (0%)
BWYV & CaMV	5	0 (0%)	5	1 (20%)	10	1 (10%)
BWYV & TuMV	5	0 (0%)	5	0 (0%)	10	0 (0%)
CaMV	5	0 (0%)	5	0 (0%)	10	0 (0%)
TuMV	5	2 (40%)	5	2 (40%)	10	4 (40%)
TuMV & CaMV	5	0 (0%)	5	4 (80%)	10	4 (40%)
Uninoculated	10	0 (0%)	10	0 (0%)	20	0 (0%)
Tipburn						
BWYV	5	0 (0%)	5	0 (0%)	10	0 (0%)
BWYV & CaMV	5	2 (40%)	5	0 (0%)	10	2 (20%)
BWYV & TuMV	5	0 (0%)	5	0 (0%)	10	0 (0%)
CaMV	5	1 (20%)	5	0 (0%)	10	1 (10%)
TuMV	5	0 (0%)	5	0 (0%)	10	0 (0%)
TuMV & CaMV	5	1 (20%)	5	2 (40%)	10	3 (30%)
Uninoculated	10	0 (0%)	10	0 (0%)	20	0 (0%)

Due to the large number of zero scores recorded in the assessments for cigar burn and tipburn following storage, the distribution of the data was skewed and statistical analyses were not carried out, however, some trends were observed from an examination of the mean scores. The scores for cigar burn (Table 15) were mostly higher in cultivar *Polinius* than cultivar *Impala* and there appeared to be no major effect of storage regime on the appearance of cigar burn symptoms. The highest mean scores were observed with inoculations of either, TuMV and CaMV in combination or with TuMV alone. This, combined with the data at harvest suggests TuMV is responsible for the development of cigar burn symptoms, especially as the mean symptom score observed with inoculation of CaMV alone was low. The data also indicated the possibility of virus interactions. The mean symptom score for inoculation with the combination of BWYV and TuMV was considerably lower than for the other two treatments involving TuMV, and similar to the mean score for inoculation with BWYV and CaMV in combination. Taken together with the lower mean scores recorded for inoculation with BWYV alone and with CaMV alone, this indicated that BWYV interacted with both other viruses. Interaction of BWYV with CaMV appeared to promote symptom development slightly but interaction of BWYV with TuMV appeared to dramatically reduce symptom development.

Table 15. Mean scores for cigar burn symptoms

	Sealed store				Unsealed store	
	17 weeks		32 weeks		24 weeks	
	Impala	Polinius	Impala	Polinius	Impala	Polinius
BWYV	0.0	0.0	0.1	0.0	0.0	0.0
BWYV & CaMV	0.1	0.1	0.1	0.1	0.3	0.4
BWYV & TuMV	0.2	0.1	0.0	0.0	0.1	0.3
CaMV	0.0	0.0	0.0	0.0	0.0	0.0
TuMV	0.7	1.1	0.3	0.7	0.5	0.9
TuMV & CaMV	0.1	0.9	0.4	1.1	0.2	1.3
Uninoculated	0.0	0.0	0.0	0.0	0.0	0.0
Grand mean	**0.15**	**0.28**	**0.11**	**0.25**	**0.11**	**0.35**

Tipburn symptoms were mostly higher in cultivar *Impala* than in cultivar *Polinius*, and a marked increase in symptoms was seen after 32 weeks in the sealed store compared to those recorded after 17 weeks (Table 16). This increase was attributed to symptom development over time rather than any effect of the storage temperature since this remained relatively unchanged over this period. (Appendix Figs 1 and 2). The highest mean scores were recorded with the inoculations involving BWYV. Intermediate mean scores were also recorded in inoculations with CaMV alone and with CaMV and TuMV in combination. These observations indicated the possibility of both BWYV and CaMV producing the symptoms known as tipburn although possibly to different extents. It should be noted however that the mean scores for inoculation with CaMV alone and with CaMV and TuMV in combination were similar to those for the uninoculated control heads.

Table 16. Mean scores for tipburn symptoms

	Sealed store				Unsealed store	
	17 weeks		32 weeks		24 weeks	
	Impala	Polinius	Impala	Polinius	Impala	Polinius
BWYV	0.2	0.0	1.2	0.5	0.8	0.0
BWYV & CaMV	0.3	0.3	1.0	0.5	0.2	0.0
BWYV & TuMV	0.0	0.0	1.5	1.1	0.1	0.0
CaMV	0.1	0.1	0.3	0.0	0.1	0.2
TuMV	0.1	0.0	0.1	0.0	0.1	0.0
TuMV & CaMV	0.2	0.1	0.3	0.0	0.1	0.0
Uninoculated	0.2	0.0	0.3	0.0	0.1	0.0
Grand mean	**0.19**	**0.07**	**0.63**	**0.31**	**0.19**	**0.04**

Virus detection and recovery

Virus detection in the samples taken from the heads at assessment showed no cross contamination of the viruses from different treatments. Levels of detection of BWYV and CaMV remained unchanged relative to the pre-harvest analysis, however detection of TuMV in ELISA indicated a lower incidence than prior to harvest. Where TuMV was detected, the samples coincided with those showing cigar burn symptoms. Where virus was recovered from leaves showing cigar burn symptoms Table 17, TuMV was predominant. CaMV was recovered from treatments where it had been inoculated. Virus recovery from leaves showing tipburn symptoms indicated that both CaMV and TuMV were present where inoculated.

Table 17. Virus recovery from heads with symptoms

	Cigar burn			Tipburn		
	Plants with symptoms tested	Plants from which virus recovered		Plants with symptoms tested	Plants from which virus recovered	
Treatment		CaMV	TuMV		CaMV	TuMV
BWYV	0	-	-	8	0	0
BWYV & CaMV	4	3	0	10	4	0
BWYV & TuMV	8	0	8	13	0	0
CaMV	1	0	0	4	2	0
TuMV	23	0	21	4	0	3
TuMV & CaMV	13	4	10	7	3	1
Uninoculated	0	0	0	8	0	0

Appendix

Fig. 1. Temperature of the unsealed store

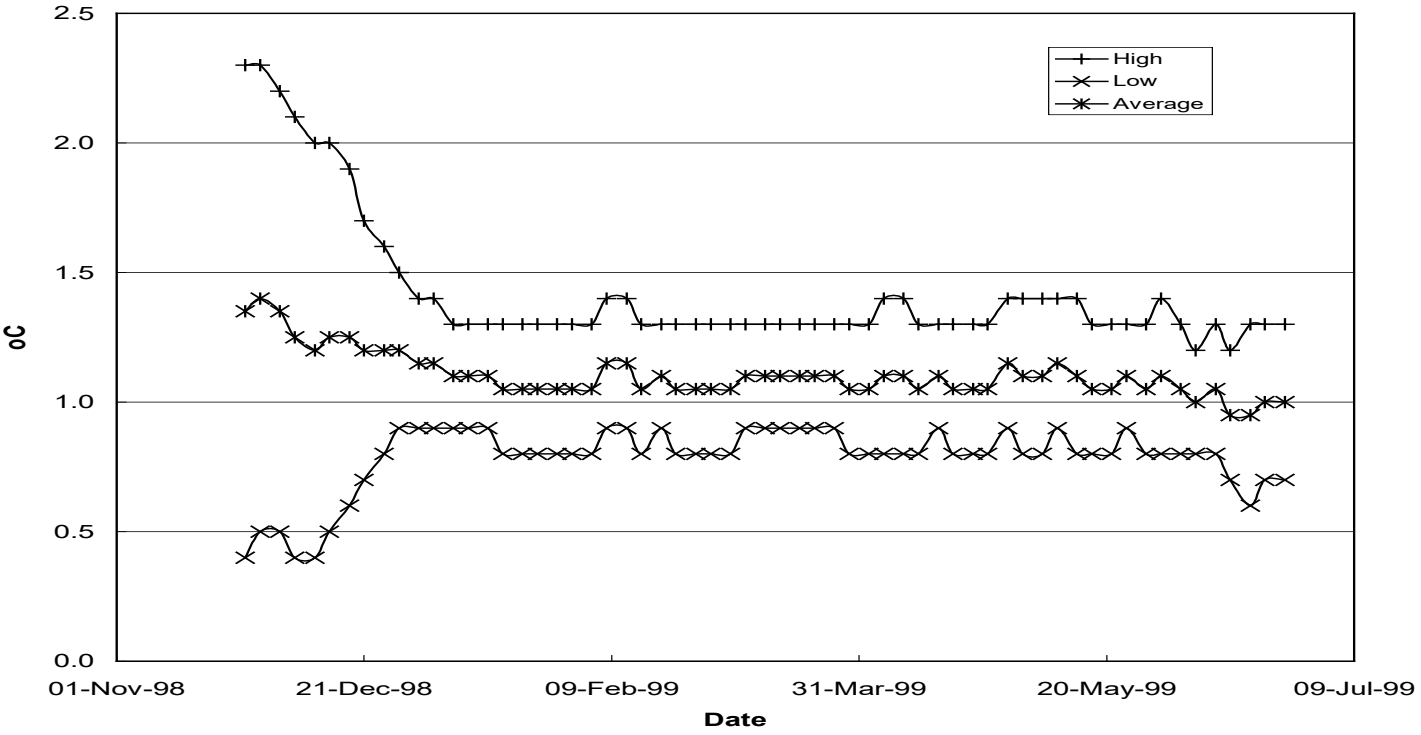


Fig. 2. Temperature of the sealed store

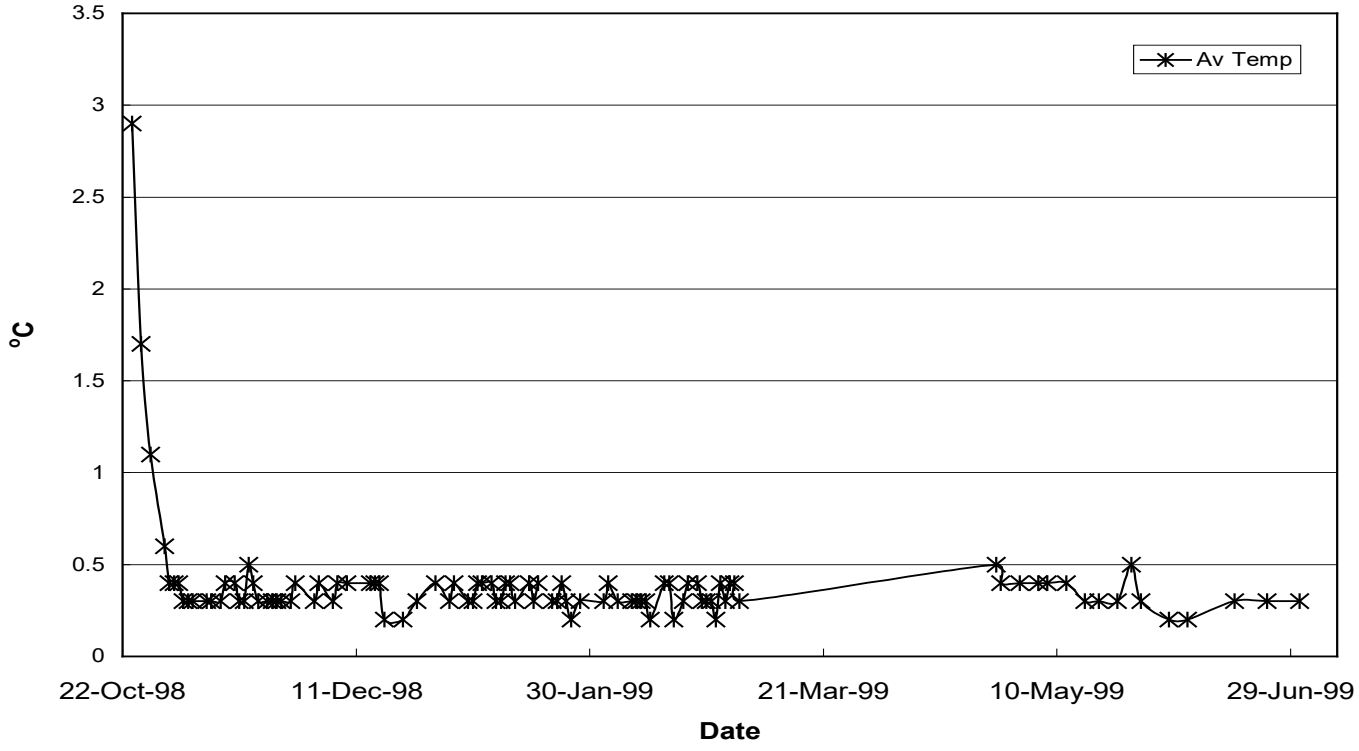


Fig. 3. Oxygen concentration of sealed store

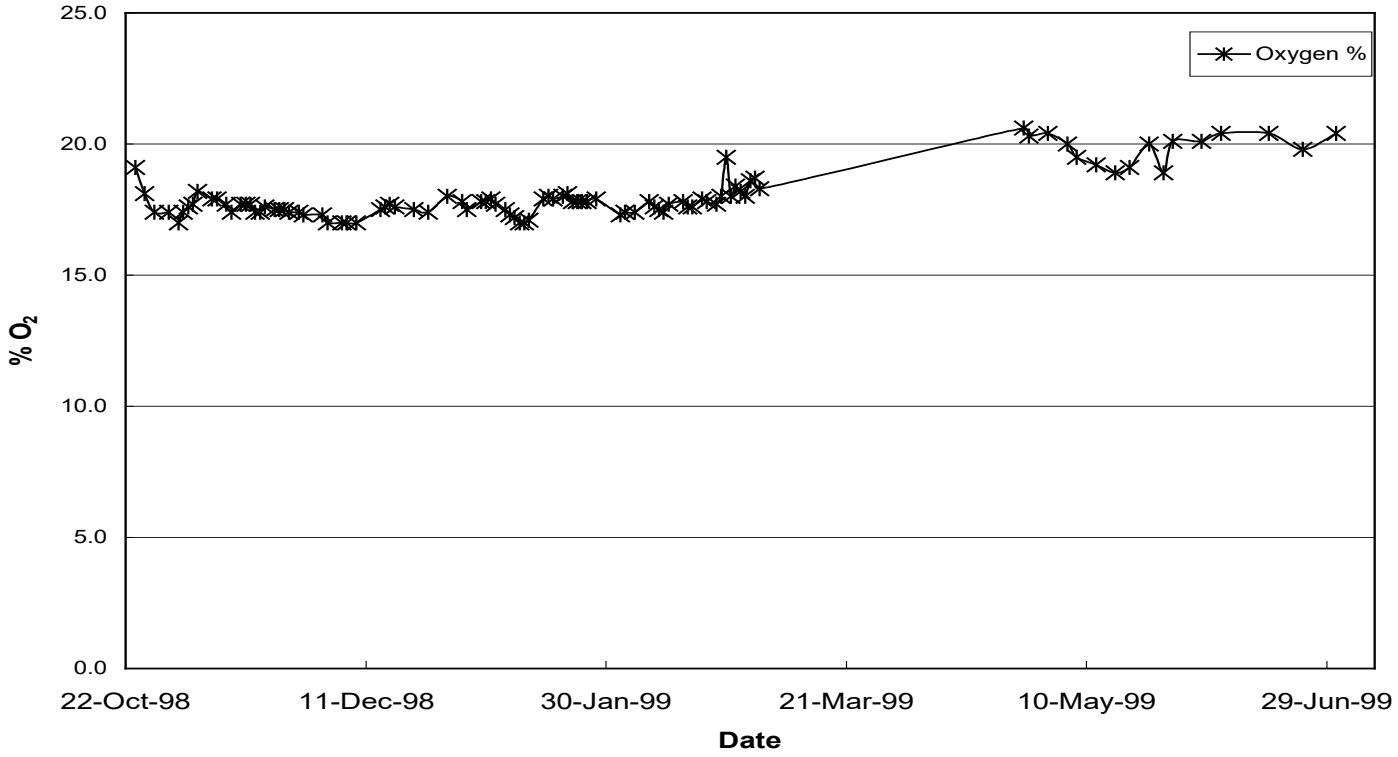


Fig. 4. Carbon dioxide concentration of sealed store

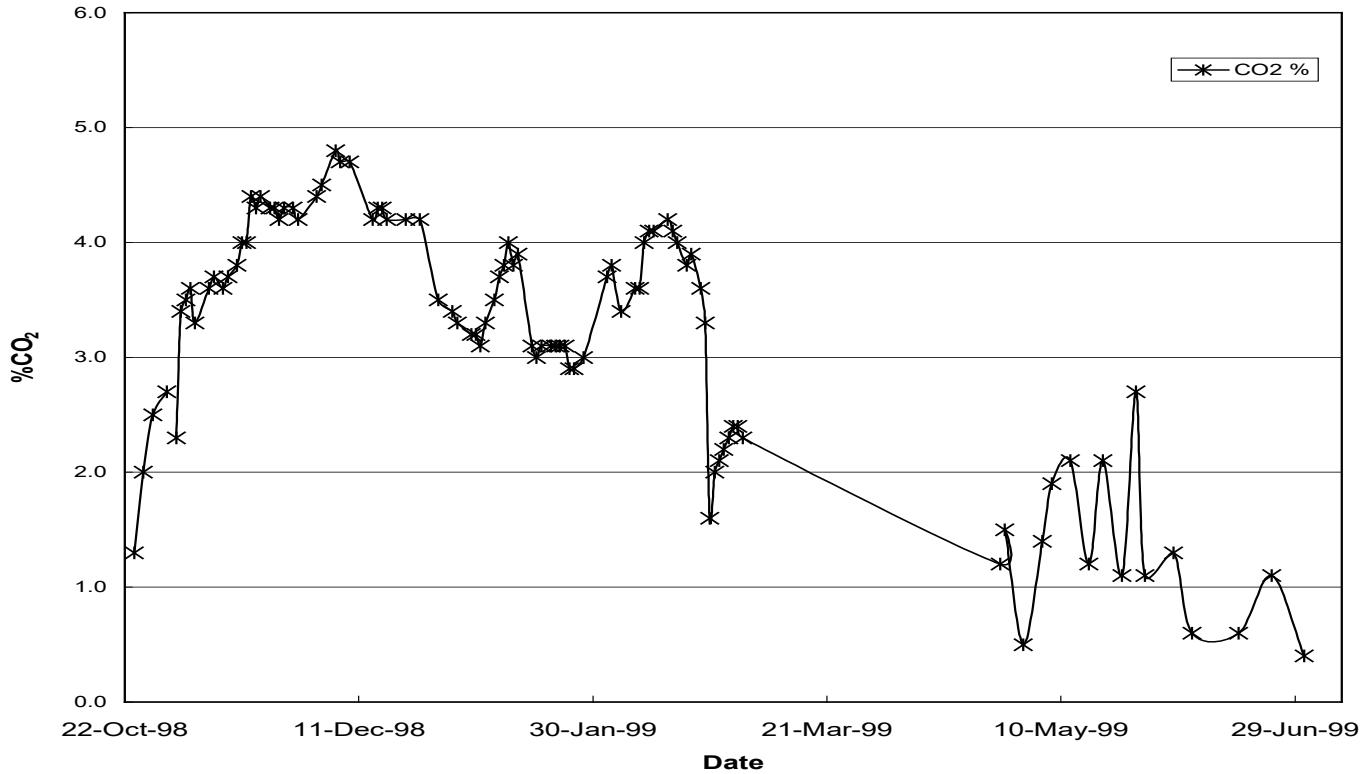


Fig. 5. Tipburn symptoms.

Fig. 6. Pepper spot.

Fig. 7. Cigar burn.