# Distribution, Variability and Overwintering of *Zucchini Yellow Mosaic Virus* in the Czech Republic

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**Abstract**

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The incidence of *Zucchini yellow mosaic virus* (ZYMV) was monitored in the south Moravian region of the Czech Republic during 1997–2001. Crops of gherkin, squash, zucchini and cucumbers were found infected with ZYMV, manifested by symptoms of severe stunting and yellowing with deformed leaves and fruits. Twenty to eighty percent of infected plants were recorded. Six isolates of ZYMV from four localities were differentiated on indicator plants; three of them were maintained as being typical for differences in pathogenicity. Overwintered weed species were tested for the presence of the virus. ZYMV was detected by ELISA in one plant of *Tripleurospermum maritimum* out of 46 tested, and in two plants of *Stellaria media* out of 29 tested in 2001. Such tests were repeated in 2002, and ZYMV was detected in three plants of *T. maritimum* out of 45 tested, in three plants of *S. media* out of 52, and in two plants of *Trifolium repens* out of 17 tested. The virus was successfully transmitted from *T. maritimum, S. media* and *T. repens* to indicator plants of *Cucurbita pepo* convar. *giromontiina*. Back-transmission of ZYMV was proved by ELISA, electron microscopy and symptoms. *T. maritimum* was found to be a new natural host of ZYMV.

**Keywords**: ZYMV; incidence; vegetables; weeds; virus reservoirs; differentiation of strains

*Zucchini yellow mosaic virus* (ZYMV) infects plants of the family *Cucurbitaceae* and is a virus of economic importance that has been described more recently in Europe. *Cucumber mosaic virus* (CMV), W*atermelon mosaic virus* 2 (WMV2), *Papaya ringspot virus type* W (PRSV-W) syn. WMV1, *Squash mosaic virus* (SqMV) and *Melon necrotic spot virus* (MNSV) have caused prevalent damage on cucurbit vegetables until the 1970-ies (LOVISOLO 1980). In 1973, a severe viral disease was observed in zucchini plants in Northern Italy; the symptoms were different from those caused by hitherto known cucurbits viruses on zucchini. LISA *et al.* (1981) identified the causal agent as a new potyvirus that they named *Zucchini yellow mosaic virus*. Infected plants exhibited severe stunting and yellowing symptoms, with leaf and fruit deformations. At the same time ZYMV was also observed in France, where it was named as *Muskmelon yellow stunt virus* (LECOQ *et al*. 1981). From an epidemiological point of view it is interesting that within 5 years the virus was reported worldwide in the most important cucurbit growing areas.

In former Czechoslovakia, the presence of ZYMV was first reported in 1991 (CHOD & JOKEŠ 1991). However, the incidence of ZYMV on cucurbit crops was sporadic until 1997. In the following years ZYMV caused considerable losses in cucumbers and squashes in Southern Moravia. The State Phytosanitary Administration of the Czech Republic monitors the distribution of the disease in the Czech Republic every year. In 2001, intensive research was initiated at the Research Institute of Crop Production Prague-Ruzyně with the aim to control the spread of ZYMV and thus reduce the production losses in cucurb-

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its. In this work we are submitting an overview of the distribution of ZYMV in Southern Moravia, results of attempts on virus transmission, its variability and differentiation of isolates. Another aspect of our work was the observation of ZYMV in overwintering weeds, from where the virus is spread by aphids into young cucurbits in spring. Further research on ZYMV in the Czech Republic will be focused on the spread of the virus by seeds, persistence of the virus in plant debris and ascertaining resistance of cucurbit species to ZYMV.

## MATERIALS AND METHODS

***Occurrence of ZYMV in the Czech Republic***. The State Phytosanitary Administration of the Czech Republic has regularly monitored the occurrence and spread of ZYMV since 1997. Infected species of vegetables (cucumber, gherkin, squash, pumpkin and zucchini), infected area, percentage of infected plants and the intensity of disease symptoms were determined by ELISA at the sites of occurrence of the virus. So far the results have not been summarised and published. Details from different districts were kindly provided by the State Phytosanitary Administration to synthesise data on the occurrence of ZYMV in the Czech Republic.

***Differentiation of ZYMV isolates***. In 2001, six leaf samples of cucumbers and squash with different symptoms of virus infection were selected from 73 samples collected at four localities with a possible occurrence of ZYMV (Křepice, Lednice, Podivín and Hrušky). The presence of ZYMV in the six samples was confirmed by ELISA. The virus was transmitted mechanically and by aphids, *Myzus persicae* (Sulzer), on to indicator plants of zucchini, *Cucurbita pepo* L. convar. *giromontiina* Grebenščikov cv. Zelená, *C. ficifolia* C. Bouché and cucumber hybrids Melody F1 and Musica F1. Each of the six isolates gave one of three distinct reactions on the indicator plants, and three isolates were maintained as being representative.

For mechanical transmission 1 g of leaves with symptoms of ZYMV was homogenised in 3 ml of 0.03M $Na_2HPO_4$ and 0.2% DIECA, pH 9.3 (MAHGOUB *et al.* 1997). Carborundum powder 200 mesh was added to the homogenate before mechanical inoculation of the indicator plants. For transmission of ZYMV by non-viruliferous aphids, *Myzus persicae* aphids were starved for 4 h prior to acquisition feeding of 5 min on infected plants, then 10 aphids were transferred to each test plant and inoculation feeding was allowed for 4 h. After inoculation feeding the aphids were killed by Pirimor insecticide. The indicator plants differentiated between three isolates of ZYMV with different pathogenicity and they were further characterised as strains of ZYMV. The presence of ZYMV in indicator plants was verified by ELISA. The specific polyclonal antiserum and procedure from Biochemica, Loewe was used. The presence of ZYMV particles in the isolates was verified by electron microscopy. Leaf samples were homogenised by mortar and pestle in 0.01M HEPES buffer pH 8.2 (1 g leaves + 2 ml buffer); the homogenate was filtered and negatively stained with phosphotungstic acid (pH 6.9) in a ratio of 1:1, placed on electron microscope grids and these were inspected by electron microscope Philips 208 S.

***Research on reservoirs and overwintering of ZYMV in the Czech Republic***. In spring of 2001 and 2002 overwintered plants of different weed species (Table 3) were collected at the margins of fields where ZYMV had been found the previous year. The presence of ZYMV in the plants of weeds was detected by ELISA, and through tests of mechanical transmission by crude sap and aphid transmission by *M. persicae* onto indicator plants of *C. pepo* convar. *giromontiina* cv. Zelená. The procedure of mechanical transmission was the same as for differentiation of ZYMV isolates. For inoculation by aphids a larger number of aphids was used and acquisition feeding extended: after 45 min acquisition feeding 50 aphids were transferred from the weed plant to one indicator plant and inoculation feeding was allowed for 4 h. Virus symptoms were evaluated on the indicator plants, and the presence of virus was tested by ELISA and electron microscope.

## RESULTS AND DISCUSSION

***Occurrence of ZYMV in the Czech Republic***. The incidence of ZYMV on cucurbit crops in the Czech Republic was sporadic until 1997, restricted to districts Břeclav and Znojmo in Southern Moravia, where less than 30 ha of gherkin and squash were infected. In 1998–2000, high incidence of ZYMV was observed in both districts with over 200 ha of cucurbit vegetables infected (ŠINDELKOVÁ 2002 – person. commun.). In 2001, the presence of ZYMV on cucurbit vegetables was proved in the four districts Břeclav, Znojmo, Brno, and Hodonín. The main infected crops were gherkins (Fig. 1), zucchini (Fig. 2) and buttercup squash. Symptoms of severe stunting and yellowing with leaf and fruit deformations (Fig. 3) appeared in infected fields in summer. The occurrence of ZYMV in cucurbit vegetables in districts of Southern Moravia is shown in Table 1.

***Differentiation of ZYMV isolates***. In 2001, three isolates of ZYMV with different pathogenity were selected from 73 samples collected on cucurbits. Isolate ZYMV-Křepice (ZYMV-K) was obtained from cucumber, isolate ZYMV-Lednice (ZYMV-L) from squash and isolate ZYMV-Hrušky (ZYMV-H) from squash. The presence of ZYMV in all samples was proved serologically by ELISA and the presence of filamentous particles (750 nm) of

Fig. 1. Gherkins infected with ZYMV

Fig. 2. Zucchini plant infected with ZYMV

ZYMV was confirmed by electron microscope (Fig. 4). The virus isolates were transmitted by aphids and mechanically on indicator plants. The presence of ZYMV in the indicator plants was confirmed by ELISA. *Cucumber mosaic virus* (CMV) was not detected by ELISA in any indicator plant with ZYMV symptoms. The differentiation of three isolates of ZYMV on indicator plants is presented in Table 2. Isolate ZYMV-K was characterised as mild pathogenic, isolate ZYMV-L as medium severe pathogenic, and ZYMV-H as severe pathogenic.

***Reservoirs and overwintering of ZYMV in the Czech Republic***. In the springs of 2001–2002 altogether 409 weed samples were collected and tested for the presence of ZYMV (Table 3). In 2001, the virus was detected by ELISA in one of 46 tested plants of *Tripleurospermum maritimum* and in two of 29 plants of *Stellaria media.* In 2002, ZYMV was proved in two of 45 plants of *T. maritimum*, in three of 52 plants of *S. media* and in two of 17 plants of *Trifolium repens.* ZYMV was successfully transmitted by *Myzus persicae* on zucchini, *Cucurbita pepo* convar. *giromontiina* cv. Zelená. Its leaves showed typical ZYMV symptoms, and presence of the virus was confirmed by both ELISA and electron microscope.

Research in other countries on the host range of ZYMV has shown that it includes members of 11 families of dicotyledons (LECOQ *et al*. 1981; LISA *et al*. 1981). Among them are the following weeds which might be considered natural reservoirs of ZYMV in the Czech Republic: *Ranunculus sardous* Cr. (PERRING *et al*. 1992), *Senecio vulgaris* (DESBIEZ & LECOQ 1997), *Stellaria media, Matricaria discoidea* and *Trifolium repens* (FLETCHER *et al*. 1999).

Our results proved that in the Czech Republic, ZYMV overwinters in *Tripleurospermum maritimum*, *Stellaria media* and *Trifolium repens* of which Sea Mayweed, *T. maritimum,* is a new natural host. Infected weeds are a source of infection on cucurbits in springtime. We are continuing the research on the epidemiology of ZYMV in the Czech Republic and on transmission of ZYMV by seeds of cucurbit plants.

Fig. 3. Zucchini fruit infected with ZYMV

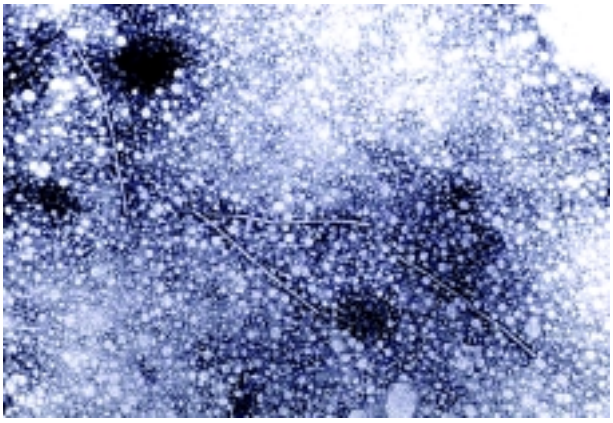

Fig. 4. Filamentous particles of ZYMV

Table 1. Occurrence of ZYMV on cucurbit crops in Southern Moravia during 1998–2001

| Year | District | Vegetable species | No. of localities | Infected area (ha) |
|---|---|---|---|---|
| 1997 | Břeclav | Gherkins | 3 | 25 |
| | Znojmo | Buttercup squash | 1 | 4 |
| 1998 | Břeclav | Gherkins | 5 | 40 |
| | | Cucumbers | 1 | 1 |
| | | Buttercup squash | 3 | 15 |
| | Znojmo | Gherkins | 12 | 139 |
| | | Buttercup squash | 4 | 18 |
| | | Zucchini | 4 | 5 |
| 1999 | Břeclav | Gherkins | 1 | 13 |
| | Znojmo | Gherkins | 7 | 61 |
| | | Buttercup squash | 5 | 59 |
| 2000 | Břeclav | Gherkins | 3 | 15 |
| | | Cucumbers | 3 | 5 |
| | Znojmo | Gherkins | 2 | 9 |
| | | Buttercup squash | 4 | 133 |
| 2001 | Brno-country | Buttercup squash | 3 | unknown |
| | Břeclav | Gherkins | 2 | 12 |
| | | Zucchini | 2 | 7 |
| | | Buttercup squash | 3 | 120 |
| | Hodonín | Buttercup squash | unknown | unknown |
| | Znojmo | Buttercup squash | 3 | 26 |
| | | Cucumbers | 1 | 6 |
| | | Gherkins | 3 | 7 |

Table 2. The differentiation of three Czech isolates of *Zucchini yellow mosaic virus* on indicator plants

| Indicator plant | | Virus isolate | | |
|---|---|---|---|---|
| | | ZYMV-K | ZYMV-L | ZYMV-H |
| *Cucurbita pepo L.* convar. *giromontiina* Grebenščikov, cv. Zelená | leaf | mild leaf-spots, mosaic, tapering | severe spots, sinuated lamina | extreme reduction in the size of leaf lamina, filamentary and blisters |
| | fruit | straight, smooth | malformation | no fruits |
| *Cucurbita ficifolia* C. Bouché | leaf | mild mosaic | mild mosaic | severe chlorotic leafspot, mosaic |
| *Cucumis sativus L.*, leaf deformation cv. Melody | leaf | no symptoms | spots, deformation | severe spots, blisters; |
| | fruit | no symptoms | mild deformation | extreme deformation |
| *Cucumis sativus L.*, cv. Musica leaf deformation | leaf | no symptoms | spots, deformation | severe spots, blisters; |
| | fruit | no symptoms | mild deformation | extreme deformation |

Table 3. List of plant species tested for presence of ZYMV

| Species | Number of plants | | Species | Number of plants | |
|---|---|---|---|---|---|
| | tested | ELISA positive | | tested | ELISA positive |
| *Sambucus nigra* | 1 | 0 | *Chenopodium hybridum* | 16 | 0 |
| *Hyoscyamus niger* | 1 | 0 | *Prunus cerasifera* | 39 | 0 |
| *Datura stramonium* | 1 | 0 | *Artemisia vulgaris* | 1 | 0 |
| *Phaseolus vulgaris* | 5 | 0 | *Thlaspi arvense* | 1 | 0 |
| *Matricaria discoidea* | 2 | 0 | *Glechoma hederacea* | 2 | 0 |
| *Matricaria inodora* | 2 | 0 | *Stellaria media* | 81 | 5 |
| *Tripleurospermum maritimum* | 91 | 3 | *Polygonum aciculare* | 1 | 0 |
| *Pisum sativum* | 3 | 0 | *Veronica hederifolia* | 3 | 0 |
| *Lamium purpureum* | 6 | 0 | *Achillea millefolium* | 1 | 0 |
| *Trifolium pratense* | 1 | 0 | *Malva neglecta* | 3 | 0 |
| *Trifolium repens* | 19 | 2 | *Senecio vulgaris* | 24 | 0 |
| *Geranium pusillum* | 2 | 0 | *Galium aparine* | 40 | 0 |
| *Melandryum album* | 2 | 0 | *Convolvus arvensis* | 1 | 0 |
| *Urtica urens* | 2 | 0 | *Medicago lupulina* | 1 | 0 |
| *Amaranthus retroflexus* | 18 | 0 | *Tanacetum vulgare* | 1 | 0 |
| *Atriplex patula* | 1 | 0 | *Vicia cracca* ssp. *Vulgaris* | 1 | 0 |
| *Chenopodium album* | 36 | 0 | | | |

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## Souhrn

SVOBODA J., POLÁK J. (2002): **Rozšíření, variabilita a přezimování viru žluté mozaiky cukety v České republice**. Plant Protect. Sci., **38**: 125–130.

Přítomnost viru žluté mozaiky cukety (ZYMV) byla poprvé v Československu publikována v roce 1991. Výskyt ZYMV byl na tykvovitých plodinách sporadický až do roku 1997. Ještě v roce 1999 bylo na jižní Moravě infikováno méně než 30 ha okurek a dýní. V letech 1998–2000 byl pozorován vysoký výskyt ZYMV ve dvou moravských okresech: Břeclav

a Znojmo. V roce 2001 se infekce rozšířila do dalších dvou okresů: Brno a Hodonín. V těchto letech byly napadeny zejména okurky nakládačky, ale také dýně, cukety a salátové okurky přibližně na dvou stech hektarech. Na napadených rostlinách se v létě objevily příznaky silné zakrslosti a žloutnutí listů spolu s deformacemi plodů. Infikováno bylo 20–80 % rostlin. Z různých lokalit bylo odebráno šest izolátů ZYMV, které byly dále diferencovány na indikátorových rostlinách. Izoláty ZYMV byly přeneseny mšicemi a mechanicky na rostliny cukety, okurky a tykve fíkolisté s cílem charakterizovat kmeny viru vyskytující se v České republice. Z lokalit Hrušky, Křepice a Lednice byly vybrány izoláty viru, lišící se svojí patogenitou na indikátorových rostlinách. Na jaře byly sbírány plevelné rostliny z okrajů polí tykvovitých plodin infikovaných ZYMV. Z plevelů, kde byl virus stanoven pomocí ELISA, byl přenesen pomocí mšic na indikátorové rostliny. Mechanický přenos ZYMV z plevelů nebyl úspěšný. V roce 2001 byl ZYMV stanoven pomocí ELISA v jedné rostlině *Tripleurospermum maritimum* (L.) Sch. Bip. ze čtyřiceti šesti testovaných a ve dvou rostlinách *Stellaria media* (L.) Vill. z dvaceti devíti testovaných. V roce 2002 byly pokusy opakovány. ZYMV byl prokázán ve třech rostlinách *Tripleurospermum maritimum* (L.) Sch. Bip. ze čtyřiceti pěti testovaných, ve třech rostlinách *Stellaria media* (L.) Vill. z padesáti dvou testovaných a ve dvou rostlinách *Trifolium repens* L. ze sedmnácti testovaných. Virus byl úspěšně přenesen ze *Tripleurospermum maritimum* (L.) Sch. Bip., *Stellaria media* (L.) Vill. a *Trifolium repens* L. na indikátorové rostliny *Cucurbita pepo* L. convar. *giromontiina* Grebenščikov. Zpětný přenos ZYMV byl prokázán pomocí ELISA, elektronové mikroskopie a příznaků. Jako nový přírodní hostitel ZYMV, ve kterém virus v České republice přezimovává, byl zjištěn *Tripleurospermum maritimum* (L.) Sch. Bip.

**Klíčová slova:** ZYMV; výskyt; zelenina; plevely; rezervoáry viru; diferenciace kmenů

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