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Tospoviruses, weeds and thrips associated with chrysanthemum (*Dendranthema grandiflora* Tzvelev cv. Polaris)

(*Keywords: Dendranthema grandiflora*, impatiens necrotic spot virus (INSV), thrips, tomato spotted wilt virus (TSWV), tospoviruses)

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Abstract. Under field conditions, impatiens necrotic spot (INSV) and tomato spotted wilt tospoviruses (TSWV) were detected by the dot blot technique in chrysanthemum (*Dendranthema grandiflora* Tzvelev cv. Polaris) plants having concentric rings and line patterns, from Villa Guerrero and Coatepec de Harinas, State of Mexico. There were 15 species of thrips associated with chrysanthemum in the region, 10 already known and five being described as new species. The known species were: *Frankliniella adadusta, F. aurea, F. helianthi, F. inutilis, F. minuta, F. simplex celata, F. occidentalis brunnescens, F. occidentalis dubia, F. occidentalis* and *Thrips tabaci*. From among the aforementioned species, only the last two have been recognized as vectors of tospovirus. Twenty-six species of weeds were found associated with chrysanthemum, of which, 16 were positive for the TSWV and none for the INSV.

1. Introduction

The main flower producing area in Mexico is the State of Mexico, with the municipality of Villa Guerrero being the main producer within that State. Chrysanthemum (*Dendranthema grandiflora* Tzvelev cv. Polaris) is the most important field-grown flower crop, and the second most important greenhouse crop (Sedagro, 1993). In Villa Guerrero, Polaris is the most commonly grown chrysanthemum due to the high market price compared with other locally cultivated varieties. In the late 1980s a disease was first observed on this cultivar in the region, and losses approached 60%. Symptoms consisted of chlorotic and necrotic foliar spots, yellowing, necrosis of stems, growth reduction, defoliation, and plant death. Occasionally, the chlorotic and necrotic foliar spots showed a pattern of concentric rings or lines. Foliar deformation and thickening of veins have also been observed. Affected plants commonly did not produce flowers, or they were small. It has been reported that the causal agent of the disease is the tomato spotted wilt tospovirus (TSWV) (Cárdenas, 1994).

Recent classification of plant viruses placed TSWV in the *Tospovirus* genus along with impatiens necrotic spot virus (INSV) (German *et al.*, 1992); previously they were considered the 'L' and 'I' strains of TSWV, respectively. Therefore, it is unknown if both viruses are present in the area. The objectives of this study were: (1) to determine if both Tospoviruses, TSWV and INSV, are present in the Villa Guerrero area affecting the chrysanthemum cv. Polaris, and (2) to know the species of thrips and weeds in the region, which might carry the virus associated with this crop.

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2. Materials and methods

Plants of chrysanthemum cv. Polaris showing symptoms of the disease were collected in San Miguel, Buenavista, and Santiago Oxtotitlán communities of Villa Guerrero. Three plants were brought in from each locality and they were defoliated separately. Leaves showing concentric rings, line pattern, foliar deformation, and thickening of veins were collected from the plants. Additionally, small pieces of stem with necrotic spots were obtained from two plants from San Miguel. There were 21 samples in total, 18 of them consisted of two leaves (approx. 5 cm long) and three consisted of four small pieces of stem (each one approx. 1·5 cm) with necrotic spots. Part of the tissue was subjected to the dot blot technique modified by Delgadillo (1992). Antisera and positive controls for TSWV and INSV were obtained from Agdia Inc. Part of the foliar tissue (two or three leaves from each locality) showing symptoms was used to observe viral inclusions by staining epidermal strips with bromephenol blue, and a last portion of tissue, six leaves, each approx 3–5 cm long, with symptoms from Buenavista was mechanically inoculated in 10, 45-day-old tomato plants (*Lycopersicon esculentum* Mill.) cv. Río Grande.

To determine which thrips were associated with chrysanthemum cv. Polaris, from May to June 1993, chrysanthemum flowers were collected in San Miguel, Buenavista, Coatepec de Harinas, Zacango, and Santiago Oxtotitlán. In the laboratory, they were placed in the refrigerator at −10°C to arrest the thrips' activity. Ten minutes later, flowers were taken from the refrigerator and shaken over a dark surface to facilitate the observation and capture of the thrips with a fine hair paintbrush or aspirator (a tip of a pipette connected to a short piece of plastic tube). The thrips collected were placed in small glass flasks with 70% alcohol for their subsequent identification.

On 13 May 1993, weeds, with or without symptoms, from two chrysanthemum fields were collected in Coatepec de Harinas and Zacango. Weeds growing inside the fields and on the borders were collected. The collected plants were dried and after 25 days they were sampled to carry out the

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serological test, which was done twice to corroborate the results.

3. Results and discussion

Of the 21 chrysanthemum samples serologically tested, 18 were positive for INSV and three were positive for TSWV. Approximately 12 days after the mechanical inoculation of the tomato plants, eight of the 10 inoculated plants showed light green areas of variable form and size on the leaves. In three plants, some necrotic areas in the upper foliar surface were present. All plants with symptoms showed a light bronze coloration on the lower foliar surface. Although these symptoms are mentioned in the literature as being caused typically by tospovirus, the possibility of other viruses exists since no other virus antisera were tested. The presence of viral inclusions in the tissue of chrysanthemum with symptoms was rare. In contrast, in the inoculated tomato plants the inclusions were observed frequently. Viral inclusions were granular and spherical, and localized in cytoplasma near the nucleus. The largest inclusions were vacuolated; these inclusions were very similar in chrysanthemum and tomato, and resembled those described by Urban *et al.* (1991). From the inoculated tomato plants that showed symptoms, five were used for serological tests, and each gave positive reaction to the INSV.

There were 15 species of thrips associated with chrysanthemum cv. Polaris. Ten of them were already known species (Table 1), and five are being described as new. Only two species shown in Table 1 (*Thrips tabaci and Frankliniella occidentalis*) have been reported as vectors of tospoviruses (German *et al.*, 1992). It has to be pointed out that the *occidentalis* complex in Mexico includes 30 species, some already known and some new, which have polymorphism in colour, a characteristic which can create confusion in identification (Johansen, personal communication). On the other hand, there are regionally specific assemblages and, consequently, it is not rare that similar complexes would be sympatric and sometimes synchronous in the Eje Volcánico Transversal (a series of volcanic elevations that extends transversally from the Gulf of Mexico to the Pacific coast at the middle of the country), to which the area of study belongs (Johansen, personal communication). The species found most frequently were: *F. occidentalis, F. occidentalis brunnescens, F. aurea and F. occidentalis dubia*, with 50, 18, 15 and 11 individuals, respectively; these species belong to the *occidentalis* complex in Mexico. Even though sampling was not done throughout the year, these results serve as an indicator for the species associated with chrysanthemum in the region and their relative abundance.

Twenty-six weed species were found associated with

Table 1. Species of thrips associated with chrysanthemum cv. Polaris in different localities of the municipality of Villa Guerrero and in Coatepec de Harinas, State of Mexico

Species	Sex	No. of individuals[a]	Locality[b]
Frankliniella adadusta	♀	2	SM
F aurea	♀	5	SM
F aurea	♀	10	Z
F. helianthi[c]	♀	5	B
F. helianthi	♂	1	B
F. inutilis	♀	2	C
F. minuta	♂	2	SM
F. minuta	♀	4	SM
F. occidentalis	♀	1	B
F. occidentalis	♀	48	Z
F. occidentalis	♂	1	Z
F. occidentalis brunnescens	♀	3	SM
F. occidentalis brunnescens	♀	6	CM
F. occidentalis brunnescens	♀	9	Z
F. occidentalis dubia	♂	1	C
F. occidentalis dubia	♀	2	CM
F. occidentalis dubia	♀	2	SM
F. occidentalis dubia	♀	2	Z
F. occidentalis dubia	♀	4	C
F. simplex celata	♀	1	Z
Thrips tabaci	♀	1	C
Thrips tabaci	♀	1	CM

[a]From three samplings.
[b]Z: Zacango; C: Coatepec de Harinas; SM: San Miguel; B: Buenavista; CM: Municipal head-board.
[c]Valid species (despite the Bryan and Smith (1956) synonymy.

Table 2. Weeds associated with chrysanthemum cv. Polaris in two localities of Villa Guerrero and in Coatepec de Harinas, State of Mexico, showing their serological reaction to TSWV and INSV

Species	No. of plants	Locality[a]	TSWV	INSV
Allium sp.	1	Z	+	–
Amaranthus hybridus	1	C	–	–
Amaranthus hybridus	1	Z	+	–
Anagalis arvensis	1	Z	+	–
Anagalis arvensis	1	C	–	–
Bidens aurea	1	C	–	–
Bidens aurea	1	SM	–	–
Bidens aurea	3	Z	+	–
Bidens pilosa	3	C	–	–
Bidens pilosa	6	SM	–	–
Bidens pilosa	4	Z	+	–
Brassica campestris	1	Z	+	–
Capsella bursa-pastoris	2	Z	+	–
Chenopodium album	1	Z	+	–
Commelina sp.	1	SM	–	–
Euphorbia sp.	2	C	–	–
Euphorbia sp.	3	Z	+	–
Galinsoga parviflora	4	Z	–	–
Galinsoga parviflora	2	SM	–	–
Lepidium virginicum	3	C	+	–
Malva sp.	6	C	+	–
Medicago denticulata	1	C	–	–
Melilotus sp.	4	C	–	–
Oxalis sp.	2	Z	+	–
Oxalis sp.	2	C	–	–
Physalis sp.	1	C	–	–
Polygonum amphibium	1	C	–	–
Polygonum amphibium	3	Z	–	–
Polygonum aviculare	3	Z	–	–
Polygonum sp.	1	C	–	–
Polygonum sp.	2	Z	+	–
Rumex crispus	1	Z	+	–
Rumex crispus	4	C	–	–
Sida rombifolia	1	C	+	–
Sida sp.	1	C	–	–
Sonchus sp.	2	Z	+	–
Spergulla arvensis	2	C	–	–
Taraxacum officinale	2	C	–	–

[a]C: Coatepec de Harinas; SM: San Miguel; Z: Zacango.

chrysanthemum cv. Polaris, of which the most frequent were: *Bidens pilosa, Bidens aurea, Malva* sp., *Galinsoga parviflora, Rumex crispus and Euphorbia* sp. (Table 2). Sixteen of the 26 weeds identified, gave a positive reaction for TSWV; none were positive for INSV.

The role of weeds in the development of the disease is complex because it is possible that one plant species might be a host for the virus, but at the same time it might not be preferred by the thrips. Consequently, virus dissemination from these plants would be minimal. However, if the weed species is a good host for both virus and thrips, its influence on virus dissemination might be important; although this will depend on the density (number of individuals per surface unit) of that particular weed and on its seasonal frequency throughout the year. Some weeds found associated with chrysanthemum in this study have been mentioned before as hosts for viruses and thrips, particularly *Capsella bursa-pastoris, Chenopodium album, Rumex crispus and Taraxacum officinale* (Cho *et al.*, 1986; Stobbs *et al.*, 1992). The weeds *Allium* sp., *Amaranthus hybridus, Anagalis arvensis, Bidens aurea, Lepidium virginicum, Sida rombifolia* and *Sonchus* sp., which also gave a positive reaction to TSWV, have not been mentioned previously as hosts of tospoviruses. It would be interesting to find out their potential as hosts for thrips since transmission efficiency of TSWV depends greatly on the host susceptibility to the virus, and on the preference of the vector for the host (Allen and Broadbent, 1986). The fact that some of the collected weeds reacted negatively to the serological test does not necessarily mean that they are not hosts or reservoirs of the virus. It is possible that the plant had not been infected with virus by the time it was collected or that viral concentration was not sufficient for detection. On the other hand, we were somewhat surprised by the fact that INSV was not detected in weeds, since as was mentioned previously, this virus showed the highest frequency in the chrysanthemum samples.

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