Effects of Tobacco- and Tomato Ringspot Viruses on the Reproductive Tissues of Pelargonium × hortorum

B. A. Scarborough and S. H. Smith

Graduate Student and Professor, respectively, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802. Present address of senior author: Star Roses, The Conard Pyle Co., West Grove, PA 19390.

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ABSTRACT

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Virus infection reduced the number of florets and increased the number of aborted buds per inflorescence. Florets of geraniums infected with tomato ringspot virus contained more aborted anthers and a greater percentage of nonviable pollen grains than did florets of healthy and of tobacco ringspot virus-infected plants. Plants infected with tomato ringspot virus when self-pollinated, produced fewer fruit per pollination, seed per pollination, and viable seed than did healthy self-pollinated plants. Though both tobacco and tomato ringspot viruses were transmitted to the seed through the maternal tissue, only tomato ringspot virus was transmitted by the pollen.

Tobacco ringspot (TRSV) and tomato ringspot (TomRSV) viruses have been isolated from geranium (*Pelargonium* × *hortorum* Bailey) (19, 20). These viruses are transmitted by the nematode *Xiphinema americanum* (11, 32). Seed transmission is characteristic of most nematode-borne viruses (22, 23), and TRSV is seed-transmitted in soybean (9), petunia (16), tobacco (33), and *Gomphrena globosa* (29). Tomato ringspot virus is seed-transmitted in soybean (17), red clover (15), strawberry, and raspberry (3, 24).

Viruses may become seed-borne via the female gametophyte tissue or pollen (28). Pollen transmission has been reported for raspberry bushy dwarf virus in raspberry (25), raspberry ringspot virus in strawberry (23), and for necrotic ringspot and prune dwarf viruses in cherry (12, 34). Certain strains of TRSV have caused pollen sterility (28). Flowers produced on petunia infected with TRSV appeared normal, but were sterile (16). Tomato ringspot virus caused 45% pollen abortion as compared with 32% in virus-indexed raspberries (10). Pollen germination in TomRSV-infected grapes was about 12%, whereas the germination percentage of the healthy pollen was 51% (13). Seed set in TRSV- and TomRSV-infected soybeans was less than in healthy soybeans (9, 18). Seed maturity was delayed in TRSV-infected soybeans (8).

Tobacco- and tomato ringspot viruses reduced emergence of soybean seedlings; germination of controls and of TRSV- and TomRSV-infected seeds was 86, 44, and 32%, respectively (17). In contrast, a recent report indicated that germination of seed from TRSV-infected soybeans was not significantly different from healthy seed (26).

Tobacco ringspot virus-infected petunias exhibited stunting and reduced flower production (16). The flowers of TomRSV-infected hydrangeas were generally smaller than those from noninfected plants, and tended to open in an irregular manner (4). Tomato ringspot virus in gladiolus caused stunting and shortening of the floral spike (2).

The objective of this study was to determine the effects of TRSV and TomRSV on the reproductive tissues of *Pelargonium* × *hortorum*.

MATERIALS AND METHODS

The TRSV was isolated from soil around peach trees showing stem pitting (30), and the TomRSV was obtained originally from A. F. Ross of Cornell University, Ithaca, N.Y. Both isolates were maintained in *Cucumis sativus* L. The geraniums, cultivar Nittany Lion Red, were grown from seed in a steam-treated (1) mixture of soil, sphagnum peat, and perlite (1:1:1, v/v) and fed daily through a plastic tube irrigation system (Chapin Watermatics, Inc., Watertown, NY 13601) with 15 g of Peters 15-15-15 geranium fertilizer (Robert B. Peters Co., Inc., Allentown, PA 18105) per liter of tap water. Each month, supplements of 0.03 g/liter sodium borate and 0.54 g/liter magnesium sulfate were added to the nutrient solution.

Geraniums were indexed for virus by grinding 0.1 g of tissue in a mortar containing 0.5 ml of 0.05 M phosphate buffer (pH 7.1) plus 4% polyethylene glycol 6000 (buffer +

PEG), and mechanically inoculating 7- to 10-day-old cucumber cotyledons dusted with Carborundum. The identity of the viruses producing local lesions or systemic infection was confirmed serologically (14). The experimental geraniums were inoculated using techniques similar to those used for indexing except that healthy, TRSV-, or TomRSV-infected cucumbers were ground only in phosphate buffer.

The cultivar Nittany Lion Red was chosen because it produced uniform progeny from seed. In an attempt to simulate conditions of commercial propagation, successive vegetative cuttings from the seedling geraniums were used. Therefore, noncharacteristic plant responses resulting from the shock phase of virus infection were eliminated. Two-month-old seedling geraniums were inoculated with TRSV and TomRSV or were treated only with buffer. These inoculated seedlings were designated as Generation One. Vegetative cuttings from these seedlings were taken to establish the experimental geraniums designated as Generation Two. Seedlings of a second group were inoculated and utilized in examining the shock effect (i.e., initial plant response to virus infection) on pollen viability and anther abortion.

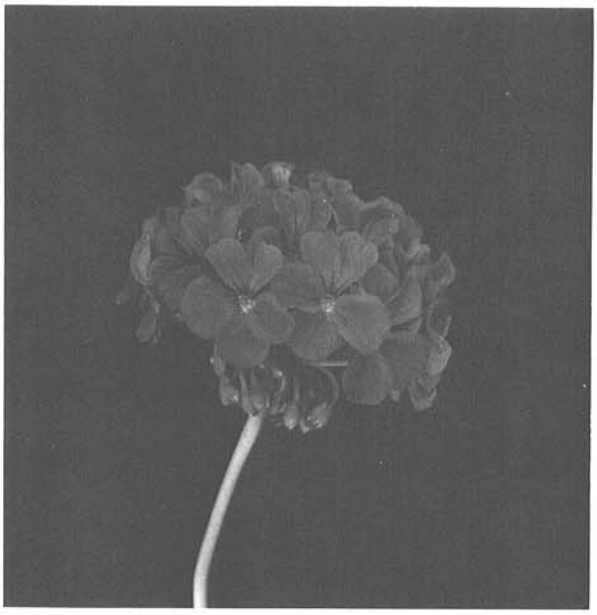

Fig. 1. Appearance of inflorescence from healthy *Pelargonium* × *hortorum*.

Fig. 2. Aborted buds on mature inflorescences of *Pelargonium* × *hortorum* infected with tobacco ringspot virus.

Establishment of TRSV and TomRSV in Nittany Lion Red, Generation One.—Sets of 23 plants were mechanically inoculated with either TRSV, or TomRSV, or were treated only with phosphate buffer to serve as controls. All plants were indexed monthly for the presence of the virus. Six months after inoculation, 13 plants of each treatment were harvested and the remaining 10 were utilized in cross-pollination trials.

Establishment of Generation Two geraniums.—Before initiating cross-pollinations on the first generation plants, cuttings were taken for the second-generation experiments. One cutting, 3.5 internodes in length, was taken from each 8-mo-old seedling geranium, rooted, and potted in the (1:1:1, v/v) soil medium. The cuttings were watered daily with the nutrient solution and were disbudded as necessary to insure 6 wk of vegetative growth.

Floret production.—The number of florets and aborted buds in the inflorescence was counted for each of the first five inflorescences on each of the 23 plants in both generations. Buds that were necrotic and did not develop into expanded florets (Fig. 1) were designated aborted buds (Fig. 2). The number of potential florets per inflorescence was calculated by adding the number of florets per inflorescence to the number of aborted buds per inflorescence.

Pollen viability and anther abortion.—Pollen viability and anther abortion were determined for three successive inflorescences on three sets of plants. First generation plants (age, 14 mo) were used for the first set of pollen viability and anther abortion trials, second generation plants were used for the second set of readings, and the second group of seedlings (age, 4 mo) were used to demonstrate the virus shock effect on anther abortion and pollen viability.

Anthers that dehisced less than 24 hr prior to testing were utilized for the pollen viability experiments. Pollen viability was measured by a modified peroxidase reaction test (21, 27). Two drops of a 0.3% hydrogen peroxide solution in 50% ethyl alcohol containing 0.2 g of benzidine were placed on filter paper 0.5 cm × 1.5 cm. Pollen grains were applied to the filter paper with a cotton swab. After 5 min, the pollen grains were examined under the microscope at a magnification of ×400. Pollen grains were designated viable when they were entirely blue or black or their centers or germ tubes were blue or black. Yellow, orange, and clear pollen grains were considered nonviable. Heat-killed pollen grains appeared yellow, orange, or clear to pale green. All the pollen grains in five random fields were counted and categorized.

Six florets per inflorescence were examined for aborted anthers. Aborted anthers were yellow-brown and shriveled, whereas viable anthers were plump and had a reddish pigmentation (5).

Cross-pollinations, self-pollinations, and seed production.—Cross-pollinations of geraniums were

made to determine if TomRSV or TRSV was seed- or pollen-transmitted. Cross-pollinations were made between healthy seed parents and TRSV- or TomRSV-infected pollen parents, and between TRSV- or TomRSV-infected seed parents and healthy pollen parents.

Ten plants of each treatment in Generation One were retained for the pollination trials. Four TRSV-infected plants, four TomRSV-infected geraniums, and two healthy plants were used as pollen parents. These were maintained apart from the seed parents. Eight control plants, five TRSV-infected geraniums, and five TomRSV-infected geraniums were used as seed parents. The florets of the seed parents were emasculated and after 24 hr the mature stigmas pollinated (6). The forceps were disinfested after each cross by heating for 12 hr at 80 C.

The cross-pollinations were: five TRSV-infected seed parents × two healthy pollen parents, five TomRSV-infected seed parents × two healthy pollen parents, four healthy seed parents × four TRSV-infected pollen parents, and four healthy seed parents × four TomRSV-infected pollen parents. Pollen from control plants was pooled to obtain enough pollen to pollinate the 10 infected seed parents.

Each pollinated floret was labeled with the date of pollination and the seed and pollen source. At harvest, the number of pollinations, fruit, and seed from each inflorescence was recorded. This information was used to determine the number of seed per pollination, the number of seed per fruit, and the percentage of fruit developed per pollination. Fruit set occurred if the receptable elongated after pollination. Date of the first seed harvested per inflorescence was recorded so the number of days to maturity, from first pollination to first mature seed, could be determined.

When approximately 150 seeds were collected from each cross, the pollen parents were self-pollinated. The self-pollinated florets were emasculated to standardize the method of pollination between the self-pollinations and the cross-pollinations. Pollen from another inflorescence on the same plant was applied to the mature stigmas. The variables examined for the cross-pollinations also were recorded for the self-pollinations.

Approximately 150 seeds of each cross and all the seed from the self-pollinations were scarified and sown individually in 5.7 cm diameter pots. Each seed was scarified with a pin (one pin per seed) just enough to expose the endosperm (7). The seeds were sown in sphagnum peat media. The number of seeds that germinated from each cross was recorded. Each seedling was indexed at the six-leaf stage.

Statistical analysis.—An analysis of variance was conducted for all the data unless otherwise stated (31). Treatment means were separated by Duncan's modified (Bayesian) least significant difference test (DLSD). Values for the number of aborted buds per inflorescence were transformed by $\log_{10}[x(1)+1.0]$ because treatment effects seemed to be more multiplicative than additive (R. Craig, *personal communication,* 1973). Pollen viability and aborted anther data were arcsin-transformed to

TABLE 1. Flower quality measurements on inflorescences of *Pelargonium* × *hortorum* inoculated with tobacco ringspot virus (TRSV) or tomato ringspot virus (TomRSV)

Variable	Inflorescence no.	Treatment means[y]		
		Control	TRSV	TomRSV
Generation One[z]:				
Florets per inflorescence	1	55.1 a	14.2 a	18.0 b
	2	52.4 a	24.2 b	25.5 b
	3	49.6	35.2	47.2
	4	76.6 a	37.4 b	43.4 b
	5	86.2 a	47.6 b	62.9 b
Aborted buds per inflorescence	1	9.8 a	47.9 b	39.5 b
	2	4.3 a	35.0 b	27.5 b
	3	4.5 a	25.8 b	28.4 b
	4	9.6 a	35.2 b	38.5 b
	5	2.3 a	27.0 b	13.1 b
Generation Two:				
Florets per inflorescence	1	73.3	63.6	70.4
	2	81.4 b	62.4 b	74.4 ab
	3	73.4	62.0	67.7
	4	69.8 b	49.0 b	57.4 b
	5	56.0	48.0	53.7
Aborted buds per inflorescence	1	3.1	14.2 b	8.4 b
	2	4.9 a	22.0 b	13.9 b
	3	4.7 a	18.7 b	10.1 b
	4	7.2 a	18.9 b	9.6 ab
	5	4.1 a	18.1 b	6.3 a

[y]Means followed by the same letter or no letter are not significantly different [Duncan's modified (Bayesian) least significant difference test, $P = 0.05$]. Comparisons may be made in a horizontal direction for each generation.

[z]Inoculated geraniums are designated as Generation One, whereas cuttings from these plants are Generation Two.

equalize the variability among treatments. Seed production and germination data on the seven treatments, namely the four cross-pollinations and the three self-pollination trials, were examined with a one-factor analysis of variance using the data for each inflorescence per plant as an observation (31).

RESULTS

Floret production.—The total number of potential florets per inflorescence was not affected by the virus treatments in either generation. The number of florets per inflorescence that completed their development was greater in the healthy plants than in the virus-infected geraniums. The number of aborted buds was greater in the virus-infected plants than in the controls for both generations (Table 1).

TABLE 2. Viability of pollen of *Pelargonium* × *hortorum* collected from inoculated seedlings of Generation One and Generation Two cuttings

Pollen source	Viable pollen[z] (%)		
	Control	TRSV	TomRSV
Generation One (8 mo)	77.6 a	68.6 ab	61.2 b
Generation One (14 mo)	65.5 a	57.4 ab	47.3 b
Generation Two Shock phase (4 mo)	69.5 a	55.8 b	45.4 c

[z]Means followed by the same letter are not significantly different [Duncan's modified (Bayesian) least significant difference test, $P = 0.05$]. Comparisons may be made in a horizontal direction.

TABLE 3. Percentage of aborted anthers of *Pelargonium* × *hortorum* as affected by tobacco ringspot virus (TRSV) and tomato ringspot virus (TomRSV)

Anther source	Anther abortion[z] (%)		
	Control	TRSV	TomRSV
Generation One (16 mo)	11.8 a	12.2 a	21.4 a
Generation Two Shock phase (4 mo)	13.9 a	15.8 a	27.6 b
	9.6 a	13.8 a	28.2 b

[z]Means followed by the same letter are not significantly different [Duncan's modified (Bayesian) least significant difference test, $P = 0.05$]. Comparisons may be made in a horizontal direction.

Pollen viability and anther abortion.—A significant reduction in pollen viability occurred in the TomRSV-infected geraniums (Table 2). Only in the second-generation cuttings did TRSV-infected geraniums have a significant decrease in pollen viability.

Tomato ringspot virus-infected geraniums had a higher incidence of anther abortion than did either the healthy or TRSV-infected plants (Table 3). Anther abortion in tobacco ringspot virus-infected geraniums was slightly greater than in the controls, but the increase was not significant in any of the experiments.

Seed production.—Results of the seed production and germination trials are listed in Table 4. Variances for all but the seed per pollination analyses were heterogeneous; therefore, a *t*-test for unequal variances and unequal number of observations (R. Craig, *personal communication,* 1973) was performed with all treatments compared to the control. Much of the variability that occurred within treatments accounting for the heterogeneity of variances was a result of crosses using the TomRSV-infected plants.

The percentage of fruit per pollination for the TomRSV-infected self-pollinated plants (TomRSV-self), the TomRSV-infected seed parents × control pollen parents (TomRSV × C) and the control seed parents × the TomRSV-infected pollen parents (C × TomRSV) was significantly lower than the percentage of fruit produced by the self-pollinated controls. The number of seed produced per pollination with the TomRSV-self was lower than the control self and control seed parents × TRSV-infected pollen parents (C × TRSV). However, the cross between the TRSV-infected seed parents and control pollen parents (TRSV × C) also had a reduction in seed set. Differences among treatments for the number of seed per fruit were not statistically separable. The number of days to seed maturity did not differ significantly among treatments. Seeds from the TomRSV-self had a germination percentage of only 34.1% as compared to 65.0% of the controls. None of the

TABLE 4. Seed production and germination data for the self- and cross-pollinated geraniums *(Pelargonium* × *hortorum)* inoculated with tobacco ringspot virus (TRSV) and tomato ringspot virus (TomRSV)

Variable	Treatment means[x]						
	Control-selfed	TRSV-selfed	TomRSV-selfed	TRSV[y] × Control	TomRSV × Control	Control × TRSV	Control × TomRSV
Fruit/Pollination (%)[z]	80.0	72.1	53.8*	70.3	64.0*	77.1	66.1*
Seed/Pollination	2.0 a	1.7 ab	0.9 ab	1.1 b	1.5 ab	1.7 a	1.2 ab
Seed/Fruit[z]	2.4	2.3	1.4	1.5	2.0	2.2	1.9
Days to seed maturity[z]	33.3	35.2	34.3	37.9	33.4	37.2	35.6
Germination (%)[z]	65.0	66.3	34.1*	72.1	74.1	74.2	68.5

[x]Means followed by the same letter do not differ significantly [Duncan's modified (Bayesian) least significant difference test, $P = 0.05$]. Comparisons may be made in a horizontal direction.

[y]Cross-pollinations are expressed as female × male. Plants treated only with buffer solution served as control.

[z]Asterisk (*) indicates that means are significantly different from the control treatment, $P = 0.05$ (*t*-test for unequal variances). Comparisons may be made in a horizontal direction.

TABLE 5. Seed transmission in self- and cross-pollinated, healthy and tobacco ringspot virus (TRSV)- or tomato ringspot virus (TomRSV)-infected geraniums *(Pelargonium × hortorum)*

Treatment (female × male)	Ratio of infected seedlings to indexed seedlings	Virus transmission (%)
Control × Control	0/162	0.0
TRSV × TRSV	4/69	5.8
TomRSV × TomRSV	1/27	3.7
TRSV × Control	18/486	3.7
TomRSV × Control	64/551	11.6
Control × TRSV	0/452	0.0
Control × TomRSV	3/393	0.8

other treatments affected seed germination.

Seed transmission.—Tomato ringspot virus was transmitted to the seed of geranium by the pollen and the ovule (Table 5). Tobacco ringspot virus was transmitted by the ovule but not the pollen in any of the 452 seedlings. Seed transmission in self-pollinated TomRSV- and TRSV-infected plants was 3.7% and 5.8%, respectively. No seed transmission occurred in the self-pollinated controls. The number of seeds indexed in each treatment is listed in Table 5. The total number of seedlings indexed for the TomRSV-self treatment was only 27. The reduction in viable anthers, reduction in fruit set, and the reduction of almost 50% in germination account for the small number of seedlings. Similarly, the number of seedlings indexed from the TRSV-self treatment was low owing to the effect of the virus on the reproductive tissues. The viruses never infected the seed parents.

DISCUSSION

Symptoms of TRSV and TomRSV infection appeared primarily in the reproductive tissues. Although reductions in viable reproductive structures were examined individually, they should be combined and compared to the noninoculated plants to illustrate the full effects of these viruses. Tobacco ringspot virus infection incited abortion in 34.1% of the florets. Of the remaining florets, 15.1% had aborted anthers and only 86% of these anthers produced viable pollen. Seed production also was reduced; the self-pollinated TRSV-infected geraniums produced 15% fewer seeds per pollination. Following TomRSV infection, 77.2% of the florets matured; the viability of the anthers and pollen was 45.9% and 73% respectively. Seed was produced by 45% of the remaining functional tissues that were pollinated and only 52.5% of the seed germinated. Because of this extreme loss of seed productivity, the seed industry would benefit from using virus-indexed seed and pollen parents.

Pollen transmission, pollen sterility, and seed transmission are important aspects for a plant breeder to consider. Breeding programs may be adversely affected when virus infection reduces viable reproductive tissue or alters phenotypic traits which could be mistaken as genotypic changes. A plant considered to be genetically male-sterile merely might be expressing symptoms of virus infection. Pathological aspects of seed transmission should be considered when developing new cultivars.

Symptoms are not expressed only during the shock phase of virus infection. Symptoms generally were more pronounced in the second-generation plants than in the mechanically inoculated seedling geraniums. The variability encompassed in the first generation as well as the more uniform and pronounced symptoms in the second generation suggest that further studies on the effects of viruses on plants should include several generations.

The wide variation in plant response to TRSV and TomRSV infection was striking. For example, the variation in numbers of fruit produced per pollination for virus-infected plants was more than 100 times that determined for the controls. Consequently, even though treatment means often appeared to be different, a statistical separation of them was not readily achieved, even with transformation of the data. Since this variation also would be expressed as a lack of uniformity in plantings of virus-infected geraniums, this nonuniformity is possibly of as much commercial importance as the symptoms on any single plant.

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