HDC FINAL REPORT
FV/132

CONTROL OF ZUCCHINI YELLOW MOSAIC VIRUS BY MILD-STRAIN PROTECTION

N.J. SPENCE, A. MILLER & E.D. SHAW
October, 1995

HDC FINAL REPORT

Final Report	October, 1995
Project Number	FV/132
Project Title	Control of zucchini yellow mosaic virus by mild-strain protection
Project Leader	Dr N J Spence
Location of Project	Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK
Project Co-ordinator	Mr Peter Emmet
Date Project commenced	1 June, 1992
Date project completed	31 May, 1995
Key words	Courgettes, cucumbers, marrows, squash, mild-strain protection

CONTENTS **PAGE**

RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

Application

Objective of the project : To carry out polythene-house and field trials at Wellesbourne to determine the effect of the mild strain of zucchini yellow mosaic virus (ZYMV:WK) on yield in courgette and marrow crops and the stability of ZYMV:WK under commercial conditions. To evaluate the control of ZYMV by mild strain protection in protected cucumber crops.

Key results: The stability of the mild-strain of zucchini yellow mosaic virus (ZYMV:WK) under commercial growing conditions was confirmed and it was effective in controlling the spread of severe ZYMV. In polythene-house and field trials the accumulative yield from courgette and marrow crops which were inoculated with ZYMV:WK was 4 to 38% less than uninoculated plants, depending on the cultivar and site, although in one field trial the yield was 7% higher from inoculated plants. Infection by severe strains of ZYMV can cause 100% losses. Mild-strain protection was effective in controlling ZYMV in protected cucumbers and caused a yield depression of 8%, compared with uninoculated plants.

Opportunity for application : Mild-strain protection is the only control method currently available for ZYMV infection and has immediate application for growers of all cucurbit species (courgette, cucumber, marrow, pumpkin and squash). The inoculum is classified as a biological pesticide and its use is regulated by the Pesticide Safety Directorate. Before it can be made available for commercial use it must be registered under EEC directive 94/414.

Summary

Scope and objective of the project : To carry out polythene-house and field trials at Wellesbourne to determine the effect of the mild strain of zucchini yellow mosaic virus (ZYMV:WK) on yield in courgette and marrow crops and the stability of ZYMV:WK under commercial conditions. To evaluate the control of ZYMV by mild-strain protection in protected cucumber crops.

Summary of results

- Courgette and marrow seedlings could be rapidly and reliably inoculated with the minimum of training and equipment.
- In polythene-house trials, mild strain inoculated courgette plants were up to 10 days later in flowering and their accumulative yield was 5 to 26% less than uninoculated plants depending on the cultivar. Of the cultivars, tested cv Acceste performed best.
- In field trials, accumulative yield from courgette and marrow inoculated plants was 4 to 38% less than uninoculated plants depending on the site, although in one trial the yield was 7% higher from inoculated plants.
- In each experiment, courgette and marrow fruit harvested from inoculated plants was symptomless and indistinguishable from that harvested from uninoculated plants.
- Where there were natural outbreaks of severe ZYMV infection in courgette, fruit from inoculated plants remained unaffected whilst that from uninoculated plants was severely distorted and unmarketable.
- Mild-strain protection was effective in controlling severe ZYMV in cucumbers and yield depression in cucumbers inoculated with ZYMV:WK in polytunnel and gauze-house experiments was 8%.

Practical and financial anticipated benefits

- For a small potential reduction in yield by using ZYMV:WK, growers eliminate the potential risk of 100% losses if there is an outbreak of severe ZYMV.
- Plants inoculated with ZYMV:WK can have a more open growth habit and are therefore easier to harvest which reduces labour costs.
- If plants are protected by ZYMV:WK, growers could return to harvesting fruit with cutting knives thereby reducing time taken for harvest and saving labour costs.
- There is no need to spray for aphids so savings are made in the cost of pesticides and the crop is more environmentally acceptable.

EXPERIMENTAL SECTION

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 at five sites and serious losses in a marrow crop at a sixth site. In 1992, the first outbreak of the disease in the Vale of Evesham 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 in the season. Further outbreaks of the virus in the Vale of Evesham followed in 1993, 1994 and 1995.

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 severe strains of ZYMV, it does not cause fruit distortion in courgette or marrow plants.

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 in this report, no precise information has been available of the effects of ZYMV:WK strain infection on courgette and marrow yields. 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 cause a severe form of the disease.

In the summer of 1992, trials were carried out at Wellesbourne in a polythene-house and at three farmers' sites, 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 1993 and 1994, further field trials were carried out at four sites.

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 sap 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 c. 24°C. After three, two-week incubation periods in cv. Goldrush, virus inoculum was prepared to infect the test seedlings.

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 1 g leaf to 2 ml solution. Inoculum for the Polythene-house and field trials was prepared in the same way, but diluted to 1 in 5 (1 g leaf to 4 ml of solution). The cotyledons of the test courgette and marrow seedlings were inoculated with the diluted sap using a muslin pad soaked in inoculum, 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.

Experimental design of commercial trials

Polythene-house trials, Wellesbourne.

Two trials were carried out in a 24 x 9 m 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 c. 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 daily 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. Fruit was harvested three times a week 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.

Field trials

At each site various numbers of ZYMV:WK inoculated plants were planted as a part of the commercial crop and their yield was compared with uninoculated plants of the same age. Seed was usually sown in modular trays and seedlings planted with a 1 m spacing between plants and rows. All trials were irrigated by overhead, spray irrigation.

Field trials, 1992.

Three separate trials were carried out on farmers' fields and at each site c. 1000 ZYMV:WK inoculated plants were planted. At the 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. Marrows were harvested weekly, starting on 5 August and finishing on 12 September. At each harvest the total number of fruits from the inoculated and uninoculated plants was recorded. The marrows were normally in the range of 20-25 cm long, however towards the end of the season they were often larger than 30 cm.

At the site at Cropthorne, Worcestershire, in the Vale of Evesham, inoculated and uninoculated plants of the courgette cv. Acceste were grown in separate adjacent blocks with a 3 m space between blocks. Seeds were sown on 5 May, seedlings were inoculated on 29 May and transplanted on 11 June. 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 site at Wadborough, Worcestershire, in the Vale of Evesham, seeds of the courgette cv. Ambassador were sown in pots on 8 June, seedlings inoculated on 18 June and transplanted on 25 June. The inoculated and uninoculated plants were planted in two adjacent unseparated blocks. Courgettes were harvested three times a week starting on 21 July and finishing on 25 August. Only fruits of marketable size (c. 14 cm) and shape were harvested and the total weight of fruit from the two trial blocks was recorded at each harvest date.

Field trials, 1993.

Four separate trials were carried out on farmers' fields, three at the same sites as 1992 and one at another site in Cropthorne (Cropthorne 2). At most sites *c.* 5000 ZYMV:WK inoculated plants were planted as a part of the commercial crop and their yield was compared with the same number of uninoculated plants of the same age with a 1 m spacing between plants and rows.

At the Chipping Campden site, seed of the marrow cv. Tiger Cross was sown on 19 May, the seedlings inoculated on 4 June and transplanted on 10 June. The inoculated and uninoculated plants were planted in two adjacent blocks with a 3 m space between blocks. Marrows were harvested weekly, starting on 30 July and finishing on 11 October, although the first fruit from the inoculated plants was harvested until 3 August. At each harvest the total number of fruits from the inoculated and uninoculated plants was recorded.

At the original Cropthorne site (Cropthorne 1), inoculated and uninoculated plants of the courgette cv. Acceste were grown in separate adjacent blocks with a 3 m space between. Seeds were sown on 4 May, seedlings inoculated on 21 May and transplanted on 1 June. 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 13 July and finished 4 October. At each harvest the number of crates (*c.* 22 Kg per crate) of fruit from the inoculated and uninoculated blocks was recorded.

At the Cropthorne 2 site plants of the courgette cv. Acceste were grown in separate adjacent unseparated blocks. Seeds were sown on 2 June, seedlings inoculated on 11 June and transplanted between 24 and 29 June. Courgettes were harvested daily during the maximum period of crop production, and two or three times a week at the beginning and towards the end of the production period. Harvesting started on 16 August and finished 10 October. At each harvest the number of boxes (*c.* 10 lbs) of fruit from the inoculated and uninoculated blocks was recorded.

At the Wadborough site the experiment was divided into two plantings of the courgette cv. Acceste, with 1000 and 2000 inoculated and uninoculated plants in the first and second plantings only. For the first planting seeds were sown in pots on 27 May, seedlings inoculated on 9 June and transplanted on 16 June; for the second planting seeds were sown in pots on 10 June, seedlings inoculated on 24 June and transplanted on 1 July. The inoculated and uninoculated plants were planted in two adjacent unseparated blocks for each planting. Courgettes were harvested every day, with fruit from inoculated and uninoculated plots being harvested on alternate days, starting on 23 July and finishing on 16 September. Initially, fruit was harvested from the first planting, later from the first and second plantings and towards the end of the season from the second planting only. Only fruits of marketable size (c. 14 cm) and shape were harvested and the total weight of fruit was recorded at each harvest date.

Field trials, 1994

Four separate trials were carried out on farmers' fields, three at the same sites as 1992 and one at another site at Eckington, Worcestershire. Between 1,000 and 6,000 of ZYMV:WK inoculated plants were planted at different sites as a part of the commercial crop with a 1 m spacing between plants and rows. Yield from the inoculated block was compared with the same number of uninoculated plants of the same age. At the Chipping Campden site, seed of the marrow cv. Tiger Cross was sown on 3 June, 2,500 seedlings were inoculated on 13 June and transplanted on 20 June. The inoculated plants were planted in a block adjacent to an uninoculated block with a 3 m space between. Marrows were harvested weekly, starting on 4 August and finishing on 6 September. At each harvest the total number of fruits from the inoculated and uninoculated plants was recorded.

There were two separate trials at the Cropthorne 1 site, inoculated and uninoculated plants of the courgette cv. Acceste were grown in separate blocks with 6,000 inoculated plants (Cropthorne 1a) in one trial and 3,000 in the other (Cropthorne 1b). In each trial the inoculated block was planted adjacent to the uninoculated block with a 3 m space between. Seeds were sown on 3 and 21 May (1a and 1b respectively) and seedlings inoculated on 13 and 31 May and transplanted on 20 May and 7 June. Harvesting started on 12 July and finished 12 September and 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. At each harvest the number of crates (*c.* 22 Kg per crate) of fruit from the inoculated and uninoculated blocks was recorded.

At the Eckington site, seed of cv Acceste plants was sown on 3 May, 1,000 plants were inoculated on 13 May and transplanted on 20 May. The inoculated plants were planted in a block adjacent to an uninoculated block with a 3 m space between. Courgettes were harvested daily during the maximum period of crop production, and two or three times a week at the beginning and towards the end of the production period. Harvesting started on 8 July and finished 27 September. At each harvest the number of boxes of fruit weighing 10lbs from the inoculated and uninoculated blocks was recorded.

Polythene-house and gauze house experiments

Seeds of the cucumber cv Pepinex were sown on moist paper wadding in plastic boxes and germinated at 26°C. The germinated seedlings were potted into soil in an insect-free glasshouse and grown at approximately 24°C. One experiment was carried out in a 24 x 9m polythene-house with insect-proof gauze at either end and consisted of 30 ZYMV:WK inoculated and 30 uninoculated plants of each cultivar, each planted in four randomised blocks (five blocks per treatment). Seedlings were inoculated with ZYMV:WK in the glasshouse and transplanted into grow-bags in the polythene-house.

The second experiment was carried out in two quarantine gauze houses. The experiment consisted of 32 ZYMV:WK and 32 ZYMV:FA inoculated plants, 32 ZYMV:WK inoculated plants which were then infected with ZYMV:FA 15 days later and 32 uninoculated plants. The experimental design consisted of four randomised plots in each of four blocks. Seedlings were inoculated with ZYMV:WK in the glasshouse and transplanted into grow-bags in the gauze houses. The severe strain ZYMV:FA was transmitted to plants 15 days after they were infected with ZYMV:WK by placing viruliferous aphids on the third true leaf inside an aphid-proof bag. Plants were sprayed with heptenophos (Hostaquick) 24 hours after transmission.

All trials were irrigated by drip irrigation with liquid feed to each plant.

Glasshouse experiments

A hand pump sprayer (Hozelock Polyspray 2) was evaluated for use in inoculating courgette seedlings with ZYMV:WK. Several experiments were carried out to determine if the spray was effective alone, whether an abrasive (carborundum) should be included in the inoculum or whether brushing the plants with a range of brushes after spraying was necessary.

Virus indexing

ELISA was used to test for cucumber mosaic cucumovirus (CMV) infection 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 (severe) strain was used to detect the two viruses. Absorbence 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 did not differentiate between the two. It was necessary, therefore, to confirm the presence of a severe strain of ZYMV by isolating the virus in seedlings of cv. Goldrush and monitoring the development of severe leaf symptoms and fruit distortion. In addition, immunocapture polymerase chain reaction followed by RFLP analysis (Barbara *et al.*, 1995) was used to differentiate ZYMV:FA and ZYMV:WK in field samples.

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 plants. Inoculated plants of both cultivars developed obvious leaf mosaic symptoms compared with uninoculated plants, but these symptoms were much milder than symptoms in the same cultivars induced by a severe strain of ZYMV (ZYMV:FA) in separate, glasshouse grown plants. 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 fruit yield from the inoculated plants of both cultivars was lower than that of the respective, uninoculated control plants. It was interesting to note, however, that the accumulative 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 difference in accumulative yield between inoculated and uninoculated plants of both cultivars was c. 60%. Ten days after the first harvest the difference in yield had fallen to below c. 30% and after 20 days to below c. 20%. By the last harvest on 14 August (63 days after the first harvest), the difference in accumulative yield was c. 5% for cv. Acceste and c. 15% for cv. Ambassador. It should be noted that the difference in accumulative yield for the cv. Ambassador increased slightly towards the end of the trial from c. 10% to c. 15% (Figure 1). There was little difference in the mean number of fruit per plant from uninoculated and inoculated plants of cvs Ambassador or Acceste and the mean fruit weight per plant of each treatment was similar for Acceste but 20% lower from inoculated plants of cv Ambassador compared with uninoculated plants (Table 1). Fruit harvested from the ZYMV:WK infected plants were blemish-free and indistinguishable from that harvested from the healthy, uninoculated plants.

Also the intensity of 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 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 and the yield from inoculated plants was always lower. The difference in accumulative yield between inoculated and uninoculated plants of cv. Diamant was c. 10% after 59 days of harvesting, and that of cv. Ambassador was c. 30% (Figure 2). There was a greater difference in the mean number of fruit per plant from uninoculated and inoculated plants of Ambassador compared with the first experiment but the difference in mean fruit number per plant for cv Diamant was similar to that of cv Acceste in the first experiment. The mean fruit weight per plant from inoculated plants was 8% lower for cv Diamant but 26% lower for cv Ambassador compared with uninoculated plants (Table 1). Harvesting was terminated at the end of November because the plants were adversely affected by low temperature and it is possible that the greater yield depression recorded for the cultivar 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 the field trials

Fruit was harvested separately from inoculated and uninoculated plants and weight of fruit, number of fruit (marrows) or number of boxes or crates of a fixed weight was recorded for each harvest by individual growers. A summary of total accumulative fruit weight for each trial is given in Table 2.

Yield data from the 1992 field trials

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. In common with the trial at the Wadborough site, a relatively low reduction in accumulative yield (c. 13%) was recorded for the ZYMV:WK inoculated treatment at the first harvest on 10 July (Figure 3). The difference in accumulative yield remained relatively constant at c. 14% for all harvests 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 (70 m) 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 strains of ZYMV. ZYMV:WK infected plants in the vicinity of the plants infected with severe strain(s) showed no fruit deformity and appeared to be protected from infection by the severe strain(s).

Fruit harvested from the inoculated plants at each site was blemish-free, marketable and indistinguishable from that harvested from the uninoculated plants. There was no visual change in the intensity of the symptoms caused by the mild-strain at any of the sites during the trial.

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 usually assessed as being c. 10 to 15% smaller than the uninoculated plants. Flowering of the uninoculated plants at this site was c. 6 days ahead of the ZYMV:WK infected plants and the initial difference in yield between inoculated and uninoculated plants was c. 86% lower from inoculated plants. This fell after 10 days of harvesting to c. 55% and after 20 days to c. 30%. By the end of harvesting, the difference in accumulated yield was c. 14% from inoculated plants (Figure 4).

A number of uninoculated plants at the margin of the uninoculated 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.

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 reduction in accumulative yield was markedly less than in the Cropthorne or Wellesbourne trials. At the first harvest the accumulative yield from the ZYMV:WK inoculated plants was c. 30% lower than that of the uninoculated plants but after 2 weeks the difference in accumulative yield fell to c. 9% (Figure 5). However, the difference in accumulative yield had risen to 26% at the final harvest on 25 August. A similar reduction in fruit yield was also observed towards the end of the harvesting period for cv Ambassador in both polythene-house trials at Wellesbourne.

Yield data from 1993 field trials

Chipping Campden site. The inoculated plants appeared to be rather stunted compared with uninoculated plants and the symptoms of ZYMV:WK were more severe than at other sites. There was a delay of five days in the harvest of fruit from the inoculated plot which caused an early yield reduction but about 10 days after the first harvest the yield from each plot was similar. Later, the yield from the inoculated plants started to decline more rapidly than that from uninoculated plants and by the last harvest the reduction in yield was about 6%. There was little spread of ZYMV:WK from the inoculated plot and a few uninoculated plants in the rows adjacent to the inoculated plants became infected (Figure 6).

Cropthorne site. Plants in the inoculated and uninoculated plots became quickly established and almost 100% of the plants in the inoculated plot were infected. Symptoms remained mild and there was no visual evidence that inoculated plants were smaller than plants from the uninoculated plot. There was little spread of the mild strain and only three plants were observed with symptoms in the uninoculated plot. There was no difference in the yield from inoculated and uninoculated plots in the early harvests and after 10 days the yield from the inoculated plants was higher and by the end of the trial was 7% higher (Figure 7).

Cropthorne 2 site. The delay in transplanting, due to poor weather, resulted in the seedlings being severely stressed. The stress particularly affected the inoculated plants and they had difficulty in becoming established and and resulted in many dying. The plot of inoculated plants therefore had far fewer plants than the uninoculated plot. Consequently, the yield from the inoculated plot was *c.* 38% lower than that from the uninoculated plot (Figure 8).

Fruit harvested from the inoculated plants at each site was blemish-free, marketable and indistinguishable from that harvested from the uninoculated plants.

Wadborough site. Inoculated plants became well established and did not appear to be stunted compared with the uninoculated plants. Initially, the yield from the inoculated plot was higher than that from the uninoculated plot but after *c.* 20 days this trend was reversed. However, the overall yield reduction in the inoculated plants was low (*c.* 12%) and the change coincided with the start of colder night temperatures observed on day 225 (Figure 9).

Yield data from 1994 field trials

Chipping Campden site. The inoculated plants appeared to be rather stunted compared with uninoculated plants and the symptoms of ZYMV:WK were more severe than at other sites. There was little spread of ZYMV:WK from the inoculated plot and a few uninoculated plants in the rows adjacent to the inoculated plants became infected. There was no difference in yield from inoculated and uninoculated plots in the early harvests and by the final harvest the yield from the inoculated plots was only 4% lower (Figure 10).

Cropthorne sites (1a and 1b). Plants in the inoculated and uninoculated plots became quickly established and almost 100% of the plants in the inoculated plot were infected. Symptoms remained mild and there was no visual evidence that inoculated plants were smaller than plants from the uninoculated plot. There was little spread of the mild strain and only a few plants were observed with symptoms in the uninoculated plot. The results at sites 1a and 1b were similar with no difference in the yields from inoculated and uninoculated plots in the early harvests and by the final harvest the yields from the inoculated plots were approx. 10 and 12% lower respectively (Figures 11 and 12).

Eckington site. Plants in the inoculated and uninoculated plots became quickly established and almost 100% of the plants in the inoculated plot were infected. Symptoms remained mild and there was no visual evidence that inoculated plants were smaller than plants from the uninoculated plot. There was little spread of the mild strain and only a few plants were observed with symptoms in the uninoculated plot. Initially there was no difference in yield from inoculated and uninoculated plots and by the final harvest the yield from the inoculated plots was approx. 8% lower (Figure 13).

An outbreak of severe ZYMV developed in a plot of uninoculated plants close to the inoculated block. Severe fruit distortion developed in uninoculated plants but incoulated plants remained free of infection and fruit were unaffected. Towards the end of the season there was an apparent recovery in plants infected by severe ZYMV. Older fruit on the plants were severely distorted but new fruit developing at the top of the plant were normal in appearence.

Fruit harvested from the inoculated plants at each site was blemish-free, marketable and indistinguishable from that harvested from the uninoculated plants.

Polythene-house and gauze house experiments

Cucumber fruits were harvested three times a week from each experiment at Wellesbourne starting on 21 July and finishing on 11 October, 1993. All fruits over 30 cm in length were harvested and individually weighed.

In the gauze house experiment, fruits from plants inoculated with ZYMV:FA only were small, severely distorted and unmarketable. In contrast, the fruits from plants inoculated with ZYMV:FA which had been protected by ZYMV:WK were blemish-free and indistinguishable from fruit from uninoculated plants. Similarly, fruit from plants inoculated with ZYMV:WK

only were indistinguishable from uninoculated plants.

There was no marketable yield from plants infected with ZYMV:FA only. The yield from protected plants was very similar to that from uninoculated plants, with the yield from plants inoculated with ZYMV:WK only being initially similar but by the end of the experiment was approximately 8% lower than from uninoculated plants (Figure 14).

In the polythene-house experiment there was initially no yield difference between plants inoculated with ZYMV:WK and uninoculated plants. By the end of the experiment the yield depression was only approximately 8%, a similar result to that obtained in the gauze house experiment (Figure 15).

Glasshouse experiments

The results showed that the method of spray inoculation and brushing the plants with a plastic brush after spraying was very effective (Table 3). The use of abrasive added to the inoculum mixture was less effective, unless a brush was used as well, so there was no advantage in using an abrasive. Spraying alone was not effective, probably because the cuticle of the cotyledon is quite tough and the pressure of the spray was not sufficient to break it. Brushing appeared to be effective in breaking the cuticle to allow entry to the virus but without damaging the plant too much. When a larger plant was sprayed on its true leaves there was a higher rate of infection by spray alone suggesting that the cuticle of true leaves can be penetrated more easily by the spray thus allowing entry of the virus (Table 4). However, it was more practical to spray plants at the cotyledon stage. A plastic brush appeared to be more effective than a natural bristle brush for inoculating plants (Table 5)

CONCLUSIONS

The Polythene-house and field trials showed that courgette plants inoculated with the mild ZYMV:WK strain could be 7 to 10 days later in flowering than uninoculated plants. 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 difference in accumulative yield between the inoculated and uninoculated plants for all harvests ranged from 4% to 27% lower from inoculated plants, depending on the cultivar. The cv. Ambassador appeared to be more sensitive to ZYMV:WK infection and other adverse growing conditions at the end of its fruit production season. This resulted in a higher difference in accumulative yield for this cultivar from mid-season onwards. In contrast, the difference in accumulative yield fell gradually throughout the season for the cv. Acceste and remained relatively constant for the marrow cv. Tiger Cross. It was interesting to observe that the final difference in accumulative yield for the cv. Diamant in the Wellesbourne trial was c. 11% lower from inoculated plants, a value equivalent to the 11% reported for the same cultivar in a polythene-tunnel experiment in France (Lecoq *et al.*, 1991).

In the field trials the difference in yield between inoculated and uninoculated plants ranged from an increase of 7% to yield reductions of 4 to 38% depending on the trial. In most trials there was a yield reduction of 10 -15% from inoculated plants. In one case where the yield reduction was 38% this could be explained by exceptional conditions of plant stress before transplanting. The other case where the yield reduction was high (26%) was in the one trial where cv Ambassador was grown. This is equivalent to the reduction in yield of the same cultivar in the first polythene-tunnel experiment at Wellesbourne. The trials confirmed the stability of the ZYMV:WK strain under commercial growing conditions. Fruit harvested from mild-strain inoculated plants was blemish-free, marketable and indistinguishable from that 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. Where there were outbreaks of severe ZYMV infection, fruits from inoculated plants remained symptomless whilst those from uninoculated plants were severely affected and unmarketable which suggested that inoculated plants were protected against severe ZYMV.

The 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.

Polythene-house and gauze-house experiments successfully demonstrated the use of mild-strain cross-protection for the control of ZYMV in cucumber. There are reports of occasional outbreaks of ZYMV in this crop so the method could easily be adapted to control problems in cucumbers in the future. The yield loss caused by ZYMV:WK was less than 10% overall, a figure comparable with that of courgettes or marrows.

After discussions with growers a successful method of inoculation was developed using a hand pump spray and a nylon brush. The method is effective, rapid and requires the minimum of equipment and training to use.

ACKNOWLEDGEMENTS

We thank Dr H. Lecoq for providing ZYMV:WK and for helpful discussions, Mr Matthew Mitchell for technical assistance, Mr Richard Sampson for photography, Grower Marketing Services, Pershore for their co-operation and all the growers who participated in the trials (Mr Mike Freemantle, Mr Mike Gardner, Messrs Martin and William Haines, Mr Carmello Masulo, Mr Vito Pilade and Mr Don Southall). The study was funded by the Horticultural Development Council. Trials were conducted under experimental permit 92/00333 from the Pesticide Safety Directorate of MAFF.

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.

Mild-strain 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.

REFERENCES

Al-Musa A.M. (1989). Severe mosaic caused by zucchini yellow mosaic virus in cucurbits in Jordan. *Plant Pathology* **38**, 541-546.

Barbara, D. J., Morton, A., Spence, N.J. and Miller, A. (1995). Rapid differentiation of closely related isolates of two plant viruses. *Journal of Virological Methods* **55**, 121-131.

Clark M.F. & Adams A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay (ELISA) for the detection of plant viruses. *Journal of General Virology* **34**, 475-483.

Lecoq H., Pitrat M. & Clément M. (1981). Identification et caractérisation d'un potyvirus provoquant la maladie du rabougrissement jaune du melon. *Agronomie* **1**, 827-834.

Lecoq H., Lemaire J.M. & Wipf-Scheibel C. (1991). Control of zucchini yellow mosaic virus in squash by cross protection. *Plant Disease* **75**, 208-211.

Lisa V., Boccardo G., D'Agostino G., Dellavalle G. & d'Aquilio M. (1981). Characterisation of a potyvirus that causes zucchini yellow mosaic. *Phytopathology* **71**, 667-672.

Nameth S.T., Dodds J.A., Paulus A.O. & Kishaba A. (1985). Zucchini yellow mosaic virus associated with severe diseases of melon and watermelon in Southern California desert valleys. *Plant Disease* **69**, 785-788.

Provvidenti R, Gonsalves D. & Humaydan H.S. (1984). Occurrence of zucchini yellow mosaic virus in cucurbits from Conneticut, New York, Florida and California. *Plant Disease* **68**, 443-446.

Ullman D.E., Cho J.J. & German T.L. (1991) Occurrence and distribution of cucurbit viruses in the Hawaiian Islands. *Plant Disease* **75**, 367-370.

Walkey D.G.A., Lecoq H., Collier R. & Dobson S. (1992). Studies in the control of zucchini yellow mosaic virus in courgettes by mild strain protection. *Plant Pathology* **41**, 762-771.

Wang H.L., Gonsalves D., Provvidenti R & Lecoq H. (1991). Effectiveness of cross protection by a mild strain of zucchini yellow mosaic virus in cucumber, melon and squash. *Plant Disease* **75**, 203-207.

Year 1
1992

Table 1 Yield from courgette cultivars uninoculated or inoculated with ZYMV:WK grown in polythene tunnel experiments

Cultivar	Mean fruit no. per plant		Mean fruit wt. (kg) per plant		Total accumulative yield (kg)		Percentage difference in total accumulative yield
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	
Expt 1							
Ambassador	28.6	24.8	2.08	1.65	48.29	39.83	- 19%
Acceste	29.3	31.8	2.95	2.8	71.56	68.33	- 4%
SED (95 df)	3.3		0.27				
Expt 2							
Ambassador	7.5	5.9	0.46	0.34	11.29	8.18	- 27%
Diamant	8.3	7.5	0.48	0.44	11.57	10.33	- 11%
SED (95df)	0.66		0.043				

Table 2 Yield difference from courgette and marrow cultivars uninoculated or inoculated with ZYMV:WK grown in field trials

Year	Trial site	Cultivar	Number of plants inoculated	Yield from plants uninoculated	Yield from plants inoculated	Percentage difference
1992	Chipping.. Campden	Tiger Cross[a]	1,000	1538 fruit	1317 fruit	- 14%
	Cropthorne 1	Acceste	1,000	4269 lb	3653 lb	- 14%
	Wadborough	Ambassador	1,000	3410 lb	2509 lb	- 26%
1993	Chipping Campden	Zebra Cross[a]	5,000	9275 fruit	7754 fruit	- 16%
	Cropthorne 1	Acceste	5,000	590 crates	630 crates	+ 7%
	Cropthorne 2	Acceste	5,000	561 boxes	348 boxes	- 38%
	Wadborough	Acceste	1,000	2554 lb	2248 lb	- 12%
1994	Chipping Campden	Zebra Cross	2,500	471 fruit	454 fruit	- 4%
	Cropthorne 1a	Acceste	6,000	360 crates	323 crates	- 10%
	Cropthorne 1b	Acceste	3,000	13240 lb	11624 lb	- 12%
	Eckington	Acceste	1,000	600 boxes	550 boxes	- 8%

[a] marrow cultivars

Year 2 1993

Table 3 Evaluation of methods for the inoculation of courgette seedlings with ZYMV:WK inoculum

Experiment	Percentage plants infected with ZYMV:WK			
	Spray only	Spray and plastic brush	Spray and abrasive	Spray, plastic brush and abrasive
1	20	100	52	93
2	45	100	70	95

Year 2 1993

Table 4 Evaluation of plant size for the inoculation of courgette seedlings with ZYMV:WK inoculum

Experiment	Percentage plants infected with ZYMV:WK			
	Spray only		Spray and plastic brush	
	Small plant[a]	Large plant[b]	Small plant	Large plant
1	40	78	100	100

[a] Cotyledons only
[b] Three true leaves

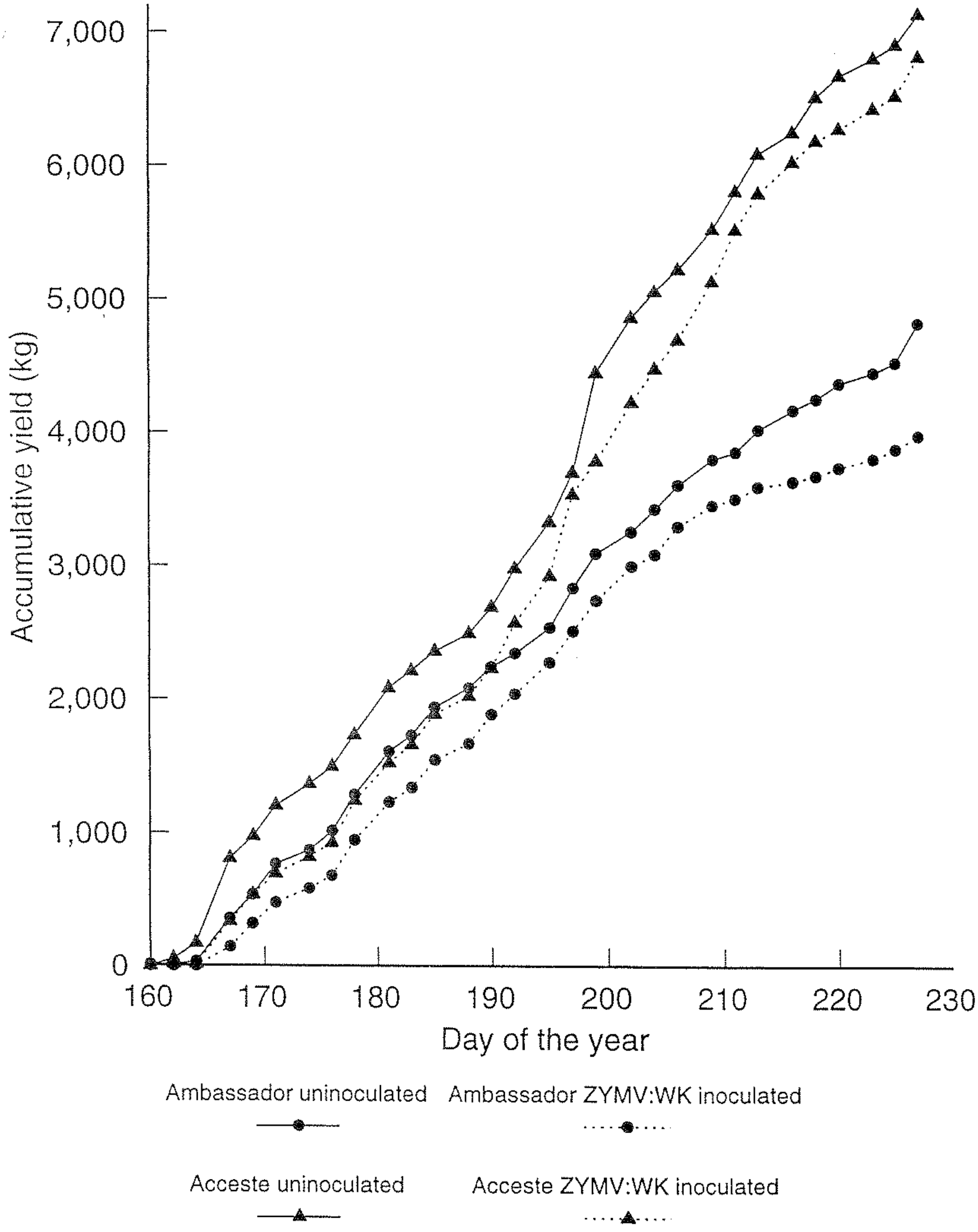
Year 2 1993

Table 5 Evaluation of brushes for the inoculation of courgette seedlings with ZYMV:WK inoculum

Experiment	Percentage plants infected with ZYMV:WK			
	Spray only	Spray and natural bristle broom	Spray and natural bristle brush	Spray and plastic brush
1	0	82	77	91

Yaer 1 1992

Figure 1
Polythene-house experiment 1



Year 1 1992

Figure 2
Polythene-house experiment 2

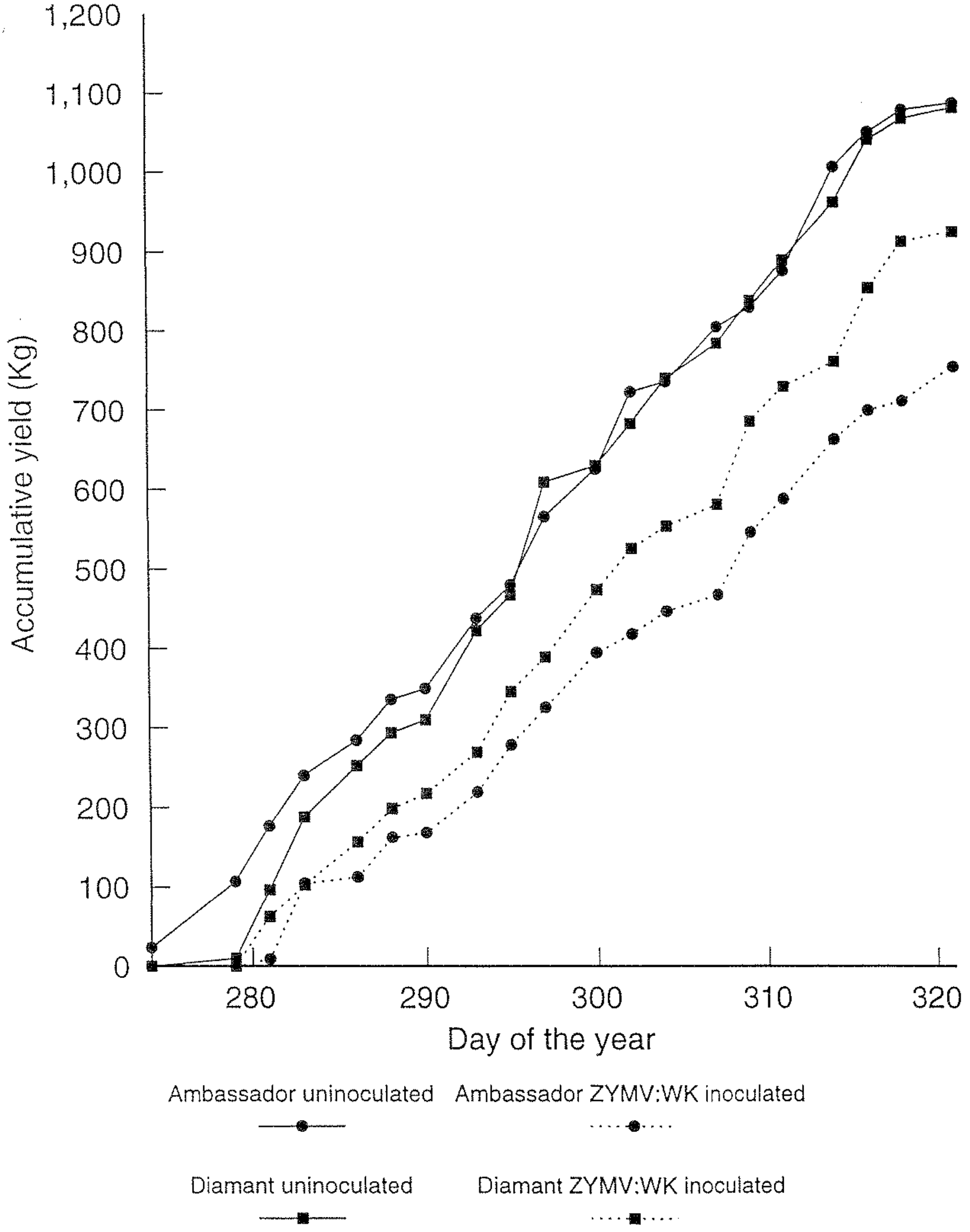


Figure 3
Chipping Campden site, 1992

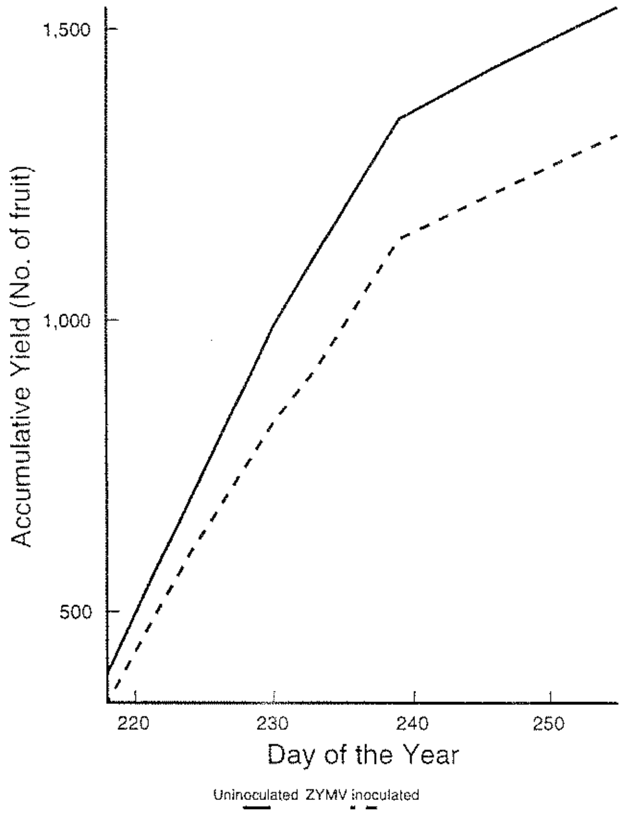


Figure 4
Cropthorne 1 site, 1992

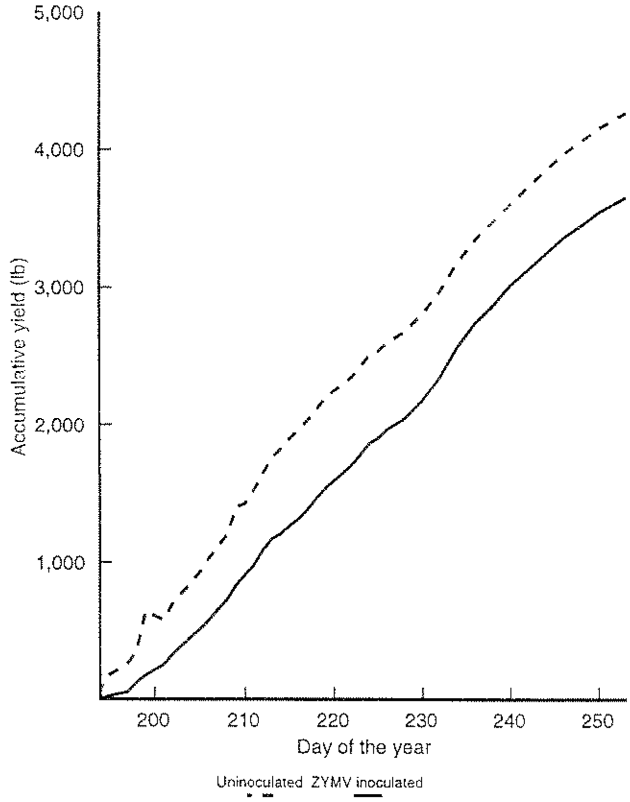


Figure 5
Wadborough site, 1992

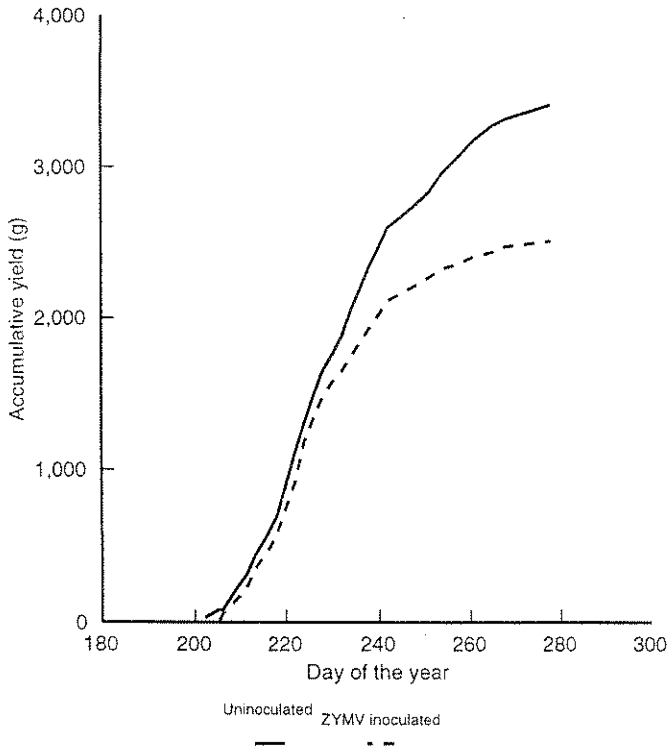


Figure 6
Chipping Campden site, 1993

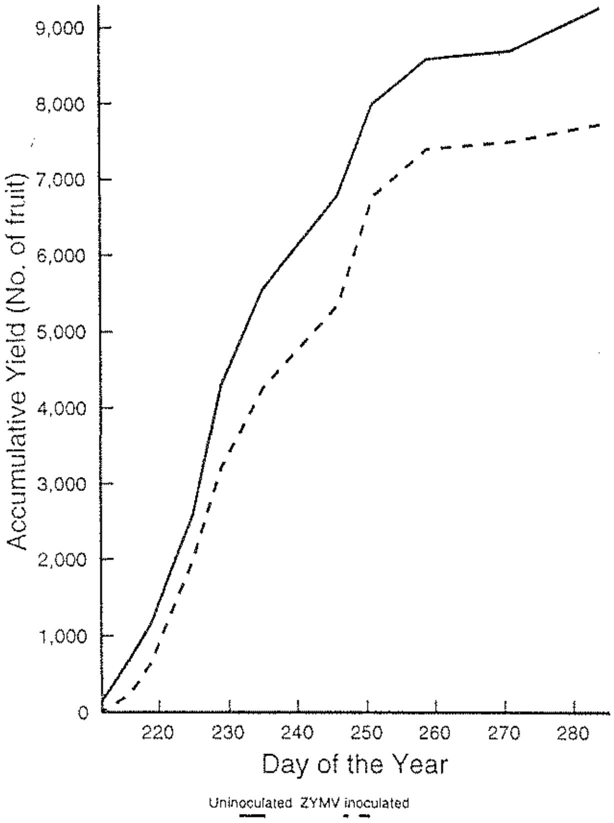


Figure 7
Cropthorne 1 site, 1993

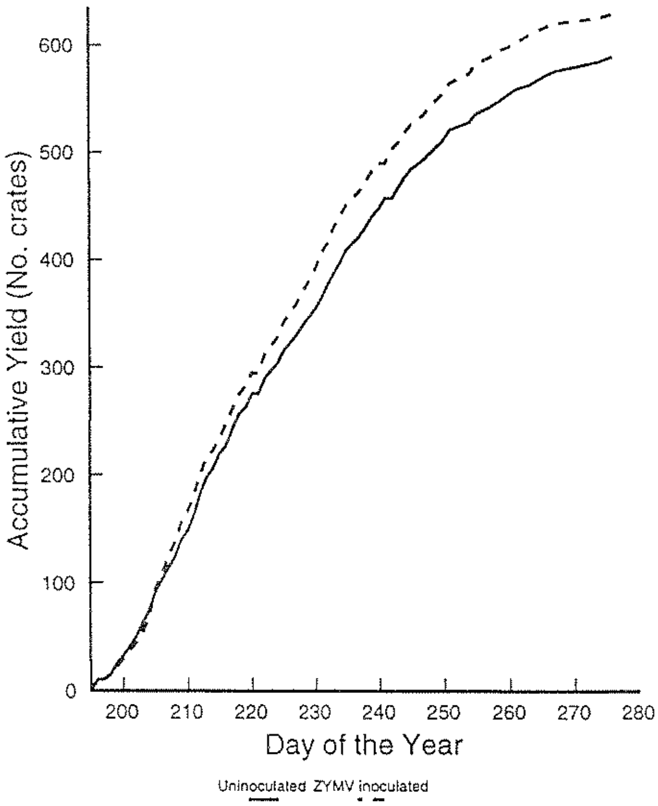


Figure 8
Cropthorne 2 site, 1993

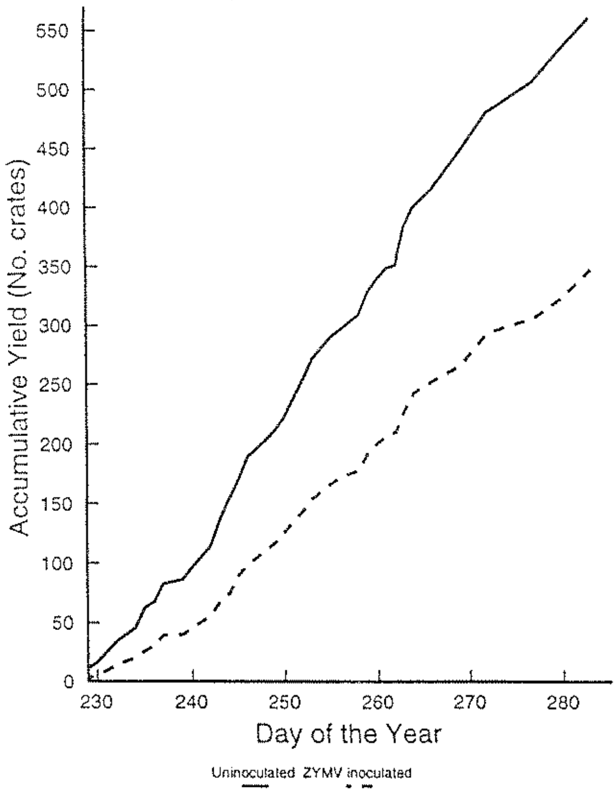


Figure 9
Wadborough site, 1993

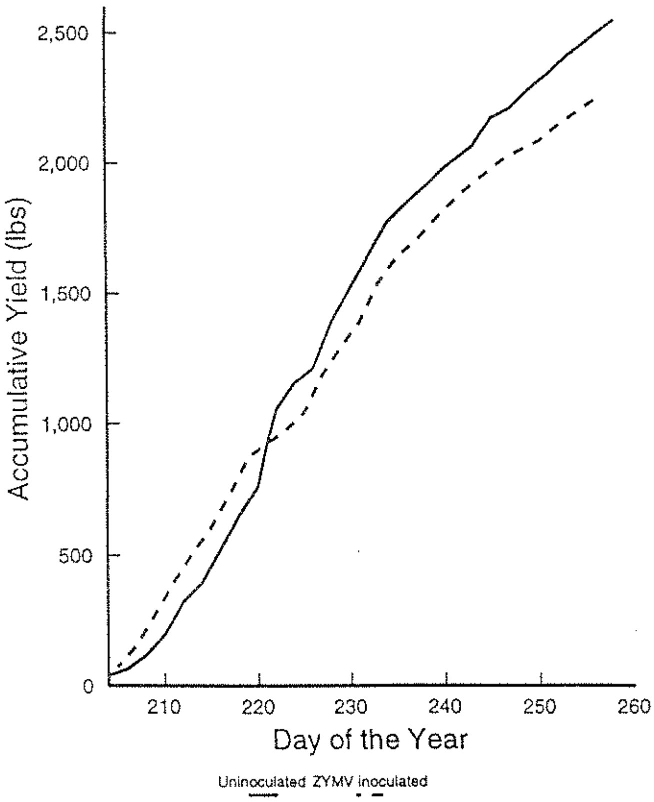


Figure 10
Chipping Campden site, 1994

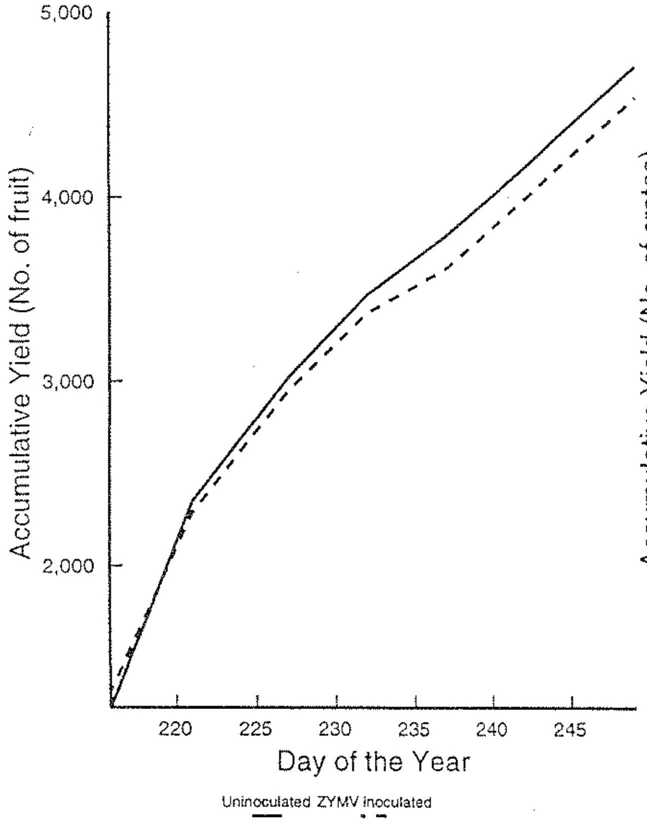


Figure 11
Cropthorne 1a site, 1994

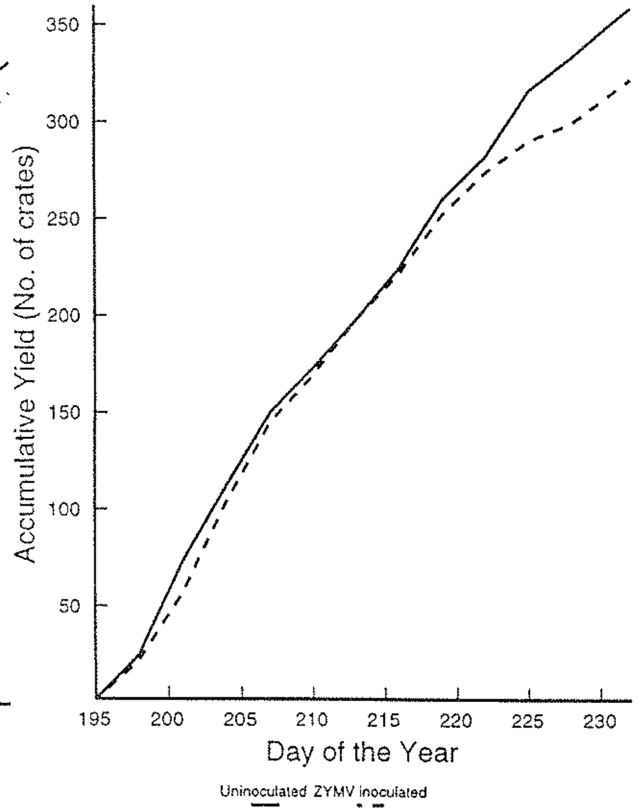


Figure 12
Cropthorne 1b site, 1994

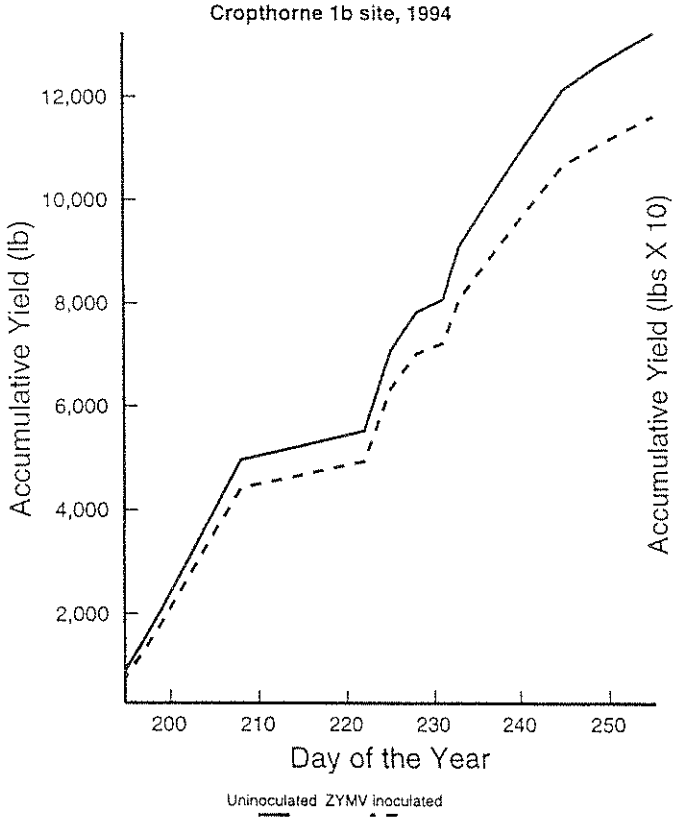
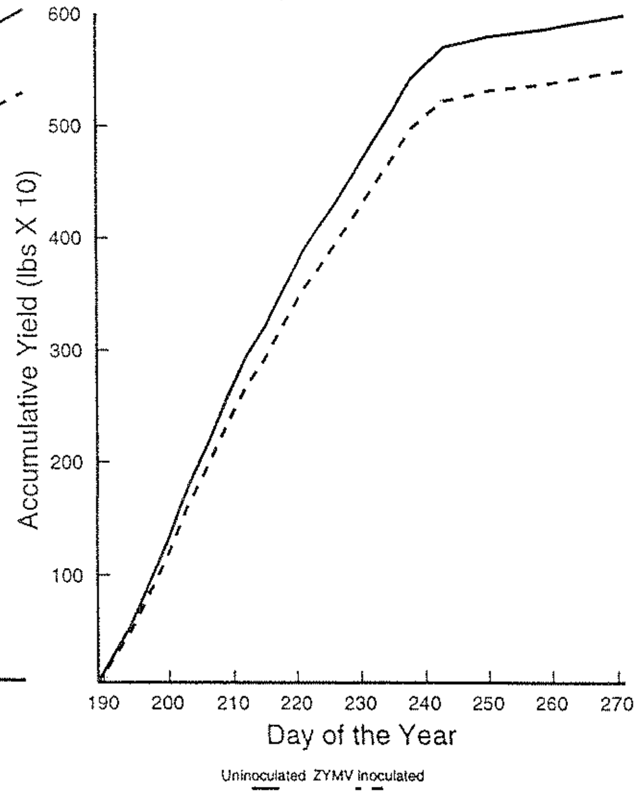
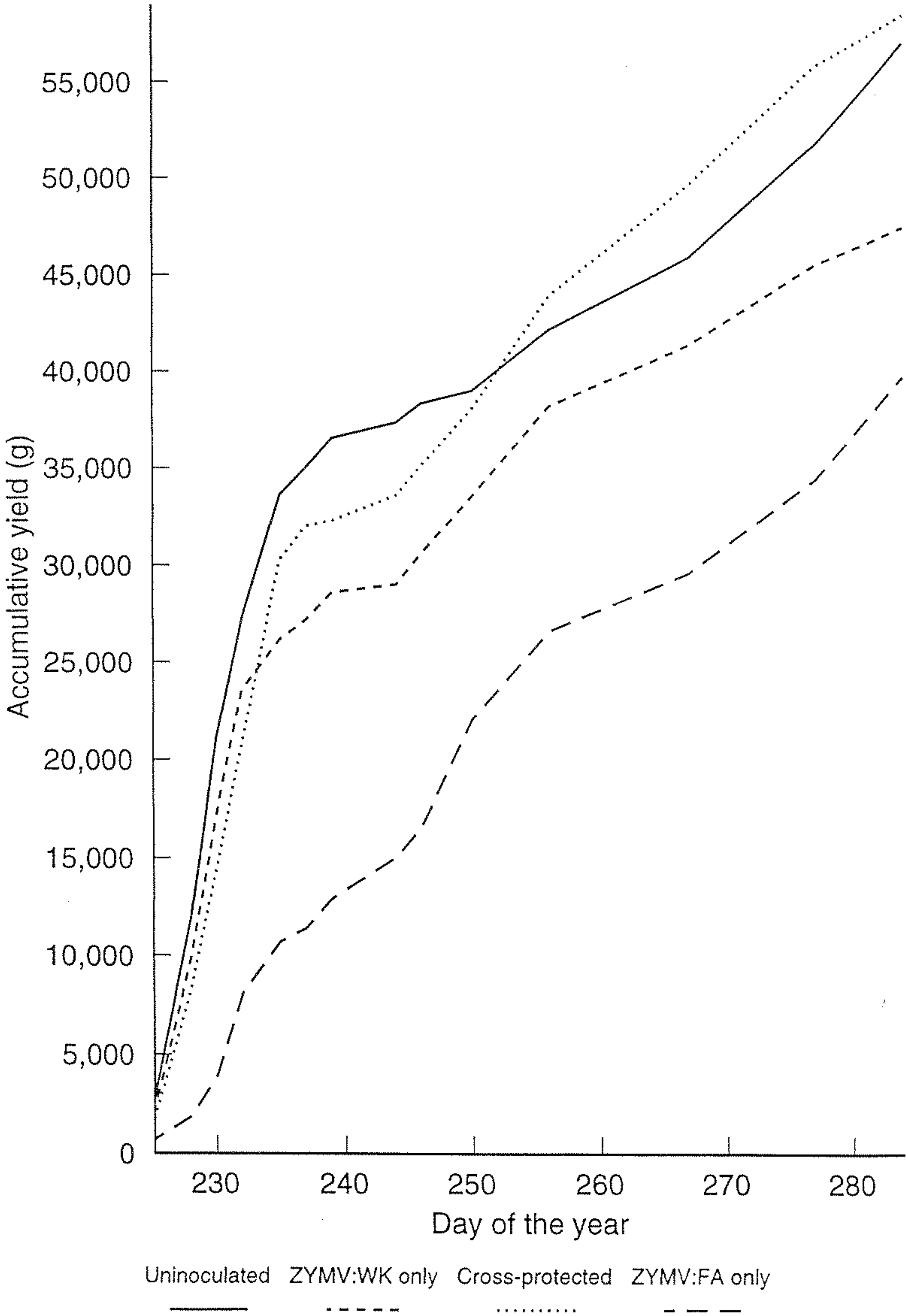


Figure 13
Eckington site, 1994



Year 2   1993

Figure 14
Cross-protection in cucumbers



Year 2 1993

Figure 15
Cucumber polytunnel experiment