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CONTROL OF ZUCCHINI YELLOW MOSAIC VIRUS
BY MILD-STRAIN PROTECTION

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## SUMMARY FOR GROWERS

Yield depression in courgette and marrow (*Cucurbita pepo*) crops resulting from inoculating plants with the mild-strain (ZYMV:WK) of the virus, has been determined in polythene-house and outdoor, commercial field trials. ZYMV:WK infected plants were up to 10 days later in flowering than healthy plants and their yield was from 5 to 23% less depending on the cultivar. All courgette and marrow fruits harvested from ZYMV:WK infected plants were marketable, blemish-free and indistinguishable from fruits harvested from healthy plants. The mild leaf symptoms induced by ZYMV:WK infection did not intensify to cause severe leaf symptoms.

The delay in flowering resulting from the use of mild-strain inoculation suggests that cross-protection should not be used to protect plants in very early courgette or marrow crops, as any delay in fruit production in these crops would cause the grower to miss the higher early season prices. It is suggested that mild-strain cross-protection should be used only in the mid and late season crops, which experience has shown to be the most vulnerable to infection by severe strains of ZYMV.

The results of the controlled environment cabinet experiments confirmed that all the courgette and marrow cultivars tested, which were the most commercially important in the UK, were susceptible to both the ZYMV:WK and ZYMV:FA (severe) strains of the virus and that the symptoms caused by the ZYMV:WK strain were mild in all cultivars tested.

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## INTRODUCTION

Zucchini yellow mosaic potyvirus (ZYMV) has caused severe losses in cucurbit crops throughout the world since the late 1970s (Lisa *et al.*, 1981; Lecoq *et al.*, 1981; Provvidenti *et al.*, 1984; Nameth *et al.*, 1985; al-Musa, 1989; Ullman *et al.*, 1991; Walkey *et al.*, 1992). In the United Kingdom ZYMV has continued to cause disease outbreaks in courgette and marrow (*Cucurbita pepo*) crops, with numerous crops being lost in the 1991 and 1992 growing seasons. In 1991, the disease first appeared in the Vale of Evesham area in mid-season (July and August) and caused complete loss of the late sown courgette crop on five farmers' holdings and serious losses in a marrow crop at a sixth site. In 1992, the first outbreak of the disease was observed in mid-August and serious losses in courgette crops occurred at two sites. A small number of ZYMV infected plants were also observed in a marrow crop late (4 September) in the season.

At present there are no ZYMV-resistant courgette or marrow cultivars available to the grower to prevent the disease, but its control by mild-strain cross-protection has been successfully used in France (Lecoq *et al.*, 1991), Taiwan (Wang *et al.*, 1991) and the UK (Walkey *et al.*, 1992). The mild-strain, designated ZYMV:WK, used in the cross-protection experiments, was originally isolated in France from a naturally-infected melon (*Cucumis melo*) plant (Lecoq *et al.*, 1992). The ZYMV:WK strain is readily sap-transmitted, but reported to be poorly aphid-transmitted (Lecoq *et al.*, 1991). It causes mild mosaic symptoms in the leaves of inoculated plants, but in contrast to the severe strains of ZYMV, it does not cause fruit distortion in courgette or marrow plants.

For commercial exploitation of ZYMV mild-strain protection to control severe ZYMV disease outbreaks in courgette and marrow crops, it is essential to know what yield depression the mild strain causes. It is also important to determine that plants inoculated with the mild strain do not develop any other adverse symptoms under commercial growing conditions and that the mild strain remains stable and does not mutate to a severe form of the disease.

Observations made in France (H. Lecoq, unpublished data) suggested that the main effect of mild-strain infection was a 10% reduction in the fruit maturation rate, but prior to the results reported here, no precise information has been available of the effects of ZYMV:WK strain infection on courgette and marrow yields.

In the summer of 1992, trials were carried out at Wellesbourne in a Polythene-house and out-doors on farmers' holdings in the Vale of Evesham, to determine the yield depression caused by the mild strain in courgette and marrow crops and the stability of the ZYMV:WK strain under commercial conditions. In addition, the results of controlled environment (CE) cabinet experiments to investigate the effects of ZYMV:WK strain infection in a number of commercially important courgette and marrow cultivars are reported.

## MATERIALS AND METHODS

### Virus isolates and virus transmission

The ZYMV:WK strain was originally supplied by H. Lecoq and stored at Wellesbourne in liquid nitrogen. Two months prior to its experimental use, the stored inoculum was defrosted and inoculated to seedlings of the marrow cv. Goldrush at the cotyledon stage. The inoculated seedlings were maintained in a separate compartment of an insect-free glasshouse at approximately 24°C. After three, two-week incubation periods in cv. Goldrush, virus inoculum was prepared to infect the seedlings in the field trials. The severe strain of ZYMV (FA) used in the CE cabinet experiments was isolated in 1989 from a naturally infected courgette plant growing in the field in the Vale of Evesham.

Virus inoculum for glasshouse sap-transmissions was prepared by grinding infected leaves (14 to 21 days after infection) in $K_2HPO_4$ (10 g/l) solution containing $Na_2SO_3$ (1 g/l) at the rate of 1g leaf to 2 ml solution. Inoculum for the polythene-house and field trials was prepared in the same way, but further diluted to 1/5 (1 vol. of sap: 4 vol. of solution). The cotyledons of the test courgette and marrow seedlings were inoculated with the diluted sap using a muslin-soaked pad, as soon as they were fully expanded. The uninoculated and ZYMV:WK inoculated seedlings for the polythene-house trial were kept in an insect-free glasshouse compartment for 2 weeks before they were transplanted.

The ZYMV severe isolate found in diseased marrow plants growing adjacent to the experimental plants in the Chipping Campden trial was isolated and maintained in seedlings of cv. Goldrush.

### Experimental design of commercial trials

**Polythene-house trials, Wellesbourne.** Two trials were carried out in a 24 x 9m Polythene-house with insect-proof mesh ends and door. In the first trial, seeds of courgette cvs. Ambassador and Acceste was sown on 24 April on moist paper wadding in plastic boxes and germinated at 26°C. The germinated seedlings were potted into soil in an insect-free glasshouse on 28 April and grown at approximately 25°C. The seedlings were inoculated with ZYMV:WK on 7 May and transplanted to the Polythene-house on 13 May. The trial consisted of 24 ZYMV:WK inoculated and 24 uninoculated plants of each cultivar, each planted in four randomised blocks (four inoculated and four healthy blocks). Each block contained 12 plants, six of each cultivar planted in adjacent plots. Within each plot the plants were planted in two rows of three plants with 1 m spacing between plants and rows. There was a 2 m spacing between blocks.

The trial was irrigated by drip irrigation to each plant and plants were sprayed with heptenophos (Hostaquick) 1 wk after transplanting.

The second trial was planted in the same Polythene-house immediately the plants from the first trial were removed. Seed of cvs. Ambassador and Diamant for this trial was sown on 3 August, potted up on 14 August and transplanted on 20 August. The experimental design was similar to the first trial except that the eight blocks were planted in a different randomised arrangement.

**Field trials.** Three separate trials were carried out on farmers' holdings. At each site 1000 ZYMV:WK inoculated plants were planted as a part of the commercial crop and their yield was compared with 1000 uninoculated plants. At the first site at Cropthorne, Worcestershire, in the Vale of Evesham, inoculated and healthy plants of the courgette cv. Acceste were grown in separate blocks in 10 rows of approximately 100 plants per row, with a 1m spacing between plants and rows. The inoculated block was planted adjacent to the healthy block with a 3m space between. Seeds were planted on 5 May, seedlings were inoculated on 29 May and transplanted on 11 June.

At the second site at Wadborough, Worcestershire, in the Vale of Evesham, seeds of the courgette cv. Ambassador were sown on 8 June, seedlings inoculated on 18 June and transplanted on 25 June. The inoculated and healthy plants were planted in two adjacent unseparated blocks. Each block contained 1000 plants arranged in 15 rows with approximately 67 plants per row at a spacing of 1 m between plants and rows.

At the third site at Chipping Campden, Gloucestershire, seed of the marrow cv. Tiger Cross was sown on 1 June, the seedlings inoculated on 11 June and transplanted on 18 June. The inoculated plants were grown in the centre of a 4 hectare crop in 4 adjacent rows with c. 300 plants in rows 1 and 2, and 200 plants in rows 3 and 4. The remainder of rows 3 and 4 were planted with uninoculated plants. The plants were grown at a spacing of 1m between plants and rows.

The three sites were irrigated by overhead, spray irrigation.

**Virus indexing**

ELISA (enzyme-linked immunosorbent assay) was used to test for cucumber mosaic cucumovirus (CMV) and to confirm the presence of ZYMV in uninoculated plants that became infected during the trials. The direct antibody sandwich (DAS) test (Clark & Adams, 1977) was used with Nunc-immuno I (A/S Nunc, Denmark) plates. Coating globulin (applied at 1μg/ml) and conjugate (used at 1/1000) prepared from antiserum against CMV or the ZYMV:FA strain was used to detect the two viruses. Absorbance values ($A_{405nm}$) were measured with a Titertek Multiskan MCC/340 reader. The ZYMV:FA polyclonal antiserum detected both ZYMV:WK and severe strains of ZYMV and does not differentiate between the two. It was necessary, therefore, to confirm the presence of a severe strain of ZYMV by isolating the virus in marrow seedlings of cv. Goldrush and to monitor the development of severe leaf symptoms and fruit distortion.

**Harvest data**

Fruits were harvested three times a week from the Polythene-house experiment at Wellesbourne starting on 12 June and finishing on 14 August in the first experiment, and starting on 30 September and finishing on 27 November in the second. At each harvest all fruits over 10 cm in length were individually weighed.

At the Cropthorne site, the courgettes were harvested daily during the maximum period of crop production, and two or three times a week towards the end of the production period. Harvesting started on 10 July and finished 9 September. At each harvest the total weight of fruit from the inoculated and uninoculated blocks was recorded. At the Wadborough site, the courgettes were harvested three times a week starting on 21 July and finishing on 25 August. Only fruits of marketable size (approximately 14 cm) and shape were harvested and only the total weight of fruit from the two trial blocks was recorded at each harvest date. At the Chipping Campden site the marrows were harvested weekly, starting on 5 August and finishing on 12 September. At each harvest the total number of fruits from the 1000 inoculated and 1000 uninoculated plants was recorded.

**Controlled environment cabinet experiments**

Two experiments were made in Saxcil CE cabinets, to determine symptom reactions and yield reductions (measured by fresh leaf weights) caused by the mild (ZYMV:WK) and severe (ZYMV:FA) strains when inoculated to a range of different courgette and marrow cultivars. The courgette cvs. Ambassador, President, Acceste and Diamant were tested in the first experiment and courgette cvs. Ambassador and Elite, and marrow cvs. Tiger Cross and Early Gem in the second. In both experiments, seeds were germinated as described above, potted-up four days later in Levington's Universal compost and grown in an insect-free glasshouse at 24°C. As soon as the cotyledons were fully expanded 10 seedlings of each cultivar were inoculated with either the ZYMV:WK or ZYMV:FA strains and 10 seedlings of each cultivar were left uninoculated as healthy controls. Immediately after inoculations the seedlings were transferred to identical CE cabinets and grown as single plant randomised plots at a constant temperature of 22°C at 120 $Wm^{-2}$ (16h light/8h dark). The ten ZYMV:WK inoculated and five healthy seedlings of each cultivar were grown in one cabinet and the ten ZYMV:FA inoculated seedlings with five healthy controls of each cultivar in the second.

After 19 days' culture the plants from both experiments were removed from the cabinets and scored for virus symptoms. The fresh weight of each plant was recorded by cutting the stem at soil level and weighing the stem and leaves. The five oldest leaves of each plant were then sampled to determine the relative virus concentrations of the different treatments. A 20 cm-diameter disc was removed with a sterile cork-borer from each leaf and the five discs were combined and ground in buffer to provide the infected sap for an ELISA test.
Leaf symptoms and stunting were scored on a 0 to 5 scale: 0, no visible symptoms; 1; very mild mosaic, symptoms just visible; 2, mild but distinct symptoms; 3, moderately severe mosaic with veinal chlorosis; 4, chlorotic veins merged to form chlorotic blotches between the main veins; 5, chlorotic veinal blotches and leaf "shoestring" distortion; 0, no stunting; 1, up to 10% stunting; 2, 10 to 20% stunting; 3, 20 to 50% stunting; 4, 50 to 70% stunting; 5, >75% stunting.

## RESULTS

### Yield data from Polythene-house trials

ZYMV:WK inoculated plants of cvs. Ambassador and Acceste grown under commercial conditions in a Polythene-house, were up to 10 days later in flowering and in producing marketable courgette fruits than uninoculated healthy plants. Inoculated plants of both cultivars developed obvious mosaic symptoms, but these symptoms were much milder than symptoms in the same cultivars induced by the severe, FA strain of ZYMV in separate, glasshouse grown plants. The ZYMV:WK plants also lost the silver-grey leaf marking that are characteristic of both cultivars. In addition, inoculated plants were visually assessed as being c. 15 to 20% smaller than uninoculated healthy plants and had a more "open" growth habit. In contrast, the uninoculated plants had larger leaves which were frequently congested in the centre of the plant.

The accumulative fruit yield from the inoculated plants of both cultivars was lower than that of the respective, uninoculated healthy control plants (see Figure 1). It was interesting to note, however, that the accumulated yield of ZYMV:WK infected plants of the cv. Acceste, became greater than that of the uninoculated healthy plants of the cv. Ambassador 30 days after the first harvest. The delay in flowering and development of marketable fruits in the ZYMV:WK inoculated plants was apparent during the early harvests from the trial, when the accumulative yield depression (the reduction in accumulative yield from inoculated plants compared with uninoculated plants) of both cultivars was approximately 60%. Ten days after the first harvest the yield depression had fallen to below 30% and after 20 days to below 20%. By the last harvest on 14 August (63 days after the first harvest), the accumulative yield depression was approximately 5% for cv. Acceste and 15% for cv. Ambassador. It should be noted that accumulative yield depression for the cv. Ambassador rose slightly towards the end of the trial from approximately 10% to 15%. All the fruits harvested from the ZYMV:WK infected plants were blemish-free and indistinguishable from those harvested from the healthy, uninoculated plants. Also the leaf mosaic symptoms induced by the ZYMV:WK strain remained mild throughout the trial and there was no indication of any symptom change that might suggest any instability of the ZYMV:WK strain.

Several plants of both ZYMV:WK inoculated and uninoculated treatments became infected with CMV during the course of the trial. These plants developed leaf symptoms typical of CMV, and their fruits became deformed with typical depressed lesions. The yield data from these plants has been excluded from the result, from the date that the CMV symptoms were observed.

The pattern of accumulative yield depression shown by the cvs. Ambassador and Diamant in the second Polythene-house trial in which harvests started on 30 September and finished on 27 November (59 days after the first harvest), was generally similar to that recorded in the first experiment (see Figure 2). The accumulative yield depression of cv. Diamant started at 100% and fell to below 10% after 59 days of harvesting, and that of cv. Ambassador also started at 100% and fell to below 30%. Harvesting was terminated at the end of November because the plants were adversely affected by low temperature and it is possible that the higher yield depression recorded for the cv. Ambassador in the second trial compared with the first, resulted from this cultivar being particularly susceptible to the combined effects of low temperature and ZYMV:WK infection.

**Yield data from field trials**

**Cropthorne site.** Symptom expression in the ZYMV:WK inoculated cv. Acceste plants grown at this site, was identical to that observed in the Polythene-house trial at Wellesbourne. The mild-strain inoculated plants were visually assessed as being approximately 10 to 15% smaller than the uninoculated plants and had lost their silver-grey leaf markings. Flowering of the uninoculated plants at this site was approximately 6 days ahead of the ZYMV:WK infected plants and the initial accumulative yield depression of the inoculated plants was approximately 86% (see Figure 3). This fell after 10 days of harvesting to approximately 55% and after 20 days to 30%. By the end of harvesting on 9 September (61 days after the first harvest), the accumulative yield depression was approximately 15%.

A number of initially uninoculated plants at the margin of the healthy control block, adjacent to the inoculated block, became infected with the ZYMV:WK strain during the first 4 weeks after transplanting. It is thought that this spread may have been caused by sap mechanically transmitted on machinery. All the fruits harvested from the ZYMV:WK inoculated plants remained free of the protuberances and deformity typical of plants infected with severe strains of ZYMV, and were indistinguishable from those harvested from uninoculated plants. A few plants at this site became infected with CMV and these developed typical leaf symptoms, were severely stunted and their fruits were deformed with characteristic sunken lesions.

**Wadborough site.** The symptoms and plant growth observed in ZYMV:WK inoculated plants of cv. Ambassador at this site, were similar to the ZYMV:WK infected Ambassador plants grown in the Polythene-house trial at Wellesbourne. At this site, the initial depression in accumulative yield was markedly less than in the Cropthorne or Wellesbourne trials. At the first harvest the yield of the ZYMV:WK inoculated plants was only 30% lower than that of the uninoculated plants but after 2 weeks the yield depression fell to approximately 9% (see Figure 3). However, the accumulative yield depression rose to 23% at the final harvest on 25 August (35 days after the first harvest). A similar reduction in fruit yield was also observed towards the end of the harvesting period for the cv. Ambassador in the polythene house trial at Wellesbourne (see Figures 1 & 2).

All the fruits harvested from the inoculated plants at this site were blemish-free, marketable and indistinguishable from those harvested from the uninoculated plants. There was no visual change in the intensity of the symptoms caused by the mild strain at this site during the trial, but by the end of the trial the development of Powdery Mildew was noticeably less in the ZYMV:WK inoculated plants than in the adjacent block of uninoculated plants.

**Chipping Campden site.** The symptoms and plant growth observed in the ZYMV:WK inoculated plants of the marrow cv. Tiger Cross at this site, were generally similar to those of the inoculated plants of the courgette cvs. Acceste and Ambassador grown at the other sites, except that healthy plants of cv. Tiger Cross do not have silver-grey leaf markings. In common with the trial at the Wadborough site, a relatively low yield depression (approximately 13%) was recorded for the ZYMV:WK inoculated treatment at the first harvest on 10 July (see Figure 3). The accumulative yield depression remained relatively constant at 14 to 15% for all harvests (the final harvest was made on 9 September, 61 days after the first). There was no visual increase in symptom severity in ZYMV:WK infected plants during the course of the experiment and all the fruits harvested were blemish-free and indistinguishable from those produced by the healthy, uninoculated plants.

There was, however, a very considerable spread of the ZYMV:WK infection at this site from the inoculated plants to adjacent rows of healthy plants. In addition, a small area (50 to 60 plants) of healthy plants, growing some distance (70m) away from the original ZYMV:WK inoculated trial plants, became infected with a severe strain(s) of ZYMV. These plants developed severe leaf symptoms and the typical fruit deformity characteristic of the severe (S) strains of ZYMV. ZYMV:WK infected plants in the vicinity of the ZYMV:S infected plants showed no fruit deformity and appeared to be protected from infection by the ZYMV:S strain.

**Controlled environment experiments**

The effects of inoculating seedlings of the main UK courgette and marrow cultivars with the mild (ZYMV:WK) and severe (ZYMV:FA) strains were determined in two separate, but identical CE cabinet experiments at 22°C (see Table 1). The results showed that all the cultivars tested were susceptible to the ZYMV:WK strains; but that the leaf symptoms and stunting induced by this strain were considerably milder than those caused by the ZYMV:FA strain.

The relative virus concentration in leaves infected with the ZYMV:WK and ZYMV:FA strain (as measured by ELISA) was variable. In some cultivars the results indicated a higher virus content in plants infected with the severe strain and in others a higher concentration in mild-strain infected plants (see Table 1).

## DISCUSSION

The Polythene-house and field trials showed that courgette plants inoculated with the mild ZYMV:WK strain were generally 7 to 10 days later than healthy, uninoculated plants in producing flowers. This resulted in a corresponding delay in marketable, courgette fruit formation on the ZYMV:WK inoculated plants. However, after this initial delay, fruit production from the mild-strain protected plants proceeded normally and the accumulative yield difference between the inoculated and uninoculated plants for all harvests ranged from approximately 5% to 23%, depending on the cultivar. The cv. Ambassador appeared to be more sensitive to ZYMV:WK infection and other adverse growing conditions (such as powdery mildew infection) at the end of its fruit production season. This resulted in a higher accumulative yield depression for this cultivar from mid-season onwards (see Figures 2 and 3). In contrast, the accumulative yield depression fell gradually throughout the season for the cv. Acceste (see Figures 2 and 3) and remained relatively constant for the marrow cv. Tiger Cross (see Figure 3). It was interesting to observe that the final depression in accumulative yield for the cv. Diamant in the Wellesbourne trial was approximately 9%, a value very similar to the 11% reported for the same cultivar in a Polythene-house experiment in France (Lecoq *et al.*, 1991).

## CONCLUSIONS

The trials confirmed the stability of the ZYMV:WK strain under commercial growing conditions. All the fruits harvested from mild-strain inoculated plants were blemish-free, marketable and indistinguishable from those harvested from healthy, uninoculated plants. The mild mosaic symptoms caused by the ZYMV:WK strain did not intensify as the leaves of the infected plants matured and did not develop the "shoe-string" deformity that is characteristic of plants infected with severe strains of ZYMV.

The three growers who participated in these trials, indicated that a yield-loss of between 10 and 20% was acceptable to them, if it insured against a possible 100% loss that might occur should the crop become infected with a severe ZYMV strain. The delay in flowering resulting from the use of mild-strain inoculation suggests that cross-protection should not be used to protect plants in very early courgette or marrow crops, as any delay in fruit production in these crops would cause the grower to miss the higher early season prices. It is suggested that mild-strain cross-protection should be used only in the mid and late season crops, which experience has shown to be the most vulnerable to infection by severe strains of ZYMV.

It is also possible that the yield depression of courgette fruits resulting from the use of the mild strain, might be reduced by increasing the amount of nitrogen fertilizer applied to the crop. It was notable that the slower fruit growth rate in marrow plants of the cv. Tiger Cross inoculated with the ZYMV:WK strain, resulted in smaller fruits. These were preferred by the grower than the faster maturing fruits produced by the uninoculated plants.

The results of the CE cabinet experiments confirmed that all the courgette and marrow cultivars tested, which were the most commercially important in the UK, were susceptible to both the ZYMV:WK and ZYMV:FA strains of the virus and that the symptoms caused by the ZYMV:WK strain were mild in all cultivars tested.

## ACKNOWLEDGEMENTS

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## GLOSSARY

**Accumulative yield**: the addition of the fruit weight at each harvest date to that of the previous harvest date so that the yield accumulates with time.

**Accumulative yield depression**: the reduction in accumulative yield from inoculated plants compared with uninoculated plants.

**Cross-protection**: the application of a mild strain of a virus to a plant to protect it from subsequent infection by a severe strain of the same virus.

**Enzyme-linked immunosorbent assay (ELISA)**: a serological test in which the sensitivity of the antibody-antigen reaction is increased by attaching an enzyme to one of the two reactants.

**Polyclonal antiserum**: serum which contains a population of antibodies.

**Figure 1 Accumulative fruit yield from courgette cultivars inoculated with ZYMV:WK compared with uninoculated plants in a polythene house**

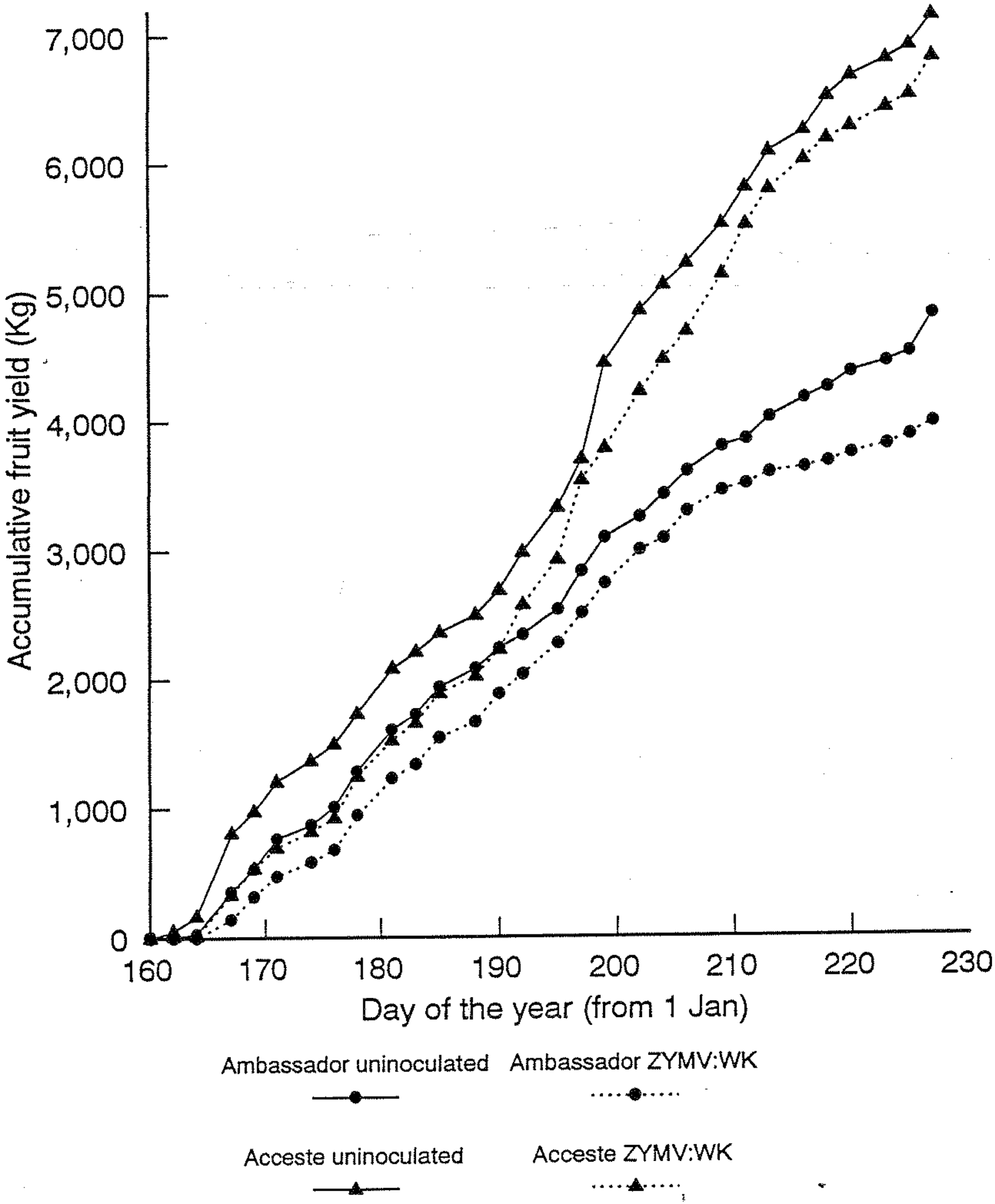
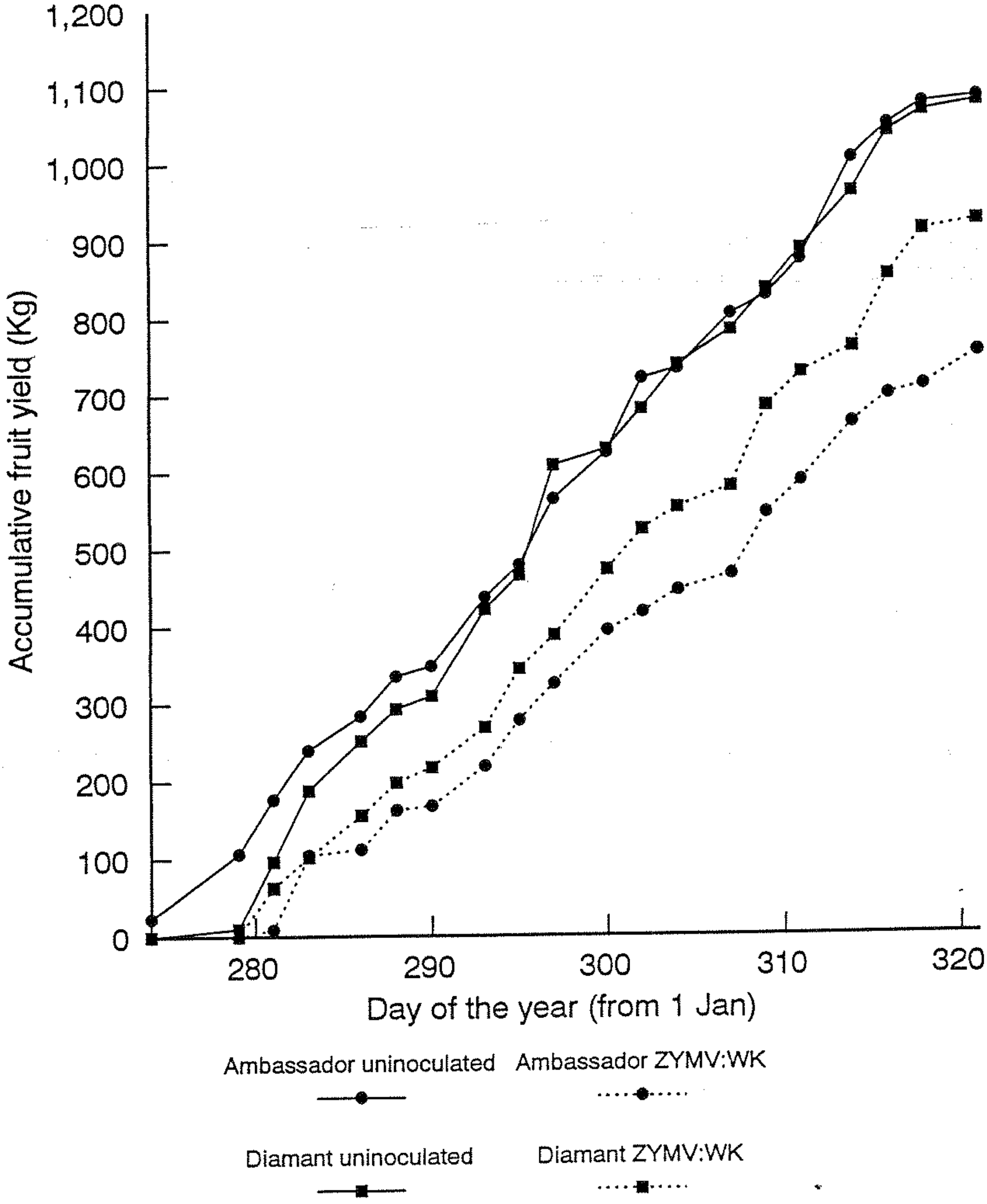


**Figure 2 Accumulative fruit yield from courgette cultivars inoculated with ZYMV:WK compared with uninoculated plants in a polythene house**



## Figure 3 Accumulative fruit yield from courgette and marrow cultivars inoculated with ZYMV:WK compared with uninoculated plants at field sites

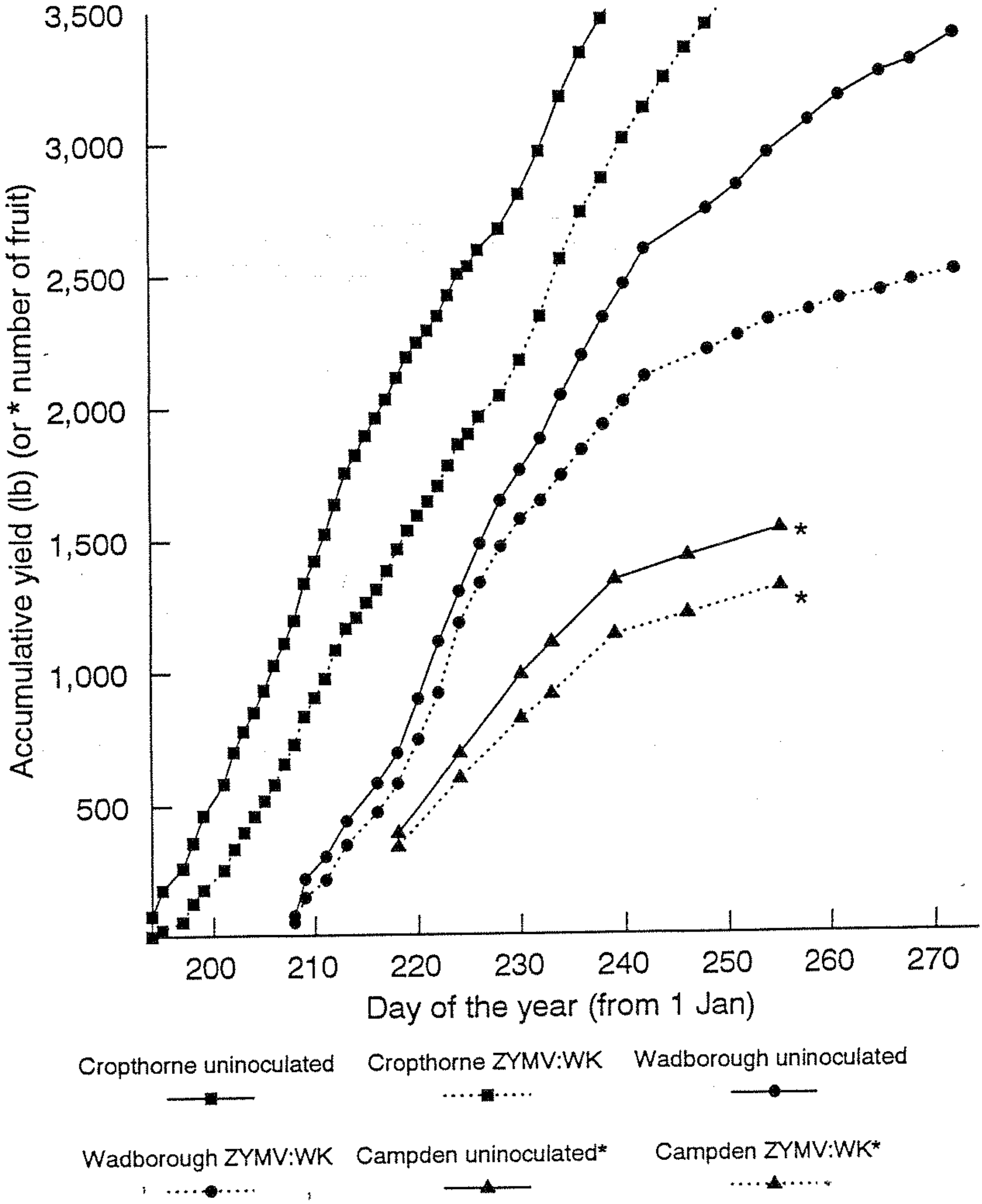


**Table 1** **Symptom scores, stunting and leaf-weight reduction resulting from inoculation with ZYMV:FA and ZYMV:WK in different *C. pepo* cultivars grown at 22°C in CE cabinets**

| | Cultivar | ZYMV:FA infected plants | | | | ZYMV:WK infected plants | | | | Healthy plants | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean symptom and stunting scores | Mean leaf weight (g) | % weight loss | ELISA[b] | Mean symptom and stunting scores | Mean leaf weight (g) | % weight loss | ELISA | Mean leaf weight (g) | ELISA |
| Experiment 1 | Ambassador | 4.4 : 4.0 | 29.35 | 72.25 | 0.110 | 0.7 : 1.3 | 76.34 | 27.83 | 0.050 | 105.78 | 0.021 |
| | President | 2.7 : 4.0 | 55.61 | 64.97 | 0.227 | 1.0 : 1.5 | 132.01 | 16.85 | 0.146 | 158.78 | 0.021 |
| | Acceste | 4.2 : 4.6 | 31.19 | 75.15 | 0.233 | 1.3 : 1.9 | 86.16 | 31.37 | 0.060 | 125.56 | 0.021 |
| | Diamant | 3.4 : 4.2 | 39.69 | 70.45 | 0.216 | 1.0 : 1.5 | 95.43 | 28.96 | 0.275 | 134.34 | 0.021 |
| Experiment 2 | Ambassador | 3.9 : 2.7 | 22.94 | 53.39 | 0.358 | 2.2 : 2.3 | 33.21 | 32.52 | 0.264 | 49.22 | 0.118 |
| | Elite | 4.2 : 2.8 | 24.66 | 57.26 | 0.514 | 2.4 : 2.3 | 37.49 | 35.02 | 0.580 | 57.70 | 0.118 |
| | Tiger Cross[a] | 4.8 : 5.0 | 11.71 | 76.28 | 0.275 | 2.0 : 1.7 | 31.41 | 36.38 | 0.319 | 49.38 | 0.118 |
| | Early Gem[a] | 3.8 : 2.5 | 32.80 | 54.92 | 0.616 | 2.2 : 1.0 | 55.72 | 23.41 | 0.537 | 72.76 | 0.118 |

[a] denotes marrow cultivars, all other cultivars are courgettes

[b] ELISA results were considered positive if the mean absorbance value ($A_{405nm}$) was twice that of the mean healthy control