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Zucchini yellow mosaic virus; two outbreaks in the Netherlands and seed transmissibility

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Abstract

In 1983 and 1987/88 two limited outbreaks of zucchini yellow mosaic virus in cucumber and zucchini squash occurred in the Westland glasshouse district in the Netherlands, mainly in glasshouses. The disease could be eradicated and has not recurred so far. In both cases a relatively mild but still highly pathogenic strain of the virus was involved. Diseased plants of zucchini yielded severely distorted or no fruits and it was difficult to obtain seeds from infected plants. Two out of 4196 seedlings grown in isolation from seed from inoculated zucchini plants showed symptoms and contained the virus, indicating that the virus can be transmitted via seed but at very low rate. This explains the erratic incidence and international distribution of the virus.

Additional keywords: cucumber, *Cucumis sativus*, *Cucurbita pepo*, eradication, prevention, zucchini squash.

Zucchini yellow mosaic virus (ZYMV) was first described in northern Italy in 1981 from zucchini squash (*Cucurbita pepo*) as a new potyvirus related to, but different from watermelon mosaic viruses (WMV) 1 and 2 (Lisa et al., 1981). The muskmelon yellow stunt virus, concurrently described in southern France from muskmelon (*Cucumis melo*) (Lecoq et al., 1981), was soon found to be identical (Lecoq et al., 1983). The disease was known in zucchini squash in northern Italy since 1973 (Lisa et al., 1981), and a virus isolate obtained from Israel in 1982 as WMV-2 (our code KB5) was identified at IPO as ZYMV (Huijberts and Bos, unpublished results 1982). The virus has meanwhile appeared in several other countries, including Great Britain, Turkey, Lebanon, Jordan, Egypt, Japan, Taiwan, Australia, Canada, and several parts of the USA (e.g. Sammons et al., 1989). Once established somewhere, it usually develops into a devastating disease of several cucurbitaceous crops and causes serious concern wherever it appears (Nameth et al., 1986). Its wide distribution and erratic incidence suggest seed transmission.

In 1983 and 1987/88 independently two localized, but damaging epidemics developed in the Westland glasshouse district in the Netherlands. During fall 1983 20 glasshouse crops of cucurbit (*Cucumis sativus*) became infested with leaf mosaic and malformation and fruit reduction and distortion, making the produce unmarketable (Fig. 1). In one instance c. 50% of the plants were affected. The disease was also observed on four zucchini holdings. During late 1987 and early summer 1988 the

Fig. 1. Severe reduction in size and distortion of fruits of cucumber after natural infection by zucchini yellow mosaic virus.

disease recurred in a limited number of zucchini crops in glasshouses and in the open. Mosaic, leaf malformation up to leaf narrowing, and plant stunting were severe, and affected plants stopped producing fruits (Fig. 2). In both cases, systematic removal of diseased plants with help of the Plant Protection Service led to eradication of the disease. Since then it has not recurred in the country.

Two Dutch virus isolates, viz. Cu38 from cucumber (1983) and Cu61 from zucchini (1987), were compared with a number of ZYMV isolates, viz. E9 and E15 described

Fig. 2. Fruit reduction and malformation in zucchini squash after inoculation with zucchini yellow mosaic virus.

by Lecoq et al. (1981), the Connecticut and Florida strains described by Provvidenti et al. (1984), and the 'watermelon mosaic' isolate (KB5) from Israel. For comparison, WMV-2, provided by Dr M. Pitrat, Montfavet, France, was used. For serology, an antiserum to the French isolate E9, and antisera produced to our own ZYMV isolate Cu61 and the Israelian KB5, with titres of 1024 and 4096, respectively, were used. Flexuous potyvirus particles could easily be detected with the electron microscope in crude leaf sap from diseased plants, either naturally or artificially infected. The particles decorated readily with antisera to ZYMV. On test plants, the Dutch isolate Cu61 resembled the Israelian isolate KB5 and differed from WMV-2. When both Dutch isolates were compared on eight cucumber genotypes with all foreign isolates mentioned above, the two Dutch isolates were mutually identical, and both were similar to the relatively mild French isolate E15 and the US Florida strain. The Israelian KB5, the US Connecticut strain and the French E9 closely resembled each other in causing systemic necrosis on a number of cucumber genotypes. The French strains E9 and E15 had been distinguished by Lecoq et al. (1981) as two pathotypes of the virus differing in causing wilting, necrosis and death, and no wilting on melon 'Doublon', respectively. The Dutch isolates thus are of a relatively mild, though still highly pathogenic strain of the virus.

In growing-out tests by the Plant Protection Service, Wageningen, using 13 000 seeds from the imported commercial zucchini seed used for growing affected crops, no evidence of seed transmission was obtained (A. Visser, personal communication 1984). ZYMV-infected plants produce practically no fully developed fruits and few viable seeds. We could harvest 5161 viable seeds from inoculated glasshouse-grown plants of 14 zucchini cultivars. When 15 freshly harvested seeds were tested with the electron microscope, virus particles could easily be detected and decorated with ZYMV antiserum in the seed coats of more than half of them, but not in their embryos. The virus could also be mechanically transmitted from fresh seed coats, but not from the embryos. In another experiment, virus antigen was also easily detectable with ELISA in seed coats of 18 out of 26 seeds tested, but not with ELISA and ISEM in their embryos. In growing-out tests under insect-proof conditions and repeated insecticide treatments for additional safety, a total of 4196 seedlings finally developed. ELISA of 818 individual seedlings and of 1211 seedlings tested in groups of 5 was negative. All remaining seedlings were visually examined. Of these, two out of the 648 seedlings of 'Black Beauty', obtained from seed over 4 months after harvesting, showed symptoms characteristic of ZYMV from their first foliage leaves onwards. The presence of the virus in them could be confirmed by decoration electron microscopy and mechanical back inoculation onto cucumber seedlings.

ZYMV is thus able to pass to offspring of infected plants via the seed. Even though the seed was harvested from inoculated plants, the rate of seed transmission (0.047%) was very low. From early infected plants practically no seed could be harvested. In plants infected with seed-borne viruses after flowering generally no transmission via seed results. ZYMV is therefore more or less self-eliminating in commercial seed production, and the chance of spread through commercial seed must be extremely low. This observation may explain the negative results of detailed seed transmission tests with cucurbitaceous hosts reported so far (Lecoq et al., 1981; Nameth et al., 1985; Greber et al., 1987; Provvidenti and Robinson, 1987; Gleason and Provvidenti, 1990), and the haphazard international distribution and erratic spread of the virus. Seed transmission of ZYMV has been mentioned for button squash in one out of 100 seedlings without specifying the growing conditions (Greber et al., 1988), and for the ranunculaceous weed host *Ranunculus sardous* in Jordan (Al-Musa, 1989b). Seed transmission is not uncommon for a potyvirus, as for the recently described *Telfairia* mosaic virus in four cultivars of the cucurbitaceous *Telfairia occidentalis* in Africa (Anno-Nyako, 1988). Earlier reports about the presence of ZYMV-like particles in seedlings of zucchini squash in low concentration decreasing from the cotyledon upwards to undetectable in the upper leaves and associated with very weak or no symptoms (Davis and Mizuki, 1986; unpublished results 1988), and of such particles, detectable in seedlings by ISEM and ELISA but not transmissible in sap or by *Myzus persicae* (Walkey, 1989), remain puzzling. Plants of zucchini squash, once infected, do not recover from disease, and the virus remains readily transmissible from them.

Embryo-infection by potyviruses, and the inability of these viruses to survive in maturing seed coats and to reach seedlings from these after germination, makes heat treatments as for the removal of cucumber green mottle virus from within the seed coats of cucumber (Van Dorst, 1967) ineffective. Heat treatment of lettuce seeds with lettuce mosaic virus even after PEG imbibition has proved insufficient (Walkey and Dance, 1979). Routine testing and certification of commercial seed will be ineffective. To ensure the virtual absence of lettuce mosaic virus in commercial lettuce seed in California samples of 30 000 seeds must prove free of the virus (Kimble et al., 1975).

Although the chances of seed infection remain very low, crops grown for seed production should be free of the disease. In nurseries, the seedlings should be grown under near quarantine conditions of isolation, aphicide treatment and regular inspection. In nurseries and crops, suspected plants should be immediately removed. Since a few weed species have been found susceptible (Lecoq et al., 1981; Al-Musa, 1989a,b), weed control seems also imperative. Dutch successes with eradication, with the then prevailing lack of knowledge about the virus and its ecology, suggest the possibility of rapid elimination in the event of re-entry of the virus. For countries where the virus is well established, resistance is urgently needed. Although some resistant and tolerant genotypes have been found (Provvidenti, 1987), the emergence of virulent new pathotypes of the virus (Lecoq et al., 1981; Lecoq and Pitrat, 1984) is causing concern. Recent experiments in France (Lecoq et al., 1991) and Taiwan (Wang et al., 1991) have indicated possibilities for cross protection with a newly selected mild strain of the virus.

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