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TOSPOVIRUSES IN CHRYSANTHEMUM MOTHER STOCK PLANTS IN POLAND

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Abstract

Tomato spotted wilt virus (TSWV) was detected with DAS-ELISA test in chrysanthemum mother stock plants in each of inspected greenhouses during years of surveys: 1999–2002. The highest level of infection of chrysanthemum plants with TSWV was noted in 1999 (91–93%) in two greenhouses on the south of Poland. The lowest infection with TSWV (up to 16.3%) was noted in the greenhouse where many plants originate from in vitro production. Other tospoviruses were detected only in individual samples. Impatiens necrotic spot virus (INSV) was detected in three of five inspected greenhouses (in 2–12 samples out of 300 tested in 2001 and 2002). Iris yellow spot virus (IYSV) was detected only in two samples of chrysanthemum plants in 2001. It is the first report of IYSV in chrysanthemum plants. Differences in TSWV infection level were noted between some of the cultivars tested.

Key words: chrysanthemum, Tomato spotted wilt-, Impatiens necrotic spot-, Iris yellow spot-tospoviruses

Introduction

Among several tospoviruses detected in Europe Tomato spotted wilt virus is the most important and widespread. TSWV infects over 900 (Peters 1998) or even 1090 (Parrella et al. 2003) plant species, mainly vegetable (tomato, pepper, lettuce, groundnut) and ornamental plants (chrysanthemum, impatiens, cyclamen, kalanchoe) grown in greenhouses. It can be spread by eight species of thrips of which Frankliniella occidentalis Pergande is the most important (Verhoeven and Roenhorst 1994, van de Wetering 1999). Florist’s chrysanthemum (Dendranthema grandiflora (D.C.) Desmoul.) is one of the most important vegetatively propagated ornamental plants grown in Poland.
TSWV symptoms in chrysanthemum plants are frequently very distinct and they consist of chlorotic and necrotic spots or large necroses on leaves and stems, as well as stunting and death of young plants. Infected chrysanthemum plants may also remain symptomless, which is typical for many cultivars (Matteoni and Allen 1989, Dal Bo et al. 1995). TSWV infection significantly reduces flower fresh weight, petal number, and number of flowers on the stem (Matteoni and Allen 1989).

*Impatiens* necrotic spot virus (INSV) appeared in Europe in 1989 in The Netherlands (de Avila et al. 1992) and since then it was noted in France (Marchoux et al. 1991), Italy (Vaira et al. 1993), Spain (Lavina and Battle 1994) and other countries, mainly in ornamental plants. It can also be spread by *F. occidentalis* (Law and Moyer 1990, de Avila et al. 1992). In Poland INSV was first identified in naturally infected *Schefflera actinophylla* Harms plants (Kamińska and Rudzińska-Langwald 1996) and it was also found in *D. grandiflora* plants simultaneously infected with TSWV (Kamińska et al. 1997).

In 1994 in Sao Paulo region new, unknown tospovirus was found in chrysanthemum plantations. It differed from previously known tospoviruses by serological properties and caused stem necrosis in chrysanthemum plants (Duarte et al. 1995). *Frankliniella occidentalis* and *F. schultzei* Trybom were proved to be its vectors. So far, Chrysanthemum stem necrosis virus has been found in chrysanthemums only in Brazil and in The Netherlands (Alexandre et al. 1996, Verhoeven et al. 1996, Nagata and de Avila 2000) and tomato plants in Brazil (Nagata et al. 1998).

Iris yellow spot virus (IYSV) initially was found only in monocotyledonous plants belonging to *Liliaceae* family. It was noted in *Iris hollandica* Tub. in The Netherlands (Cortes et al. 1998) and in onion plantations in the USA, Israel, and Brazil (Hall et al. 1993, Gera et al. 1998, Pozzer et al. 1999). In 2000 IYSV was found also in dicotyledonous plant – *Eustoma russelianum* (Don) Griseb (Kritzman et al. 2000). This virus is spread mainly by *Thrips tabaci* Lindeman (Nagata and Almeida 1999).

*Tomato spotted wilt virus* has not been detected in Poland until 1994 in any of chrysanthemum mother plants tested (Kryczyński and Stawiszynska 1996, Kryczyński 1998), but in 1997 it was detected in 3.8% of plants tested only in one of the three greenhouses surveyed (Kryczyński 1998, Balukiewicz et al. 1999). Increasing level of infection with TSWV in Europe in chrysanthemum plants connected with the introduction of a new vector – *F. occidentalis* were the main inspirations to this work.

**Material and methods**

Leaf samples were collected twice a year in spring and fall from 1999 to 2002 in five big greenhouses (designated here as A, B, C, D and E) producing chrysanthemum cuttings and growing mother stock plants. All these enterprises import chrysanthemum propagative material mostly from The Netherlands, France and United Kingdom. They reproduce this material and sell cuttings to many chrysanthemum growers in Poland. Only one greenhouse (D) was specialised in producing chrysanth-
themum cuttings only, while two other greenhouses (B and E) produced also cut flowers and pot chrysanthemums. In two other greenhouses other ornamental plants were also grown (A) or even vegetable plants including tomatoes (C).

Leaf samples were collected from individual chrysanthemum mother stock plants. Randomly 10–15 samples were collected from each of 20–30 cultivars in each of the greenhouses surveyed in each inspection time. In greenhouses C and D in 1999 composite leaf samples were collected but testing was repeated using the samples from individual plants. Leaf samples were collected directly to Bioreba AG plastic bags. They were brought to the laboratory, frozen and stored in freezing (−20°C) until ELISA test. Leaf samples were tested for Tomato spotted wilt virus (TSWV), Impatiens necrotic spot virus (INSV) and Iris yellow spot virus (IYSV) with DAS-ELISA test conducted according to the original protocol of Clark and Adams (1977) using commercial kits produced by Loewe Biochemica GmbH. The absorbance (A 405) values were determined using Multiscan photometer at 405 nm. Readings were regarded positive when they were higher than the mean value of the healthy control plus ×3 standard deviation. DAS-ELISA test was used as a convenient method of testing plants for all these viruses since it is considered to be the best tool for routine testing for plant viruses (Spiegel et al. 1993) and it was efficiently used in our previous research (Balukiewicz et al. 1999).

**Results**

*Tomato spotted wilt virus* was detected in all greenhouses inspected. In enterprise A level of TSWV infection was variable (from 0 up to 88% infected plants) and it was lower during fall tests (Table 1).

In B enterprise TSWV was not detected only during first test in 1999. The highest level of infections was noted during spring tests in year 2000 and 2001 (49.6% and 51% plants infected). The last two tests, conducted in fall 2001 and spring 2002, shown a decrease of infection with TSWV.

**Table 1**

<table>
<thead>
<tr>
<th>Enterprise</th>
<th>Fall 1999</th>
<th>Spring 2000</th>
<th>Fall 2000</th>
<th>Spring 2001</th>
<th>Fall 2001</th>
<th>Spring 2002</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>88.3</td>
<td>8.0</td>
<td>33.3</td>
<td>4.0</td>
<td>0</td>
<td>22.9</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>49.6</td>
<td>21.6</td>
<td>51.0</td>
<td>0.6</td>
<td>0.3</td>
<td>21.1</td>
</tr>
<tr>
<td>C</td>
<td>91.2</td>
<td>47.6</td>
<td>nt</td>
<td>0.33</td>
<td>3.6</td>
<td>31.6</td>
<td>26.9</td>
</tr>
<tr>
<td>D</td>
<td>93.2</td>
<td>29.6</td>
<td>nt</td>
<td>nt</td>
<td>10.6</td>
<td>4.6</td>
<td>25.0</td>
</tr>
<tr>
<td>E</td>
<td>nt</td>
<td>4.0</td>
<td>16.3</td>
<td>0</td>
<td>1.6</td>
<td>2.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Total</td>
<td>30.5</td>
<td>43.8</td>
<td>15.3</td>
<td>21.2</td>
<td>4.1</td>
<td>7.7</td>
<td>19.8</td>
</tr>
</tbody>
</table>

nt – not tested.
During two first tests conducted in C enterprise in 1999 and in spring 2000 the level of infection was measured as 91% and 47.6%. In 2001 only few plants were found infected with TSWV – 0.3% and 3.6% but in 2002 significant increase of TSWV infection was noted – till 31%.

The level of infection in chrysanthemums in enterprise D was similar to that in enterprise C – the highest infections were noted during the first tests in years 1999 and 2000 (93% and 29.6% plants infected).

The enterprise E was included to the research in spring of 2000. The level of infection in this enterprise was the lowest comparing to the other enterprises (Fig. 1). In the fall of 2000 the highest infection was noted – 16.3% of plants were found to be infected with TSWV. During the next tests TSWV was not detected at all (spring 2001) or was detected only in few plants (1.6–4%).

Two other tospoviruses were detected only in single samples. In enterprise A INSV was detected in 12 samples in 2002 and IYSV in two samples in 2001. In enterprise B INSV was detected in the year 2002 in six samples. INSV was also detected in chrysanthemum plants in enterprise E (two samples in 2001).

We tried to analyze the infection of particular cultivars with Tomato spotted wilt virus. TSWV was not detected in cultivars: ‘Fiji Yellow’, ‘Jewel Time’ and ‘Reagan White Elite’. The highest number of plants infected with TSWV (25–39%) were found in cultivars: ‘Blanche’, ‘Divalis’, ‘Intrepid’, ‘Puma’ and ‘Sheena Yellow’ (Table 2).

![Fig. 1. Average chrysanthemum stock plants infection with TSWV in greenhouses surveyed from 1999 to 2002](image-url)
Table 2

Infection of chrysanthemum cultivars with TSWV

<table>
<thead>
<tr>
<th>No TSWV found</th>
<th>Rare TSWV infection (up to 5%)</th>
<th>High TSWV infection (25–39%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Fiji Yellow'</td>
<td>'Bislet'</td>
<td>'Blanche'</td>
</tr>
<tr>
<td>'Jewel Time'</td>
<td>'Brill Time'</td>
<td>'Divalis'</td>
</tr>
<tr>
<td>'Reagan White Elite'</td>
<td>'Eleonora Lilac'</td>
<td>'Intrepid'</td>
</tr>
<tr>
<td></td>
<td>'Mirage Gel'</td>
<td>'Sheena Yellow'</td>
</tr>
<tr>
<td></td>
<td>'Ibera'</td>
<td>'Puma'</td>
</tr>
</tbody>
</table>

Discussion

As it was expected *Tomato spotted wilt virus* was the most common tospovirus detected in chrysanthemum mother stock material grown in Poland. TSWV is also common in chrysanthemums in The Netherlands (Verhoeven and Roenhorst 1994, van de Wetering 1999), Italy (Vaira et al. 1993), USA (Stack et al. 1997), Canada (Matteoni and Allen 1989) and South America (Alexandre et al. 1996, Dal Bo et al. 1995). It was also detected earlier in Polish chrysanthemum crops (Kaminska and Korbin 1991, 1994) but was not as common as our present research indicates (Kryczyński and Stawiszynska 1996, Kryczyński 1998, Balukiewicz et al. 1999). The significant increase in TSWV incidence is probably caused by the increase of the population of its main vector – *F. occidentalis* and by the lack of adequate control procedures (Goldbach and Peters 1994). At present, TSWV infection causes one of the biggest problems in many crops, including chrysanthemums.

The second tospovirus that could be commonly found in chrysanthemum growing greenhouses is Chrysanthemum stem necrosis virus which is also transmitted by thrips and is specific for chrysanthemum plants (Duarte et al. 1995, Alexandre et al. 1996, Verhoeven et al. 1996, Nagata and de Avila 2000) but we were not testing plants for this virus since we used ELISA test and there is no antiserum against CSNV.

We have detected also *Impatiens necrotic spot virus* in some of the tested plants and cultivars. INSV has already been reported in chrysanthemum plants in European countries (Marchoux et al. 1991, Vaira et al. 1993, Lavina and Battle 1994). In Polish chrysanthemums this virus was always found together with TSWV (Kaminska et al. 1997). In our present research we detected INSV without coinfection with TSWV in some cultivars – ‘Bislet’, ‘Buccin d’Oré’, ‘Creamist Golden’, ‘Mermaid’ and ‘Intrepid’ but this virus is not a real problem in commercial chrysanthemum crops in Poland.

Iris yellow spot virus was detected in two individual plant samples from greenhouse A in 2001. This seems to be the first report on this virus in chrysanthemum plants. IYSV is rather limited to monocotyledonous plants, although it has already been reported in *Eustoma russelianum* (Kritzman et al. 2000). In our present research IYSV was detected in chrysanthemum plants only by ELISA test, but A 305 readings were so high that they have left no doubts as to the identity of the virus. Of course
we tried to confirm our diagnosis but we could not detect IYSV in any other chrysanthemum plant tested later. It was impossible to find again the two individual plants in which the virus was detected. Our research was conducted in commercial greenhouses and we had to follow the procedures that were used there.

Similarly, it is difficult to indicate the primary source of tospoviruses in Polish chrysanthemum mother stock plantations, because the owners or the managers of the plantations were not willing to indicate the origin of the starting material and to give information about the history of the plantings. We could assume that the main source of the primary infections were the other crops grown in the neighbouring greenhouse blocks, as it happened in the case of chrysanthemum cultivars grown in the greenhouse of our Department of Plant Pathology (Balukiewicz and Kryczyński 2001). Such opinion would be justified by the very high level of TSWV infection in greenhouse C, where various crops, including tomatoes, were grown in the vicinity of chrysanthemum mother stock plantations and by the relatively high level of TSWV infection in greenhouse A, where many ornamental plants were grown besides chrysanthemums. On the other hand, very high level of TSWV infection was observed also in greenhouse D, which was the only one specialising in chrysanthemum propagative material only. But we suspected, that the starting material in greenhouse D was obtained not directly by importation from abroad, but by the channel of greenhouse C.

The possibility of importing infected cuttings originating from not adequately tested mother stocks cannot be excluded. This type of infection source has already been proved in Poland in the case of PNRSV-infected sour cherry rootstocks (Kryczyński et al. 1994), ACLSV-infected apple-tree propagative material (Kryczyński et al. 1995) and CVB-infected chrysanthemum propagative material (Kryczyński 1998). During the present research at least once we tested plant material which arrived few days before directly from one of the Western European countries and we detected several TSWV-infected plants. Nevertheless, TSWV spread by thrips vectors plays an important role in propagation of the virus infection within the crop. When the owners of greenhouses C and D replaced highly infected plant material in fall of 2000 by the newly imported stock material, the plant infection dropped to the very low level in 2001 (see Table 1).

Another adequate method of controlling tospovirus infection is to start chrysanthemum production from in vitro obtained stock material. Growing chrysanthemums from meristem-tip culture proved to be a good method for eliminating Tomato spotted wilt virus (Balukiewicz and Kryczyński 2001) and in our present research program greenhouse E, the only one using this method, was the one with the lowest TSWV infection level.

We tried to analyse the level of TSWV infection in particular chrysanthemum cultivars, since in our previous research (Balukiewicz et al. 1999) some old cultivars (‘Divalis’, ‘Intrepid’, ‘Jacob Lane’, ‘Passionament’ and ‘Royalis’) proved to be the most often TSWV-infected. This was confirmed in our present research program for some old cultivars (‘Blanche’, ‘Divalis’, ‘Intrepid’) but on the other hand some newly introduced cultivars (‘Puma’ and ‘Sheena Yellow’) proved to be highly infected.
Streszczenie

TOSPOWIRUSY W UPRAWACH MATECZNYCH CHRYZANTEM W POLSCE

W pięciu gospodarstwach szklarniowych testowano rośliny testem DAS-ELISA na obecność wirusa brązowej plamistości pomidora (Tomato spotted wilt virus, TSWV), wirusa nekrotycznej plamistości niecierpek (Impatiens necrotic spot virus, INSV) oraz wirusa żółtej plamistości kosaćca (Iris yellow spot virus, IYSV). W czasie czteroletnich badań przetestowano 7342 rośliny i stwierdzono dość wysoki poziom porażenia chryzantem TSWV. W 1999 roku w dwóch obiektach szklarniowych na pięć badanych ponad 91% roślin było porażonych tym wirusem. Jesienią 2001 roku stwierdzono jego występowanie we wszystkich pięciu obiektach szklarniowych, a porażenie wynosiło od 0,6% w gospodarstwie B do 10,6% w gospodarstwie D. Najmniej roślin porażonych TSWV wykrywano w gospodarstwie E – od 0 do 16%. W gospodarstwie tym są prowadzone próby uzyskiwania zdrowego materiału rozmnożeniowego m.in. przez stosowanie kultur in vitro. Zarówno INSV, jak i IYSV wykrywano sporadycznie. INSV wykryto w 2002 roku w gospodarstwie A w 12 próbkach (na 300 zebranych), w gospodarstwie B w sześciu próbkach (na 300 zebranych) oraz w gospodarstwie E w dwóch próbkach (na 300 zebranych). IYSV wykryto w gospodarstwie A w dwóch próbkach (na 300 zebranych). Jest to pierwsze doniesienie o występowaniu IYSV na chryzantemach.

Literatura


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