# Field Resistance of Six Cultivars of Winter Oilseed Rape against *Turnip Yellow Mosaic Virus*

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**Abstract**

ŠPAK J., KUBELKOVÁ D. (2002): **Field resistance of six cultivars of winter oilseed rape against *Turnip yellow mosaic virus***. Plant Protect. Sci., **38**: 73–75.

The resistance of six cultivars of winter oilseed rape (SL 509, SL 507, Darmor, Solida, Jet Neuf, Silesia) against *Turnip yellow mosaic virus* was studied. The number of over-wintering plants and plants with symptoms of TYMV infection were monitored. All plants were tested by the double diffusion test in agar and by DAS-ELISA to prove infection. ELISA was the most sensitive method, revealing 32–76% of latent virus infections in individual cultivars. The results clearly illustrated that monitoring of plant symptoms is not sufficient to prove TYMV infection in the field and that sensitive, large scale methods like ELISA must be employed to obtain reliable data.

**Keywords:** *Turnip yellow mosaic virus*; oilseed rape; *Brassica*; ELISA; serology

**Abbreviations: DAS-ELISA** = double antibody sandwich enzyme-linked immunosorbent assay; **PBS** = phosphate-buffered saline; **TYMV** = *Turnip yellow mosaic virus*

*Turnip yellow mosaic virus* (TYMV) is a seed (DE ASSIS & SHERWOOD 2000) and beetle transmitted virus infecting a wide range of brassicas world-wide (RAYBOULD *et al*. 1999). In our previous studies we estimated losses caused by artificial TYMV infection in several winter oilseed rape and fodder turnip rape cultivars (POLÁK *et al*. 1989) and proved seed transmission of TYMV in these crops (ŠPAK *et al*. 1993). We also searched for natural sources of the virus (PELIKÁNOVÁ *et al*. 1990; ŠPAK *et al*. 1991) and found 50–90% incidence of TYMV in the fodder turnip rape cultivar Perko PVH in the field (ŠPAK & POLÁK 1985). In contrast to Germany (SCHRÖEDER 1994), where TYMV has been observed in low percentage in spring and winter oilseed rape, we have never found naturally TYMV infected winter oilseed rape in the Czech Republic. Also, infected seedlings raised from TYMV-infected seed in our previous experiments remained symptomles (ŠPAK *et al*. 1993).

The aim of this study was to induce an artificial field infection in six oilseed cultivars, to thus test their resistance and the reliability of symptoms, serology and ELISA for the diagnosis of the virus.

## MATERIALS AND METHODS

The seed of six winter oilseed rape cultivars (Table 1) were purchased from the Research and Breeding Station Slapy at Tábor. One hundred seeds of each cultivar were sown at the end of August in experimental plots. At the five true leaf stage the number of plants in each plot was reduced to 50. Plants were dusted with carborundum and inoculated with strain TYMV 2 (ŠPAK & POLÁK 1988) using a glass rod. The inoculum was prepared from 1 g of leaves with symptoms of infection ground in a mortar in 2 ml of 0.1 M phosphate buffer pH 7.0 In May of the following year, the number of over-wintering plants and

Supported by Grant No. Z 50561902 of the Academy of Sciences of the Czech Republic.

plants with symptoms of TYMV infection were monitored. All plants were tested by the double diffusion test in agar and by DAS-ELISA to prove the infection.

Double diffusion tests were performed in 0.9% Difco Noble agar in 0.01 M McIlvaine buffer pH 7.0 with 0.02% sodium azide in a Petri dish. Antiserum diluted 1:200 was placed in a central well and antigens (sap from infected plants) in surrounding wells. Antigens were prepared by homogenisation of leaves in 0.01 M McIlvaine buffer pH 7.0 with 0.02% sodium azide, in a 1:1 ratio (w/v). After 24–48 h serological reactions were evaluated and stained with amido black 10B. Antiserum with the titre 1:2048, prepared in our laboratory against the TYMV 2 strain (ŠPAK & POLÁK 1988) was used. DAS-ELISA was performed as described in (ŠPAK *et al.*1993). Samples with the absorbance at 405 nm higher than $x + 3s$ ($x$ = value of healthy control, s = standard deviation) were scored as positive.

## RESULTS AND DISCUSSION

The results summarised in Table 1 show that the overwintering of all cultivars was similar, being the best in cv. Silesia and the worst in cv. Solida. Symptoms of virus infection, not very distinct pale yellowish-green mosaic on leaves, appeared in all cultivars. The number of symptomatic plants was lowest in Silesia. In cv. Solida false symptoms were scored as positive in two plants, as shown later by serology and ELISA. No spur formation was observed in double diffusion serological tests. This indicates that there had been no natural infection of the experimental plants by another strain of the virus occurring in nature (PELIKÁNOVÁ *et al.* 1990; ŠPAK *et al.* 1991) and transmitted e.g. by Phyllotreta beetles (PROESLER 1971).

Although the match between diagnostic methods was best between symptoms and serology (Table 2), ELISA was the most sensitive method, revealing 32–76% of latent virus infections in individual cultivars. Mean absorbance values in positive, negative and healthy control plants were 1.39, 0.18 and 0.08, respectively. We did not observe a significant difference in mean absorbance values (standard deviations) among individual cultivars, which were in cvs SL 509 1.50 (0.15), SL 507 1.15 (0.30), Darmor 1.42 (0.34), Solida 1.29 (0.24), Jet Neuf 1.58 (0.04), Silesia 1.41 (0.29). Although the level of infected plants was the lowest in SL 509, we found no distinct difference in the resistance of the cultivars. The high frequency of latent infections could explain why natural infection of winter oilseed rape has not yet been observed in the Czech Republic.

Recently, seed transmission of brassica viruses (DE ASSIS & SHERWOOD 2000) and their occurrence in natural populations (RAYBOULD *et al.* 1999; MASKELL *et al.* 1999) has attracted interest with respect to risk evaluation of large scale utilisation of genetically modified brassica crops in the field. Similar studies are confronted with a lack of epidemiological data obtained in field studies with cultivated and naturally infected brassicas including weeds. Our results clearly illustrated that monitoring of plant symptoms is not sufficient to prove TYMV infection in the field and that in similar studies sensitive, large scale methods like ELISA must be employed to obtain reliable data.

Table 1. Overwintering and detection of *Turnip yellow mosaic virus* in oilseed tape cultivars

| | SL 509 | | SL 507 | | Darmor | | Solida | | Jet Neuf | | Silesia | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | (%) | N | (%) | N | (%) | N | (%) | N | (%) | N | (%) |
| Autumn | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 |
| Spring | 38 | 76 | 38 | 76 | 39 | 78 | 34 | 68 | 37 | 74 | 46 | 92 |
| Symptoms | 13 | 26 | 16 | 32 | 12 | 24 | 7 | 14 | 12 | 24 | 5 | 10 |
| Serology positive | 16 | 32 | 26 | 52 | 25 | 50 | 5 | 10 | 27 | 54 | 6 | 12 |
| ELISA positive | 32 | 64 | 49 | 98 | 48 | 96 | 43 | 86 | 46 | 92 | 43 | 86 |

N = number of plants

Table 2. Match of diagnostic methods of *Turnip yellow mosaic virus* in oilseed rape (% of plants)

| | SL 509 | SL 507 | Darmor | Solida | Jet Neuf | Silesia |
|---|---|---|---|---|---|---|
| Symptoms + serology | 82 | 62 | 48 | 72 | 44 | 84 |
| Symptoms + ELISA | 40 | 32 | 25 | 16 | 26 | 12 |
| Serology + ELISA | 50 | 56 | 52 | 12 | 58 | 14 |

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Received for publication May 24, 2002
Accepted after corrections June 20, 2002

**Abstrakt**

ŠPAK J., KUBELKOVÁ D. (2002): **Polní rezistence šesti odrůd řepký ozimé vůči *Turnip yellow mosaic virus***. Plant Protect. Sci., **38**: 73–75.

V polním pokusu bylo testováno 6 odrůd řepky ozimé (SL 509, SL 507, Darmor, Solida, Jet Neuf, Silesia) na odolnost k viru žluté mozaiky vodnice (TYMV). Výsledky přezimování rostlin, příznaků na listech, sérologie a ELISA testů a vzájemné korelačních vztahy těchto faktorů (%) prokázaly vlastnosti jednotlivých odrůd a hlavně spolehlivost diagnostických metod. Nejlépe přezimovala odrůda Silesia. Příznaky na listech nelze považovat za spolehlivého ukazatele infekce. Jako nejspolehlivější metoda detekce TYMV se osvědčila DAS-ELISA.

**Klíčová slova**: virus žluté mozaiky vodnice; řepka ozimá; *Brassica*; ELISA; sérologie

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