

# Petunia as an Indicator Plant for Use by Growers to Monitor for Thrips Carrying the Tomato Spotted Wilt Virus in Greenhouses

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## ABSTRACT

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Petunia, gloxinia, globe amaranth, tomato, pepper, and three species of tobacco (*Nicotiana tabacum*, *N. glutinosa*, and *N. benthamiana*) were tested under growth room and greenhouse conditions as indicator plants for monitoring for western flower thrips (*Frankliniella occidentalis*) carrying the tomato spotted wilt virus. The percentage of leaves with feeding scars was consistently the greatest for petunia. Petunia and gloxinia developed the greatest number of viral lesions, and lesions developed as early as 2-3 days after exposure to thrips. The percentage of plants that became infected was greatest for petunia, followed by gloxinia. Thrips attraction and virus transmission to petunia were enhanced when yellow boards without adhesive were placed in the pots at plant height.

Indicator plants have been used successfully as monitors for the presence of viruliferous insects among both incoming and resident insect populations (22). The degree of infection in indicators may be used as a relative index of the "inoculum pressure" (14) at a given time in various locations. In Canada and elsewhere, the tomato spotted wilt virus (TSWV) is becoming more prevalent in greenhouse ornamental and vegetable crops (15,17). This virus is not easily detected or identified in many crops before symptom development without sensitive laboratory tests, and symptoms may take several weeks or longer to develop (2,3). Therefore, an indicator plant that could quickly monitor the presence of viruliferous thrips in greenhouses would be a useful early alert to growers, especially with crops such as *Cyclamen*, *Brassica*, *Schefflera*, and *Fatsia* species in which the virus has a latent period of several months (Allen and Matteoni, unpublished data [3]), during which time the virus continues to spread unnoticed. Consequently, most of a crop may have to be discarded after many months of costly production.

Candidate species for monitoring studies were chosen on the basis of their susceptibility to all isolates of the virus collected across Canada (13,18,27) and isolates tested in other countries (7,9,10, 23-25), and because several of the candidates are sensitive local lesion hosts of the virus (23,25). Information was not available on the relative attractiveness of the candidates to the western flower thrips (WFT), *Frankliniella occidentalis*

(Pergande), which is the principal vector of TSWV in Canadian greenhouses (2,4). This paper reports the results of an evaluation of the relative attractiveness of indicators to thrips and reactivity to the virus and discusses selection criteria for an indicator plant as they relate to epidemiological considerations.

## MATERIALS AND METHODS

### Virus isolates, thrips, and test plants.

A culture of the western flower thrips was established in a growth room on TSWV-infected chrysanthemum (*Dendranthema grandiflora* Tzvelev 'Palisade,' 'Icecap,' 'Mellow,' and 'Polaris') obtained from a commercial greenhouse near Freelon, Ontario. Periodically (four times, 5 days apart), second instar thrips were collected from a laboratory culture (2) and placed onto the virus-source plants at a rate of 10-30 thrips per plant. To facilitate thrips survival during their soil phase (6), the chrysanthemums were grown in 15-cm-diameter pots placed into a shallow pan (35-cm wide and filled to a depth of 2 cm with soil), which ran the length of the growth room bench.

Test plants included petunia (*Petunia × hybrida* Hort. Vilm.-Andr. 'Calypso'), tomato (*Lycopersicon esculentum* Mill. 'Glamour'), globe amaranth (*Gomphrena globosa* L. 'Dwarf Buddy'), green pepper (*Capsicum annuum* L. 'California Wonder'), gloxinia (*Sinningia speciosa* (Lodd.) Hiern. 'Imperial Red Velvet'), and three tobacco species (*Nicotiana benthamiana* Domin., *N. tabacum* L. 'Harrow Velvet,' and *N. glutinosa* L.). All species except gloxinia were grown from seed and potted into a loam:peat:sand (1:1:1) mix in clay pots. Gloxinias were grown from 6-cm plugs in commercial potting medium (Sunshine Mix No. 1) in 15-cm-diameter plastic azalea pots.

Plant age at testing ranged from 3 to 6 wk old, when there were about 10 leaves per plant. To facilitate evaluation, the side shoots and flower buds were removed before testing.

**Evaluation of indicator plants by thrips-mediated inoculation.** Seven tests were conducted in which both the amount of apparent feeding activity of the WFT and infection of indicator plants were assessed. In six of the tests, 24 plants per species were randomly placed into the growth room. Half of each species were located on each side of a double row of the virus-infected and thrips-infested chrysanthemum plants, which served as source plants, and occupied a central position over the length of the bench at a density of 16 plants per linear meter. To provide competitive food sources for thrips and to simulate a greenhouse situation, a variety of healthy commercial foliage plants (*Brassaia actinophylla* Endl., *Schefflera arboricola* Hayata, *Fatsia japonica* (Thunb.) Decne. & Planch., and *Hedera* L. spp.) and potted flowering plants (*Antirrhinum majus* L., *Saintpaulia ionantha* H. Wendl., *Sinningia speciosa*, and *Zinnia elegans* Jacq.) were used randomly to fill the bench. All of these species are fed upon by the WFT under commercial greenhouse conditions and are naturally infected by the TSWV (Allen and Matteoni, unpublished data [13,27]). The seventh test was conducted in an 8 × 12 m greenhouse containing randomly located virus-infected and thrips-infested chrysanthemum plants and the aforementioned flowering and foliage plants.

Test plants were exposed to the TSWV-WFT complex for 7 days in the growth room or for 14 days in the greenhouse. The numbers of adult thrips trapped on a double-sided, yellow sticky board (10.2 × 20.3 cm) ranged from five to 22 over randomly selected 24-hr periods in the growth room and from 10 to 30 thrips over 72-hr periods in the greenhouse. After exposure, plants were then held for an additional 20-24 days in an isolated greenhouse before final symptom readings were taken. During the latter period, insecticidal sprays (20) were used to control thrips and to limit further virus transmission.

The association of the TSWV with local lesions in petunia and *N. glutinosa* was tested by triturating lesions in an inoculation buffer and using the suspen-

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sion to inoculate *Datura stramonium* L. in which the virus was easily detected by enzyme-linked immunosorbent assay (ELISA [16]). ELISA was performed directly on systemically infected leaves of the other indicator species.

To assess the apparent feeding preference of the WFT for the indicator plants, the numbers of leaves with feeding scars were recorded at day 3 and/or day 7 after exposure to the thrips. Only scars that could be seen without magnification were counted, and successive counts were done by the same personnel. Because gloxinia, tobacco, and pepper developed considerably larger leaves than the other indicator species, half-leaves were tallied separately. For tomato, the individual leaflets were tallied separately. Percentages of leaves with feeding scars were calculated for each plant, and square root-arc sine transformations were used to compare means of plant species in a completely randomized design by the LSD test (26). The mean square root-arc sine values were then untransformed to mean percentages (12) for reporting. The relative amount of leaf area with feeding scars was determined by ranking from least damaged (rank = 1) to most damaged (rank = 8 [26]), and the mean ranks were compared by LSD in a completely randomized design.

Indicator plants were evaluated for TSWV infection at approximately 3, 7, or 21 days after exposure to the thrips. The numbers of plants that were infected, numbers of local lesions per plant or per leaf, and the time required for symptoms to develop were recorded. The percentage values for infected plants were

compared by means of the normal deviate, with correction for continuity for values of 0 and 100% (26).

**Sensitivity of indicator plants to TSWV by mechanical inoculation.** To determine the relative sensitivity of indicator species to TSWV, mechanical inoculation trials were conducted on all eight species. Because of the significant variation in numbers and types of local lesions and response times of each plant species, a subjective evaluation was made at two different times after inoculation. For each of 10 tests, groups composed of one plant of each species were mechanically inoculated with the chrysanthemum isolate of TSWV obtained from systemically infected leaves of *D. stramonium* triturated in a buffer consisting of 0.01 M TRIS, 0.01 M sodium sulfite, and 0.1% cysteine hydrochloride, pH 7.8, at a ratio of 1 g of leaf to 7 ml of buffer (16). Approximately 1.5 ml of inoculum was applied to each of four leaves per plant. Fresh inoculum was used for each group. The order of inoculation of the plant species in each group was randomized to minimize variations related to the short half-life of the virus inoculum. Plants were evaluated for symptom development 4 and 16 days after inoculation. The eight plants from each test were ranked from least damage (rank = 1) to most damage (rank = 8 [26]), and the mean ranks were compared by LSD in a completely randomized design.

This test was repeated with isolates of the same serotype from cyclamen (*Cyclamen persicum* Mill.) from British Columbia (13), from tomato from Ontario (2), and with an isolate of a dif-

ferent serotype (19) from New Guinea impatiens (*Impatiens wallerana* J. D. Hook) from Ontario to determine if the indicators responded similarly to a variety of virus isolates.

**Evaluation of yellow boards as thrips-attractants to indicator plants.** Tests in greenhouses and growth rooms containing a wide variety of ornamental and vegetable crops infested with the WFT demonstrated that over a 24-hr period, petunia plants consistently had fewer thrips than yellow sticky boards placed within 6 cm of the plants. Therefore, the usefulness of the boards (without adhesive) in increasing the attraction of thrips to indicator plants was tested. Petunia plants were potted separately and placed as pairs equidistant (60 cm) from TSWV-infected and WFT-infested chrysanthemum source plants in the growth room. One pot of each pair received a yellow board attached vertically to a stake so that the bottom of the board was within 2 cm of the nearest leaf. All plants had been trimmed to approximately nine expanded leaves after removal of side shoots. Each of three tests consisted of 20 pairs of plants. An evaluation of the percentage of leaves per plant with virus lesions was done at 3 and 7 days after exposure. Paired data were analyzed by the Student's *t* test (12) using the described transformations.

## RESULTS

**Apparent feeding preference of the western flower thrips.** Feeding scars were evident on all indicator species at the first evaluation (3–5 days [Table 1]). At that time, petunia consistently had the greatest ( $P < 0.01$ ) percentage of leaves

**Table 1.** Feeding activity of the western flower thrips (*Frankliniella occidentalis*) on indicator species under growth room conditions

Test no.	Exposure to thrips (days)	Leaves with feeding scars (%) <sup>v,w</sup>								
		Petunia	Pepper	Gloxinia	Amaranth	Tomato	<i>Nicotiana</i> species			
							<i>tabacum</i>	<i>glutinosa</i>	<i>benthamiana</i>	
1	3	86.1 a <sup>x</sup>	... <sup>y</sup>	24.1 b	42.9 b	34.9 b	...	...	...	...
	7	95.1 a	...	50.2 b	58.6 b	45.1 b	...	...	...	...
2	3	45.5 a	...	26.4 b	19.9 b	21.5 b	30.2 a	15.7 b	...	...
	7	94.0 a	...	52.8 bc	56.4 bc	47.7 c	65.6 b	25.6 d	...	...
3	3	82.6 a	51.5 b	44.4 b	17.1 c	47.0 b	...	...	...	...
	7	100.0 a	93.6 b	60.5 c	56.2 c	91.2 b	...	...	...	...
4	3	98.7 a	47.1 b	...	...	...	8.8 c	6.3 c	...	...
	4	97.3 a	74.7 b	43.7 cd	47.5 c	73.5 b	14.1 e	27.8 de	...	22.2 e
5	7	100.0 a	80.8 b	69.5 b	70.3 b	83.7 b	28.1 c	38.5 c	...	24.1 c
	3	90.3 a	62.7 b	52.6 b	54.2 b	...	...	...	...	22.1 c
6	7	100.0 a	93.6 b	51.8 c	86.4 b	...	...	...	...	36.4 c
	5	48.3 ab	23.0 cd	7.9 ef	1.5 f	63.8 a	...	32.2 bc	...	14.4 de
7	7	84.2 a	37.9 b	20.9 c	10.6 c	89.1 a	...	43.3 b	...	20.0 c
	12	95.2 a	88.2 a	40.1 c	24.6 c	96.4 a	...	62.0 b	...	32.9 c
Mean <sup>z</sup>	3–12	97.6	80.7	54.2	58.8	72.8	34.2	33.1	...	31.1

<sup>v</sup> *Petunia* × *hybrida*, *Sinningia speciosa*, *Gomphrena globosa*, *Lycopersicon esculentum*, *Capsicum annuum*, and tobacco species as indicated; 24 plants per species per test.

<sup>w</sup> Square root-arc sine transformations of raw data were analyzed; mean square root-arc sine values were untransformed to percentages.

<sup>x</sup> Mean percentages in the same row followed by the same letter are not significantly different as determined by LSD at the  $P = 0.01$  level.

<sup>y</sup> Species not included in a particular test.

<sup>z</sup> Means of the longest exposure time for which data were recorded in all tests.

with feeding scars (mean = 78%). In three tests, all of the leaves on petunia had feeding scars by day 7. However, when the indicators were ranked as to the extent of feeding damage (relative leaf area affected), pepper consistently ranked the highest, followed by petunia, tomato, amaranth, gloxinia, *N. glutinosa*, *N. benthamiana*, and *N. tabacum* ( $P < 0.01$ , mean ranks 7.8a, 7.0b, 6.1c, 4.6d, 4.0e, 2.5f, 2.4f, and 1.5g, respectively). Feeding scars were difficult to detect on gloxinia because of the density of leaf hairs.

**Infection of indicator plants after exposure to thrips.** The percentage of

plants that became infected was consistently greatest for petunia. Significant infection was observed rarely in amaranth, tomato, pepper, or the tobacco species at or before the second evaluation (7 days [Table 2]). None of the flowering or foliage plants used as competitive food sources expressed symptoms in 7 days but did several weeks or months later.

There was considerable variation in the numbers of local lesions among plants of the same species. The mean number of local lesions on petunia and gloxinia (16.1 and 13.4, respectively [Table 2]) was consistently greater than

numbers on all other species. Further, lesions developed on petunia and gloxinia as early as 2–3 days after exposure to the TSWV-WFT complex, indicating that thrips moved quickly to the indicators.

Whereas distinct viral lesions were readily detected on petunia, gloxinia, and the *Nicotiana* spp., lesions were more difficult to identify on pepper, tomato, and amaranth during the early stages of infection. All lesions were clearly associated with thrips feeding scars. TSWV eventually became systemic in all test species, except petunia, after inoculation by thrips. Lesions in petunia were

**Table 2.** Incidence of indicator plants infected with the tomato spotted wilt virus, after exposure to viruliferous western flower thrips (*Frankliniella occidentalis*)

Test no.	Days after initial exposure <sup>s</sup>	Infected plants (%) <sup>t</sup>								
		Petunia	Gloxinia	Amaranth	Tomato	Pepper	<i>Nicotiana</i> species			
							<i>tabacum</i>	<i>glutinosa</i>	<i>benthamiana</i>	
1	3	16.7 <sup>i</sup>	8.3	0.0	0.0	...	...	...	...	
	7	87.5 a	50.0 b	0.0 c	0.0 c	...	...	...	...	
	21	91.6 a	70.8 ab	83.3 ab	58.3 b	...	...	...	...	
2	3	25.0 a	16.7 ab	0.0 b	0.0 b	...	0.0 b	0.0 b	...	
	7	75.0 a	54.2 a	0.0 b	0.0 b	...	0.0 b	0.0 b	...	
	21	79.2 ab	62.5 bc	96.0 a	41.2 c	...	50.0 c	58.3 bc	...	
3	3	25.0 a	37.5 a	0.0 b	0.0 b	0.0 b	...	...	...	
	7	95.8 a	66.7 b	0.0 c	0.0 c	0.0 c	...	...	...	
	21	100.0 a	66.7 b	29.2 c	20.8 c	45.8 bc	...	...	...	
4 <sup>v</sup>	3	8.3	...	...	...	0.0	0.0	0.0	...	
	7	58.3 a	...	...	...	0.0 b	0.0 b	0.0 b	...	
	21	58.3	...	...	...	37.5	54.2	33.3	...	
5	4	95.8 a	54.2 b	0.0 c	0.0 c	0.0 c	0.0 c	12.5 c	0.0 c	
	7	100.0 a	91.7 a	0.0 c	4.2 c	4.2 c	20.8 c	50.0 b	4.2 c	
	14	100.0 a	95.8 a	45.8 bc	20.8 c	37.5 bc	62.5 b	58.3 b	54.2 b	
	21	100.0 a	95.8 a	87.5 ab	83.3 ab	87.5 ab	79.2 ab	75.0 b	70.8 b	
	21	100.0 a	95.8 a	87.5 ab	83.3 ab	87.5 ab	79.2 ab	75.0 b	70.8 b	
6	3	54.2 a	25.0 b	0.0 c	...	0.0 c	...	...	0.0 c	
	7	100.0 a	95.8 a	0.0 b	...	0.0 b	...	...	0.0 b	
	21	100.0 a	95.8 ab	100.0 a	...	70.8 c	...	...	78.5 bc	
7 <sup>w</sup>	5	12.5	0.0	0.0	0.0	0.0	...	0.0	0.0	
	7	25.0 a	12.5 b	0.0 b	0.0 b	0.0 b	...	4.2 b	0.0 b	
	12	66.7 a	45.8 ab	4.2 d	45.8 ab	16.7 cd	...	29.2 bc	4.2 d	
	21	83.3 a	58.3 ab	45.8 b	45.8 b	50.0 b	...	33.3 b	41.7 b	
Mean no. of infected plants										
	3–5	33.9	23.6	0.0	0.0	0.0	0.0	3.1	0.0	
	7	77.4	61.8	0.0	0.8	0.8	6.9	13.6	1.4	
	21	87.5	75.0	73.6	49.9	58.3	61.1	50.0	63.7	
Mean no. of leaves per plant <sup>x</sup>		12.6	11.4	10.4	19.2	14.4	8.7	9.5	12.8	
Mean no. of lesions per plant <sup>y</sup>		16.1	13.4	7.7	2.8	3.9	5.2	3.2	1.8	
Mean no. of lesions per leaf <sup>z</sup>		1.2	1.2	0.7	0.1	0.2	0.6	0.4	0.3	

<sup>i</sup> *Petunia* × *hybrida*, *Sinningia speciosa*, *Gomphrena globosa*, *Lycopersicon esculentum*, *Capsicum annuum*, and tobacco species as indicated, respectively; 24 plants per species per test.

<sup>s</sup> Plants were exposed to viruliferous western flower thrips for 7 days, after which insecticide was applied and plants were maintained in isolation until day 21.

<sup>t</sup> Percentages without letters or followed by the same letter were not significantly ( $P < 0.05$ ) different, as determined by comparing in pairs the means of the normal deviate (26).

<sup>u</sup> Species not included in a particular test.

<sup>v</sup> Thrips feeding activity was particularly high in test 4, so plants were exposed to viruliferous thrips for only 3 days, and plants were evaluated twice.

<sup>w</sup> Test conducted in a greenhouse for 14 days, after which insecticide was applied and plants were maintained in isolation until day 21.

<sup>x</sup> Mean number of leaves per plant for all seven tests, representing half-leaves for gloxinia, pepper, and *Nicotiana* spp. and leaflets for tomato.

<sup>y</sup> Values for local lesions per plant taken after a 21-day exposure to viruliferous thrips. In test 5 and 6, lesions were too numerous to count for petunia and gloxinia, so values at 7 days after exposure were used for these species.

<sup>z</sup> Mean is weighted for the actual numbers of leaves per plant for each test.

associated only with infections caused by the TSWV.

**Sensitivity of indicator plants to TSWV by mechanical inoculation.**

Lesion numbers on plants in the mechanical inoculation tests varied considerably (2.5–150 lesions per leaf) from group to group. However, of the species tested, petunia and one tobacco species (*N. glutinosa*) were consistently the most sensitive to TSWV after 4 days (Table 3). Local lesions also developed on *N. tabacum* and gloxinia by day 4, but they were fewer in number and less conspicuous, respectively. By day 16, systemic symptoms developed on all *Nicotiana* spp., and stem cankers and wilting symptoms were evident. Systemic infection was evident in a few tomatoes and most gloxinias by day 16, but systemic symptoms were just beginning in pepper and had not yet begun in amaranth.

Mechanical inoculation tests conducted with the cyclamen, the tomato, and the impatiens isolates yielded similar results in all test species.

**Influence of yellow boards on the attraction of thrips to petunia.** The proportion of petunia leaves that developed viral lesions after 3 days was significantly ( $P < 0.05$ ) greater (approximately six-fold) on plants with yellow boards without adhesive than on adjacent plants without boards. The difference fell to about two-fold after 7 days. The increased attraction of thrips to petunia with yellow boards was confirmed in thrips-infested greenhouses where limited tests were conducted adjacent to some 30 species of ornamental or vegetable crops in which the virus was not present (*data not presented*). Feeding scars on petunia were usually detected within 24 hr.

**DISCUSSION**

The ideal indicator species for the detection of thrips carrying the TSWV was considered to be one that is highly attractive to the vector even when in competition with a diverse range of host crops; expresses characteristic disease symptoms quickly; reacts similarly to a

wide range of virus strains; does not become systemically infected during the monitoring period, and, therefore, is not a significant source of virus; retains vectors so that they can be collected for identification or testing; reacts quickly and characteristically to feeding injury; and is widely available and easily propagated and maintained. Of the indicator species tested, petunia most closely met these criteria. Its most useful attributes are the rapid attraction of thrips that feed heavily and cause easily recognizable scars and the development, within 2–3 days, of viral lesions that are easily distinguishable from feeding damage. Western flower thrips were often found on petunia within 1 hr of positioning plants among the variety of ornamental plants used in the growth room study.

Petunia reacted similarly to all isolates of TSWV obtained from across Canada, and communications from other workers in Maryland, Ohio, New York, North Carolina, Texas, Hawaii, and Holland indicated that petunia was susceptible to all isolates tested, including a different serotype isolated from impatiens (19). For this reason, petunia is used in this laboratory for assays on samples of all ornamental and vegetable crops suspected of carrying the TSWV.

Gloxinia also developed lesions within a few days of exposure to thrips but was not as good an indicator of thrips activity in that feeding sites were not readily evident. Also, it became systemically infected within 2 wk and was more costly to produce. The other indicator species required unsatisfactorily long periods (more than 7 days) for symptoms to develop and the plants became systemically infected.

Other cultivars of petunia have not been tested; therefore, it is possible that cultivars are available that are both more susceptible to infection and more attractive to thrips than cv. Calypso. This consideration is under study.

Results from greenhouse and growth room tests indicated that attraction of the WFT to petunia, and subsequent

virus transmission, were considerably enhanced if yellow boards (without adhesive) were placed on stakes in the petunia pots. Other colors, such as blue (5), may be even more attractive but have not yet been tested. The boards also serve as convenient markers of the location of the indicator plants. This monitoring system is being used in commercial greenhouses in Ontario where tomato transplants are grown for field use (21).

A review of viruses that may infect greenhouse ornamental and vegetable crops (8,11), especially insect-borne viruses, indicated that few viruses induce symptoms on petunia similar to those caused by TSWV. Those that may induce similar symptoms in 2–4 days are the soil-borne tobacco mosaic, cucumber necrosis, tobacco necrosis, and tomato bushy stunt viruses, which may occasionally splash from contaminated soil onto host leaves where infections can occur naturally (1). However, exposure to these viruses is now rare in commercial greenhouses where soilless mixes are routinely used.

Because the TSWV disease is not easily identified visually in many greenhouse crops, especially during the initial week(s) after infection, and because detection of the virus in plants or in thrips requires laboratory facilities and experienced personnel, the use of an indicator plant, such as petunia, may be the most practical and expedient way for growers to monitor for the introduction of viruliferous thrips or to assess their virus-control programs. In addition to the routine use of indicators throughout production ranges of a greenhouse complex, indicators should be used in propagation areas and especially in isolation houses where incoming stock is assessed for disease and insect contamination. Indicator plants also would be useful for monitoring the persistence of viruliferous thrips in greenhouses before introducing successive crops.

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**Table 3.** Relative sensitivity of indicator plants to tomato spotted wilt virus by mechanical inoculation

Species	Mean rank <sup>1</sup> of sensitivity	
	4 days	16 days
Petunia ( <i>Petunia × hybrida</i> )	7.5 a <sup>2</sup>	4.2 d
Tobacco ( <i>Nicotiana glutinosa</i> )	7.3 a	6.5 b
Tobacco ( <i>N. tabacum</i> )	6.0 b	4.5 c
Gloxinia ( <i>Sinningia speciosa</i> )	4.5 c	5.5 c
Tobacco ( <i>N. benthamiana</i> )	3.7 c	6.8 a
Pepper ( <i>Capsicum annuum</i> )	2.7 d	4.3 d
Tomato ( <i>Lycopersicon esculentum</i> )	2.3 d	1.7 f
Globe amaranth ( <i>Gomphrena globosa</i> )	2.3 d	2.6 e

<sup>1</sup>Four and 16 days after inoculation, all plants in each group were subjectively placed in order from least sensitive to the virus (rank = 1) to most sensitive (rank = 8). Tests were replicated 10 times using freshly prepared inoculum for each test.

<sup>2</sup>Mean ranks in the same column followed by the same letter are not significantly different as determined by LSD mean separation ( $P < 0.01$ ).

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