HDC ANNUAL REPORT
FV/132

CONTROL OF ZUCCHINI YELLOW MOSAIC VIRUS BY MILD-STRAIN PROTECTION

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July, 1994

HDC ANNUAL REPORT

Annual Report	Year 2, July 1994
Project Number	FV/132
Project Title	Control of zucchini yelllow mosaic virus by mild-strain protection
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Location of Project	Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK
Project Co-ordinator	Mr Peter Emmet
Date Project commenced	1 June, 1992
Date completion due	31 May, 1995
Key words	Courgettes, cucumbers, marrows, mild-strain protection

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INTRODUCTION

Zucchini yellow mosaic potyvirus (ZYMV) continues to cause severe losses in cucurbit crops throughout the world. Last year we reported that the use of the mild-strain (ZYMV:WK) under commercial conditions was successful but that there was a yield reduction of 10-20% in cougette and marrow crops in field trials at three sites in the Vale of Evesham. Although this yield reduction was considered acceptable to growers, compared with the potential of 100% yield loss caused by a severe strain of ZYMV, any reduction in yield is undesirable.

In 1992 the trials were limited to 1000 inoculated plants at each of three sites because of restrictions in the experimental licence from Pesticedes Safety Directorate (PSD) so in 1993 the licence was modified and trials were extended to 5000 inoculated plants at each of four sites. This allowed greater flexibility in the trials and allowed us to examine ways of improving yield from inoculated plants and to continue monitoring the effect of mild strain inoculation on courgette and marrow crops.

Polythene-house and gauze house experiments were also conducted to determine if mild-strain cross-protection was effective in cucumber crops and whether the mild strain causes any yield depression in this crop.

The results of glasshouse experiments to investigate the methods of application of ZYMV:WK to courgettes are also 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 thawed 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. The severe strain of ZYMV (FA) used in other 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 in 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.

Field trials

Trials were carried out on farmers' fields at the same three sites as 1992. An additional trial was carried out at another site in Cropthorne (Cropthorne 2). At each site *c.* 5000

ZYMV:WK inoculated plants were planted as a part of the commercial crop and their yield was compared with *c.* 5000 uninoculated plants of the same age.
At the Cropthorne 1 site; inoculated and uninoculated plants of the courgette cv. Acceste were grown in separate blocks in 30 rows of *c.* 150 plants per row, with a 1 m spacing between plants and rows. The inoculated block was planted adjacent to the healthy block with a 3 m space between. Seeds were sown in modular trays on 4 May, seedlings inoculated on 21 May and transplanted on 1 June.

At the Wadborough site the experiment was divided into two plantings of the courgette cv. Acceste, each with c. 2000 inoculated and uninoculated plants. 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. Each block had 10 rows of c. 200 plants with a spacing of 1 m between plants and rows.

At the Chipping Campden site, seed of the marrow cv. Tiger Cross was sown in modular trays on 19 May, the seedlings inoculated on 4 June and transplanted on 10 June. The inoculated plants were planted in 11 rows of c. 450 plants per row. The plants were grown at a spacing of 1 m between plants and rows and there was a 3 m space between inoculated and uninoculated rows.

At the Cropthorne 2 site plants of the courgette cv. Acceste were grown in separate blocks in 41 rows of c. 120 plants per row, with a 1 m spacing between plants and rows. The inoculated block was planted adjacent to the uninoculated block with no space between. Seeds were sown in modular trays on 2 June, seedlings inoculated on 11 June and transplanted between 24 and 29 June.

The four sites were irrigated by overhead, spray irrigation.

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 (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.

RESULTS

Harvest data from field trials

Cropthorne site. 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.

Wadborough site. 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.

Chipping Campden site. 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. 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.

Cropthorne 2 site. 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 trays of fruit from the inoculated and uninoculated blocks was recorded.

Yield data from field trials

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 approximately 5% higher and it remained higher for the rest of the experiment (Figure 1).

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 yield reduction in the inoculated plants was very low (< 10%) and the change co-incided with the start of colder night temperatures observed on day 225 (Figure 2).

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 10% (Figure 3). 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.

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 which had difficulty in becoming established and many died. The plot of inoculated plants therefore had far fewer plants than the uninoculated plot. Consequently, the final yield from the inoculated plot was *c.* 40% lower than that from the inoculated plot (Figure 4).

All the fruits harvested from the inoculated plants at each site were blemish-free, marketable and indistinguishable from those 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. 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 5).

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 6).

Glasshouse experiments

The results showed that the method of spray inoculation and brushing the plants with a stiff nylon brush after spraying was very effective (Table 1). 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 alllow 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 2). 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 3)

DISCUSSION AND CONCLUSIONS

The trials confirmed the stability of the ZYMV:WK strain under commercial conditions. All the fruits harvested from mild-strain inoculated plants were blemish-free, marketable and indistinguishable from those harvested from healthy, uninoculated plants.

The yield depression was generally lower than that in 1992 (except at the Cropthorne 2 site). This may reflect more accurately the true yield depression as the trials in 1993 were much larger than in 1992 and more representative of the commercial situation. After grower's experience in 1993 they were more willing to grow the inoculated plants on better ground and include the inoculated plants as part of the crop rather than an isolated trial. It is also possible that the yield depression of courgette fruits resulting from the use of the mild strain, might be reduced further by increasing the amount of nitrogen fertilizer applied to the crop as top dressing.

Growers are now confident in using the mild-strain inoculum and many would like to be able to inoculate their whole crop. They are also keen to continue to seek ways in

which to improve the yield from inoculated plants. There is an urgent need to pursue full PSD registration when sufficient funds are raised to cover the cost.

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. The method will be tested on a commercial basis in 1994.

ACKNOWLEDGEMENTS

Trials were conducted under trials permit 92/00333 from the Pesticide Safety Directorate. We thank Mr Mike Freemantle, Mr Mike Gardner, Messrs Martin and William Haines, Mr Carmello Masulo, Mr Vito Pilade and Mr Don Southall who participated in the field trials for their co-operation.

PUBLICATIONS

Spence, N.J (1993). Self control : ZYMV. *Grower* **120** 27-28.

Spence, N.J., Walkey, D.G.A. and Miller, A. (1993). Mild-strain cross-protection of zucchini yellow mosaic virus. Abstracts of the 6th International Congress of Plant Pathology, Montreal, July 28-August 6, 1993.

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.

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
Cropthorne courgette harvest

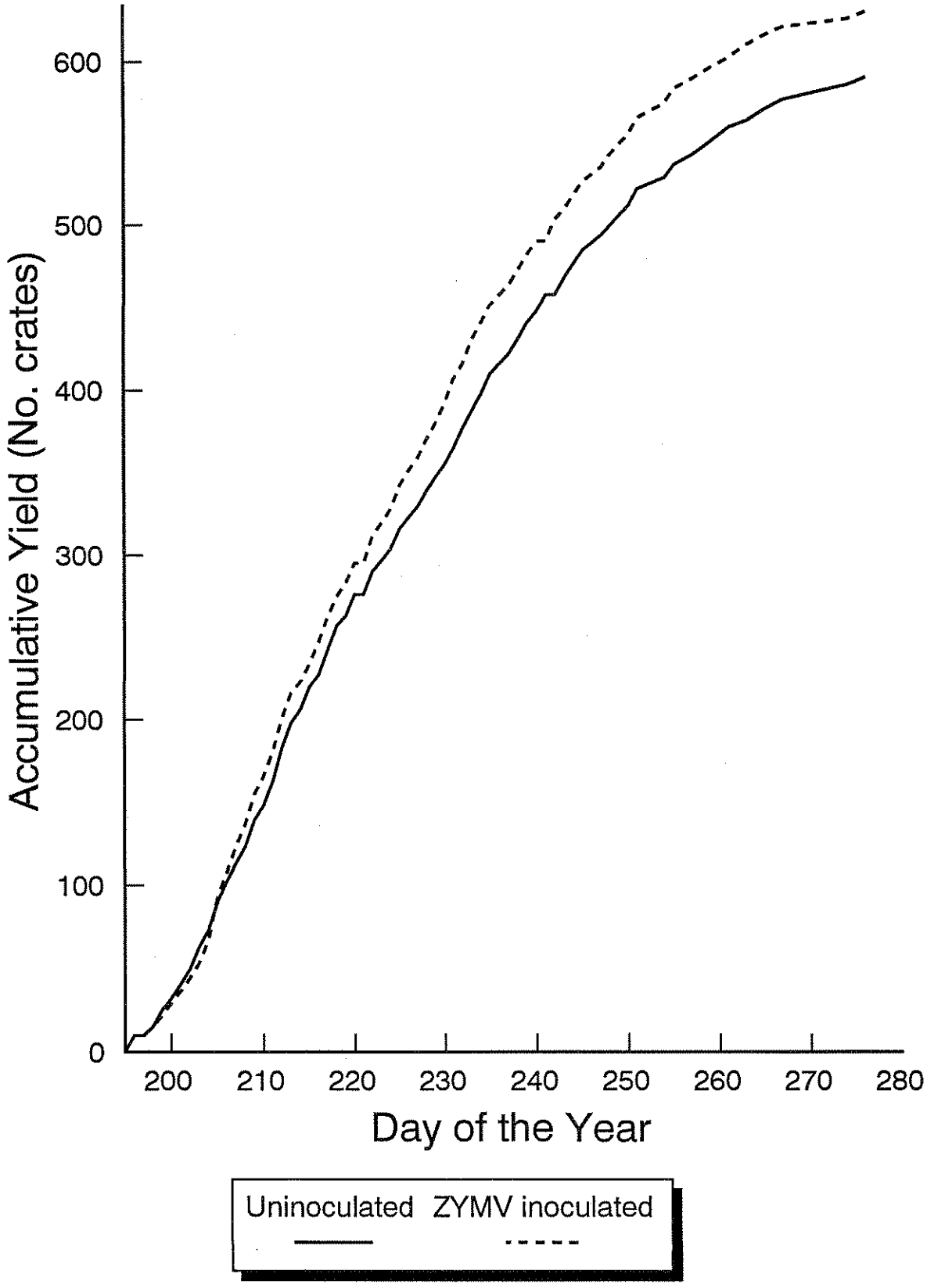

Figure 2
Wadborough courgette harvest

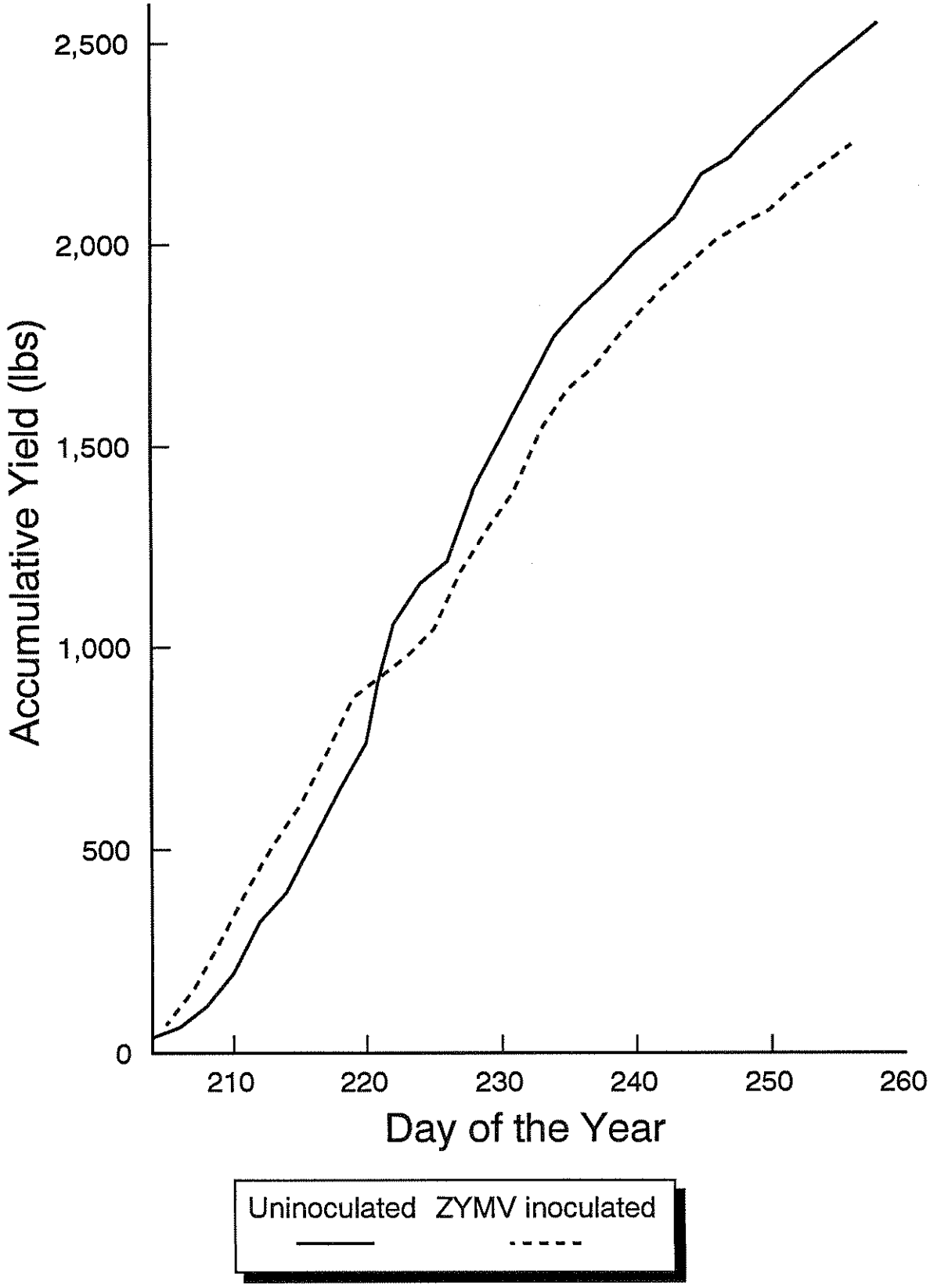

Figure 3
Chipping Campden marrow harvest

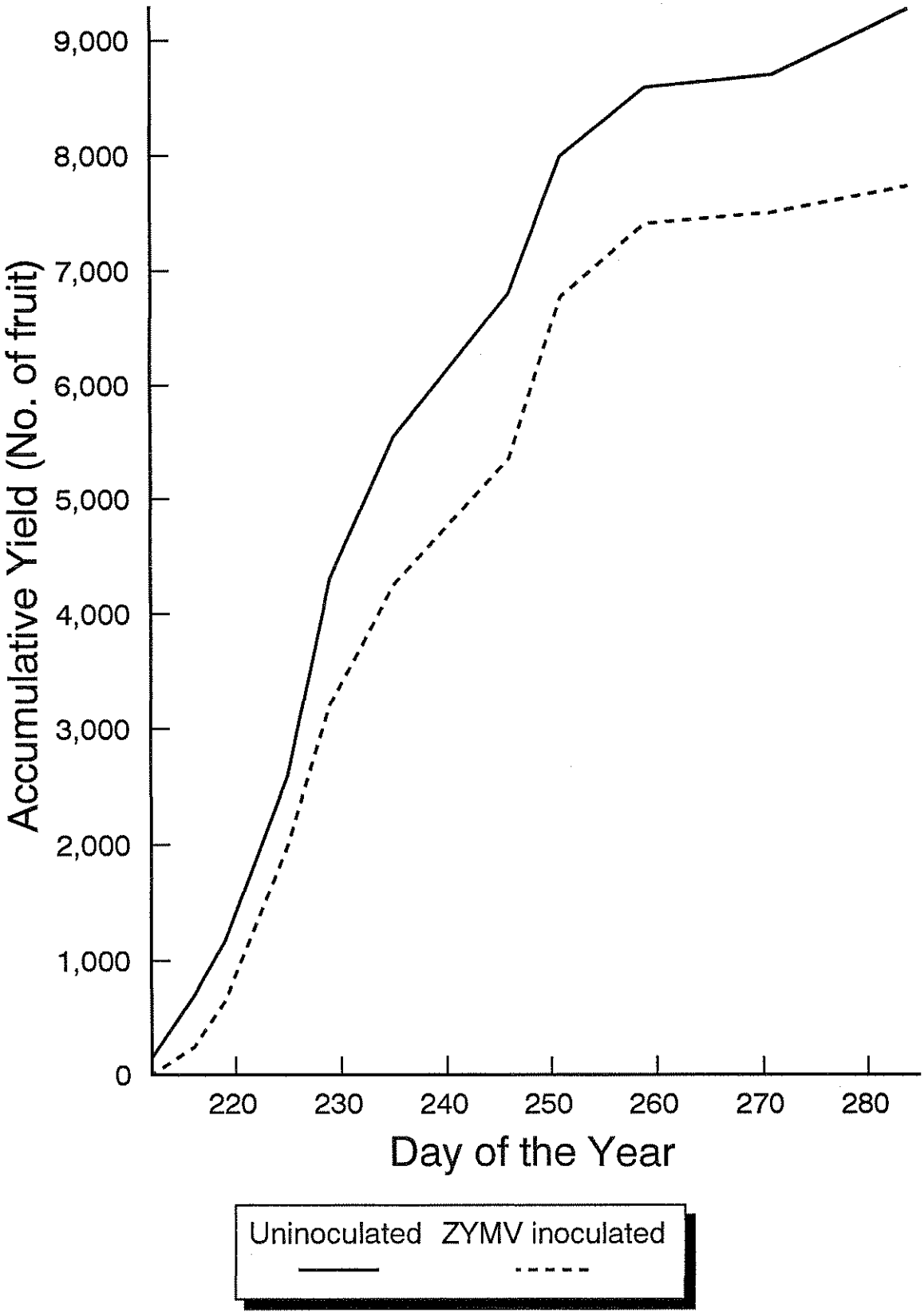

Figure 4
Cropthorne 2 site courgette harvest

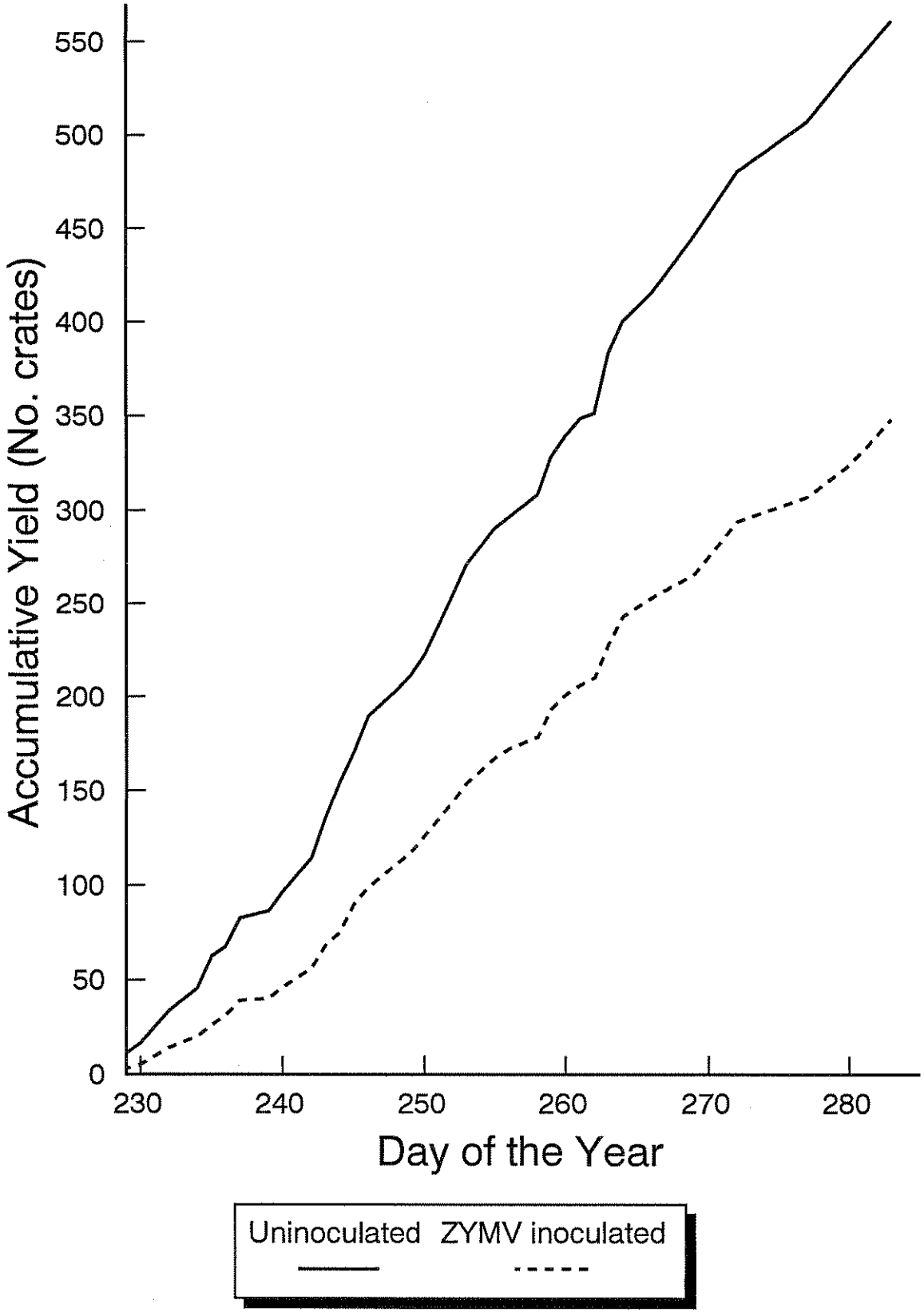

Figure 5
Cross-protection in cucumbers

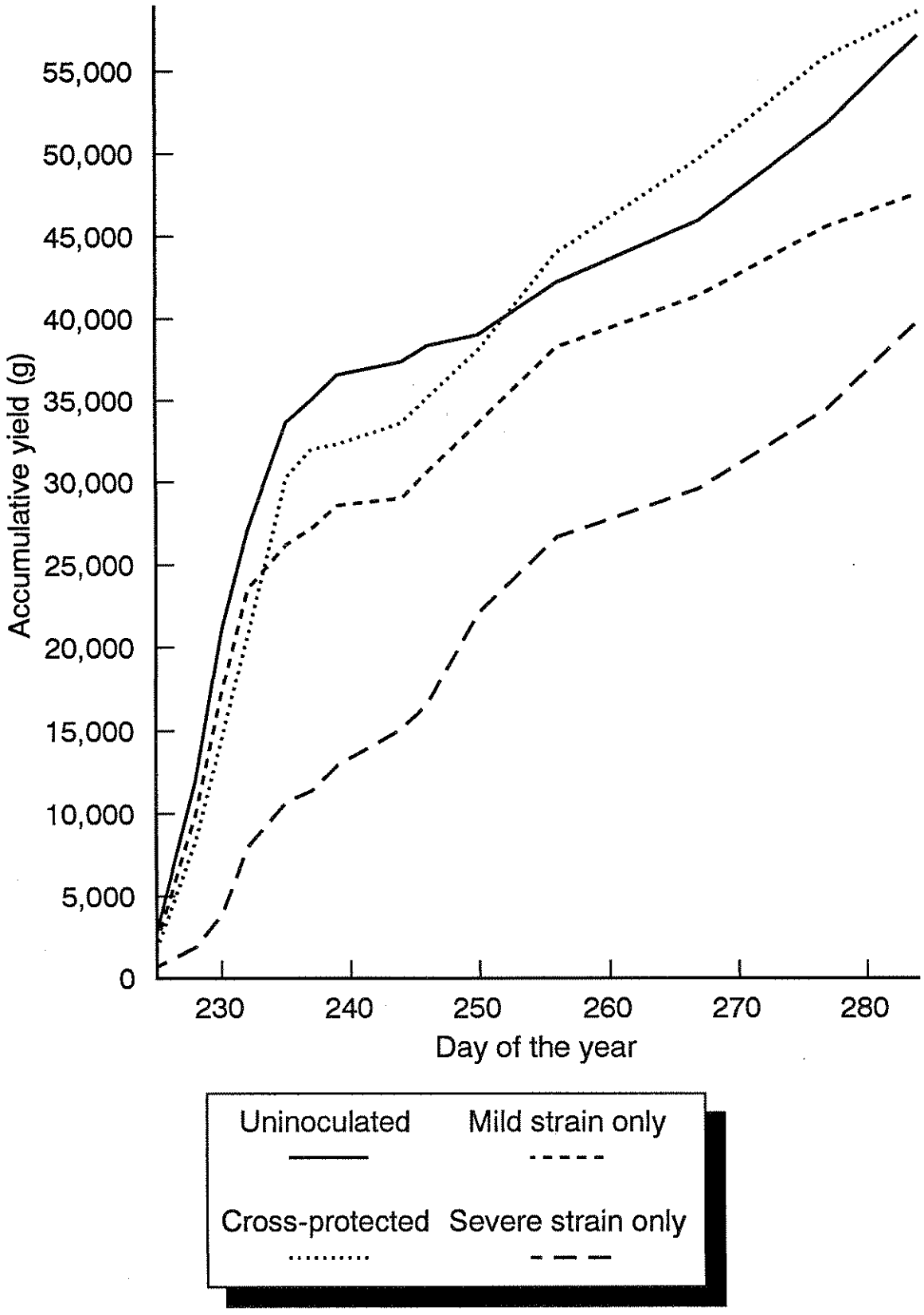

Figure 6
Cucumber polytunnel harvest

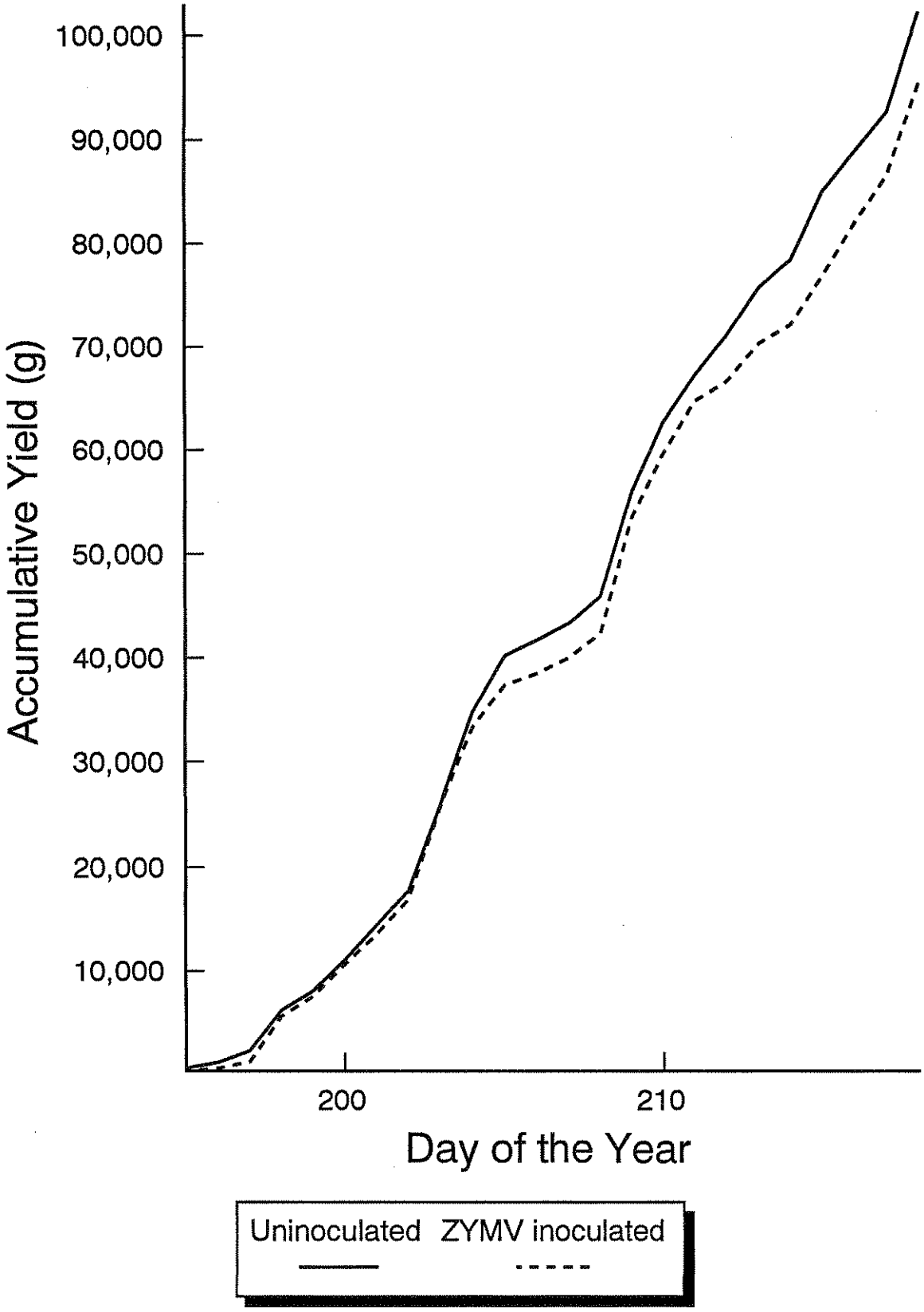

Table 1 Evaluation of methods for the inoculation of courgette seedlings with ZYMV:WK inoculum

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

Table 2 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

Table 3 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