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Zucchini yellow mosaic virus – incidence and sources of virus infection in field-grown cucumbers and pumpkins in the Spreewald, Germany

Zucchinigelbmosaikvirus – Verbreitung und Herkunft an Feldgurken und -kürbissen im deutschen Spreewald

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Summary

In a survey in field-grown cucumber (*Cucumis sativus* L.) of the Spreewald cultivation area, Germany, from 2001 to 2004, infection with zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), cucumber mosaic virus (CMV) and cucumber green mottle mosaic virus (CGMMV) were recorded. ZYMV was of most relevance, owing to its damaging effects and frequency. Pumpkins (*Cucurbita pepo* L. and *Cucurbita maxima* Duchesne) were also found to be infected.

In glasshouse experiments with 27 fresh and pickle cucumber cultivars frequently cultivated in the area, all cultivars proved (after mechanical inoculation) to be susceptible to ZYMV. ZYMV-infected cucumbers showed leaf chlorosis and mosaic, as well as deformation of leaves and fruits. Mechanical inoculation with ZYMV, in the early developmental stages of cucumber plants, reduced yields by up to 70%. Mixed infection with ZYMV and CMV resulted in synergistic effects, increasing the severity of damage. Fruit and leaf deformation also occurred in cucumber cultivars exhibiting a documented tolerance to CMV.

The widespread weeds fat hen (*Chenopodium album* L.) and hoary alison (*Berteroa incana* (L.) DC.) were identified as possible sources of natural ZYMV infection in the Spreewald region. ZYMV transmission through seed seems to be of no importance, and possible only if fresh seeds from diseased cucumbers or pumpkins are used (commercially available seed is not contaminated). Uptake of ZYMV through contaminated soil is not a major mode of natural virus transmission, even though it proved possible in baiting experiments. Epidemiologic and economic implications of ZYMV infections of cucumber and pumpkin plants are discussed.

Key words: contamination, cucumber mosaic virus, seed, soil, transmission, virus source, weeds

Zusammenfassung

In Untersuchungen zum Auftreten von Viren im Freilandanbau von Gurken im Spreewald wurden in den Jahren 2001 bis 2004 Zucchinigelbmosaikvirus (ZYMV), Wassermelonenmosaikvirus (WMV), Gurkenmosaikvirus (CMV) und das Grünscheckungsmosaikvirus der Gurke (CGMMV) nachgewiesen. Von besonderer Bedeutung erwies sich ZYMV im Untersuchungszeitraum durch sein häufiges Auftreten und die großen Schäden. Das Virus war sowohl in Gurken (*Cucumis sativus* L.) als auch in Kürbissen (*Cucurbita pepo* L. und *Cucurbita maxima* Duchesne) nachzuweisen.

Versuche mittels mechanischer Inokulation ergaben für alle 27 geprüften Gurkensorten eine Anfälligkeit gegenüber ZYMV. Die Blätter ZYMV-infizierter Pflanzen zeigten Chlorosen und Mosaik, die Früchte waren deformiert. Pflanzen, die in frühen Entwicklungsstadien durch mechanische Inokulation infiziert wurden, reagierten mit Ertragsdepressionen von bis zu 70 Prozent. Bemerkenswert ist, dass eine Mischinfektion von ZYMV und CMV sichtbare Schäden an Sorten verursacht, welche als tolerant gegenüber CMV beschrieben werden.

Auf der Suche nach möglichen Infektionsquellen von ZYMV in der Region Spreewald wurden 23 verschiedene Unkrautarten getestet. Natürliche Infektionen mit dem Virus wurden in Weißem Gänsefuß (*Chenopodium album* L.) und Graukresse (*Berteroa incana* (L.) DC) gefunden. Eine Übertragung von ZYMV durch kontaminiertes Saatgut wurde bisher nur dann nachgewiesen, wenn frisches Saatgut aus erkrankten Gurken bzw. Kürbissen verwendet wird. An kommerziell erhältlichem Saatgut wurde dagegen keine Kontamination festgestellt. Die Übertragbarkeit von ZYMV durch kontaminierten Boden wurde durch Fangpflanzen belegt, da in virusfreien Testpflanzen, die in ZYMV kontaminiertem Boden kultiviert wurden, später ZYMV nachgewiesen werden konnte. Die epidemiologische und wirtschaftliche Bedeutung einer ZYMV-Infektion von Gurken und Kürbissen wird diskutiert.

Stichwörter: Boden, Gurkenmosaikvirus, Kontamination, Saatgut, Übertragung, Unkräuter, Virus-Reservoir

1 Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important crops in Brandenburg, Germany, with the Spreewald region, where field-grown cucumbers cover c. 630 ha, as the main area of cultivation. Since 1999, virus infections of cucumbers in open land of the Spreewald region has gained increased attention. In 2000, for example, severe virus outbreaks were observed, affecting several cucumber cultivars and resulting in yield losses up to 50%. Initial serological tests of diseased cucumber plants by ELISA revealed infections with two different viruses: cucumber mosaic virus (CMV) and zucchini yellow mosaic virus (ZYMV).

During the last few decades, CMV has occurred sporadically in field-grown cucumbers in the Spreewald, although infected plants did not exhibit distinct symptoms and severe yield losses did not occur. ZYMV has not been observed previously in this area.

ZYMV was first detected in summer squash (*Cucurbita pepo* L.) in northern Italy (Lisa et al. 1981). Subsequently, the virus spread worldwide (Desbiez and Lecoq 1997), especially infecting summer squash, pumpkin (*Cucurbita maxima* Duchesne) and melons (*Cucumis melo* L.) and causing significant yield reductions in these crops (Nameth et al. 1985). However, little is reported on fruit symptoms and damage due to ZYMV infection of cucumbers, even though the virus was repeatedly detected in cucumber plants. Possible causes for the rapid distribution of ZYMV have been discussed, but these remain

unclear. According to NAMETH et al. (1985) and DAVIS and MIZUKI (1986), seed transmission of the virus might be responsible, but changes in cultural practice (DESBIEZ and LECOQ 1997) and the introduction of ZYMV by infected fruits (LECOQ et al. 2003) may also be relevant. Furthermore, ZYMV belonging to the genus Potyvirus is readily transmitted by several aphid species (LISA et al. 1981).

In order to evaluate viral incidence and distribution, surveys for ZYMV and other viruses infecting cucumber were carried out over four consecutive years (2001 to 2004) in field-grown cucumbers and pumpkins in the Spreewald region of Germany. Additionally, epidemiological studies were initiated, to determine sources of ZYMV infection. Several pickling cucumber cultivars were inoculated with ZYMV to investigate their virus susceptibility. Furthermore, experiments were done on transmissibility of ZYMV through soil, seed and weeds.

2 Materials and methods

2.1 Survey and virus detection in cucumber, pumpkins and weeds

From 2001 to 2004, annual surveys covered three fields. In total, 1,920 cucumber plants (including 11 different cultivars), 500 pumpkin plants and 350 weeds (comprising 23 different species) were collected. Pumpkin cultivars (*Cucurbita pepo* L., *Cucurbita maxima* Duchesne) were grown on cucumber field margins, whereas only weeds close to ZYMV-infected cucumber and pumpkin plants were included in samples. Virus detection was performed by DAS-ELISA, according to CLARK and ADAMS (1977). Specific antibodies against zucchini yellow mosaic virus (ZYMV), cucumber mosaic virus (CMV), cucumber green mottle mosaic virus (CGMMV), watermelon mosaic virus (WMV) and papaya ringspot virus (PRSV) were obtained from DSMZ (Braunschweig. Germany), Bioreba (Reinach, Switzerland) and Loewe Biochemica (Sauerlach, Germany), respectively, and applied as indicated by the supplier. Virus-specific antibodies were conjugated with alkaline phosphatase, and the enzyme-catalyzed accumulation of nitrophenol was determined spectrophotometrically at 405 nm, using an ELISA reader (Dynax Opsys MR). Each sample was measured in triplicate, and mean values exceeding twice those of negative controls (healthy plant material) were considered virus-positive.

2.2 Susceptibility tests of cucumber cultivars

Experiments were carried out in the glasshouse, on 27 different pickling and slicing cucumber cultivars, under standard conditions (light 16 h, temperature day 21–25°C – night 17°C, and 60% humidity). Experiments covered pickling cucumber cultivars `Adam`, `Anne`, `Aubade`, `Aztek`, `Berdine`, `Cabaret`, `Capra`, `Carine`, `Clementine`, `Conny`, `Dirigent`, `Dolomit`, `Emmy`, `Melody`, `Minos`, `Montreal`, `Nadine`, `Parka`, `Placido`, `Profi`, `Salinas`, 12-18 RZ, RS 30303080 and RS 30303640, as well as slicing cucumber cultivars `Carnito`, `Tine` and `Travito`. For this purpose, cotyledons or newly developed first leaves of young potted plants were mechanically inoculated with ZYMV-infected *Chenopodium quinoa* leaf material, homogenized in 0.1 M phosphate buffer, pH 7.0, according to WETZEL (1984). Consecutively, to estimate effects of a CMV and ZYMV mixed infection of cucumbers, ten plants each of the cultivars `Berdine`, `Capra`, `Melody`, `Nadine`, `Profi` and `Carnito` were inoculated additionally with CMV-infected leaf material. Inoculated plants were inspected for visual symptoms twice a week and also tested weekly by DAS-ELISA. Cucumber plants with ZYMV or with ZYMV/CMV mixed infection were also assessed six weeks after infection by counting the number of leaves as well as the number and quality of fruits.

2.3 Virus contamination of soil samples

Soil samples were taken close to ZYMV-infected cucumber plants in several fields. The samples were each subdivided into two parts and tested according to the method described by BÜTTNER and NIENHAUS (1989). The first part of each sample was used in baiting experiments immediately after collection; the second part was preserved for 4 months at 6°C before use. *C. sativus* `Berdine` and *C. quinoa* were used as bait plants. Development of symptoms in bait plants was monitored; plants were also inspected by DAS-ELISA, as described above.

2.4 ZYMV particle uptake and release from soil

Virus infection of plants through soil was investigated by planting two *C. sativus* plants in sterilized soil (in quintuplicate), the plants having been mechanically inoculated with ZYMV as described previously. After 10 weeks of cultivation under standard glasshouse conditions, three young healthy plants replaced infected plants. In each case, one plant was removed after 4, 8 and 12 weeks and homogenates of roots, older leaves and shoots were subjected to ZYMV-specific DAS-ELISA.

To study the release of ZYMV to soil, *C. sativus* `Berdine` and *C. quinoa* were mechanically inoculated with the virus. After confirmation by DAS-ELISA of successful infection, plants were planted in soil-containing pots and cultivated in a glasshouse under standard conditions. After irrigation with tap water, drainage water was collected in plastic mats. A fortnight after inoculation, drainage water was sampled for the first time and tested repeatedly for 12 weeks at intervals of 4 days. Each sample of drainage water was mixed with phosphate buffer, pH 7.0 (1:2; w/v) and used to inoculate ten *C. quinoa* plants in a biotest. Inoculated plants were visually inspected every 3 days and, if characteristic leaf symptoms were seen, samples were tested by DAS-ELISA and biotest. Finally, DAS-ELISA was conducted four weeks after inoculation, the roots, older and youngest leaves of inoculated plants being tested separately.

2.5 Assessment of ZYMV transmission through seed

To examine virus transmission through seed, 2,600 seeds were harvested from naturally ZYMV-infected pumpkins (*C. maxima* Duchesne). Seeds were subjected to various treatments: i) 100 seeds were tested directly by DAS ELISA (using ZYMV-specific antibodies) ii) 100 eight-day-old seedlings or iii) 1,000 pumpkin plants were tested in two different developmental stages (4 weeks after cultivation, 1- to 2-leaf stage and 6-week-old plants, 3- to 5-leaf stage) cultivated in the glasshouse under standard conditions. Also, 100 seeds were investigated after surface disinfection with 2% (v/v) MENNO-Florades (Menno Vertriebsgesellschaft mbH, Norderstedt, Germany) for 1 min. and after storage for four months at 6°C. Additionally, 100 seedlings and 500 older pumpkin plants (both 1- to 2-leaf stage and 3- to 5-leaf stage) from disinfected and stored seeds, respectively, were tested by DAS-ELISA. Positive ELISA reactions were confirmed by mechanical inoculation of *C. quinoa* plants and subsequent monitoring of the development of symptoms.

Finally, 3,000 seeds from six different commercially available *C. sativus* cultivars (`Capra`, `Dolomit`, `Melody`, `Nadine`, `Profi`, `Salinas`), 400 seeds from *C. maxima* `Gelber Zentner` and 200 seeds from *C. pepo* cultivars `Little indian mix` and `Mandarin` were investigated. For this purpose, plants were grown from seed in the glasshouse and tested at the 3- to 5-leaf stage by DAS-ELISA, using ZYMV-specific antibodies.

3 Results

3.1 Virus infections in field-grown cucumber and pumpkins

From 2001 to 2004, infection of *C. sativus* with the cucumber viruses CMV, WMV and ZYMV was detected with a clear prevalence of the last-mentioned virus. Only in the first year was a single CGMMV infected plant identified by DAS-ELISA. Overall, 27% of plants proved to be virus affected; in detail, 17% were infected by ZYMV alone, and 10% exhibited a mixed infection of ZYMV and CMV. In 2002, virus infections were found neither in visual inspections nor in ELISA tests. However, the survey in 2003 showed 26% of plants to be virus-infected, exclusively with ZYMV; in the final year of the survey, 25% of sampled cucumber plants were infected by ZYMV or WMV. In 19% a mixed infection with both viruses was found, whereas 6% of plants were affected by a single cucumber virus. Results of the four-year survey of three different cucumber fields are summarised in Fig. 1. ZYMV-infected plants exhibited leaf mosaic and malformation of fruits. Fruits from plants with mixed infections (either with ZYMV/CMV or with ZYMV/WMV) were severely malformed and reduced in size, as indicated in Fig. 2.

In 2003, 80 pumpkin plants of five different cultivars were cultivated in the margin of a cucumber field (Fig. 3). During summer inspections, typical symptoms of virus infection (such as chlorotic rings and linepattern on the leaves) were observed. Testing of pumpkins by ELISA revealed ZYMV infection in 24 plants. Additionally, in the following year, 50 pumpkin plants were investigated in a second field with pumpkins adjacent to cultivated cucumbers, and 44 of the pumpkin plants proved to be virus infested. More specifically, 13 plants were infected by ZYMV, 9 by WMV and 22 by both viruses.

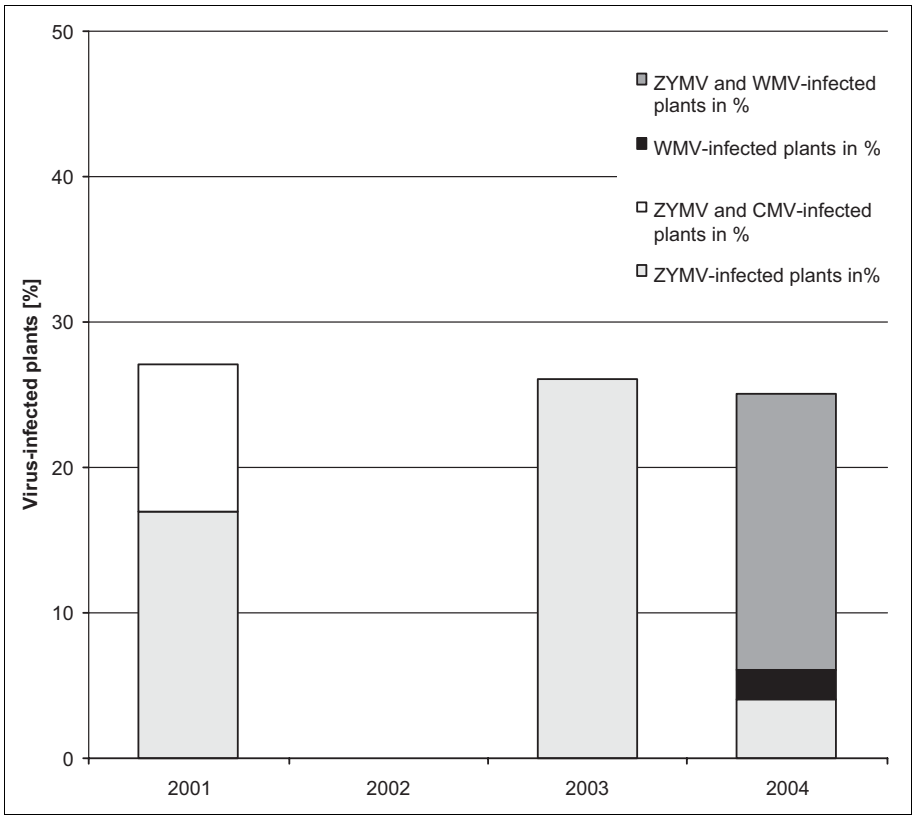

Fig. 1: Average proportion in percent of virus-infected cucumber plants in three different fields in the Spreewald growing area, Germany, throughout four consecutive years (n = 400 – 550/year).

Fig. 2: Virus-induced symptoms on curcurbits grown in the Spreewald, Germany. (A) Mosaic symptoms on zucchini yellow mosaic virus-infected pumpkin leaf. (B) Malformed cucumber (left) of a CMV- and ZYMV-infected *Cucumis sativus* 'Capra' in comparison with a fruit (right) from a virus-free plant.

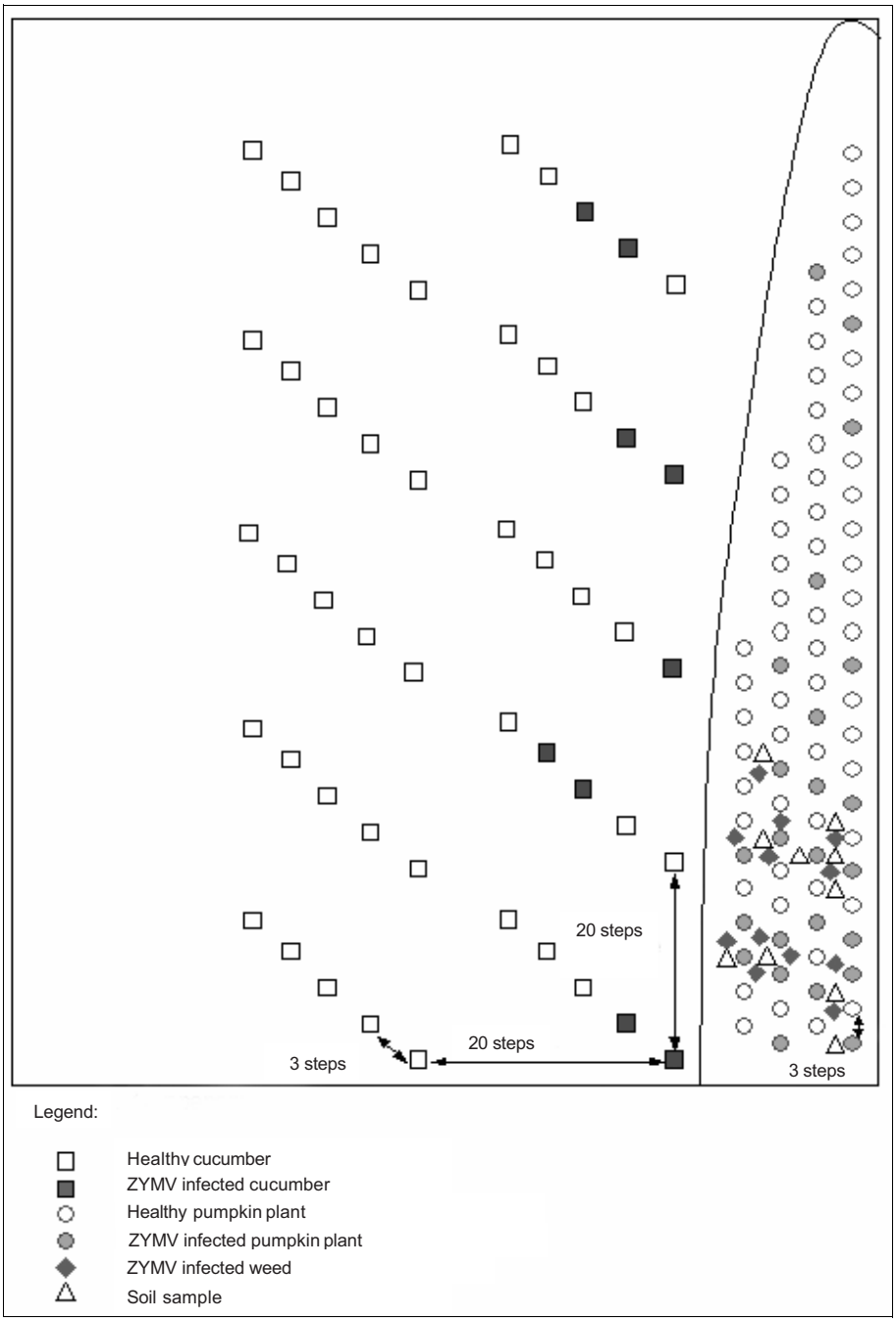

Fig. 3: Field plan outlining the sampling raster and reported ZYMV-infected cucumbers, pumpkins and weeds in the Spreewald growing area, Germany.

3.2 ZYMV incidence, transmission and virus reservoirs

In 2003, zucchini yellow mosaic virus (and possible virus reservoirs) was investigated in detail by sampling c. 1,500 m^2 of a field with cultivated cucumbers according to a specific raster (Fig. 3). In parallel, 70 weed plants of 11 species were tested by ELISA and 10 soil samples were collected. ZYMV was detectable in nine out of 50 cucumber plants tested, most of the infected ones adjoining the diseased pumpkins (Fig. 3).

By use of bait plants, zucchini yellow mosaic virus was confirmed in test plants derived from three out of 10 different soil samples. However, the pathogen was found only in soil samples tested directly after collection. Bait plants which were placed into soil samples stored for 4 months at 6°C prior to planting remained virus free. Eight weeks after cultivation of test plants in ZYMV-contaminated soil, the virus was detectable by ELISA in the roots of two out of five plants. After 12 weeks, the pathogen was not only present in roots of every test plant but also in older and younger leaves. The release of ZYMV to soil was also investigated by inoculating *C. quinoa* and *C. sativus* with drainage water from ZYMV-infected *C. quinoa* plants, but test plants developed no typical symptoms of the disease and virus infection was not detectable by DAS-ELISA. Even *C. quinoa*, which was irrigated with drainage water from ZYMV-infected plants, did not become infected.

In weeds collected from cucumber fields, naturally infected by ZYMV, the virus was found in two species: fat hen (*Chenopodium album* L.) and hoary alison (*Berteroa incana* (L.) DC). Moreover, CMV infections were detected in common chickweed (*Stellaria media* (L.) Vill.) and pineapple weed (*Matricaria discoidea* DC), whereas WMV was found in small-flowered crane's-bill (*Geranium pusillum* Burm. f. ex L.).

Additionally, contamination of cucumber and pumpkin seeds was investigated. Seeds harvested from ZYMV-infected *C. maxima* germinated at a rate of 96%. DAS-ELISA detected ZYMV in 56 out of 100 tested samples and in 51 out of 100 seedlings grown from seeds without prior treatment. In older

Table 1: Detection of ZYMV in seeds, seedlings and young plants grown from ZYMV-infected *Cucurbita maxima*

Seed treatment		Plant material	n	ZYMV infection [%][3]
Disinfection[1]	Storage[2]			
–	–	Seeds	100	56
		Seedlings	100	51
		Young plants (1–2 leaves)	1000	0.2 [4]
		Plants (3–5 leaves)	1000	0
+	–	Seeds	100	31
		Seedlings	100	0
		Young plants (1–2 leaves)	500	0
		Plants (3–5 leaves)	500	0
–	+	Seeds	100	0
		Seedlings	100	0
		Young plants (1–2 leaves)	500	0
		Plants (3–5 leaves)	500	0

[1] 2% (v/v) MENNO-Florades, 1 min.
[2] 4 months, 6°C.
[3] Detection by DAS-ELISA and biotest.
[4] Detection by DAS-ELISA only.
(–) Untreated.
(+) Treated.

developmental stages of plants (1-2 leaf stage and 3-4 leaf stage) no virus infection could be detected by DAS-ELISA with the exception of two samples – derived from cotyledons – out of 1,000 plants investigated, as indicated in Table 1. However, no development of virus induced symptoms was observed and sap inoculations from these two plants also caused no further infections of test plants.

In seed samples, which were disinfected for two minutes using MENNO-Florades and then subjected to ELISA, ZYMV was detected in 31 out of 100 samples. However, plants mechanically inoculated with a homogenate of these seeds showed no subsequent virus infection and, also, none of the 100 seedlings and 500 plants grown from disinfected seeds was infected with ZYMV. No ZYMV was found in seeds, seedlings and young plants cultivated and tested after 4 months of seed storage. Consistent with these findings, which are summarised in Table 1, ZYMV was neither found in plants grown from 3,000 commercially available seeds of *C. sativus*, nor in 400 seeds of *C. maxima* or 200 seeds of *C. pepo* (data not shown).

3.3 ZYMV susceptibility of cucumber cultivars

Infection experiments with zucchini yellow mosaic virus, including 27 different cucumber cultivars which were frequently cultivated in the region, showed that all cultivars were susceptible following mechanical inoculation. ZYMV-infected cucumbers developed leaf chlorosis and mosaic; leaves and fruits were also distorted. Mechanical inoculation at the early developmental stages of the plants reduced yields by up to 70%. A mixed infection with ZYMV and CMV resulted in particularly severe damage (namely, malformation of leaves and fruits), which also affected cucumber cultivars with a reported tolerance to CMV. In a more detailed comparison, five different cucumber cultivars were grown for 40 days until maturation of the first fruits. To evaluate yield, plants were cultivated until completion of the seventh harvest of mature fruits. Specifically, total numbers of harvested fruits per plant were counted and both marketable and unmarketable fruits were recorded. The analyses were based on the relative average harvest of 10 plants per cultivar, whereas harvested fruits from healthy plants are given as the reference value 1.0 in Fig. 4.

Relative harvest of CMV-infected plants ranged between 0.8 (`Nadine`) and 1.0 (`Melody`) that of healthy controls, and no fruits showed characteristic symptoms. Conversely, plants of all cultivars infected with ZYMV produced noticeable fewer fruits. In comparison with the CMV-infected cucumbers, these plants yielded between 0.6 (`Berdine`) and 0.4 (`Nadine`), and some fruits were heavily distorted, causing the quotient of marketable crop to drop to 0.5 (`Berdine`) and 0.3 (`Nadine`), respectively. Mixed infection of CMV and ZYMV reduced yields (in `Capra` and `Melody`) between 0.3 and 0.2 of relative harvest of the healthy plants, respectively) and most strongly decreased fruit production. The majority of fruits were deformed, with necrosis and, therefore, unmarketable.

4 Discussion

During a survey of field-grown cucumber (*Cucumis sativus* L.) in the Spreewald growing area in Germany, from 2001 to 2004, infections with zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), and cucumber mosaic virus (CMV) were observed. Similar findings were reported from ZYMV-infected vegetable fields in the Rhineland (Doradzillo et al. 2000; Doradzillo et al. 2001), where CMV was also detectable and fruit deformation and leaf distortion had caused yield losses in several cucumber cultivars as well as in spinach plants.

ZYMV predominated in the period under investigation and was also detected in pumpkins (*Cucurbita pepo* L. and *Cucurbita maxima* L.). Cucurbits were often grown in the immediate vicinity of cucumber fields or in the margins of cucumber fields. The risk of overlooking virus-infected plants is considerable, particularly for pumpkins, because some cultivars exhibit heritable leaf variegations or variable fruit forms and colours which may disguise virus symptoms.

Tests of weeds, soil and seeds were done to gain more information on possible virus sources. In the Spreewald cropping area, 350 individual weed plants of 23 species were investigated, revealing fat hen and hoary alison as ZYMV-infected. The latter is a biennial weed and could be a potential reservoir of the pathogen in this growing area. To date in other temperate regions, Lecoq (unpublished in Desbiez and Lecoq 1997) did not find natural reservoirs of ZYMV, despite extensive searching. Under tropical and subtropical climates, cultured as well as wild curcurbits (which are potential hosts of ZYMV) were grown throughout the year (Yuki et al. 2000). In this regions the virus was detected in wild Cucurbitaceae, for instance in *Melothria pendula* L. (Adler et al. 1983), *Cayaponia tibiricae* Cogn. (CT) (Yuki et al. 1999), *Momordia charantia* L. and *Cucumis dipsaceus* Ehrenb. ex Spach (Ullman et al. 1991), *Moluccella laevis* and *Luffa cylindrica* (L.) M. Roem. (Al-Musa 1989a, b) and *Lagenaria siceraria* (Molina) (Verma et al. 2004; Ullman et al. 1991).

ZYMV could be isolated from soil, using bait plants. Referring to the literature, this appears to be the first report of ZYMV-contaminated soil, our experiments demonstrating the uptake of ZYMV from soil into the roots of healthy plants. However, it was not possible to demonstrate the release of ZYMV particles from the roots of infected plants to soil by applied serological assays. Hence, the infections of healthy plants were possibly caused by infected root remains of previously cultivations.

Desbiez and Lecoq (1997) suggested seed transmission of ZYMV as a possible reason for the rapid spread of ZYMV, but screening of cucurbit seeds from the growing years 2001 to 2004 (inclusive) showed no ZYMV infections. Different

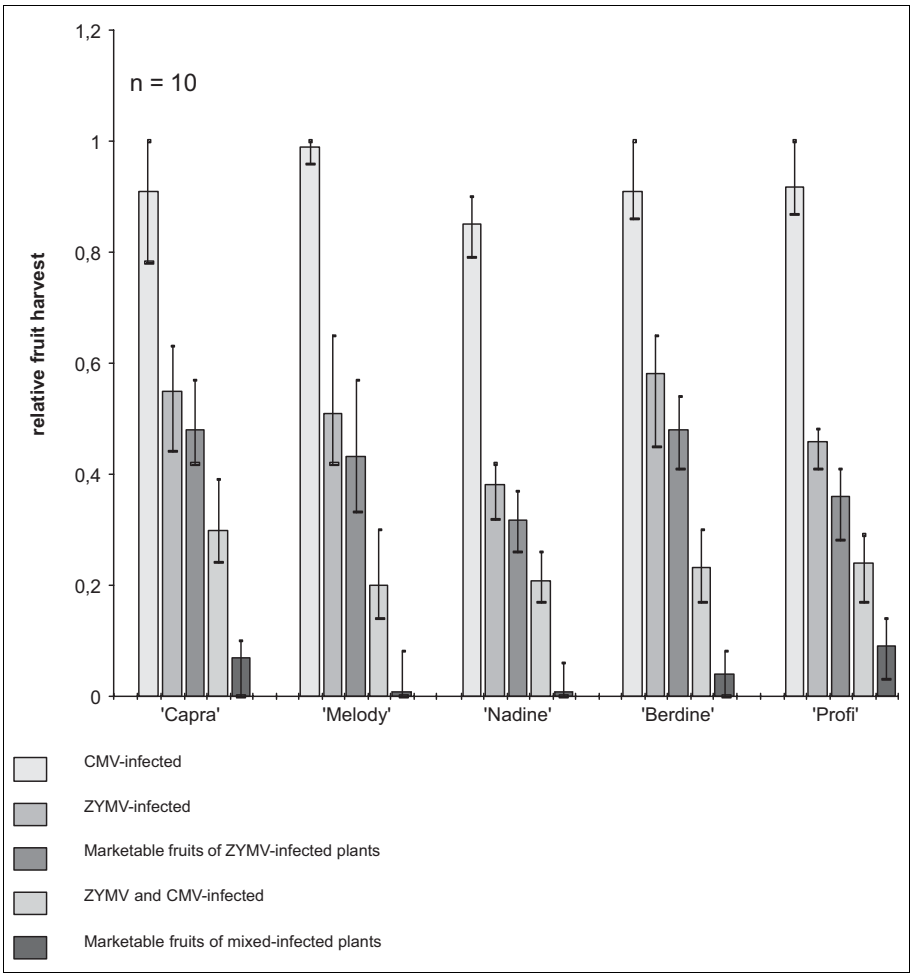

Fig. 4: Diagram of relative harvest of virus-infected (ZYMV and CMV in single and mix infection after mechanical inoculation) *Cucumis sativus* cultivars 'Berdine', 'Capra', 'Melody', 'Nadine' and 'Profi', and the proportion of marketable fruits in the Spreewald growing area, Germany. Average relative yield of every variant including ten plants is indicated as are minimum and maximum values.

working groups also reported opposing results. Lecoq and Wipf-Scheibel (unpublished in Lecoq et al. 2003) could not confirm any virus contamination in 70,000 seedlings from ZYMV-infected Cucurbitaceae of various cultivars (of different species) and ZYMV transmission was neither found in *C. pepo* (Nameth et al. 1985), *Cucumis melo* L. (Gleason and Provvidenti 1990; Wong et al. 1994), *C. sativus*, *C. moschata* (Robinson et al. 1993) nor in *C. maxima* (Greber et al. 1987) seed. In contrast, Davis and Mizuki (1986) found ZYMV seed transmission in seedlings of *C. pepo*, at a maximum rate of 80%.

Tests of seeds derived from ZYMV-infected pumpkins indicated a contamination of the seed coat raised from untreated seed. After seed-disinfection with MENNO-Florades, some of the tested seeds were still ZYMV-positive, but the pathogen could not be found in seedlings or plants. Furthermore, mechanical inoculation of the homogenate from the ELISA-positive seeds did not cause an infection in test plants. Therefore, we conclude that although the antigen might be still present on the seed coat, the virus is no longer infectious. This is supported by our observation that ZYMV was detectable in homogenates of cotyledons from two *C. maxima* plants grown directly after seed harvest, but mechanical transmission to test plants was unsuccessful. Also, later testing of the same plants at the 3- to 5-leaf stage did not reveal ZYMV, either in any plant or in the two plants whose cotyledons were ZYMV-positive in the first DAS-ELISA test. These inconsistent results could therefore be due to contamination of the cotyledons with non-infectious particles; this is supported by investigations from Walkey (1989) who detected ZYMV in summer squash seedlings, using ELISA, although transmission through mechanical inoculation was not successful.

After four months of seed storage, the virus was found neither in the seeds of ZYMV-infected *C. maxima* nor in the seedlings or plants grown from them. This result confirms the assumption that ZYMV contaminates the seed coat and that the pathogen is inactivated during maturation and storage, as has also been demonstrated for other viruses, e.g. pea streak virus (PSV) (Ford 1966) and squash mosaic virus (SqMV) (Powell and Schlegel 1970).

From our findings we conclude that ZYMV transmission through seed can, at best, be very low. The tests indicate that infected seed loses its infectivity through storage; hence, spread of ZYMV within the Spreewald region through commercial seed is considered highly unlikely. Transmission by insect vectors remains as the most likely potential source of ZYMV. Therefore, more attention towards putative aphid vectors of ZYMV, and further studies on their occurrence in the cucumber cultivation area are most relevant. Also, for further epidemiological studies, virus isolates from different regions should be characterized in detail.

All 27 cucumber cultivars tested proved to be susceptible to ZYMV infection, resulting in leaf chlorosis and mosaic as well as the production of malformed fruits. None of the cucumber cultivars exhibited resistance or tolerance towards ZYMV, even though some of them were tolerant to other virus infections, such as CMV. Because the use of virus-resistant cultivars is the best way to control virus diseases (Desbiez and Lecoq 1997) it is of great importance to develop cucumber cultivars with resistance against ZYMV, especially considering the synergistic effects of mixed infections.

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